3rd International Conference on Neglected and Underutilized Species: for a Food-Secure Africa

Accra, Ghana, 25-27 September 2013

Proceedings

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Cover photo: Nutritious finger millet in the rain, Kolli Hills, India. Credit: M. S. Swaminathan Foundation/O. King

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Foreword

The past decade has seen growing interest in research and development linked to value chains of neglected and underutilized species (NUS). Their nutritional value, their adaptability to marginal environments and hence usefulness for adaptation to climate change, their commercial prospects, as well as their cultural and conservation values have elevated NUS visibility in food and agriculture policies and programmes.

Today, an increasing number of scientists, many of whom are young and forging their careers, carry out research on such crops and trees, often as a part-time activity alongside their work on major commodity and staple crops. But in spite of this, the total body of research on NUS is still limited, as are opportunities to publish results. International scientific conferences can contribute to meeting this need. They serve the multiple purposes of sharing scientific results, nurturing collaboration and networking, and providing a forum for reflection on what the latest scientific evidence means for policy processes.

The *3rd International Conference on Neglected and Underutilized Species for a Food-Secure Africa* was held in Accra, Ghana on 25-27 September 2013 (NUS 2013). This Conference represented the culmination of a three-year project funded by the ACP-EU Science and Technology Programme, 'Building human and institutional capacity for enhancing the conservation and use of neglected and underutilized species of crops in West Africa, and Eastern and Southern Africa'. The project, implemented by six African and two European organizations, held regional meetings in 2010 that identified priority underutilized crops with potential for commercialization in the two African sub-regions. Subsequently, the project trained young African researchers who were working on these priority crops. In ten training workshops, about 250 scientists strengthened their capacity in research project proposal preparation, scientific communication, food and nutrition, experimental design and data management, and value chain development. Many of the trainees took the opportunity to share their research results at this conference, in oral or poster presentations (NUS 2013 Book of Abstracts).

At the NUS 2013 Conference, all presenters were asked to include a slide on the key policy messages emerging from their research. Combined with the discussions at the various sessions these were synthesized into two policy documents: the 'Accra statement for a food-secure Africa', and a more detailed 'Policy Brief: realizing the potential of neglected and underutilized species'.

These Conference Proceedings contain 31 papers presented at NUS 2013. It adds to the existing library of proceedings from two similar international symposia focusing on NUS: Arusha, Tanzania in 2008 and Kuala Lumpur, Malaysia in 2011.

It is our hope that the NUS 2013 conference and these Proceedings contribute both to developing capacity for NUS research among young scientists and to the wider sharing of evidence-based results in this exciting field of science.

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Content

Agronomic constraints to the development of fonio millet (*Digitaria exilis* Stapf) in Senegal

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Abstract

In Senegal, fonio millet (*Digitaria exilis* Stapf) is grown mainly in Casamance and Eastern Senegal, agro-ecological zones located in the southern and eastern parts of the country respectively. Fonio consumption is in high demand in the urban centres of the country and in some developed countries. However, grain yields are still very low (200 - 700 kg/ha). A field survey was conducted in farmers' fields in 88 villages to identify the major constraints of low grain yields of fonio in the main producing areas: Sedhiou and Kolda provinces in Casamance and Tambacounda and Kedougou provinces in Eastern Senegal. The results showed that fonio is grown on sandy clay, sandy or gravelly soils. The fonio fields of 80% farmers were less than 0.5 ha in size. Groundnut is the best preferred previous crop for fonio in a rotation system. Most farmers (70%) used medium maturing landraces (90-day cultivars). Soil was ploughed or scratched before sowing. The sowing techniques consisted of broadcasting seeds which were then introduced later into the soil by scraping or by hand using branches. Fonio fields receive little or no fertilizer while plant protection measures are absent. Weeds are controlled by hand pulling one month after planting. Highest grain yields (2083 kg/ha) were obtained in Kolda and Kedougou provinces while lowest yields were observed in Tambacounda province (780 kg/ha) and Sedhiou province (769 kg/ha). This field survey has allowed the identification of major constraints of fonio production in the southern and southeastern regions of Senegal and led to emphasize cultural practices that may improve fonio yield.

Key words: Fonio, *Digitaria exilis*, field survey, agronomic constraints, Senegal.

Introduction

Fonio millet (*Digitaria exilis* Stapf) is considered as a minor crop compared to rice, corn, millet and sorghum in West Africa. However, it is a very important crop for human diets because of the high methionine and cysteine content in the tiny grains. Fonio is also considered as a staple food for millions of people from Senegal to Lake Chad (Vietmeyer et al., 1996; Vodouhe et al., 2003).

In Senegal, fonio millet is grown in Casamance and Eastern Senegal, agro-ecological zones located in the south and the southeast of the country respectively. In these zones, fonio production was estimated at 1068 tons per annum with grain yields ranging from 200 to 700 kg/ha (DAPS, 2008). This fonio production accounts for less than 1% of the national cereal harvest. In spite of its marginal status, the consumption of dishes made with fonio is increasing in urban and semi-urban areas. Thus, the production of fonio millet has to satisfy this growing demand. However, the agronomic practices in the fonio cropping systems are not well documented at national and regional levels.

The aim of this study was to analyse the fonio cropping systems in the main areas of production in Senegal. The work may allow a better understanding of farming practices in these areas with a focus on the factors limiting fonio yields in farmers' fields.

Methodology

A field survey was carried out in 2008 in four provinces: Kolda and Sedhiou in Casamance, and, Tambacounda and Kedougou in Eastern Senegal. Casamance and Eastern Senegal are agro-ecological zones situated in the south and the southeast of Senegal, respectively (Figure 1). Fonio millet is mainly grown in these two zones. A total of 88 farmers' fields was randomly selected from 34 villages (Table 1). These fields were monitored during the rainy season, from July to November 2008.

The data collected included the following information: the main characteristics of the fonio field (surface, soil type, type and source of seeds, rotation system, etc.), the cultivation practices (land preparation, planting techniques, crop husbandry, etc.) and the average grain yield of fonio. The statistical calculations comprising frequencies, means and standard errors were done using the SPSS package (version 16.0).

Results

Main characteristics of fonio fields

Fonio is grown on 0.69 ± 0.03 hectares per field (Table 2). Overall, 20% of the famers' fields are between 0.5 and 1 hectares whereas only 3.4% of the farmers grow fonio on more than one hectare. The smaller fields tend to be located in the Tambacounda province while the largest are more concentrated in the Kedougou province. Table 3 shows the different types of soil texture observed in the monitored plots. Most of the fonio plots' texture is sandy to sandy-clay in Kolda and Sedhiou provinces or rocky, particularly in Kedougou province. A small proportion of the monitored plots had clay soil or hydromorphic soil (lowland).

Preceding crops of fonio

In 63% of the total plots groundnut was the major crop preceding fonio (Table 4). Other less commonly grown rotation crops are: none (2%), other cereals (6%) and other crops (28.5%).

Farmers interviewed indicated the need of good management of soil fertility, weed control, and in rare cases lack of space as the reasons for preferring groundnut as the major crop preceding fonio in the rotation system. A minority of them choose groundnut to precede fonio apparently with no reason.

Variety used and source of seed

Table 4 shows the types of fonio varieties used in the monitored plots. It was found that producers use three different types of varieties based on the length of their development cycle. The early one (75-90 days) is grown in Kolda and Sedhiou provinces. The medium duration (90-120 days) is the most widely cultivated and covers fields across the two regions. The long duration variety (more than 120 days) is the least used by farmers. It is mainly grown in the province of Kedougou. Seeds used by farmers are obtained from their personal stock, the local market or provided by the extension services or NGOs (Table 5). It was found that the most common source of fonio seeds was farmers' personal stock, except in Tambacounda where seeds are supplied by an NGO called 'ENDA Tiers Monde' with its programme to increase fonio production in this part of the country. Seeds from local markets are used in all areas and they play a very important role, especially in Kolda province.

Cultural practices

Most fonio fields do not receive fertilizers. Out of the 88 plots, only 2% located in Kolda province were fertilized with urea. Similarly, out of the 88 plots monitored, only 2.3% were fertilized with manure (139 kg/ ha). Weeding was found to be a common practice among all farmers (Table 6). In Kedougou province, weeding was almost routine (91%) whereas in Sedhiou province only 30% of the plots were weeded. Weeding is typically done manually approximately 30 days after sowing. None of the plots was treated with pesticides.

Grain yield

Farmers included in this study reported that when fonio reaches maturity the colour of the grain changes with the panicle lodging due to the weight exerted by the matured grains. They also observed that a matured fonio field attracts many birds. Harvest is done manually and takes place 3-4 days after maturity. Grain yield varied considerably among the fields under study (Table 7). Highest yields (2083 kg/ha) are observed in Kolda and Kedougou provinces while lower yields are recorded in Tambacounda province.

Discussion

This agronomic study of fonio has highlighted the variability of agricultural practices. The results on the size of fonio plots showed that it rarely exceeds one hectare. Indeed, from the point of view of the decision-makers, and for agricultural scientists, fonio is a minor crop. Fonio production is limited compared to that of other cereals (rice, maize, millet, sorghum). A decrease of areas sown with fonio has been reported by Gueye (2008). This situation can be explained by the tediousness of the post-harvest techniques and food processing in comparison with other cereals. Fonio fields of more than one hectare are located in Kedougou province. This is due to the presence of several economic interest groups (EIG) and non-governmental organizations (NGOs) which are promoting fonio value addition in this province. In Tambacounda province, the fonio fields are generally less than 0.25 hectares in size and fonio is regarded rather as a secondary crop despite the intervention of various NGOs.

Groundnut is the main crop which precedes fonio. According to farmers' knowledge, groundnut plays an important role in soil fertility management and weed control. Because of root symbiosis with rhizobia (bacteria), groundnut fixes atmospheric nitrogen and enhances nutrient uptake in the absence of fertilization (Cattan et al., 2001). It also facilitates the management of weeds since it is a root crop, thus avoiding any competition with fonio but also reduces the necessity of weeding the crop. Fonio monoculture is rarely practiced in the study area. According to Ndiaye et al. (2008), rotating cowpea with fonio reduces the density of *Macrophomina phaseolina*, a fungus which causes root rot or wilting when cowpea is grown in consecutive years.

The late-maturing landraces of fonio are less preferred by farmers due to the progressive reduction of rainfall. Indeed, late maturing varieties were not suitable for such climatic conditions. In addition, beetles that appear at the end of the rainy season damage the fonio grains, hence the need to introduce early maturing and adapted varieties.

In Kedougou and Sedhiou provinces, fonio seeds are self-produced (Fall et al., 2005). This is due to the fact that fonio has a strong ethno-cultural connotation in these parts of the country. In Kolda province, seeds are obtained from the local markets. Indeed, this is an area characterized by significant trade flows, especially with Diaobé Market which is sub-regional in scale. Most of these seeds come from Guinea (Gueye, 2008).

The highest yield (2083 kg/ha) is observed in the region of Kedougou province. This higher productivity could be explained by the quality of the soils which are more fertile. In addition, the soil texture is gravelly, which is a limiting factor for weed growth in fonio fields.

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References

DAPS. 2008. Résultats définitifs de la campagne 2007-2008. DISA/DAPS/MAE, Dakar (Sénégal), 14 pp.

Fall C.A., Fofana A., Baldé S. 2005. Recherche agronomique sur le Fonio (*Digitaria exilis* Stapf.) au Sénégal : diagnostic des systèmes de production et sélection de variétés performantes. ISRA Dakar (Sénégal), 12 pp.

Guèye O. 2008. Etude de la chaine de valeurs du fonio au Sénégal. Rencontre des acteurs et partenaires de la filière fonio organisé par l'USAID, le 26 mai 2008 à Tambacounda (Sénégal), 27 pp..

Ndiaye M., Termorshuizen J., Bruggen H.C. 2008. Effect of rotation of cowpea (*Vigna unguiculata*) with fonio (*Digitaria exilis*) and millet (*Pennisetum glaucum*) on *Macrophomina phaseolina* densities and cowpea yield. African Journal of Agriculture Research 3:37-43.

Cattan P., Letourmy P., Zagre B., Minougou A., Compaoré E. 2001. Rendement de l'arachide et du sorgho en rotation sous différents itinéraires techniques au Burkina Faso. Cahiers agricultures 10: *59-72.*

Vodouhe S.R., Zannou A., Achigan Dako E. 2003. Actes du Premier Atelier sur la Diversité Génétique du Fonio (*Digitaria exilis*) en Afrique de l'Ouest. Conakry, Guinée, du 04 au 06 Août 1998. Institut International des Ressources Phytogénétiques (IPGRI), Rome, Italie.

Vietmeyer N.D., Borlaugh N.E., Axtell J., Burton G.W., Harlan J.R., Rachie K.O. 1996. *Fonio* (Acha). National Research Council editor. In: Lost crops in Africa, Volume I, BOSTID Publication, National Academy Press, Washington D.C. pp. 59-75.

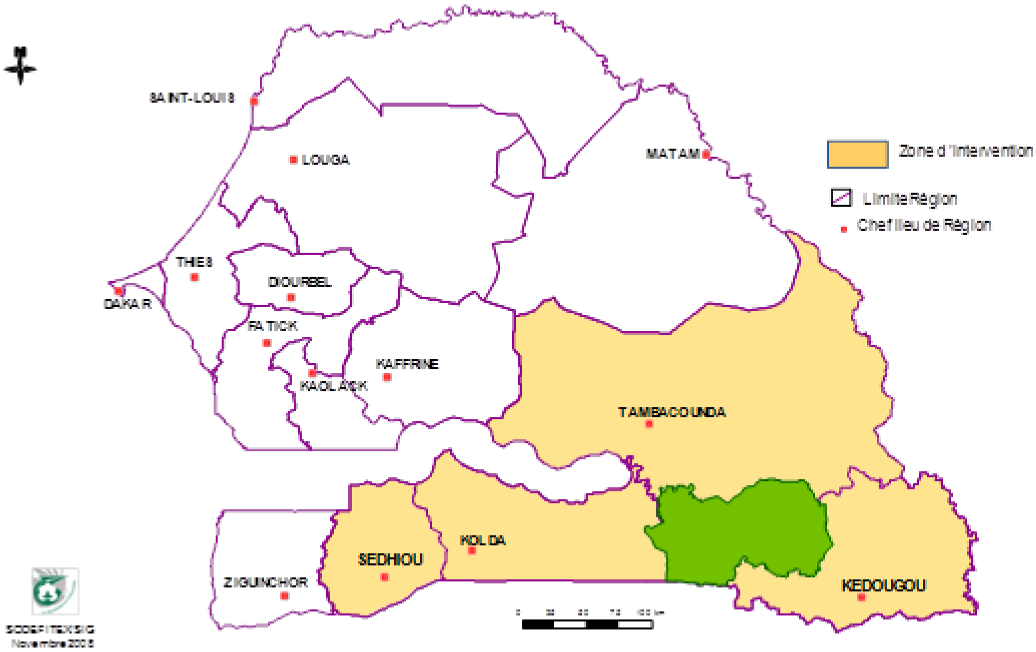

Figure 1: Localization of study area

Table 1. Number of villages and monitored farmers' fields

	Eastern Senegal		Casamance	
	Tambacounda	**Kedougou**	**Kolda**	**Sedhiou**
Number of villages	08	05	11	10
Number of fields	19	14	27	28

Table 2. Size of monitored farmers' fields

	Cultivated area (%)			
Province	**Less 0.25 ha**	**0.25 - 0.5 ha**	**0.5 -1 ha**	**More than 1 ha**
Kolda	55.6	25.9	14.8	3.7
Sedhiou	46.4	28.6	21.4	3.6
Tamba	63.2	31.5	5.3	0.0
Kedougou	28.6	35.7	28.6	7.1
Mean	50.0	29.5	17.0	3.4

Table 3. Types of soil texture in the monitored farmers' fields

Province	**Soil Texture/Soil Type**				
	Sandy	**Sandy-Clay**	**Clay**	**Gravelly**	**Inland**
Kolda	25.9	59.3	7.4	0.0	7.4
Sedhiou	57.1	39.3	0.0	0.0	3.6
Tamba	36.8	47.4	0.0	15.8	0.0
Kedougou	0.0	0.0	7.1	92.9	0.0
Mean	34.1	40.9	3.4	18.2	3.4

Table 4. Crops preceding fonio and landraces used in monitored farmers' fields

Province	Crop preceding fonio (%)				Type of landrace used (%)		
	Fonio	Groundnut	Main cereals	Other crops	Early maturing	Medium maturing	Late maturing
Kolda	0.0	65.4	7.7	26.9	30.8	65.4	3.8
Sedhiou	3.7	81.5	0.0	14.8	30.8	65.4	3.8
Tambacounda	0.0	47.4	10.5	42.1	16.7	77.8	5.6
Kedougou	8.3	41.7	8.3	41.7	0.0	81.8	18.2
Mean	2.4	63.1	6.0	28.5	23.5	70.4	6.2

Table 5. Sources of seed used in the monitored farmers' fields

Province	Source of seeds			
	Farmers' stock	Local markets	Farmers' stock and local markets	Extension services
Kolda	50.0	38.5	7.7	3.8
Sedhiou	88.5	11.5	0.0	0.0
Tambacounda	31.6	5.3	0.0	63.2
Kedougou	83.3	16.7	0.0	0.0
Mean	63.4	19.3	2.4	15.7

Table 6. Weeding and the period of weeding according to fonio farmers

Province	Plots weeded (%)	Period of weeding (days after sowing)
Kolda	73.1	25
Sedhiou	29.6	32
Tambacounda	68.4	29
Kedougou	91.7	35
Mean	60.7	30

Table 7. Fonio grain yield recorded in the monitored farmers' fields

Province	Grain yield (kg ha^{-1})		
	Maximum	Minimum	Mean
Kolda	2083	625	1160
Kedougou	2083	458	1160
Tambacounda	1687	208	780
Sedhiou	1437	354	769

Yield response of selected taro (*Colocasia esculenta*) landraces from South Africa to irrigated and rain-fed field conditions

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Abstract
Taro (*Colocasia esculenta*) is an important underutilized crop in sub-Saharan Africa with limited agronomic research. It is mainly cultivated by subsistence farmers, mostly women, using landraces of which little is known concerning their agronomy and drought tolerance. The aim of this study was to evaluate drought tolerance mechanisms of three taro landraces collected from the Umbumbulu rural area in KwaZulu-Natal under field conditions. These were Dumbe-dumbe (DD), Dumbe lomfula (DL) and KwaNgwanase (KW) landraces. Field trials were undertaken at Roodeplaat, Pretoria, in September 2010, laid out in a split-plot design arranged in a randomised complete block design (RCBD), with irrigation (irrigated vs. rain-fed) as main plots and landraces (DD, DL and KW) as sub-plots, and replicated three times. Soil water content was monitored weekly using the gravimetric sampling method. Emergence, plant height, leaf number, leaf area index, and stomatal conductance were determined weekly. Yield and yield components were determined at harvest. Emergence of taro landraces was slow and showed highly significant differences ($P<0.001$) between landraces with the DL landrace never forming a good stand. Growth of taro landraces (plant height, leaf number and leaf area index) as well as stomatal conductance were all lower under rain-fed than in irrigated conditions. The DD landrace showed moderate sensitivity to limited water availability under rain-fed conditions than the KW and DL landraces. Results of yield and yield components showed that, although not statistically significant, total biomass, harvest index, corm number and mass and final yield were lower under rain-fed compared to irrigated conditions. However, there were highly significant ($P<0.001$) differences between landraces − DL failed to form any yield under both rain-fed and irrigated conditions while the DD landrace yielded higher than the KW landrace. Overall, the DD landrace showed better adaptation to limited water availability and exhibited drought avoidance and escape mechanisms.

Introduction
Although South Africa is regarded as food secure, it still faces challenges of food insecurity and malnutrition at the household level (de Klerk et al., 2004). These challenges may be solved by encouraging research on previously neglected and underutilized crops (NUS). In South Africa, taro is one such underutilized, traditional and "indigenised" crop which has been identified as having potential to contribute to food security in the subtropics as a dryland crop (Modi and Mabhaudhi, 2013). It is 'indigenised" inasmuch as although it originated outside of South Africa (Lebot, 2009), local cultivation and natural selection over hundreds of years have produced local landraces (Schippers 2002, 2006).

In South Africa, taro is commonly known by its Zulu name '*amadumbe*' and is mainly cultivated along the coastal areas and hinterland of KwaZulu-Natal province (Modi, 2007), with little cultivation occurring inland. Its cultivation along the coastal lines may be due to the widely held perception that taro is 'water loving' (Mabhaudhi et al., 2013a). This may, in part, explain its current low levels of utilization. However, recently, there has been an increase in taro production owing to improved access to niche markets by farmers. Taro is now being promoted as a healthy alternative to the exotic and more popular potato (*Solanum tuberosum*). Recent studies have also evaluated the possibility of using taro for making chips (Mare, 2010). However, research describing such basic aspects as agronomy, water use and drought tolerance of taro landraces cultivated by subsistence farmers currently lags behind. Availability of such information would go some way in promoting and expanding taro production beyond current levels. In this study, it was hypothesized that local landraces may have evolved to acquire drought tolerance over years of farmer and natural selection, often under sub-optimal conditions. The aim of this study was therefore to identify and determine drought tolerance mechanisms in selected taro landraces collected from rural areas in KwaZulu-Natal.

Materials and methods

Plant material

Three taro landraces were collected from two locations in KwaZulu-Natal. One was from KwaNgwanase (KW) (27°1′S; 32°44′E) and two (Dumbe-Dumbe (DD) and Dumbe Lomfula (DL) were from Umbumbulu (29°36′S; 30°25′E). The KW and DL landraces are dasheen types while DD is an eddoe type (Mabhaudhi and Modi, 2013). Prior to planting, corms of all three landraces were initially selected for uniform plant size (Singh et al., 1998) in order to eliminate propagule size effects. Following this, corms were treated with a combination fungicide and bactericide (Sporekill®) and thereafter sprouted in vermiculite (30°C, 90% RH).

Experimental design

Field trials were planted at the Agricultural Research Council's Roodeplaat Research Station (25°60′S; 28°35′E; 1 160 m a.s.l.) on the 10th of September, 2010. Roodeplaat is classified as a semi-arid zone with average seasonal rainfall (November to April) of ~500 mm, which is highly variable with maximum precipitation in December and January. Daily maximum and minimum temperature averages for Roodeplaat are 34°C and 8°C, respectively, in summer (November to April) [Agricultural Research Council – Institute for Soil, Climate and Water (ARC-ISCW), undated]. The soil at Roodeplaat was classified as a sandy loam (USDA taxonomic system, undated).

The experiment was designed as a split-plot laid out in a randomized complete block design with three replications. The main factor was irrigation: rain-fed and full irrigation. The three landraces (DD, DL and KW) were the sub-factors. The main plots were 207.4 m^2 each and were spaced 10 m apart to avoid water sprays from irrigated plots from reaching rain-fed plots. Sub-plot size was 17 m^2 with an inter-plot spacing of 1 m, and plant spacing of 1 x 0.5 m. Rain-fed trials were established with irrigation to allow for maximum plant stand. Thereafter, no supplementary irrigation was supplied. For the irrigated trials, irrigation scheduling was to meet 100% reference evapotranspiration (ET_o). Temperature and rainfall data for the duration of the experiments were monitored using an automatic weather station situated within the farm.

Agronomic practices

Prior to land preparation, soil samples were taken and submitted for soil chemical and physical analyses at the ARC-ISCW. Land preparation involved disking and rotovating to achieve a smooth and even seed bed. Based on soil fertility results, an organic fertilizer, Gromor Accelerator® (30 g N kg^{-1}, 15 g P kg^{-1}, 15 g K kg^{-1}), was applied (at 5330 kg ha^{-1}) at planting. Fertilizer application was split into two, half at planting and the balance applied 10 weeks after planting. No pesticides and herbicides were used during the experiment. Routine weeding was done by hand-hoeing.

Data collection

Data collected included weekly counts of emergence until no further emergence was observed. Thereafter, plant height and leaf number were determined weekly. Leaf area index was measured using the LAI2200 Canopy Analyser (LI-COR, Inc. USA & Canada). Stomatal conductance was measured from the abaxial surface of the 2nd youngest, fully expanded and fully exposed leaf using a steady state leaf porometer (Model SC-1, Decagon Devices, USA). Yield and yield components (biomass, harvest index and corm number per plant) were determined at harvest.

Data analyses

Data were subjected to statistical analysis of variance (ANOVA) using GenStat® (Version 14, VSN International Ltd, UK). Duncan's multiple range test was used to separate means at the 5% level of significance.

Results and discussion

Time to 90% emergence was on average >49 days (Figure 1). Dumbe-dumbe emerged significantly ($P<0.001$) faster than KwaNgwanase (KW) and Dumbe Lomfula (DL). The DL landrace never established a good stand. Slow establishment has previously been reported by several authors (Mare, 2010; Mabhaudhi et al., 2013a, 2013b) who observed that taro landraces generally took up to 70 days to establish. This suggests that taro landraces may not be water-use efficient at the establishment stage. Mabhaudhi (2012) suggested that the use of headsetts/cuttings (*huli*), as opposed to corms, as planting material could result in better emergence.

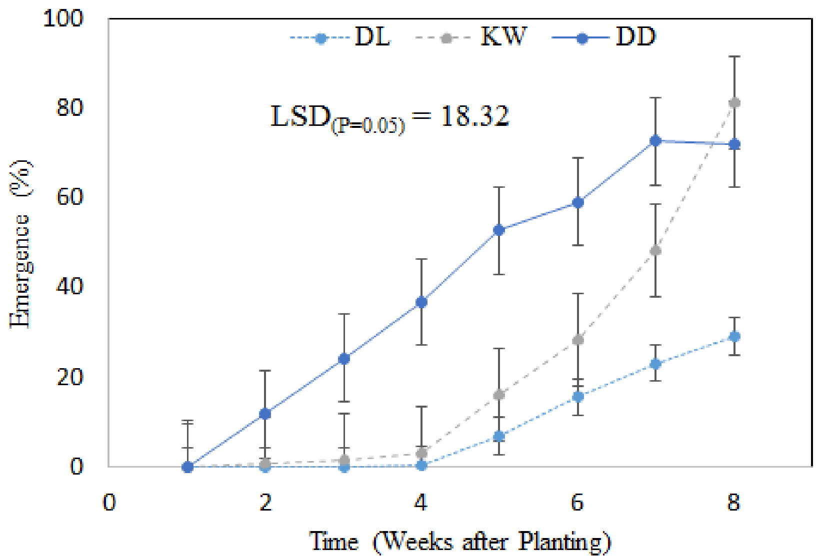

Figure 1. Weekly emergence (%) of taro landraces (DD, DL & KW) across irrigated and rain-fed conditions.

Results showed that stomatal conductance was significantly (P<0.001) higher (>100%) under irrigated than rain-fed conditions (Figure 2). The trend showed that the DD landrace had better stomatal regulation compared with the DL and KW landraces under both irrigated and rain-fed conditions. This confirmed earlier results (Mabhaudhi et al., 2013a, 2013b; Mabhaudhi, 2012) that DD was able to adapt to stress by regulating its stomatal conductance, while DL and KW landraces had lesser stomatal control. The higher stomatal conductance in DL and KW may be a function of their leaf size (data not shown). Dumbe Lomfula and KW have larger leaves, compared to DD (Mabhaudhi and Modi, 2013) which implies a larger surface area for transpiration. Stomatal regulation is a drought avoidance mechanism (Levitt, 1972; Turner, 1986); the DD landrace exhibited drought avoidance mechanisms, especially under rain-fed conditions.

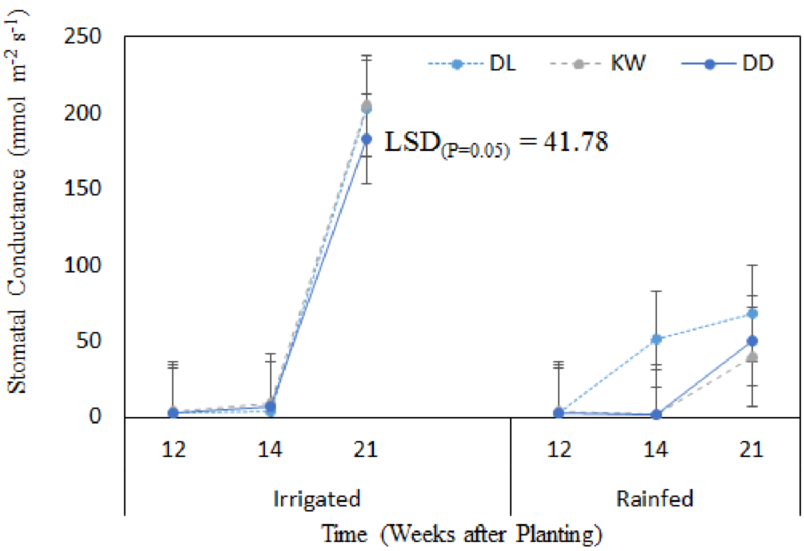

Figure 2. Stomatal conductance (mmol m^{-2} s^{-1}) of taro landraces (DD, DL & KW) under irrigated and rain-fed field conditions.

The essence of drought avoidance is to minimize crop water use, and morphological changes can assist the plant in this regard. Reduced plant height, leaf number and leaf area index contribute towards reducing plant water use (Mitchell et al., 1998), thereby avoiding drought. As such, drought avoidance mechanisms are associated with improved water use efficiency under drought conditions (Blum, 2005). Results of the current study showed that despite lack of statistical significance, with the exception of leaf area index, all canopy characteristics (plant height, leaf number and leaf area index) were lower under rain-fed relative to irrigated conditions (Figs. 3 and 4). This suggested that taro landraces avoided drought through lowering canopy size and hence surface area available for transpiration. This was clearer in the DD landrace than the DL and KW landraces which typically have large canopy sizes (Mabhaudhi and Modi, 2013). The moderate reduction in canopy size by the DD landrace under rain-fed conditions, together with its demonstrated ability to regulate stomatal aperture further suggested that the landrace had drought avoidance mechanisms. This concurred with findings from separate studies involving similar landraces (Mabhaudhi, 2012; Mabhaudhi et al., 2013a, 2013b).

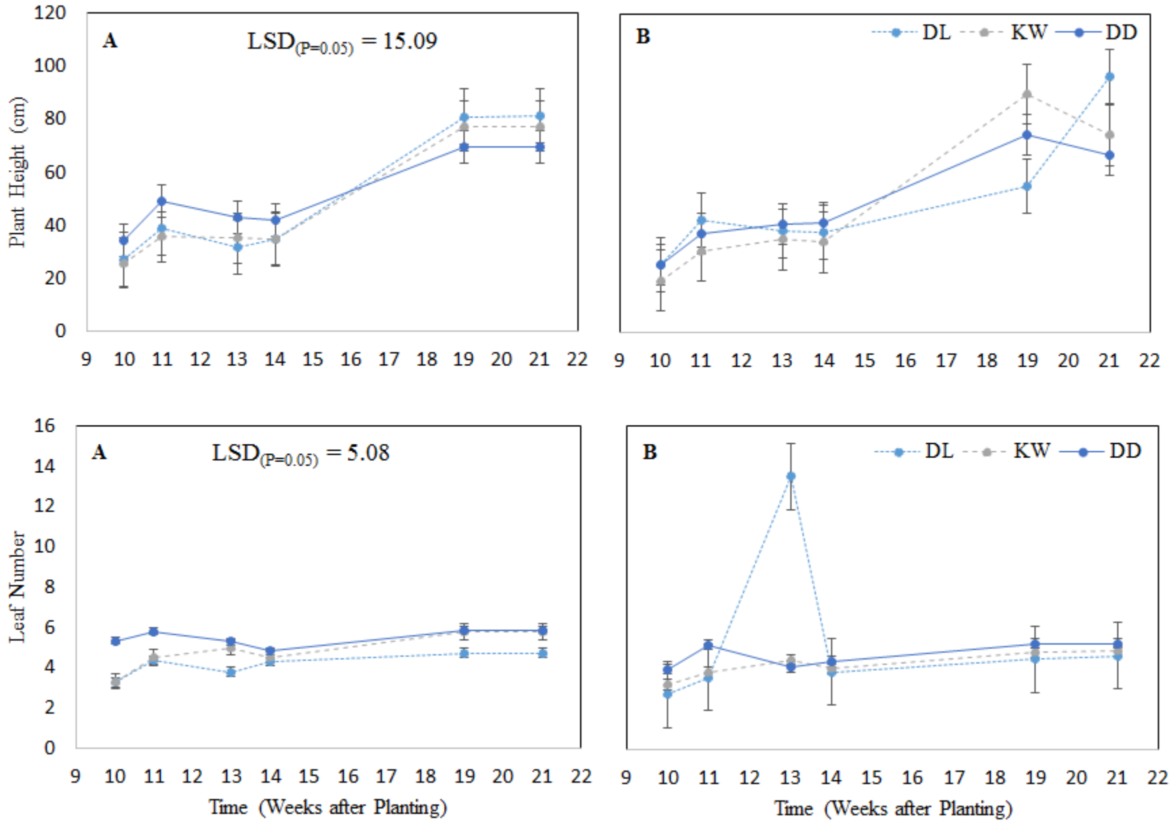

Figure 3. Plant height (cm) and leaf number of taro landraces (DD, DL & KW) under irrigated (A) and rain-fed (B) field conditions.

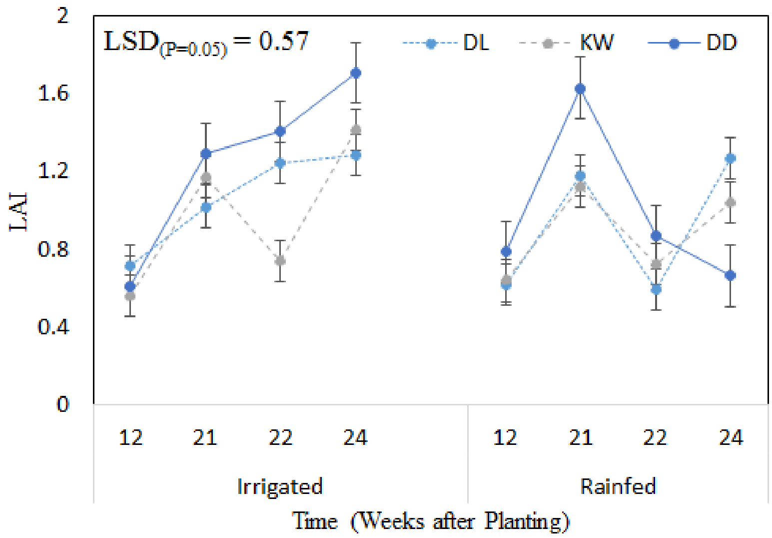

Figure 4. Leaf area index (LAI) of taro landraces (DD, DL & KW) under irrigated and rain-fed field conditions.

It has previously been argued that drought avoidance mechanisms were often at the expense of biomass production and yield attainment (Blum, 2005; Mabhaudhi, 2012). The results of this study showed that despite a lack of statistical significance, measured yield and yield components (biomass, harvest index and corm number) were lower under rain-fed than irrigated conditions (Table 1). The lack of statistical significance was probably due to the fact that rainfall received at Roodeplaat during the growth period was above normal (data not shown). There were significant differences ($P<0.05$) between landraces for measured yield components. The DL landrace, whose natural habitat is in shallow streams, failed to form any yield under both irrigated and rain-fed conditions. Dumbe-dumbe performed better than the KW landrace, especially under rain-fed conditions. This was possibly due to the landrace's ability to moderately reduce its canopy size and regulate stomatal aperture under rain-fed conditions. The performance of the DD landrace, compared with the KW landrace, concurred with reports by Uyeda et al. (2011). They reported that upland taro varieties were often more water-use efficient than varieties adapted to flooded conditions. This concurred with our initial hypothesis that certain landraces such as Dumbe-dumbe may have evolved to acquire mechanisms of drought tolerance.

Table 1. Yield and yield components (biomass, harvest index and corm number) of two taro landraces [Dumbe-dumbe (DD) and KwaNgwanase (KW)] grown under irrigated and rain-fed conditions.

Water Regime	Landrace	Biomass plant^{-1} (kg)	Harvest Index (%)	Corm number plant^{-1}	Yield (t ha^{-1})
Irrigated	Dumbe-dumbe	1.58a	72.28a	22.39a	22.77a
	KwaNgwanase	1.19ab	46.31a	7.39b	10.65a
Mean		**1.39^{a}**	**59.30^{a}**	**14.89^{a}**	**16.70^{a}**
Rain-fed	Dumbe-dumbe	1.45a	61.37a	19.83a	19.25a
	KwaNgwanase	0.98b	53.64a	4.61b	11.54a
Mean		**1.21^{a}**	**57.50^{a}**	**12.22^{a}**	**15.40^{a}**
LSD $_{(P=0.05)}$ (Water Regime*Landrace)		0.31	25.82	6.84	8.90
CV%		14.60	15.60	14.90	18.00
S.E.D.		0.13	10.55	2.80	3.64

Numbers in the same column not sharing the same letter differ significantly at LSD (P = 0.05).

Conclusions

Although taro grows best when irrigated, taro landraces also performed and produced reasonable yields under rain-fed conditions. The Dumbe-dumbe landrace, an eddoe type landrace, showed drought avoidance mechanisms under rain-fed conditions. This was achieved through stomatal regulation and adjustments in canopy size. Therefore, the Dumbe-dumbe landraces may be suitable for cultivation under rain-fed conditions in areas other than the coastal areas where it is currently cultivated. Slow emergence of taro landraces remains an issue to be dealt with in future research as it poses challenges to water use during the establishment stage. Such studies could investigate propagule size effect on establishment as well as other planting material enhancement strategies for improving crop establishment.

Acknowledgements

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References

Blum A. 2005. Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research 56:1159-1168.

de Klerk M., Drimie S., Aliber M., Mini S., Mokeona R., Randela R., Modiselle S., Vogel C., De Swart C., Kirsten J. 2004. Food security in South Africa: key policy issues for the medium term. Human Sciences Research Council, Pretoria. pp. 82.

Lebot V. 2009. Tropical root and tuber crops: Cassava, sweet potato, yams and aroids. CABI, Cambridge, UK.

Levitt J. 1972. Responses of plants to environmental stresses Academic Press, New York.

Mabhaudhi T. 2012. Drought tolerance and water-use of selected South African landraces of taro (*Colocasia esculenta* L. Schott) and Bambara groundnut (*Vigna subterranea* L. Verdc). PhD Thesis. University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Mabhaudhi T., Modi A.T. 2013. Preliminary assessment of genetic diversity in three taro (*Colocasia esculenta* L. Schott) landraces using agro–morphological and SSR DNA characterisation. Journal of Agricultural Science and Technololgy B 3:265-271.

Mabhaudhi T., Modi A.T., Beletse Y.G. 2013a. Response of taro (*Colocasia esculenta* L. Schott) landraces to varying water regimes under a rain shelter. Agricultural Water Management 121:102-112.

Mabhaudhi T., Modi A.T., Beletse Y.G. 2013b. Growth response of selected taro (*Colocasia esculenta*) landraces to water stress. 2nd International Symposium of Underutilized Food Plants – Crops for the Future, University of Nottingham, Malaysian Campus, Kuala Lumpur, Malaysia, 25 June – 1 July, 2011. Acta Horticulturae 979:327334.

Mare R.M. 2010. Taro (*Colocasia esculenta* (L.) Schott) yield and quality in response to planting date and organic fertilisation. PhD Thesis. University of KwaZulu-Natal, South Africa.

Mitchell J.H., Siamhan D., Wamala M.H., Risimeri J.B., Chinyamakobvu E., Henderson S.A., Fukai S. 1998. The use of seedling leaf death score for evaluation of drought resistance of rice. Field Crops Research 55:129-139.

Modi A.T. 2007. Effect of indigenous storage method on performance of taro (*Colocasia esculenta* (L.) Schott) under field conditions in a warm sub-tropical area. South African Journal of Plant and Soil 24:214-219.

Modi A.T., Mabhaudhi T. 2013. Water use and drought tolerance of selected traditional and indigenous crops. WRC Report No. 1771/1/13. Water Research Commission, Pretoria, South Africa.

Schippers R.R. 2002. African indigenous vegetables, an overview of cultivated species. (Revised version on CD-ROM). Natural Resources International Limited, Aylesford, UK. pp 214.

Schippers R.R. 2006. Traditional vegetables in Africa. Proc. Int. Symp. On the nutritional value and water use of indigenous crops for improved livelihoods. (Available on CD-ROM). Univ. Pretoria, Pretoria, South Africa. In: Tsuji GY, Hoogenboom G, Thornton PK, editors. Understanding options for crop production, Kluwer Academic Publishers, The Netherlands. pp 195-156.

Turner N.C. 1986. Crop water deficits: a decade of progress. Advance in Agronomy 39:1-51.

Uyeda J., Radovich T., Sugano J., Fares A., Paul R. 2011. Effect of irrigation regime on yield and quality of three varieties of taro (*Colocasia esculenta*). Hanai' Ai/The Food Provider, May-April, 2011, pp 1-3. Available at http://www.ctahr.hawaii.edu/sustainag/news/articles/V7-Uyeda-taro.pdf.

WRC (Water Research Commission, South Africa), 2009. WRC Knowledge Review 2008/09. Water Research Commission, Pretoria, South Africa.

Agromorphological characterization of *Amaranthus* species in Central Malawi

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Abstract

Twenty-seven accessions of *Amaranthus* species from the central region of Malawi (Dedza, Lilongwe and Kasungu Districts) were surveyed and characterized using agromorphological traits. A total of 26 descriptors, mainly defined by the International Plant Genetic Resources Institute (IPGRI), were used to describe stems, leaves, roots and the plant itself from December 2011 to April 2012. Qualitative descriptors included parameters such as growth habit, presence of spines in leaf axils, branching index, leaf shape, terminal inflorescence shape while the quantitative descriptors included plant height, growth habit, leaf number, seed yield, and spines. These qualitative and quantitative parameters were the most significant in delineating the uniqueness of the different accessions. Significant differences generated by ANOVA ($P<0.01$) were found in all parameters that were studied. Means were separated using Tukey's Honesty Significant Difference Test that grouped related accessions and with the Least Significant Difference (LSD). Means were compared at ($P\leq0.05$). The highest yielding accessions were BV-BF-01 (*A. hypochondriacus*) and LL-BF-04 (Green Giant) in terms of seed and vegetables respectively. Leaf yield ranged between 6554 kg ha^{-1} and 31 599 kg ha^{-1} while seed yield ranged between 450 kg ha^{-1} and 3900 kg ha^{-1}. It was clearly evident that there is a diversity of *Amaranthus* species in Malawi that are rich genetic resources for future breeding purposes but they require conservation. This work is an important step in the conservation of *Amaranthus* species in the central region of Malawi, which show distinctive and interesting morphological characters such as prostrate versus erect growth, availability of axillary inflorescence and the varying lengths of the same, length of terminal inflorescence and seed yield. Not many of the people in the local communities are involved in optimal production of *Amaranthus* species.

Key words: Agromorphology, *Amaranthus*, characterization, genetic diversity, plant descriptors

Introduction

Amaranth belongs to the Amaranthaceae family and is an extremely variable, erect to spreading plant. The height of mature plants varies between 0.3 m and 2 m, depending on the species, growth habit and environment (Rensburg et al., 2007). The plant is considered indigenous in Malawi. A wide range of indigenous vegetables are consumed in Malawi and they contribute greatly to the nutritional well being of rural people by providing the essential nutrients required for body growth and development and for prevention of diseases associated with nutritional deficiencies, such as blindness due to vitamin A deficiency. Rural families traditionally have made conscious efforts to preserve these plants around their homesteads, in crop fields and communal lands (Kwapata et al., 1992). Indigenous vegetables provide an important source of employment for those outside the formal sector in peri-urban areas of many African cities because of their generally short, labour-intensive production systems, low levels of purchased input use, and high yields (Schippers, 2000).

According to Rensburg et al. (2007), considering their potential nutritional value, indigenous leafy vegetables could greatly contribute to food security and balanced diets of rural households and possibly also urban households. Available data indicate that many of these indigenous plants are rich in vitamins and micro-elements required by the human body to grow, develop, and prevent nutritional disorders and diseases (Kwapata et al., 1992). Amaranth species of different morphologies, growth and development are found in varied agro-ecological zones throughout Malawi. *Amaranthus hypochondriacus* is thought to be a hybrid of three species: *A. hybridus, A. cruentus, and A. powelli.* It is considered the most robust and high-yielding of the grain amaranths. *A. cruentus* is used both as a grain and leafy vegetable. The grain type has white seeds while the vegetable type has dark seeds. *A. tricolor* is considered the best among the vegetable amaranths. It is succulent, low growing and compact and can be produced as a hot-season leafy vegetable in arid regions. However, *A. hybridus* is the most common leafy vegetable. It originated from the tropical Americas and displays two stem colours: red and green. It is the green type that is grown as a vegetable while the red-stemmed type is grown as an ornamental plant (Mng'omba, 2000).

Promotion of the best cultivars of *Amaranthus* species has never been considered in Malawi. Most attention is given to exotic vegetables. The aim of this study was to characterize the morphological diversity and to assess the cultivation of *Amaranthus* species in Central Malawi.

Materials and methods

Study site

The study was done in three districts: Lilongwe, Dedza, and Kasungu of the central region of Malawi where there are diverse *Amaranthus* species naturally growing in many areas. Eight accessions were established and maintained at the horticulture farm of Bunda College of Agriculture, Lilongwe. Three accessions were from Bunda college and these included *A. cruentus* (LL-BF-02), *A. hybridus* (LL-BF-05), *A. lividus* (LL-BF-01) and Green giant (LL-BF-04). Two species, *A. cruentus* (DZ-BF-01) and *A. hybridus* (DZ-BF-02) were from Dedza. From Kasungu, Zalewa and Bvumbwe the accessions *A. hybridus* (KU-BF-01), *A. cruentus* (ZW-BF-01) from Zalewa and *A. hypochondriacus* (BV-BF-01) were obtained, respectively. Successful germination was achieved from the seed established on October 10, 2011 on ridges that were 10 m long and 0.75 m apart and the spacing within a ridge was 0.4 m except for Green giant which was spaced at 1 m within the ridge. Each accession was planted on two ridges. Apart from the eight accessions established at the farm, 19 other accessions were characterized *in situ* in the study area bringing the total of studied accessions to 27.

The eight accessions planted at the farm were allocated to ridges in the plot at random. For the *in situ* characterization, the plants were studied in places that the species were growing in numbers large enough to warrant their study. Five plants were randomly selected for characterization and using destructive sampling, measurements were taken from five samples on each accession. Data on the morphology of the different varieties were collected based on the IBPGR *Amaranthus* descriptors list for both qualitative and quantitative traits. Data on leaf yield were collected three times at two week intervals on selected plants of each accession and data on seed yield were also collected on plants that were left to grow without any interference that would otherwise affect seed yield.

Data analysis

SPSS was used to analyse qualitative data and GenStat and MINITAB were used to analyse quantitative data. The scoring for the characters was done according to the Bioversity International Amaranth Descriptor List. Mean data were subjected to statistical analysis to calculate range, standard deviation, coefficient of variation and Least Significant Differences. Means were compared using Tukey's Honest Significant Difference Test in GenStat.

Results

Establishment

It was difficult to establish the plants in the cooler months, and successful establishment was in the warm parts of the year (Figure 1).

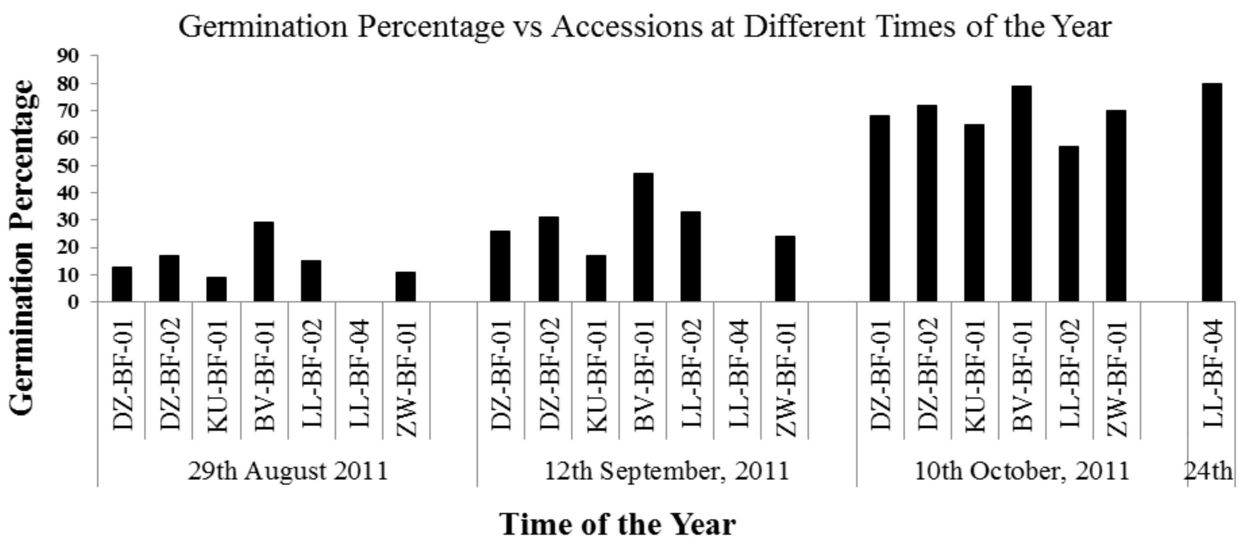

Figure **1**. Germination of accessions at different times of the year.

Qualitative traits

Twenty of the accessions were erect and seven were prostrate. Only one of the accessions, from Lilongwe (LL-BW-01), had stem pubescence and the rest were devoid of this character. Stem pigmentation also varied greatly among the plants. Fourteen were green, four of which had darker green stripes, eleven had a red stem and two had green with red stripes. Most

(81%) of the accessions had branches all along the stem and a few had either all of them near the top (7%) or near the bottom (7%) (Figure 2).

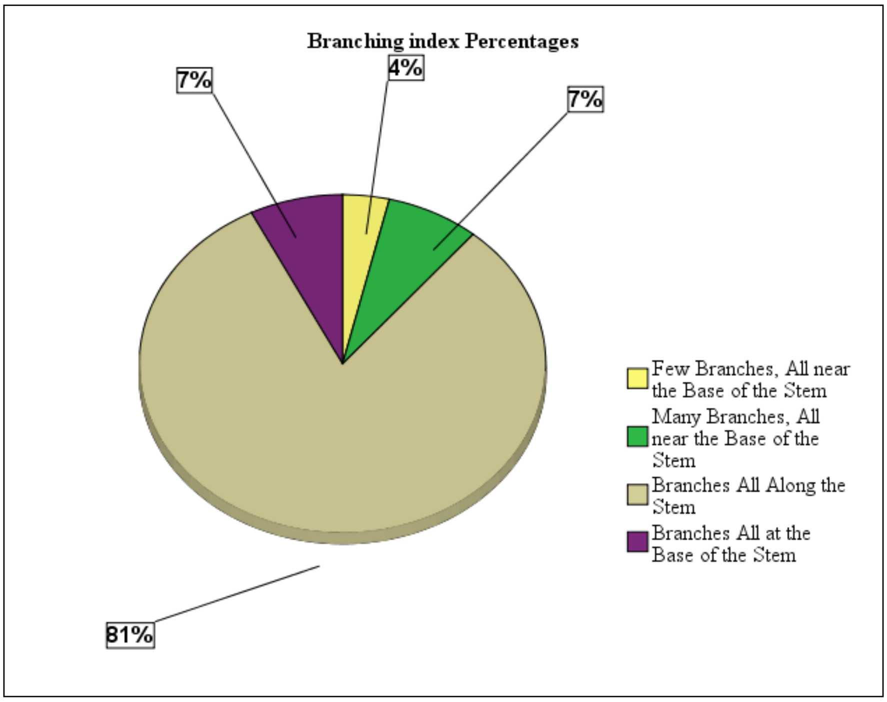

Figure **2.** Percentage of occurrence of the different branching indices displayed in the accessions

Axillary inflorescences were absent in 59.3% (16) of the plants and present in 40.7% (11). Sixteen plants had a green inflorescence, six plants had red or purple colour but most of them were typically red. Five accessions had a mixture of red and green parts of flowers (rusty look). Spines in leaf axils were present in 6 accessions and 21 accessions had no spines. No accession had leaf pubescence.

The common shapes of leaves were lanceolate (11) and ovate (11), two accessions had oblong leaves and two others had elliptical leaf shapes while only one had trullate leaves. On leaf pigmentation, 29.6% (8) of the accessions had their basal area pigmented mainly with purple, 44.4% (12) had dark green leaves, 22.2% (6) had normal green leaves, and 3.7% (1) were purple. Four (14.8%) had dark green petioles, 40.7% (11) had green, 37.0% (10) had red to purple, and only 7.4% (2) had green with red-striped petioles (Figure 3). Thirteen accessions had a tap root type representing 48.1% and fourteen had a fibrous root type representing 51.9%.

a) b) c)

Figure **3.** Different leaf pigmentations (a) DZ-03-U, (b) DZ-04-U, and (c) LL-BW-03.

There were variations in the shape and positioning of the inflorescence as shown in Figure 4. The 26% of the last bar is made of 14.8% (4) that had inflorescences that were mostly in leaf axils as well as terminal inflorescences whose side branches were short, 1/4 of, or shorter than the inflorescence length while 11.2% (3) also had axillary inflorescences as well as lateral inflorescences having long side branches, greater than 1/4 of the inflorescence length.

About 74.1% (20) of the accessions had an erect inflorescence, while 18.5% (5) had horizontal, half droop, or arch-shaped inflorescences while only two had an inflorescence whose attitude was drooping, with at least the terminal 2/3 hanging vertically down. The inflorescence density of 8 (29.6%) of the accessions was lax, of twelve (44.4%) was intermediate, and of seven (25.9%) was dense.

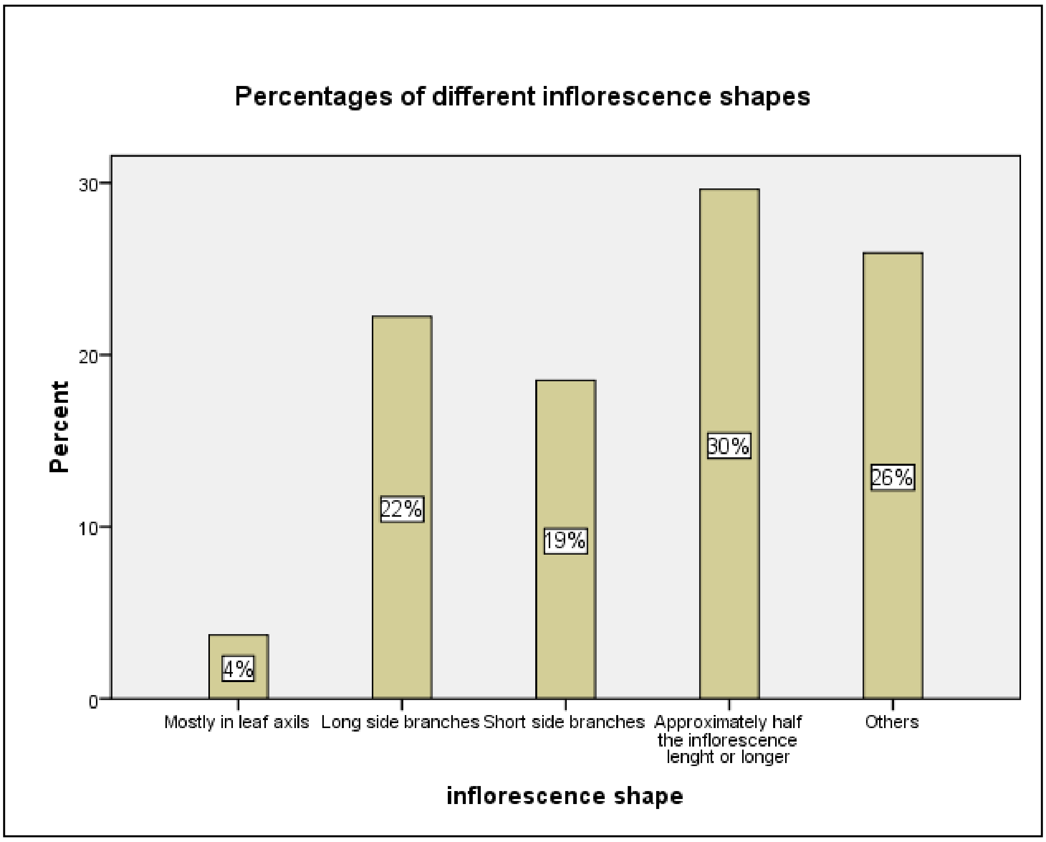

Figure 4. The occurrence of different inflorescence shapes in the accessions expressed as percentages.

Quantitative traits

Results of qualitative traits have been summarized in table 1 below.

Key to table 1:

PH: Plant height; LL: Leaf length; LW: Leaf width; BbL: Length of basal lateral branches; LN: Leaf number ISL: Terminal inflorescence stalk length; TbL: Length of top lateral branches PL: Petiole length; ILL:Terminal inflorescence laterals length; AIL: Length of axillary Inflorescence

Table 1. Quantitative parameters of the accessions

	MEANS											
	PH	**BbL**	**TbL**	**AIL**	**LL**	**LW**	**PL**	**LN**	**ISL**	**ILL**	**LL/LW**	**PL/LL**
ACCESSIONS												
BV-BF-01	115.0 abc	17.9 ab	11.5 abcd	0.0 a	22.44 hijk	10.8 j	10.4 i	394 ab	15.5 abcdef	3.3 bcd	2.13 hijk	0.47 bcde
DZ-01-L	120.0 abcd	105.0 efgh	56.7 hij	0.5 ab	5.30 b	3.1 bc	2.9 bc	2533 bcdefg	24.0 cdefgh	14.0 m	1.73 cdefgh	0.55 bcdefgh
DZ-01-U	125.6 abcd	97.0 efgh	39.8 fg	12.2 d	27.20 l	14.2 m	12.5 klm	1656 abcdef	40.0 hi	7.5 ij	1.91 defghij	0.46 bcd
DZ-02-L	80.0 ab	78.5 cdefg	20.8 cde	0.0 a	6.20 bc	5.2 fg	4.3 de	931 abc	18.0 bcdefg	5.5 ef	1.20 a	0.70 ijklm
DZ-02-U	211.8 bcdef	270.0 k	54.0 ghi	0.0 a	23.68 ijk	14.0 m	15.0 o	1724 abcdef	49.0 i	12.5 l	1.69 cdefg	0.64 fghijkl
DZ-03-L	177.0 abcdef	91.5 efgh	23.4 de	0.0 a	20.60 gh	12.6 l	10.3 i	2375 abcdefg	24.8 cdefgh	7.9 j	1.64 bcde	0.50 bcdef
DZ-03-U	59.0 ab	59.2 bcde	0.0 a	0.7 ab	6.10 bc	4.6 efg	3.5 cd	1111 abcde	10.5 abc	3.5 cd	1.33 ab	0.57 cdefghi
DZ-04-L	128.4 abcde	86.1 efgh	19.3 cde	0.0 a	16.60 f	9.3 i	11.8 jk	2228 abcdefg	20.9 bcdefg	7.3 hij	1.79 cdefgh	0.71 jklm
DZ-04-U	168.0 abcdef	173.0 ij	13.0 abcd	8.0 c	13.50 e	5.5 g	7.0 g	717 abc	29.0 defgh	5.0 ef	2.46 k	0.52 bcdefg
DZ-05-U	65.6 ab	0.0 a	69.3 j	0.6 ab	8.00 bc	4.5 def	5.0 ef	851 abc	20.0 bcdefg	11.2 k	1.79 cdefgh	0.63 fghijkl
DZ-BF-01	310.0 ef	62.0 bcdef	11.5 abcd	0.0 a	23.00 hijk	12.5 l	17.0 p	2686 cdefg	26.6 cdefgh	6.6 ghi	1.84 cdefghi	0.74 lm
DZ-BF-02	310.0 ef	97.0 efgh	13.0 abcd	0.0 a	22.50 hijk	12.2 kl	13.5 mn	3585 fgh	30.0 efgh	6.8 ghi	1.85 cdefghi	0.60 efghijk
KU-BF-01	275.0 cdef	120.0 gh	10.0 abcd	0.0 a	25.00 kl	14.2 m	13.4 mn	3159 defgh	13.0 abcde	2.5 b	1.76 cdefgh	0.54 bcdefgh
KU-CM-00	263.7 cdef	80.7 defg	14.9 bcde	0.0 a	24.50 jkl	14.0 m	14.2 no	2943 cdefgh	23.5 cdefgh	6.7 ghi	1.75 cdefgh	0.58 defghij
KU-MT-00	78.5 ab	34.7 abcd	21.2 de	0.5 ab	7.30 bc	3.7 cde	4.8 ef	1819 abcdefg	19.1 bcdefg	7.2 hij	1.97 efghij	0.66 hijkl
KU-TR-01	50.2 ab	88.8 efgh	52.5 ghi	0.0 a	11.00 de	6.7 h	7.0 g	957 abcd	41.1 hi	15.4 n	1.64 bcde	0.64 ghijkl
KU-TR-02	48.0 ab	28.6 abc	6.5 abc	0.2 ab	8.50 cd	4.8 fg	5.5 f	448 ab	11.4 abcd	3.7 d	1.78 cdefgh	0.66 hijkl
KU-TR-03	79.6 ab	0.0 a	65.0 ij	0.8 ab	7.80 bc	3.6 cd	4.0 cde	330 ab	31.3 fghi	17.6 o	2.16 ijk	0.52 bcdefg
LL-AQ-01	48.0 ab	67.5 bcdef	47.2 gh	1.0 b	5.50 b	2.5 b	4.0 cde	411 ab	13.5 abcdef	5.0 ef	2.23 jk	0.73 klm
LL-AQ-02	24.1 a	22.2 ab	3.0 ab	0.5 ab	5.10 ab	3.4 bc	2.2 ab	203 a	5.6 ab	2.6 bc	1.50 abc	0.43 ab
LL-BF-01	46.0 ab	21.0 ab	4.0 ab	0.5 ab	2.20 a	1.4 a	1.5 a	7234 i	0.0 a	0.0 a	1.58 bcd	0.69 ijkl
LL-BF-02	300.0 def	135.0 hi	16.0 bcde	0.0 a	21.30 hi	11.8 kl	12.0 jkl	5158 hi	26.0 cdefgh	6.5 gh	1.81 cdefgh	0.56 bcdefghi
LL-BF-04	195.0 abcdef	194.9 j	100.0 k	11.4 d	22.00 hij	12.5 l	18.0 p	3282 efgh	48.7 i	13.0 l	1.76 cdefgh	0.83 m
LL-BW-01	203.0 abcdef	186.3 j	61.7 ij	0.0 a	23.00 hijk	11.3 jk	7.0 g	3487 fgh	28.3 cdefgh	5.9 fg	2.04 ghij	0.31 a
LL-BW-02	189.0 abcdef	97.8 efgh	52.9 ghi	0.0 a	27.00 l	16.1 n	13.0 lm	2793 cdefg	21.4 bcdefg	4.7 e	1.68 bcdef	0.48 bcde
LL-BW-03	189.8 abcdef	111.6 fgh	28.0 ef	0.0 a	18.00 fg	9.0 i	9.0 h	1874 abcdefg	33.9 ghi	8.1 j	2.01 fghij	0.50 bcdef
ZW-BF-01	319.9 f	180.0 ij	15.8 bcde	0.0 a	25.00 kl	12.3 l	11.0 ij	4038 gh	27.2 cdefgh	6.8 ghi	2.03 ghij	0.44 abc
GRAND MEAN	**154.8**	**92.8**	**30.8**	**1.4**	**15.9**	**8.7**	**8.9**	**2182.5**	**24.2**	**7.3**	**1.8**	**0.6**
CV %	**49.4**	**22.8**	**19.5**	**27.6**	**7.70**	**4.7**	**5.3**	**42.5**	**30.7**	**5.7**	**8.10**	**9.80**
SE ±	**48.4**	**13.4**	**3.8**	**0.2**	**0.77**	**0.3**	**0.3**	**586.6**	**4.7**	**0.3**	**0.09**	**0.04**
LSD ($P \leq 0.05$)	**95.9 *** **	**26.5 *** **	**7.5 *** **	**0.5 *** **	**1.52 *** **	**0.5 *** **	**0.6 *** **	**1162.8 *** **	**9.3 *** **	**0.5 *** **	**0.18 *** **	**0.07 *** **

One way ANOVA ($P \leq 0.01$)***

Discussion

Establishment

Amaranth is photoperiod sensitive and starts to flower as soon as the day length shortens (Rensburg et al., 2007). This explains why it was difficult to establish during the cold months (Figure 1). Amaranth seeds need soil temperatures of between 18°C and 25°C to germinate and an air temperature above 25°C for optimum growth. The growth ceases at temperatures below 18°C.

Qualitative traits

The growth habit, branch arrangement and orientation vary from species to species. The environment also plays a role in branch arrangement and positioning. According to Ackerly et al. (2011), it appears that CO_2 affects branch number due to effects on the overall rate of development and not to changes in the pattern of branch production. Axillary bud initiation and branching patterns depend on the species as well as environmental conditions (Costea and DeMason, 2001)

Stem colour may vary during the same vegetative cycle (Costea and DeMason, 2001). The colour was different from tender age to maturity with the red-stemmed accessions appearing green when young and gradually turning red towards maturity. The stem and inflorescence pigmentation of the accessions was green or red with some plants showing intermediary traits giving a rusty look. This was common in sites where green and red plants co-existed owing to cross pollination. They are easily cross bred, and even weedy types will cross with the intended crop if not rogued from the field (O'Brien and Price, 2008).

The leaf shapes of amaranths can vary considerably within a single species. However, there are general shapes that distinguish the species (Legleiter and Johnson, 2013). The majority of the accessions were of the *Amaranthus palmeri* (Palmer amaranth) and *Amaranthus rudis* (waterhemp) types. Common waterhemp leaves are generally long, linear, and lanceolate. Palmer amaranth leaves are wider and ovate to diamond-shaped (Legleiter and Johnson, 2013; Nordby et al., 2013).

There were also variations in the shape and positioning of the inflorescence. For preliminary identification of *Amaranthus* species, the useful tool can be the number, thickness, orientation and density of branches in inflorescences. The flowers are arranged in small and contracted cymes, which are agglomerated, axillary and additionally arranged in racemose or spiciform terminals, and large and complex synflorescences (Janovská et al., 2012). O'Brien and Price (2008) observed that vegetable types form flowers and seeds along the stems.

Regardless of species, the choice of variety is influenced by individual preference for leaf colour and taste (Oba et al., 2008). Some of the most common commercial amaranths are selections of *A. tricolor* which come in various leaf colours such as white (light green), dark green, red, purple and variegated (Palada and Chang, 2003).

According to Tranel et al. (2002), the growth of the terminal inflorescence and its branches is indeterminate (racemose) and, thus, it may reach a length of 100 cm or even more. However, *A. edulis* is the only exception in which the inflorescence is determinate due to the presence of a terminal polymerous male flower and, therefore, the elongation of the inflorescence in this species is due to intercalary growth of the axis.

Quantitative traits

The plant height ranged from 38.3 cm to 361 cm. The mean height was 154.8 cm. The tallest accession was DZ-02-U from Dedza (elevation of 1572 masl) that was measured *in situ* and most of the plants that recorded good heights were those established at the farm while those *in situ* were growing in unfavourable environments and so their potential performance was affected. Tukey's pairwise comparisons grouped the plant height means of all the accessions and it was found that mean plant height of accession LL-AQ-02 was significantly different from those of the other accessions. However, there were some relationships among the accessions in terms of plant height since no accession was unique to it and they were not significantly different from species to species but the mean of ZW-BF-01 was significantly higher than those of the other accessions (Table 1).

Accessions KU-TR-03 and DZ-05-U did not have basal lateral branches. The maximum length of basal lateral branches was 291cm and the minimum was 12.4 cm, the average length of lateral branches being 92.83 cm. Accession DZ-03-U had 59.2 cm as its average length in basal lateral branch and was 59 cm in height on average. The mean length of basal lateral branches in accessions BV-BF-01, LL-AQ-02 and LL-BF-01 was significantly lower than the other accessions while the mean length of basal lateral branches of DZ-02-U was significantly higher.

The length of top lateral branches ranged from 2 cm to 116.7 cm; the mean length was 31.96 cm. Species growing in natural conditions had relatively longer top lateral branches than those established at the farm. Tukey's Honesty Significant Difference test showed that the mean lengths of top lateral branches of LL-BF-04 were significantly higher than the rest of the accessions. The mean length of top lateral braches of LL-AQ-02 and LL-BF-01 was significantly lower (Table 1) but just as in the other traits, there were some similarities in the mean length on the lateral branches among the accessions.

The length of axillary inflorescence ranged from 0.2 cm to 12.8 cm and the mean length was 3.08 cm. The maximum length of the leaves was 29 cm and the minimum was 1.9 cm, the mean being 15.85 cm. For leaf width the mean was 8.74 cm and the maximum and minimum values were 1.2 cm and 16.5 cm respectively. Tukey's pair wise comparisons were also conducted on these parameters and on leaf length it was found that the mean leaf length of LL-BF-01 was significantly shorter compared to the other accessions while significantly on the higher side was DZ-01-U. There was also much variability of leaf length amongst the accessions as the groupings in Table 1 show and this signified that the variability was extensive. BV-BF-01, DZ-BF-02, DZ-BF-01, and LL-BW-01 had mean leaf lengths that were insignificantly different but they were found to be significantly different from accessions DZ-03-U, DZ-02-L, KU-MT-00, KU-TR-03 and DZ-05-U. However, there were still some linkages amongst the accessions despite the significant differences.

Most accessions displayed significant differences in leaf width while some were also grouped based on similarities. Accessions LL-BF-01 and LL-AQ-01 are different but they were significantly smaller in mean leaf width compared to the rest of the accessions. ZW-BF-01 and DZ-BF-01 had wider leaves than the others. Regarding the length of axillary inflorescences, only 12 of the 27 accessions had such inflorescences and comparison of mean length of these showed that 8 accessions were significantly different from the 4 and this trait was common in prostrate growing accessions.

Leaf length and leaf width ratio showed on average that the leaf length was double the average leaf width in all accessions. The petiole length and leaf length ratio showed that the average length of the petioles was half that of the leaf length. These relationships can be seen in Table 1. The ANOVAs for these ratios also showed that there were significant differences in leaf length/width ratio and petiole length/leaf length ratio.

The variability in petiole length (PL) was also somewhat large. The maximum length was 18.5 cm and the minimum value was 1.3 cm with a mean of 8.9 cm. The number of leaves was 316 on the lower side and 7319 on the higher side and the mean was 2182. The longest inflorescence stalk length observed measured 69 cm and the shortest was 4.9 cm. The mean length was 24.16 cm. The laterals length of the inflorescence had a mean of 7.3 cm. The maximum value was 18.1 cm and the minimum value was 2.1 cm.

The highest leaf yielding accession was LL-BF-04 (considered to be Green Giant) that also was shown to have a longer vegetative phase compared to the other accessions. Its leaf yield of five plants went up to 18.53 kg and the least leaf yielding was BV-BF-01 (considered to be *A. hypochondriacus*) which is a grain amaranth and five plants yielded 1.13 kg. The leaf yield per hectare ranged between 6554 kg ha^{-1} to 31 599 kg ha^{-1} and Schippers (2000) and Rensburg et al. (2007), reported that under cultivated conditions amaranth produces fresh leaf yields of up to 40 t ha^{-1}. High yield is one of the most important characteristics for vegetable production especially during the hot and wet summer season in tropical and sub-tropical regions (Engle, 2003).

BV-BF-01 being a grain type of *Amaranthus* yielded higher in seeds than in leaves. Its vegetative phase was also very brief and the least seed yielding was DZ-BF-01. The grain yield was between 450 kg ha^{-1} and 3900 kg ha^{-1}. Amaranth grain yield strongly depends on environment, weather conditions, species, genotype, and production techniques, and varies in a wide range from 500 to 2000 kg grain ha^{-1} while it is reported that with appropriate varieties and production techniques, yields of 1500 to 3000 kg grain ha^{-1} can be expected, whilst in Europe, yield can be between 2000 and 3800 kg ha^{-1} (Mlakar et al., 2010). According to Tubene and Myers (2008), although individual seeds are very small, amaranth plants produce abundant edible seed, which have high protein and oil content. The grain amaranths are more productive seed producers than vegetable amaranths.

Analysis of variance (ANOVA) showed that there were significant differences ($P<0.01$) in all the quantitative traits (Table 1).

Conclusion

Despite the diversity of amaranth discussed, not many farmers are involved in the proper production of amaranthus species. Rather, rural families traditionally have made conscious efforts to preserve these plants around their homesteads, in crop fields and communal lands (Kwapata et al., 1992). There is a general perception problem in Southern Africa where, as with many other African leafy vegetables, people believe the plants will grow naturally (Rensburg et al., 2007). There is a need to evaluate the growth performance of *Amaranthus* species under different management practices in order to select and recommend the most appropriate ones to the farmers.

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References

Ackerly D.D., Coleman J.S., Morse S.R., Bazzaz F.A. 2011. CO_2 and temperature effects on leaf area production in two annual plant species. Ecological Society of America, Ecology, 73:1260-1269.

Costea M., DeMason D.A. 2001. Stem morphology and anatomy in *Amaranthus* L. (*Amaranthaceae*) —Taxonomic significance. Biology Faculty Publications. 74.

Engle L.M. 2003. Yield evaluation of amaranthus species. Progress Report. AVRDC – the World Vegetable Center. p. 109.

Janovská D., Čepková P.H., Džunková M. 2012. Characterisation of the amaranth genetic resources in the Czech gene bank. In: Çalişkan, M, editor. Genetic diversity in plants. InTech. Rijeka, Croatia.

Kwapata M.B., Dawa G., Maliro M. 1992. Growth and yield performance of vegetable amaranth varieties in contrasting agroecological zones. African Crop Science Conference Proceedings Journal, Pretoria, 13 – 17 January 1997. African Crop Science Society, Makerere University. Kampala, Uganda. 3:819-823.

Legleiter T., Johnson B. 2013. Palmer amaranth biology, identification and management. Purdue Extension, Purdue University.

Mlakar S.G., Turinek M., Jakop M., Bavec M., Bavec F. 2010. Grain amaranth as an alternative and perspective crop in temperate climate. Journal for Geography 5-1:135-145.

Mng'omba S.A. 2000. Performance of grain amaranths under rain-fed and irrigated conditions in three contrasting agro-ecological areas in Malawi (Thesis).University of Malawi, Bunda College of Agriculture, Lilongwe, Malawi.

Nordby D., Hartzler B., Bradley K. 2013. Biology and management of waterhemp. The Glyphosate, Weeds, and Crops Series. Purdue Extension, Purdue University.

O'Brien G.K., Price M.L. 2008. Amaranth grain and vegetable types. ECHO Technical Note. North Fort Myers, USA.

Oba S., Khandaker L., Ali B. 2008. Total polyphenol and antioxidant activity of red amaranth (*Amaranthus tricolor* L.) as affected by different sunlight level. United Graduate School of Agriculture, Gifu University, Yanagido, Gifu 501-1193, Japan.

Palada M.C., Chang L.C. 2003. Suggested cultural practices for vegetable amaranth. International Cooperators' Guide. Asian Vegetable Research and Development Centre. AVRDC publication.

Rensburg W.S.J., Averbeke W., Slabbert R., Faber M., Jaarsveld P., Heerden I., Wenhold F., Oelofse A. 2007. African leafy vegetables in South Africa. Water 33:317- 326.

Schippers R.R. 2000. African indigenous vegetables. An overview of the cultivated species. Chatham, UK: Natural Resources Institute /ALP-EU Technical Centre for Agricultural and Rural Cooperation.

Tranel P., Wasson J., Jeschke M., Rayburn A. 2002.Transmission of herbicide resistance from a monoecious to dioecious weedy amaranth species. Tag Theoretical and Applied Genetics 105:674 – 679.

Tubene S.L., Myers R.D. 2008. Ethnic and specialty vegetables handbook. Maryland Department of Agriculture.

Daylength effects on growth and seed production efficiency in Bambara groundnut (*Vigna subterranea* L.)

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Abstract

Daylength affects the reproduction of many crop species that grow away from the equator. Because daylength cannot be regulated in field conditions, it is important that crop genotypes have appropriately matched photoperiod requirements to ensure reproductive success at different latitudes and in different growing seasons. This is an issue with Bambara groundnut (*Vigna subterranea* L.), an underutilized African legume rich in protein which grows on marginal soils. Changes in daylength can delay flowering. However, for this species incorrect daylength can completely abolish pod-filling. For smallholder and subsistence farmers, where the sowing date of the crop is often determined by the timing of the rains, matching photoperiod requirement to daylength is important for reproductive success. The objective of this work was to determine photoperiod effects on production efficiency and to identify photoperiodic ideotypes for different production systems. Plants grown as crop stands in three climate-controlled glasshouses with 12/14/16 hours of daylength were examined for photoperiodic requirements for pod-filling and yield in five landraces.

The period from flowering to mature pod-set was shortest in 12 hours, intermediate in 14 hours, and longest in 16 hours in four of the five genotypes, with complete failure to fill pods in one genotype. Three photoperiodic types have been identified: (1) qualitative short-day (Ankpa 4), (2) quantitative short-day (Getso, Gresik and LunT) and (3) quantitative long-day (IITA-686). Seed number doubled in 16 hours for IITA-686, but incomplete pod-filling (due to indeterminacy in 16 hours) led to an overall reduction in average seed weight. These results support the hypothesis that manipulating photoperiod sensitivity may be an avenue to increasing yield. Parental genotypes derived from landraces that differ for photoperiod requirements have been crossed and a series of mapping populations created.

Introduction

The daylength at the equator (zero latitude) is reasonably stable, but towards the earth's poles the lengths of day and night change to become increasingly unequal. These differences in daylength provide an environmental cue that allows plants to flower in response to seasonal fluctuations (Garner and Allard, 1920; Thomas and Vince-Prue, 1997). The ability to sense and respond to changes in daylength is known as photoperiodism, and many developmental responses in plants, animals and even fungi are under the control of photoperiod (Dawson et al., 2001; Tan et al., 2004; Thomas and Vince-Prue, 1997). Photoperiod control in legumes has been reported for, e.g. flowering time and seed production in soybean *(Glycine max)* (Kantolic and Slafer, 2005, 2007; Summerfield et al., 1993); for growth and development and flowering and pod-set in Bambara groundnut

(Brink, 1997; Harris and Azam-Ali, 1993; Linnemann, 1993; Linnemann et al., 1995); flowering time in cowpea (*Vigna unguiculata*) (Ellis et al., 1994; Hadley et al., 1984; and phenological development in lablab bean (*Dolichos lablab*) (Keatinge et al., 1998. These studies indicated that the phenological developments of short-day legumes are generally under the influence of temperature and photoperiod. The exposure to long photoperiods in indeterminate soybean for example, increased the reproductive period from flowering to maturity, and this resulted in an increase in seed number (Kantolic and Slafer, 2007). In Bambara groundnut (*Vigna subterranean* L.), photoperiod usually has a stronger effect on pod-set and pod-filling than on flowering (Brink 1997).

Because daylength cannot be regulated in field conditions, it is important that crop genotypes have appropriately matched photoperiod requirements to ensure reproductive success at different latitudes and in different growing seasons. Bambara groundnut is an African native legume rich in protein and generally grown by smallholder farmers who appreciate its tolerance of drought and poor soils. Pod and seed number, and seed weight are the most important components responsible for differences in yields between different landraces and environments. These components are mainly determined during the post-flowering period and the effects extend through pod filling and maturity. While changes in photoperiod can delay flowering, incorrect daylength can completely abolish pod-filling. The sensitivity of Bambara groundnut to long photoperiod determines the limits of the sowing period for smallholder farmers, and adaptation of the crop outside its main growing season.

Bambara groundnut landraces generally have been reported to require a short day-length of a maximum of 12-13 hours in the period of pod-set and pod-filling, and there are considerable differences between landraces in their response to long photoperiods (Brink et al., 2000; Harris and Azam-Ali, 1993; Linnemann, 1991, 1993; Linnemann and Craufurd, 1994). For most landraces, short days and long days often fall below the critical photoperiodic range for successful fruit set even after flowering (Jørgensen et al., 2009). Similarly, genotypes may become female infertile and/or male sterile in response to unfavourable photoperiodic changes. These conditions can cause "blanking", an abortion of the pod formation process. In many Bambara groundnut landraces the onset of podding is retarded by long photoperiods (Brink et al., 2000; Linnemann, 1991, 1993; Nishitani and Inouye, 1981).

Since Bambara groundnut is produced and consumed in different countries in sub-Saharan Africa with daylengths varying from 12 h to 14 h, depending on the latitude and time of growth, it is important to understand the photoperiod requirements for reproductive success and improvement. The objective of this work was to determine photoperiod effects on seed production efficiency and to develop photoperiodic ideotypes for different production systems.

Materials and methods

Experimental setup and photoperiodic treatments

The experiments were conducted on plants grown as crop stands in three climate-controlled tropical glasshouses in 2011, at the School of Biosciences, Sutton Bonington Campus, University of Nottingham. For each daylength treatment, the experimental design was a complete randomized design (CRD) with eight plants per genotype. Individual plants represented each replicate. Five genotypes derived from landraces (Table 1) were subjected to 12, 14 and 16 h of constant daylength treatment in glasshouses 1, 2 and 3, respectively, with daylength in each glasshouse controlled by an automatic blackout system. While a photoperiod of 12 h d^{-1} was natural, a photoperiod of 16 h d^{-1} was considered to be extreme for a short-day plant such as Bambara groundnut. Each glasshouse condition was kept at approximately 50-60% relative humidity, 27/28^{o}C and 22/23^{o}C day and night temperatures, respectively. Lighting was supplemented in glasshouses during a period longer than 12 h natural daylength with artificial lights, when light levels were below 20,000 lux.

The Bambara groundnut landrace Ankpa 4 from Nigeria is known to be highly photoperiod sensitive for flowering and pod-set when grown under photoperiod conditions longer than 12 h (Linnemann et al., 1995), while the rest of the landraces used are poorly characterized or have not been tested for photoperiod sensitivity. Where information was available, it was often derived largely from pot experiments. Four plants per landrace in two beds (eight plants in total) were planted directly into glasshouse soil without mounding (2-3 cm planting depth) at a spacing of 25 cm by 25 cm in all three glasshouses on the 8th of August 2011, under natural light conditions. This experiment was carried out for five months and plants harvested on the 8th of January, 2012. The planting was done in a completely randomized design, to allow for at least three plants in between border guard rows per landrace in the final analysis (three replications). Irrigation was supplied by trickle tape for 15 min morning and 15 min evening throughout the experiment period. This accounted for a flow rate of 500 ml day^{-1}, supplied to 40 plants in two rows (20 plants each) over a 5 m stretch. Each of the three different daylength treatments was initiated from sowing in each of the glasshouses and data were collected according to standard IPGRI descriptors for days to emergence, days to first flower opening (anthesis), and yield determining traits (pod numbers $plant^{-1}$, seed number $plant^{-1}$ and grain weight $plant^{-1}$). Measurements were non-destructive, and direct observations at the beginning of pod set were impossible because some of the selections included in the experiment mostly buried their pods (e.g Ankpa 4, Lun T and Getso). Maturity was established when leaves began to age, and pods began to dry out in 12 h daylength. Seeds were dried to 12% moisture at 37^{o}C for two weeks and above-ground dry matter at 85^{o}C (oven dry weight) for 48 h. Data were subjected to analysis of variance with mean separation according to LSD (GenStat 15th Edition). The characterization of these genotypes derived from landraces enabled us to select divergent parents for genetic crosses and to generate segregating F_2 populations.

Plant material

Plant material consisted of five groundnut landraces collected from different locations in Africa, with contrasting growing seasons. These landraces are adapted to tropical conditions in the major Bambara groundnut-growing regions of sub-Saharan Africa and Indonesia. As a self-pollinating crop, landraces used were selected and seed obtained from single plants. Where individual plants manifested desirable traits, seeds from these individual plants were selected and grown separately, to produce several pure lines (genotypes). Given the strongly inbreeding nature of Bambara groundnut (as evidenced through very low levels of heterozygosity in individual genotypes derived from single plant landrace accessions assessed with 20 SSR markers; 2% heterozygosity (Molosiwa et al., 2015), seed derived from single plants should essentially represent unselected varieties. Details of these plant materials, country of origin and geographical distribution from the equator are listed in Table 1 below.

Table1. Background of landraces used for studies, seed colours, and countries of origin

S/N	Name	Testa colour	Origin/Remark
1	IITA 686	Dark	Tanzania; photoperiod less sensitive (Jorgensen, pers. comm.)
2	Ankpa 4	Brown	Nigeria; photoperiod sensitive (Linnemann et al., 1995)
3	Lun T	Cream	Sierra Leone
4	Gresik	Dark	Indonesia
5	Getso	Cream	Nigeria

Results

Flowering

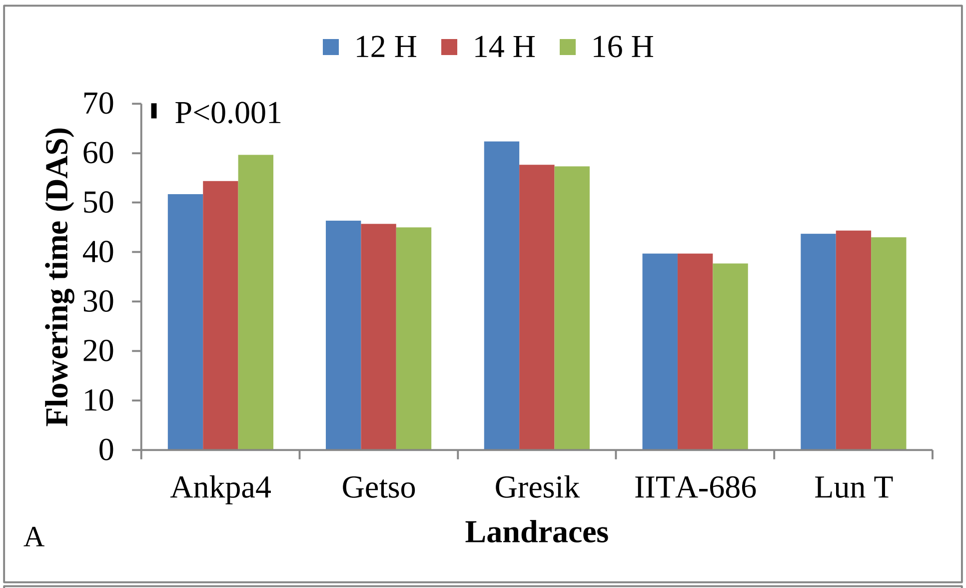
The flowering times, measured in days after emergence (DAE) were significantly affected by landrace ($P<0.01$) but not by photoperiod (Fig. 1A). However, flowering time for the three daylength treatments interacted significantly with landrace ($P<0.01$). The 12 h treatment gave the largest interaction between daylength and landrace, where Gresik and Ankpa 4 took longer to flower compared to all other landraces. This was closely followed by the 16 h treatment, where Ankpa 4 and Gresik took longer to flower than Lun T, Getso and IITA-686. In terms of numbers of flowers produced, there were significant differences between landraces, daylength and the interaction between landrace and daylength ($P<0.01$) with progressive increases in flower numbers for Getso and IITA in 16 h>14 h>12 h. The reverse was the case for Ankpa 4 with lower numbers of flowers in 16 h and 14 h compared to 12 h.

Pod number

The number of pods produced was significantly affected by landrace, daylength and an interaction between landrace and daylength ($P<0.01$; Figure 2). The pod numbers for IITA-686 increased with increasing daylength, but decreased with increasing daylength treatment for Getso and Lun T. The landrace Ankpa 4 could only produce pods in 12 h daylength. There was a two-fold yield increase in pod number in IITA 686 at 16 h because it continued to flower and produced pods even at 150 days after sowing.

Seed number and weight

Seed number and weight were significantly affected by landrace and photoperiod, as well as the interaction between landrace and daylength ($P<0.01$). The exposure to the 'extreme' daylength treatment of 16 h resulted in zero pod or seed yields for Ankpa 4, but increased the number of seed for IITA-686 (Figure 3A, 3B). The 12 h daylength treatment however, was more favourable for seed numbers and weight for the Getso and Lun T landraces compared to 14 and 16 h. The results also revealed a decrease in seed number and seed weight for IITA-686 and Gresik in 14 h.



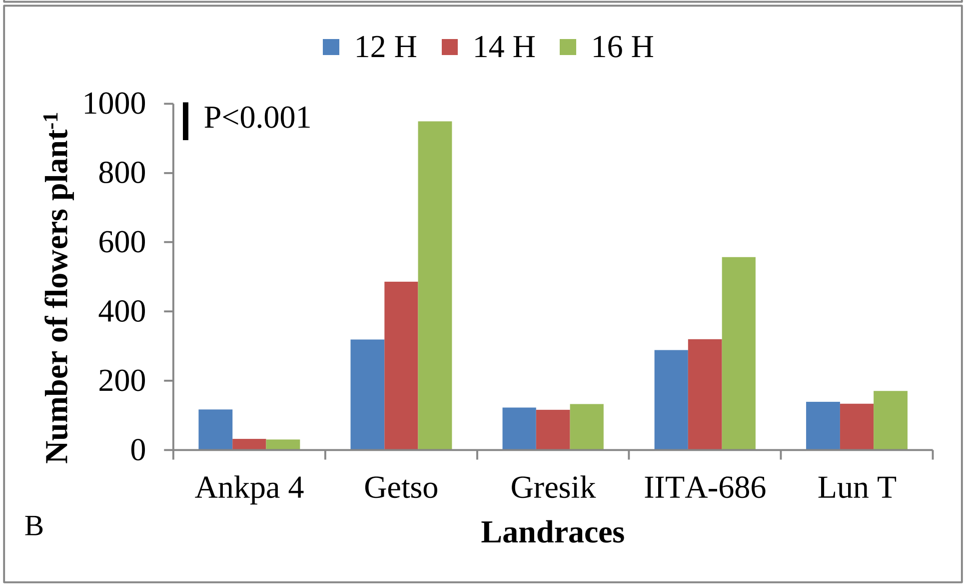


Figure 1. Effect of daylength on flowering, (A) Flowering time (B) Number of flowers

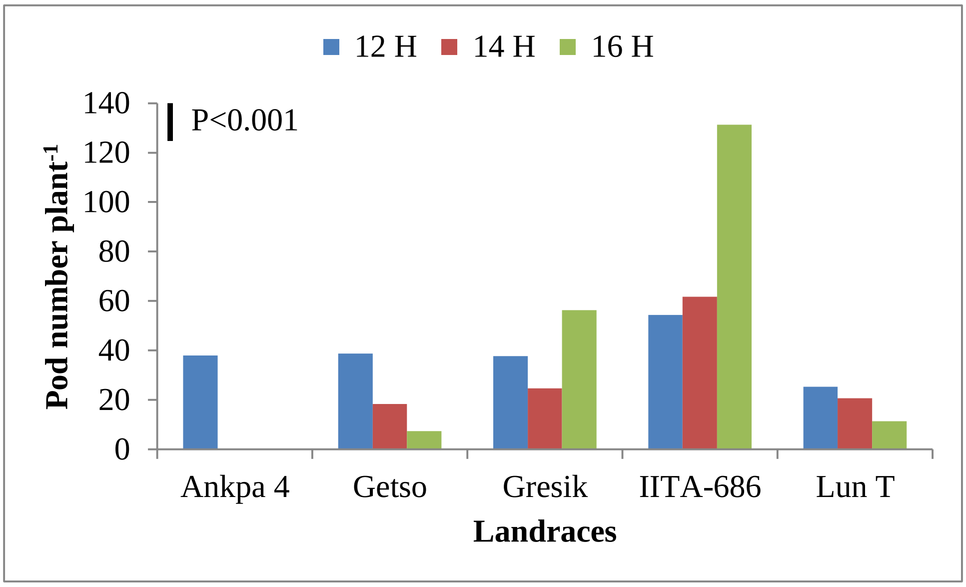


Figure 2. Effect of photoperiod on podset in Bambara groundnut

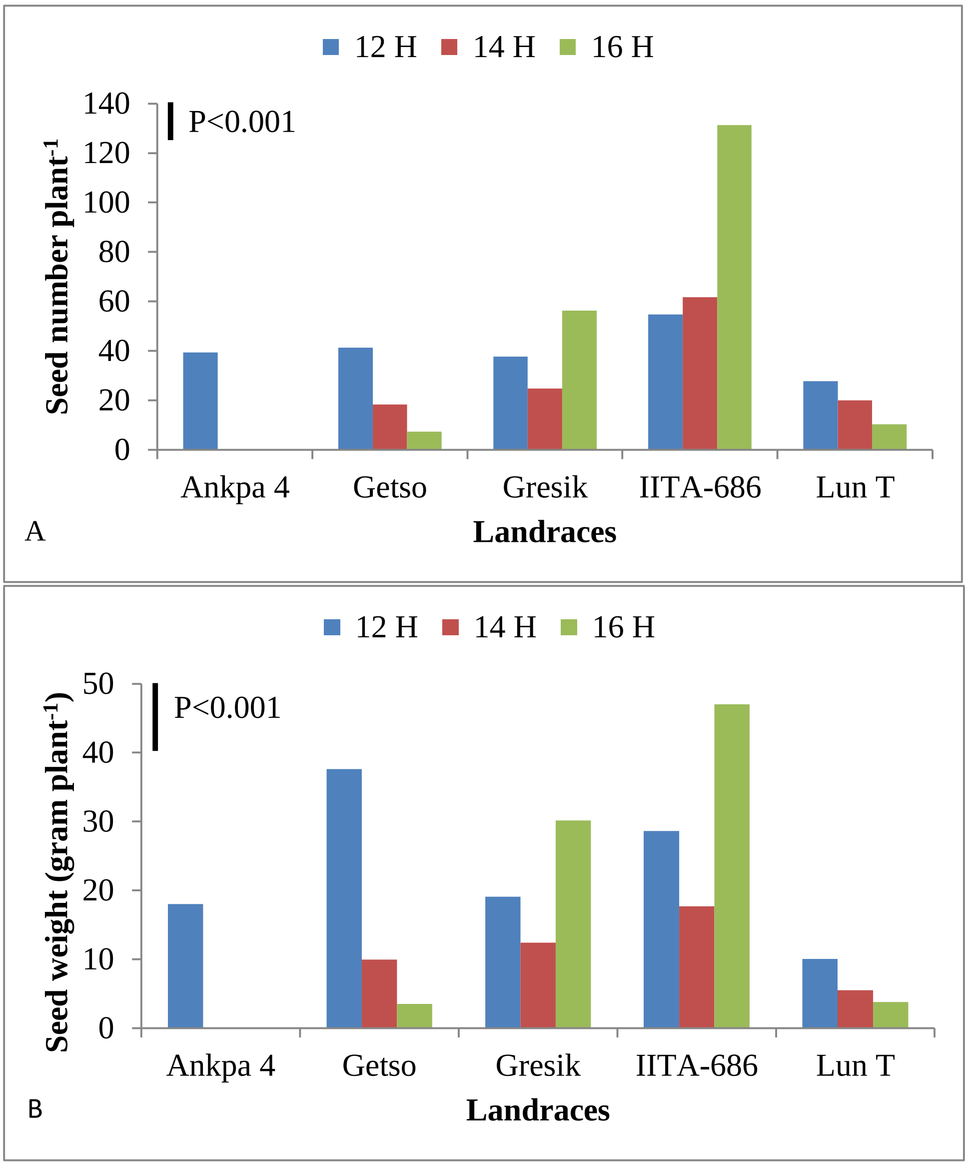


Figure 3. Effect of daylength on yield determinants in Bambara groundnut, (A) Seed number (B) Seed weight

Discussion

In general, a strong photoperiodic effect on podset, pod filling and maturity in Bambara groundnut was observed and considerable differences existed between landraces under long days. The outcome of the effect of photoperiod on flowering time (days after sowing) was in agreement with most studies on Bambara groundnut, where there has been no influence of daylength treatments on progress to flowering (Brink, 1997; Brink, 1999; Harris and Azam-Ali, 1993; Linnemann, 1991; Linneman and Craufurd, 1994; Sesay et al., 2008). However, there was a delay in flowering time for Ankpa 4 in 16 h and the number of flowers produced reduced as daylength increased from 12 h to16 h. The flowering time and the total number of flowers produced are clearly influenced by both temperature and photoperiod (Figs. 1A, 1B). The results also showed that the number of pods and seed yield from long-day treatments considered in the five Bambara groundnut landraces, were mostly influenced by photoperiod since temperature was kept constant across all three conditions (Figs. 2, 3A and 3B). The glasshouse conditions, coupled with the fact that plants were grown as crop stands, make the results from this study more comparable to natural conditions. The above results indicate clearly that long days can have a significant economic impact on the seed yield of Bambara groundnut after flowering has occurred. This is similar to what is observed in indeterminate soybean cultivated under field conditions, where sensitivity to photoperiod remained high during the reproductive period and was highly and positively coupled with the process of generation of yield (Kantolic and Slafer, 2007).

Three photoperiodic types were identified: (1) qualitative short-day type (e.g. Ankpa 4), (2) quantitative short-day type (e.g. Getso, Gresik and LunT) and (3) quantitative long-day type (e.g. IITA-686). Seed number doubled in 16 h for IITA-686, but incomplete pod-filling (due to indeterminacy in 16 hours) led to an overall reduction in average seed weight. These results support the hypothesis that manipulating photoperiod sensitivity may be an avenue to increase and stabilize yields. Parental genotypes derived from landraces, which differ for photoperiod requirement in this study, have been crossed and a series of mapping populations created.

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References

Brink M. 1997. Rates of progress towards flowering and podding in Bambara groundnut (*Vigna subterranea*) as a function of temperature and photoperiod. Annals of Botany 80:505-513.

Brink M. 1999. Development, growth and dry matter partitioning in Bambara groundnut (*Vigna subterranea*) as influenced photoperiod and shading. Journal of Agricultural Science 133:159-166.

Brink M., Sibuga K.P., Tarimo A.J.P., Ramolemana G.M. 2000. Quantifying photothermal influence on reproductive development of Bambara groundnut (*Vigna subterranean*): models and their validation. Field Crops Research 66:1-14.

Dawson A., King V.M., Bentley G.E., Ball G.F. 2001. Photoperiodic control of seasonality in birds. Journal of Biological Rhythms 16:365–380.

Ellis R.H., Lawn R.J., Summerfield R.J., Qi A., Roberts E.H., Chay P.M., Brouwer J.B., Rose J.L., Yeates S.J. 1994. Towards the reliable prediction of time to flowering in six annual crops. III. Cowpea (*Vigna unguiculata*). Experimental Agriculture 30:17-29.

Garner W.W., Allard H.A. 1920. Effect of the relative length of day and night and other factors of the environment of growth and reproduction in plants. Journal of Agricultural Research 18:553–603.

Hadley P., Roberts E.H., Summerfield R.J., Minchin F.R. 1984. Effects of temperature and photoperiod on flowering in soya bean (*Glycine max*): a quantitative model. Annals of Botany, 53:669-681.

Harris D., Azam-Ali S. 1993. Implications of daylength sensitivity in Bambara groundnut (*Vigna subterranea*) for production in Botswana. Journal of Agricultural Science 120:75-78.

Jørgensen S.T., Aubanton M., Harmonic C., Dieryck C., Jacobsen S.P., Simonsen H.L., Ntundu W.H., Stadler F., Basu S., Christiansen J.L. 2009. Identification of photoperiod neutral lines of Bambara groundnut (*Vigna subterranea*) from Tanzania. Climate Change: Global Risks, Challenges and Decisions, Copenhagen, Denmark, IOP Conf. Ser.: Earth Environ. Sci. **6** 372023 doi: 10.1088/1755-1307/6/37/372023.

Kantolic A.G., Slafer G.A. 2005. Reproductive development and yield components in indeterminate soybean as affected by post-flowering photoperiod. Field Crops Research 93:212-222.

Kantolic A.G., Slafer G.A. 2007. Development and seed number in indeterminate soybean as affected by timing and duration of exposure to long photoperiods after flowering. Annals of Botany 99:925-933.

Keatinge J.D.H., Qi A., Wheeler T.R., Ellis R.H., Summerfield R.J. 1998. Effects of temperature and photoperiod on phenology as a guide to the selection of annual legume cover and green manure crops for hillside farming systems. Field Crops Research 57:139-152.

Linnemann A.R. 1991. Preliminary observations on photoperiod regulation on phenological development in Bambara groundnut (*Vigna subterranea*). Field Crops Research 26:295-304.

Linnemann A.R. 1993. Phenological development in Bambara groundnut (*Vigna subterranea*) at constant exposure to photoperiods of 10 to 16 h. Annals of Botany 71:445-452.

Linnemann A.R., Craufurd P.Q. 1994. Effects of temperature and photoperiod on phenological development in three genotypes of Bambara groundnut (*Vigna subterranea*). Annals of Botany 74:675-681.

Linnemann A.R., Westphal E., Wessel M. 1995. Photoperiod regulation of development and growth in Bambara groundnut (*Vigna subterranea*). Field Crops Research 40:39-47.

Molosiwa O., Aliyu S., Stadler F., Mayes K., Massawe F., Kilian A., Mayes S. (2015). SSR marker development, genetic diversity and population structure analysis of Bambara groundnut [*Vigna subterranea* (L.) Verdc.] landraces. Genetic Resources and Crop Evolution DOI 10.1007/s10722-015-0226-6

Nishitani T., Inouye J. 1981. The growth habit of Bambara groundnut (*Voandzeia subterranea* Thouars) and Kersting's groundnut (*Kerstingiella geocarpa* Harms); effects of daylength and temperature on flowering. Japanese Journal of Tropical Agriculture 25:163-169.

Sesay A., Magagula C.N., Mansuetus A.B. 2008. Influence of sowing date and environmental factors on the development and yield of Bambara groundnut (*Vigna subterranea*) landraces in a sub-tropical region. Experimental Agriculture 44:167-183.

Summerfield R.J., Lawn R.J., Qi A., Ellis R.H., Chay P.M., Brouwer J.B., Rose J.L., Shanmugasundaram S., Yeates S.J., Sandover S. 1993. Towards the reliable prediction of time to flowering in six annual crops. II. Soyabean (*Glycine max*). Experimental Agriculture 29:253-289.

Tan Y., Merrow M., Roenneberg T. 2004. Photoperiodism in Neurospora Crassa. Journal of Bioligical Rhythms 19:135-143.

Thomas B., Vince-Prue D. 1997. Photoperiodism in plants, 2nd edn. Academic press, San Diego.

Enhancing the resilience of livelihoods of small millet farmers through participatory varietal selection in India

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Abstract

Small millets are nutritious but underutilized crops that are grown in heterogeneous underutilized environments ranging from hills to coastal agro-ecosystems in India. In the last two decades, these rain-fed ecosystems have been affected by various climate change-related issues, the most important being a change in the rainfall pattern. These changes vary from place to place. So there is a need for high location specificity in varietal selection with the participation of farmers in each micro agro-ecosystem, instead of broad general recommendations. With this objective, Participatory Varietal Selection (PVS) was undertaken at five sites in India in a research project 'Revalorising Small millets in Rain-fed Regions of South Asia'. The methodology adopted comprised situation analysis to identify farmers' needs in a cultivar, short-listing of the traditional as well as released varieties likely to be suitable for the changed rainfall regimes, on-farm evaluation through mother and baby trials, and promotion of identified suitable varieties through community-based channels.

In the two cycles of PVS trials, 60 traditional varieties, 45 released varieties and 12 pre-release varieties in four small millets – finger millet (*Eleusine coracana)*, little millet, (*Panicum sumatrense*), kodo millet *(Paspalum scrobiculatum)* and barnyard millet (*Echinochloa frumentacea)* – were tested with the involvement of 578 men and 333 women farmers. Based on farmers' preferences and performance in the trials, one to four potential varieties were identified in each crop for each site. The results of these studies highlighted the importance of farmers' participation in the varietal selection process, as they used a combination of traits for varietal selection. It is expected that this study will demonstrate the importance of scaled-up PVS for enhancing resilience, and will create a case for including potential traditional varieties for promotion under state-funded crop support systems.

Keywords - Varietal diversity, traditional varieties, preference analysis, particpatory varietal selection (PVS), small millets

Introduction

Small millets are nutritious but underutilized crops that are an integral part of rain-fed ecosystems in India. They are grown in marginal and heterogeneous rain-fed environments ranging from hills to coastal agro-ecosystems. In the last two decades, these rain-fed ecosystems have been affected by various climate change-related issues. The main issues faced by the small millet farmers are late onset of rainfall, changes in the dry spell pattern within the crop period and reduction in rainfall amount. These issues have led to repeated crop loss, increase of fallow lands, loss of livelihoods and migration. So it is essential to ensure effective adaptation of small millet farmers at the micro-level for climate change, to enhance the resilience of their livelihoods.

The presence of varietal diversity in crops in general is expected to aid farmers to cope with the unexpected risks due to change in climate. But in the last two decades, a decline in inter- and intra-species diversity among small millets is being observed in various degrees in India. So there is a need for enhancing varietal diversity of small millets. Though climate change is happening everywhere, it affects each location differently and the local level impact also varies across the locations (ACEDRR, 2008). So there is a need for varietal selection under each micro agro-ecosystem, with the participation of farmers to identify suitable varieties, instead of broad general recommendations. For this purpose, the participatory varietal selection (PVS), a novel approach in selection of cultivars, was tested in the study. PVS was thought to be more appropriate (Yadavendra and Witcombe, 2007) as this approach is more rapid and cost-effective in identifying farmer-preferred cultivars provided a suitable choice of cultivars exists.

This research was carried out as part of an action research project, 'Revalorising Small millets in Rain-fed Regions of South Asia', aimed at enhancing the production and consumption of small millets, by using gender-sensitive participatory approaches to address constraints related to production, distribution and consumption. This project is supported under CIFSRF by IDRC and DFATD, Canada.

Materials and methods

This study was undertaken in five distinct agro-climatic regions of India during 2011-2012, covering four small millets – finger millet (*Eleusine coracana)*, little millet, (*Panicum sumatrense*), kodo millet *(Paspalum scrobiculatum)* and barnyard millet (*Echinochloa frumentacea)*. Finger millet is cultivated extensively in three project sites namely Anchetty (Tamil Nadu), Bero (Jharkhand) and Semiliguda (Odisha), while it is also grown on a smaller scale in the Jawadhu Hills, another project site in Tamil Nadu. The main crop of the latter site is little millet, which is also an important crop in Semiliguda. Barnyard millet and kodo millet are grown at the Peraiyur site. All four crops are cultivated mainly under rain-fed conditions.

In this study a schematic approach with the following components was followed in the following sequence: 1) understanding of varietal diversity in the sites, 2) PVS and 3) establishing community-based seed systems. The execution involved continual structured involvement and interaction of the farming community and scientists through the course of the study. For establishing the status of varietal diversity, participatory methods such as transect walks, biodiversity surveys and focus group discussions were followed. The PVS method adopted included the following: 1) situation analysis to identify farmers' needs in a cultivar, 2) a search for suitable materials to test with farmers, 3) experimentation on its acceptability in farmers' fields and 4) wider dissemination of farmer-preferred cultivars. Experimentation on acceptability of suitable materials in farmers' fields included mother trials, baby trials and informal research and development.

Based on the local farmers' needs in a cultivar and the input provided by the researchers working on these crops, a set of promising varieties, comprising both traditional and released ones, were short-listed for each crop in each relevant site. The short-listed varieties were evaluated for their suitability in diverse farm situations prevalent in each site by conducting mother trials during the 2011 and 2012 crop seasons on farmers' fields. The mother trials in each crop involved 8-10 promising varieties tested across the villages as unreplicated trials. However, replicated trials with the same set of varieties, one in farmers' fields and another at the research institution, were also undertaken during 2012. In mother trials, the plot size for each variety was 40 m^2 and 12 m^2 in replicated trials. Crops in mother trials were raised following farmers' practices in the site, such as sowing seeds by broadcasting. Recommended agronomic practices like appropriate fertilizer doses and line sowing were adopted only in the replicated trials. Special care was taken to ensure distribution of trials across the villages in the site and the involvement of women in the trials. Data were collected on various plant growth and yield parameters, which were analysed using appropriate statistical procedures. In addition to quantitative assessments, farmers' preference analysis was also carried out, wherein both men and women farmers assessed varietal performance in certain field trials as individuals and as groups. They individually selected the first three preferred varieties among the tested varieties and shared the reasons for this. Individual score values of each variety (as 1st, 2nd and 3rd preference) were converted into weighted scores. The most suitable varieties of finger millet, little millet, barnyard millet and kodo millet were identified for each site based on the analysis of trial data and farmers' preferences. Such identified varieties were further tested in baby trials with a greater number of farmers in each site in the years 2012 and 2013 for further confirmation of their suitability by evaluating them in larger plot size (minimum of 200 m^2) with farmers' varieties as controls. In baby trials, frequency analysis of positive and negative opinions of trial farmers along with the analysis of yield data was followed for identifying the suitable varieties. Varieties selected in baby trials in 2012 were to be taken further for large scale trials during 2013. Seeds of most preferred varieties emerging from PVS will be mass multiplied for further promotion in the region, leading to enhanced varietal diversity of the four crops.

Results

Status of varietal diversity in project sites

Finger millet – Anchetty presented a different picture compared to the other three sites in terms of high penetration of released varieties (Table 1). Most of the area under finger millet in Anchetty is covered by two released varieties (GPU 28 and INDAF 5). The local farmers here have been cultivating many released varieties such as HR 911, INDAF series and Sharadha, for over two decades. A high degree of varietal diversity was observed in Semiliguda, with 19 traditional varieties. Most of the areas under finger millet in Semiliguda, Bero and Jawadhu Hills were with few popular traditional varieties.

Little Millet – A large number of traditional varieties of little millet were observed in the Jawadhu Hills (9) as well as at Semiliguda (8). Of these only Sittan, Karusittan and Vella samai in Jawadhu Hills and Bada saon in Semiliguda are widely cultivated (Table 1).

Barnyard millet and Kodo millet – At the Peraiyur site, not much diversity was observed either in barnyard millet or in kodo millet. Only traditional varieties were found to be under cultivation in each crop. Among them Sadai (barnyard millet) and Siru varagu (kodo millet) were more popular.
The results of the study indicated that although there were present many varieties in the sites, no more than two varieties covered the majority of the area for each of the four crops studied. Further varietal diversity at hamlet level was very limited in all the sites (Table 2). This situation clearly indicated the need for increasing varietal diversity of small millets in the sites. As the baseline survey in the study sites indicated that more than 90% of the

farmers practise using farm-saved seeds, the best strategy for enhancing varietal diversity is by creating more options regarding the preferred varieties with the involvement of farmers, and popularizing the same for reaching large number of farmers. For this PVS was attempted in the sites.

Table 1. Status of varietal diversity in small millets at the study sites

Project sites	**No. of varieties present**		**Popular varieties**	
	Traditional	**Released**	**Number**	**Name**
Finger millet				
Anchetty	2	3	2	GPU 28 (R), INDAF 5 (R)
Bero	4	-	2	*Demba (T), Lohardagiya (T)*
Jawadhu Hills	2	-	1	*Muttan kelvaragu (T)*
Semiliguda	19	2	4	*Bati (T), Mati (T), Kalakarenga (T), Sunamani (T)*
Little millet				
Jawadhu Hills	9	-	3	*Sittan (T), Karusittan (T), Vella samai (T)*
Semiliguda	8	2	1	*Bada saon (T)*
Barnyard millet				
Peraiyur	3	-	1	*Sadai (T)*
Kodo millet				
Peraiyur	4	-	1	*Siru varagu (T)*

R- Released variety; T- Traditional variety

Table 2. Status of hamlet level varietal diversity of small millets at the study sites

Site	Crop	Hamlets studied	Share of hamlets with different number of varieties (%)				
			1	2	3	4	5
Semiliguda	Finger millet	40	40	28	20	10	3
	Little millet	20	95	5	0	0	0
Bero	Finger millet	32	69	31	0	0	0
Jawadhu Hills	Little millet	36	31	47	14	8	0
	Finger millet	33	45	45	9	0	0
Anchetty	Finger millet	29	62	24	14	0	0
Peraiyur	Barnyard millet	10	70	30	0	0	0

Source: Baseline survey, RESMISA project, 2011.

Participatory varietal selection

In the two cycles of mother trials conducted in 2011 and 2012 and one cycle of baby trials conducted in 2012, 60 traditional varieties, 45 released varieties and 12 pre-release varieties of four small millet crops were tested across the five sites, with the involvement of 578 men and 333 women farmers. The results of mother and baby trials are presented in Tables 3 and 4.

Finger millet – Among the 18 varieties (7 traditional, 9 released and 2 pre-release) included in mother trials in Anchetty, 4 varieties namely Kempu, Halukuli, GPU 66 and Sharadha were identified as suitable varieties. The baby trials in 2012 validated the suitability of the first three varieties. In Anchetty, as mentioned earlier, farmers have already better performing varieties like GPU 28 and the present need is to have alternatives that perform as well as these varieties. The Kempu and Halukuli varieties fulfilled this requirement due to their yielding ability and were preferred by the farmers. Similarly the performance of *Sharadha*, an old released variety, was also as productive as the top yielders and because it is already being grown in certain pockets of the site, it was chosen for further promotion in other areas based on farmers' preference. A pre-release variety, GPU 66, was preferred by the farmers due to its large panicle size.

At Bero, among the ten varieties (four traditional, four released and two pre-release) included in mother trials, four varieties namely A 404, GPU 28, GPU 66 and GPU 67 were identified as suitable. Baby trials in 2012 reconfirmed the suitability of the first two varieties. The varieties A 404 and GPU 28 were preferred for their high-yielding ability and medium crop duration. While GPU 66 was preferred due to medium crop duration, tall plants with large semi-compact panicles, e.g. GPU 67 were preferred due to their short duration, synchronized maturity and non-lodging character. Besides, the popular traditional varieties Lohardagiya and Demba, expressed high yielding ability which needs to be exploited in further crop improvement research.

At Jawadhu Hills, among the 12 varieties (5 traditional, 5 released and 2 pre-release) included in mother trials, 4 varieties namely Kempu, Ragalli sivalli, GPU 28 and GPU 66 were identified as the suitable varieties. Considering the presence of just two traditional varieties of finger millet in the site, all the four farmers' preferred varieties were included for evaluation during 2013.

Table 3. Identification of promising varieties through PVS in small millets – 2011 and 2012

Project sites	Mother Trials		Baby Trials		No. of varieties included in mother trials			No. of varieties selected for further testing			
	No. of varieties	No. of trials	No. of varieties	No. of trials	Trad	Rele	Pre-R	Trad	Rele	Pre-R	Total
Finger millet											
Anchetty 2011 2012	 15 10	 27 14	 -- 3	 -- 34	7	9	2	2	1	1	4
Bero 2011 2012	 6 9	 25 24	 -- 2	 44	4	4	2		2	2	4
Jawadhu Hills 2011 2012	 7 10	 12 24	 -- --	 -- --	5	5	2	2	1	1	4
Semiliguda 2011 2012	 21 10	 16 21	 -- 2	 -- 67	18	5	2		1	2	3
Little millet											

Jawadhu Hills 2011 2012	 8 9	 33 22	 -- 2	 -- 35	8	5		2	1		3
Semiliguda 2011 2012	 8 10	 16 22	 -- --	 -- --	5	5		1			1
Barnyard millet											
Peraiyur 2011 2012	 19 10	 16 18	 -- 3	 -- 64	8	9		3	1		4
Kodo millet											
Peraiyur 2011 2012	 4 10	 5 14	 -- --	 -- --	5	3	4	1		1	2

Trad – Traditional variety; Rele – Released Variety; Pre-R – Pre release variety

At Semiliguda, among the 25 varieties of finger millet (18 traditional, 5 released and 2 pre-release) included in mother trials, 4 varieties namely Bhairabi, Chilika, GPU 66 and GPU 67 were identified as suitable varieties. Baby trials in 2012 validated the suitability of the Bhairabi variety and not Chilika. The variety Bhairabi was preferred due to its traits namely high yield, medium crop duration, non-lodging, ease in threshing and bold grains. The variety GPU 66 was preferred due to its bigger panicle size and high yield while GPU 67 was preferred due to its non-lodging nature and uniform maturity. The variety Chilika was not preferred by the majority of the farmers in 2012 due to its traits, namely grain loss from finger breakage during harvest, poor threshability and small grains. Kalakarenga and Mati, the two popular traditional varieties, expressed high yielding ability and could be useful in further varietal improvement.

Little millet – In the Jawadhu Hills, of the 13 varieties (8 traditional and 5 released) included in mother trials, 3 varieties namely CO 4, Koluthana and Perungolai were identified as suitable varieties. Baby trials in 2012 reconfirmed the suitability of CO 4 and the other two will be tested in 2013. The variety CO 4 was preferred because of its height (more fodder yield) and Koluthana for its semi-compact panicles. Perungolai was selected because of its large, semi-compact panicles and tall stature. The popular local cultivars, Sittan *a*nd Karusittan, expressed high yielding ability and less grain shattering at maturity, which could be exploited in breeding research.

At Semiliguda, among the ten varieties (five traditional and five released) included in mother trials, Kala saon was identified as the suitable variety. In farmers' preference analysis, Bada saon was the most preferred variety due to its high yielding ability, suitability for waterlogged lands and ease in harvesting and threshing. It could be exploited for further varietal improvement.

Barnyard millet – At Peraiyur, of the 17 varieties (8 traditional and 9 released) included in mother trials, 4 varieties namely CO 2, Mallankinaru, Mallankinaru1 and Aruppukottai were identified as suitable varieties. Baby trials in 2012 reconfirmed the suitability of the first three varieties. The local popular variety, Sadai expressed high yielding ability, which needs to be exploited for further varietal improvement.

Kodo millet– At Peraiyur, among the 12 varieties (5 traditional, 3 released and 4 pre-release) included in mother trials, 2 varieties namely Uppu varagu and TNAU 111 were identified as the suitable varieties. While Uppu varagu was the highest yielder, TNAU-111 was preferred by the farmers for its bold grain in addition to good yielding ability. Siru varagu, the local popular variety, expressed high yielding ability, which needs to be exploited for further varietal improvement.

Table 4. Performance of promising small millets varieties in baby trials, 2012

Project sites	Test variety	Local check varieties	Number of trials	Average grain yield (Kg/ac)		No. of trials with yield over the check	
				Test variety	Check variety	Increase	Decrease
Finger millet							
Anchetty	GPU 66	INDAF 5, GPU 28	12	1028	1012	7	5
	Kempu		10	768	756	4	6
	Halukuli		10	876	896	5	5
Bero	A 404	*Demba, Lohardagiya, Hybrid, Dudarice*	19	1156	952	10	1
	GPU 28		18	736	610	11	5
Semiliguda	Bhairabi	*Bodi, Bada, Dinda, Badu, Dasrabodi, Sana, Bada, Bodel*	30	784	744	19	11
	Chilika		19	856	752	16	3
Little millet							
Jawadhu Hills	CO 4	*Sittan, Siruvellai, Siru samai, IR 8*	19	337	360	10	9
Barnyard millet							
Peraiyur	CO 2	*Sadai*	15	1040	992	12	3
	Mallankinaru		16	1196	1104	15	1
	Mallankinaru 1		16	1148	1076	12	3

Discussion

The present study indicated that participatory varietal selection is a robust and efficient approach to identifying additional suitable varieties within a short period. One to four varieties were identified for each crop in each of the five sites in two PVS cycles. Joshi et al. (1997) also reported that the PVS approach provided farmers with the benefits of new genetic materials five to six years in advance of the formal system and with minimal effort. The utility of the PVS approach in understanding farmers' criteria for selecting varieties and analysing reasons for non-adoption of released varieties in finger millet has been reported by Gowda et al. (2000).

Though yield appeared to be the main criterion for assessing the superiority of a variety, the trial farmers also took into consideration several other traits/dimensions before preferring a variety. Presence of varietal trials on their own fields provided every trial farmer with an opportunity to closely observe the performance of each variety. In addition to high-yielding ability (high tillering, larger panicle size and bold grains), crop duration, non-shattering of grains at maturity, non-lodging, uniform maturity, and good fodder yield were also considered. Women farmers, in particular, were more concerned with grain quality traits, such as colour, taste, grain hardiness and storage quality. The results also revealed that some of the popular traditional varieties from the nearby area were suitable, indicating the possibility of introducing varieties from neighbouring areas. Such varieties have the advantage of being adapted to similar ecologies and hence show good adaptation. PVS also brought immediate benefits to the target farmers in having access to a large number of potential varieties and having in their possession the seeds of promising varieties identified by them. Furthermore, the study indicated the need for local community organizations to locate these participatory research efforts and to provide continuity.

The impact of PVS largely depends on the adoption of identified varieties by the local farmers in the study sites. Some of the possible next steps to build on the efforts so far made include:

1. Initiation of a decentralised system of quality seed production and distribution at the community level in each site, to promote both the existing as well as the newly identified suitable varieties on a sustainable basis[1],
2. Purification of potential traditional varieties and promoting proper seed selection among farmers in order to preserve their identity,
3. Conservation of vanishing varieties through relevant grassroots level institutions and custodian farmers, and
4. Participatory plant breeding to fully exploit the high genetic potential of traditional varieties.[2]

The project has demonstrated the effectiveness of scaled-up PVS for enhancing the resilience of small millet farmers. Some of the policy suggestions that emerge from this study are:

1. PVS to be used by national agricultural research systems for refining their districtwise recommendations of varieties, particularly where specific adaptation to agro-ecosystems is required such as in the Eastern Ghats and where quality parameters plays an important role in varietal selection,
2. The role of local community organizations in varietal improvement needs to be recognized and they need to be given state support for establishing community-based seed systems that effectively integrate on-farm conservation, varietal improvement and varietal distribution,[3]
3. Popular traditional varieties need to be included in the formal public seed system for production and distribution and also in other state-funded crop support systems, and

[1] See Walker, 2006 on the need for such decentralized seed systems.

[2] See Ceccarelli and Grando (2007) for more on the need for participatory plant breeding.

[3] See Joshi et al. (1997) and Joshi and Witcombe (1996) for institutionalization of the PVS approach and involvement of relevant grassroots level institutions for improving varietal evaluation systems.

4. Farmers' rights related to these varieties need to be recognized and secured through various ways, including through using geographical indicators.

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References

Ceccarelli S., Grando S. 2007. A model of decentralized-participatory plant breeding. In: Arunachalam V, editor. Participatory plant breeding and Knowledge management for strengthening rural livelihoods. MSSRF/PR/07/66, Chennai, India, pp.19-40.

Advanced Centre for Enabling Disaster Risk Reduction (ACEDRR). 2008. Coping with disasters–participatory designing of efficient cropping pattern for rain-fed location. http://dhan.org/acedrr/research-coping-with-disasters.html

Gowda B.T.S, Hallaswamy B.H., Seetharam A., Virk D.S., Witcombe J.R. 2000. Participatory approach in varietal improvement: A case study in finger millet. Current Science 79:366-368.

Joshi K.D., Subedi M., Rana R.B., Kadayat K.B., Sthapit R. 1997. Enhancing on-farm varietal diversity through participatory varietal selection: A case study for Chaite rice in Nepal. Experimental Agriculture 33:335-344.

Joshi A., Witcombe J.R. 1996. Farmer participatory crop improvement. II. Participatory varietal selection, a case study in India. Experimental Agriculture 32:461-477.

Walker T.S. 2006. Participatory varietal selection, participatory plant breeding, and varietal change. World Bank, Washington, DC, https://openknowledge.worldbankorg/handle/10986/9182

Yadavendra J.P., Witcombe J.R. 2007. The impact of new maize and rice varieties on the livelihoods of poor farmers in marginal agricultural areas of western India. In: Arunachalam V, editor. Participatory plant breeding and Knowledge management for strengthening rural livelihoods. MSSRF/PR/07/66, Chennai, India, pp.19-40.

The potential of castorbean as a bioenergy crop in Egypt

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Abstract
Seeds of four castorbean landraces were obtained from different locations in Egypt. Seeds were first investigated for physical properties, and later planted in the field during the 2012 season. The morpho-agronomic properties, oil concentration, and fatty acids composition were studied. Based on seed size, three main landraces of dry, dehiscent castor seed type were identified: a small-seeded landrace, a medium-seeded landrace, and a large-seeded landrace. The seed size ranged from 0.18-0.71 g by weight, 0.86-2.2 cm by height, 0.46-1.3 cm by width, and 0.27-0.57 cm by thickness. The red medium-seeded landrace had a greater plant height (320 cm).The brown medium-seeded landrace recorded the tallest main spike (38 cm), the highest number of capsules per spike (39), the maximum number of capsules per plant (743.7), the highest seed yield per plant (1.1 kg), the highest seed yield per hectare (1.75 tonnes), and maximum oil yield per hectare (648.1 kg). The grey small-seeded landrace had the highest oil percentage (44%). The brown large-seeded landrace produced the heavier grains (68.06 g) and five fatty acids were found in castor oil (palmitic, stearic, oleic, linoleic, and ricinoleic acid). A slight variation was noted in ricinoleic acid, which was the major component of oil ranging from 81.96 to 86.80%. The brown medium-seeded landrace with a high seed yield, oil content, and desirable fatty acid composition will be further investigated for biodiesel production.

Keywords: biodiesel, castorbean, fatty acids.

Introduction
Castorbean (*Ricinus communis* L.) belongs to the family Euphorbiaceae (Salamatbakhsh et al., 2012) and has been in cultivation for 4000 years (Olsnes, 2004). Castor is a fast-growing C_3 plant (Doan, 2004) and is cultivated in many tropical and subtropical regions of the world (Govaerts et al., 2000) as well as on a commercial scale in more than 31 countries. India, China, and Mozambique are the major castorbean growing countries, comprising about 98% of the world's production (FAO, 2012). The world annual production of castor oil seeds in 2012 was about 1.95 million tonnes. Castor is an important industrial oilseed crop (Sarwar et al., 2010). The seeds contain a very high oil content of approximately 50%, which can be extracted easily by pressure. The oil already has a growing international market, assured by more than 700 uses and is a basic constituent in a variety of industries, including the manufacturing of soaps, paints, dyes, coatings, inks, cold-resistant plastics, waxes and polishes, nylon, pharmaceuticals, and perfumes (Millard and Leclaire, 2007). Castorbean cultivation emerges as a promising activity for biodiesel production, providing income in resource-poor areas (Savy-Filho, 2005). The biodiesel derived from castor oil has several advantages over other vegetable oils due to the presence of 5% more oxygen, low levels of residual phosphorus and carbon, high cetane number, solubility in alcohol and absence of aromatic hydrocarbons (Scholz and Silva, 2008). The high viscosity of the castor oil is due to the high percentage of ricinoleic acid, which is a limiting factor for the use of pure castorbean diesel in engines (Pinzi et al., 2009). However, the

use of this biodiesel blended with petrodiesel can be exploited in regions with severe winters. This is a highly recommended procedure because of its low freezing point and the lubricant power afforded by castor oil (Berman et al., 2011; Singh, 2011). The castorbean plant presents a wide variation regarding vegetative traits such as leaf and stem colours, number and size of leaf lobes and presence of wax covering the stem (Savy-Filho, 2005). There are nearly 250 cultivars of castorbean (Ovenden et al., 2009). Due to the increased demand in many countries, improvement of varieties is drawing greater attention from breeders (Sujatha et al., 2008). It is necessary to characterize the genetic diversity present across *R. communis* germplasm from different geographic regions to develop a genotyping scheme that links castorbean traits to a particular germplasm source or geographic region (Hinckley, 2006). Morphological characterization is the first step in the description and classification of germplasm collections (Smith et al., 1991) to guide use enhancement. Therefore, the objectives of this study were to compare and evaluate some castorbean landraces in terms of morpho-agronomic characters and genetic diversity to explore their potential use as a bioenergy crop in Egypt.

Materials and methods

To evaluate the possibility of using castorbean (*R. communis*) as a bio energy crop in Egypt, four landraces were investigated. Collections of seeds were made from various locations in two governorates (Qalyubia and Menufia) during 2011. The seeds were planted in the experimental field of the soils and water research department, NRC, Atomic Energy Authority, Egypt, at a distance of 3 m x 3 m between plants. The soil was characterized as sandy (sand 90% silt 0.02%, and clay 0.07%) and the chemical properties were pH: 7.97, electrical conductivity (EC) (dS/m): 0.27, soil organic matter (%): 0.3, total nitrogen (%): 0.07. Plants received NPK at the rates 150, 110 and 110 kg ha^{-1} in the form of ammonium sulphate, super phosphate, and potassium sulphate respectively. Plants were irrigated with freshwater every two days and were arranged in a randomized complete block design with three replicates. The plants were harvested on August 2012. Plant height (cm), main spike length (cm), number of spikes per plant, number of capsules per spike, number of capsules per plant, 100-seed weight (g), seed yield, oil percentage (%), and oil yield were determined. The results were expressed as means with standard deviation (±S.D.) and LSD=0.05.

Results and discussion

Physical characterization of the castorbean landraces

Seeds: based on seed size, three main landraces of dry dehiscent castorbean seed type were identified within the study area (Figure 1): a small-seeded landrace (SSL), a medium-seeded landrace (MSL), and a large-seeded landrace (LSL). As described in Table 1, the seed size ranged from 0.18-0.71 g by weight, 0.86-2.16 cm by height, 0.46-1.30 cm by width, and 0.27-0.57 cm by thickness. Based on seed colours, four seed-coat colour patterns were found. The large-seeded landraces exist in white-specked brown, medium-seeded landraces in red and brown, while the small-seeded landraces exist mainly in a dark grey colour as indicated in Figure 1.

Table 1. Physical characteristics of the seeds of the four castorbean landraces

	Seed characteristics											
Castorbean Landraces	Weight (g)			Height (cm)			Width (cm)			Thickness (cm)		
	M		SD	M		SD	M		SD	M		SD
L1	0.18	±	0.01	0.86	±	0.06	0.46	±	0.06	0.27	±	0.06
L2	0.50	±	0.05	1.55	±	0.13	0.86	±	0.06	0.57	±	0.06
L3	0.48	±	0.01	1.40	±	0.10	0.85	±	0.05	0.57	±	0.06
L4	0.71	±	0.01	2.16	±	0.06	1.30	±	0.10	0.53	±	0.06
L.S.D.	0.04			0.17			0.13			0.11		

*The data represent mean ± SD of three replicates, and L.S.D. = 0.05

Plant height: this is an important indicator of ultimate yield and is a function of genetic constitution and environmental conditions under which the crop is grown (Cheema, 2011). The data in Table 2 show that the red medium-seeded landrace (RMSL) had the highest plant height of 320 cm, followed by the brown medium-seeded (BMSL) and the grey small-seeded landraces (GSSL; 288.3 and 263.3 cm). The shortest plants were observed in the case of the brown large-seeded landrace (BLSL; 228.3 cm). The difference in plant height may be attributed to the genetic make-up of landraces and adequate moisture availability. Our results corroborate the findings of Cheema (2011) who stated that plant height is a genetic trait, as well as temperature- and moisture-related traits.

Main spike length (cm)**:** data in Table 2 show that L2 produced the tallest main spike (38 cm), followed by L3 (22.3 cm). However, L4 recorded the shortest main spike (20.3 cm). Sarwar et al. (2010) reported that the trait, main spike length, is more important and can play a significant role for improving seed yield if selection is based on this trait.

Number of spikes per plant**:** as described in Table 2, the GSSL had the highest number of spikes plant^{-1} (31.5), followed by BMSL and BLSL, 19 for both. On the other hand, RMSL had the lowest number of spikes per plant (6.7). Moshkin (1967) reported that there was a close relationship between seeds and number of spikes, number of capsules, and size of seed in a castorbean.

Number of capsules per spike**:** data regarding number of capsules per spike are presented in Table 2. BMSL recorded the highest number of capsules per spike (39), whereas GSSL showed the lowest number, 18.33. Sarwar et al. (2010) reported that traits like spikes per plant and capsules per spike are more important and can play a significant role for improving seed yield if selection is based on these traits.

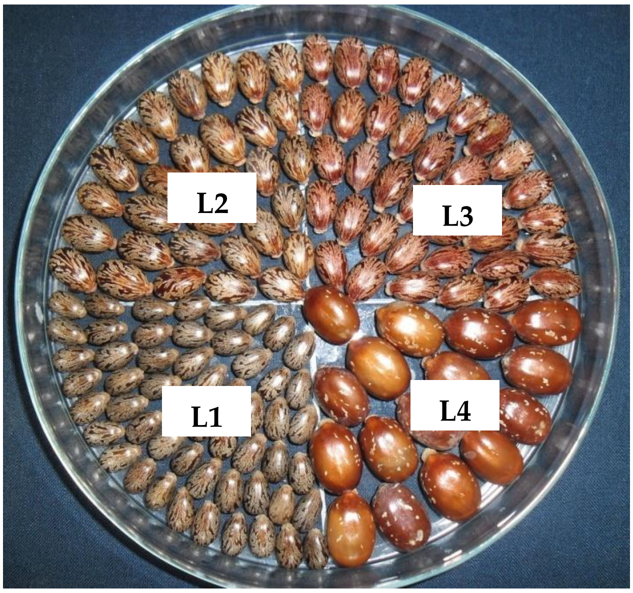

Figure 1. Representation of the variability in colour and size observed among seeds of castorbean collected from different locations in Egypt.

Table 2. Plant height, main spike length, no. of spikes per plant, and no. of capsules per spike for the four castorbean landraces

Castorbean Landraces	Plant height (cm)			Main spike length (cm)			No. of spikes per plant			No. of capsules per spike		
	M		SD	M		SD	M		SD	M		SD
L1	263.3	±	12.6	21.00	±	1.0	31.50	±	3.5	18.33	±	0.6
L2	288.3	±	10.4	38.00	±	3.5	19.00	±	1.0	39.00	±	4.0
L3	320.0	±	20.0	22.33	±	0.6	6.70	±	0.3	21.33	±	1.5
L4	228.3	±	15.3	20.33	±	2.1	19.00	±	1.0	20.33	±	1.5
L.S.D.	28.24			3.95			3.56			4.31		

*The data represent mean ± SD of three replicates and LSD = 0.05.

***Number of capsules per plant*:** this is one of the most important components contributing to the yield of castorbean crop (Cheema, 2011). As shown in Table 3, the maximum numbers of capsules per plant (743.7) were produced by L2, while the minimum numbers (142.8) were produced by L3. The number of capsules appears to be a function of the number of branches. The greater number of branches results in a higher number of capsules. Koutroubas et al. (1999) reported that the number of capsules per plant of different varieties, can be different depending on genetic characteristics and weather conditions.

***100-seed weight*:** it is obvious from the data presented in Table 3 that L4 produced heavier (68.06 g) grains followed by L2 (43.51 g), while the lighter seeds (19.62 g) were produced by L1. Similar results were observed by Akula and Reddy (1998), who reported a variation in 100-seed weight among different cultivars of castorbean. Hocking and Steer (1989) reported that seedling from small seeds produced less dry matter yield. Ahmad (2001) also found that larger seed is more robust than smaller seeds.

Table 3. Number of capsules per plant, weight of 100 seeds, seed yield per plant, and seed yield per ha (tonnes) for the four castorbean landraces.

Castorbean Landraces	No. of capsules plant^{-1}			Weight of 100 seeds (g)			Seed yield plant^{-1} (kg)			Seed yield ha^{-1} (tonne)		
	M		SD	M		SD	M		SD	M		SD
L1	578.7	±	81.1	19.62	±	3.9	0.31	±	0.04	0.49	±	0.06
L2	743.7	±	115.0	43.51	±	2.1	1.10	±	0.06	1.75	±	0.10
L3	142.8	±	10.2	41.64	±	2.8	0.21	±	0.01	0.33	±	0.02
L4	386.0	±	29.5	68.06	±	5.3	0.82	±	0.05	1.30	±	0.08
L.S.D.	135.7			6.97			0.08			0.13		

*The data represent mean ± SD of three replicates and LSD = 0.05.

Seed yield per plant: this is a complex and multifaceted trait as it represents the ultimate expression of different yield factors (Sarwar et al., 2010). As shown in Table 3, the minimum seed weight per plant (0.21 kg) was produced by RMSL. However, the maximum seed weight (1.10 kg) was obtained from BMSL. Salih and Khidir (1975) reported that the number of primary branches per plant, the number of capsules and 100-seed weight in castorbean had high positive correlations with yield per plant and with each other.

Seed yield per hectare: this is an important parameter from a farmer's point of view (Cheema, 2011). Traits like branches per plant, main spike length, spikes per plant and capsules per spike can play a significant role for improving seed yield if selection is based on these traits (Sarwar et al., 2010). L2 recorded the highest seed yield ha^{-1} (1.75 tonnes), followed by L4, 1.30 tonnes, while L3 showed the lowest seed yield per ha^{-1} (0.33 tonnes). Williams and Kittock (1969) reported that higher seed yields were associated with early planting, adequate moisture availability and early harvest. The results are in accordance with Kumar et al. (1997) who showed that the moisture adequacy index positively influenced yield and accounts for significant variation in the total yield of castorbean. Lima et al. (1998) found that castorbean seed yield was positively correlated with plant height and number of capsules per plant. Reddy et al. (1999) studying 56 castorbean germplasm lines observed correlation of seed yield with earliness, capsule weight and number of capsules per plant under rain fed and irrigated conditions. Aswani et al. (2003) reported that capsules in primary spikes, number of spikes per plant, number of days to 50 percent flowering and maturity, length of primary spikes and 100-seed weight were the major yield-contributing characters in castorbean.

Oil content: castorbean has been traditionally cultivated for the production of lubricants and paints (Berman et al., 2011; Scholz and Silva, 2008). The oil content and other characteristics are temperature and moisture dependent. The maximum oil content was demonstrated by L1 (44.01%) closely followed by L3 (40.63%), while the lowest oil percentage (31.35%) was recovered for L4. The results are in accordance with Yermanson et al. (1967) who reported that small grains have a lower percentage of skin and therefore contain a higher oil percentage. The amount of oil in castorbean seeds is a genetic trait, but is affected by environmental conditions, agricultural operations and harvesting time (Taherifard et al., 2012). Also it might be due to the conducive conditions available which led to better uptake of nutrients from the soil favouring oil synthesis. Similar results were observed by Ramos et al. (1984) who found a large variation in oil percentage of 36 castorbean cultivars, ranging from 39.6 to 59.5%.

Table 4. Oil content (%) and oil yield ha^{-1} (kg) for the four castorbean landraces.

Castorbean Landraces	Oil percentage (%)			Oil yield ha^{-1} (kg)		
	M		SD	M		SD
L1	44.01	±	4.8	215.8	±	13.3
L2	37.07	±	3.2	648.1	±	53.5
L3	40.62	±	2.3	132.8	±	16.2
L4	31.35	±	2.6	407.5	±	25.8
L.S.D.	6.32			59.29		

*The data represent mean ± SD of three replicates and LSD = 0.05

***Oil yield ha^{-1}*: this was calculated on the basis of oil content and grain yield. As shown in Table 4, BMSL recorded the highest oil yield per hectare (648.1 kg), followed by the BLSL (407.5 kg). On the other hand, L3 had the lowest oil yield per hectare (132.8 kg). Gupta et al. (1951) studied the variability of oils of 19 castorbean seed cultivars widely grown in Africa, America and Asia. They found that the oil content did not vary in most of them.

Fatty acids profile

The detailed results and discussion of individual fatty acids is given below:

1-Palmitic acid: a higher concentration of palmitic acid (2.8%) was recorded with BLSL followed by BMSL, and GSSL. On the other hand, the lowest palmitic acid content (1.5%) was found in RMSL. Our findings are in agreement with Ramos et al. (1984) who observed differences among different cultivars of castorbean for palmitic acid. Gupta et al. (1951) studied oils of 19 castor seed cultivars and found that palmitic acid was roughly over 1%.

2-Stearic acid: stearic acid is a saturated fatty acid and not desired in edible as well as industrial oil like castorbean. It is found in a minute quantity in castorbean (Cheema, 2011). The highest stearic acid (3.40%) content was recorded for GSSL, followed by BMSL (1.4%). On the other hand, the lowest stearic acid (0.6%) content was observed in the fourth landrace (BLSL). Velasco et al. (2005) reported that different varieties of castorbean showed different stearic acid levels.

3-Oleic acid: the data regarding oleic acid percentage are presented in Table 5. It is clear from the data that GSSL produced the highest oleic acid (8.3%) followed by RMSL (7.1%). On the other hand, BLSL produced the minimum oleic acid (0.1%). The results of oleic acid are in accordance with Rojas-Barros et al. (2005) who reported that the analysis of fatty acid composition of individual F2 castor seeds showed a considerable variation for oleic acid.

Table 5. Palmitic, stearic, oleic, linoleic, and ricinoleic acid concentrations of four castorbean landraces

Castorbean Landraces	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Ricinoleic Acid
L1	1.64	3.40	8.30	4.70	81.96
L2	1.80	1.40	3.70	9.30	83.80
L3	1.50	1.00	7.10	7.40	83.03
L4	2.80	0.60	0.10	9.70	86.80

*The data represent means of three replicates

4-Linoleic acid: linoleic acid is an unsaturated fatty acid (Cheema, 2011). The maximum linoleic acid content (9.7%) was obtained from L4, followed by L2 (9.3%), and L3 (7.4%). The lowest linoleic acid percent (4.7%) was produced by L1. Our study supported the results of Lanna et al. (2005) who demonstrated soybean oil variability in linoleinic acid content for different soybean varieties.

5-Ricinoleic acid: ricinoleic acid is the main component of castor oil (Jeong and Park, 2009).The quality of castor oil mainly depends on ricinoleic acid. A slight variation was noted in ricinoleic acid. BLSL had the highest ricinoleic acid content (86.8%) followed by BMSL and RMSL (83.8 and 83.03% respectively). However, GSSL produced the lowest ricinoleic acid percentage (81.96). Our study supported the results of Ramos et al. (1984) who noted slight variation in ricinoleic acid of castor oil ranging from 83.65 to 90%. Also, Rojas-Barros et al. (2005) showed considerable variation for ricinoleic acid in F2 castor seeds. Among the fatty acids, ricinoleic acid ranged between 91.4 to 94.9% while others had a lesser percentage (Gupta et al., 1951).

Conclusion

Despite being an important crop, castorbean has never been realized as a commercial crop in Egypt. Moreover, it is grown on marginalized land without receiving much care and attention. Based on seed size and colour, four castorbean landraces were identified. The brown medium-seeded and the brown large-seeded landraces with a high seed yield, oil content and desirable fatty acid composition could be a good source for biodiesel production.

References

Ahmad S. 2001. Environmental effects on seed characteristics of sunflower (*Helianthus annus*). Journal of Agronomy and Crop Science 187:213-216.

Akula B., Reddy T.B. 1998. Effect of dates of sowing on yield of castor cultivars. Journal of Oilseed Research 15:375-376.

Aswani K., Sangwan R.S., Jatasra D.S. 2003. Correlation and path coefficient analysis in castor (*R.communis*) under dry land conditions. Indian Journal of Dryland Agricultural Research and Development 18:89-91.

Berman P., Nizri S., Wiesman Z. 2011. Castor oil biodiesel and its blends as alternative fuel. Biomass & Bioenergy 35:2861–2866.

Cheema N.M. 2011. Yield and Chemical Composition of Castorbean (*R.communis*) As Influenced By Environment. Department of Agron., Fac. of Crop and Food Sci., Arid Agriculture Univ., Rawalpindi, Pakistan. PhD thesis.

Doan L.G. 2004. Ricin: Mechanism of Toxicity, Clinical Manifestations, and Vaccine Development: A Review. Journal of Toxicology 42:201-208.

FAO. 2012. Food and Agriculture Organization of United Nations. (http/faostat.fao.org).

Govaerts R., Frodin D.G., Radcliffe-Smith A. 2000. World checklist and bibliography of Euphorbiaceae (with Pandaceae). Redwood Books Limited, Trowbridge, Wiltshire.

Gupta S.S., Hilditch T.P., Riley G.P. 1951. The fatty acids and glycerides of castor oil. Journal of the Science of Food and Agriculture 2:245-251.

Hinckley A.C. 2006. Genotyping and bioforensics of *R.communis*. Lawrence Livermore National Laborry. UCRL-TH-226437.

Hocking P.J., Steer B.I. 1989. Effect of seed size, cotyledon removal and nitrogen stress on growth and yield components of oilseed sunflower. Field Crop Research 22:59-75.

Jeong G.T., Park D.H. 2009. Optimization of biodiesel production from castor oil using response surface methodology. Applied Biochemistry and Biotechnology 156:431-441.

Koutroubas S.D., Papakosta D.K., Doitsinis A. 1999. Adaptation and yielding ability of castor plant (*R. communis*) genotypes in a Mediterranean climate. European Journal of Agronomy 11:227-237.

Kumar P.V., Ramakrishna Y.S., Ramana Rao B.V., Victor U.S., Srivastava N.N., Subba Rao A.M. 1997. Influence of moisture, thermal and photoperiodic regimes on the productivity of castorbeans (*R.communis*). Agricultural & Forest Meteorology 88:279-289.

Lanna A.C., Jose A.C., Oliveira M.G.A., Barros E.G., Moreira M.A. 2005. Effect of temperature on poly unsaturated fatty acid accumulation in soybean seeds. Brazilian Journal of Plant Physiology 17:676-690.

Lima E.F., Santos J., Dos W. 1998. Genotypic, phenotypic and environmental correlations among agronomic characteristics of castorbean (*R.communis*). Revista de Oleaginosas e Fibrosas 2:147-150.

Millard C.B., Leclaire R.D. 2007. Ricin and Related Toxins: Review and Perspective.In: Romano JA, Lukey BJ, Salem H. (editors). 2007. Chemical warfare agents: chemistry, pharmacology, toxicology, and therapeutics. CRC Press. Ch. 17, pp.432-452.

Moshkin V.A. 1967. *Rukovodstvo po sektsii i semenovodst vu maslichnykh kultur* (Manual of breeding and seed production of oil crops) Moscow. In: Castor: Moshkin, V.A. (ed.). Amerind Publishing Co. (Pvt).Ltd. New Delhi 1986. p:1-315.

Olsnes S. 2004. The history of ricin, abrin and related toxins. Toxicon 44:361-370.

Ovenden S.P.B., Gordon B.R., Bagas C.K., Muir B., Rochfort S., Bourne D.J. 2009. Cultivar Determination of *R.communis* via the Metabolome: a Proof of Concept Investigation. Human Protection and Performance Division DSTO Defense Science and Technology Organization 506 Lorimer St Fishermans Bend, Victoria 3207 Australia.

Pinzi S., Garcia I.L., Lopez-Gimenez F.J., Castro M.D.L., Dorado G., Dorado M.P. 2009. The ideal vegetable oil-based biodiesel composition: a review of social, economical and technical implications. Energy & Fuels 23:2325-2341.

Ramos L.C.S., Tango J.S., Savi A., Leal N.R. 1984. Variability for oil and fatty acid composition in castorbean varieties. Journal of the American Oil Chemists' Society 61:1841-1843.

Reddy P.R., Vanaj M., Sankar G.R.M., Rao C.H., Venkateswarlu S., Eastin J.D. 1999. Yield components in castor germplasm under irrigated and rain fed conditions. Journal of Maharashtra Agricultural Universities 24:36-41.

Rojas-Barros P., de-Haro A., Fernandez-Martinez J.M. 2005. Inhabitance of high oleic / low ricin oleic acid content in the seed oil of castor mutant OLE-1. Crop Science 45:157-162.

Salamatbakhsh M.R., Tobe A., Taherifard E. 2012. Effects of foliar application of micronutrients on yield and yield components of castorbean (*R.communis*) varieties. European Journal of Experimental Biology 2:975-979.

Salih S.H., Khidir M.O. 1975. Correlations, Path Analyses and Selection Indices for Castorbean (*R.communis* L.). Experimental Agriculture 11:145-154.

Sarwar G., Ahmed H.M., Hussain J. 2010. Evaluation of Castorbean (*R. Communis*) Mutants for Genetic Parameters and Cluster Analysis. Journal of Agricultural Research 48:289-302.

Savy-Filho A. 2005. Melhoramento da mamona, In: Borem A, editor. Melhoramento de especiescultivadas. Second Edition, 429-452, Editora UFV, 85-7269-206-1, Viçosa, Brazil.

Scholz V., Silva J.N. 2008.Prospects and risks of the use of castor oil as a fuel. Biomass and Bioenergy 32:95-100.

Singh A.K. 2011. Castor oil-based lubricant reduces smoke emission in two-stroke engines. Industrial Crops and Products 33:287-295.

Smith S.E., Dos A.L., Warburton M. 1991. Morphological and agronomic variation in North African and Arabian alfalfa. Crop Science 31:1159-1163.

Sujatha M., Reddy T.P., Mahasi M.J. 2008.Role of biotechnological interventions in the improvement of *R.communis* and *Jatropha curcas*. Biotechnology Advances 26:424-435.

Taherifard E., Ghorttapeh A.H., Gerami F. 2012. Manipulation of castorbean (*R.communis*) growth and its effect on some characteristics by pruning of lateral branches. Annals of Biological Research 3:1909-1913.

Velasco L., Rojas B.P., Fernandez M.J.M. 2005. Fatty acid and tocopherol accumulation in the seeds of high oleic acid castor mutant. Industrial Crops and Products 22:201-206.

Williams J.H., Kittock D.L. 1969. Management factors influencing viability of castorbean (*R.communis* L.) seed. Agronomy Journal 61:954-958.

Yermanson D.M., Hemestreet F., Carber M.J. 1967. Inheritance of Quality and Quantity of Seed-Oil in Safflower (*Carthamus tinctorius*) Crop Science 7:417-422.

Genetic improvement of winged bean for increased productivity and nutritional security

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Abstract
Increased reliance on a few major food crops calls for new practices of agricultural diversification particularly the recognition of the role of underutilized crops. Winged bean (*Psophocarpus tetragonolobus*) is an underutilized food legume cultivated mainly in hot, humid countries in Asia and the Pacific. It is a multi-purpose crop (pulse, vegetable and root tuber) and cultivated either as a cover crop for soil management or as a sole crop. It was once identified as the 'future soybean' due to its comparable seed protein content. Winged bean attracted research and development efforts in the 1970s to explore its potential development into a more significant crop. As with many other underutilized crops, the initial efforts were not sustained and winged bean remains a crop yet to fulfill its potential. In the present study, morpho-physiological traits of 24 contrasting winged bean accessions were evaluated. Morpho-physiological characterization showed significant variation between accessions ($P<0.001$) suggesting improvement is possible through selection. Simple linear regression was used to evaluate the correlation between flowering time and yield. A number of accessions showed superior characters, e.g. S319 was superior over the other accessions with the highest harvest index and earliest flowering time and could form a basis for selection of parents to be used in future breeding programmes. Additionally, a paired-end Mi-Seq sequencing of barcoded libraries for leaf, root and reproductive tissues (based on the pooled-RNA of six Malaysian accessions) was undertaken in order to generate a high coverage sequence transcriptome assembly. Simple sequence repeat (SSR) markers were developed from the sequence assembly data for genetic diversity analysis. A combination of morpho-physiological studies and SSR marker development will greatly enhance winged bean research and development, especially in breeding programmes for increased productivity.

Keywords: winged bean, *Psophocarpus tetragonolobus,* underutilized, morpho-physiological characters, harvest index

Introduction
The world has a large reservoir of edible plant species of which over 7000 species are known to have been cultivated by man as crop plants (FAO, 2013). Most of the world relies on only a few major crop species (wheat, rice, maize and potato) to provide more than 60% of our daily dietary intake (FAO, 2013). The narrowing of the number of crop species upon which global food and nutritional security depends affects not only our ability to cope with the consequences of climate change but also our ability to supply a diversified diet to fight 'hidden hunger'. Expanding the range of crop species for food and nutrition can reduce risks associated with climate change as well as enhance nutritional security. One of the possible efforts of agricultural diversification is to bring forward potential underutilized crops, such as winged bean.

Winged bean (*Psophocarpus tetragonolobus*) is a perennial vine that grows abundantly in hot and humid countries. Winged bean is believed to have originated from the Highlands of Papua New Guinea based on the existence of diverse varieties (Khan, 1976) or from Africa based on the close resemblance to some other *Psophocarpus* species especially *P. grandiforus* from East Africa on the basis of phyto-geographical evidence, morphology and disease transferability (Harder and Smartt, 1992). It is renown as a "one species supermarket" because practically all parts (pulse, leaves, pods and root tuber) of the plants are edible. Winged bean seed is highly nutritive (National Academy Press, 1981) with protein content and amino acid composition comparable to soybean and was once identified as the 'future soybean'. In Tanzania, winged bean has been recommended as a good alternative source of plant protein (Mnembuka and Eggum, 1995). Winged bean seed oil has comparable quality to peanut oil and a comparable level of tocopherol to soybean or corn (Khor et al., 1982). High protein and oil content make winged bean seed a suitable raw material for processed food such as composite flour, milk and curd. Winged bean is also regarded as an efficient nitrogen fixer similar to the other ureide-transporting legumes such as *Desmodium*, Siratro and soybean (*Glycine max*) (Yoneyama et al., 1986).

In 1978, winged bean's strong potential for development into a more significant crop received research attention leading to the establishment of the International Council for Development of Underutilized Plants (ICDUP), a private, non-profit organisation in the United States of America. The council organized two international seminars to gather research information about winged bean (Stephenson, 1981). As with many other underutilized crops the initial efforts were not sustained and winged bean remains a crop yet to fulfill its potential. Viney and bushy growth habits of winged bean are considered as major production constraints by planters because of the need to build trellises or support. Additionally, the plants must be planted far apart to avoid intertwining between the neighbouring plants (Stephenson, 1981). As such, planters always opt for other plants that are easier to deal with. Clearly genetic improvement of winged bean is needed to develop an ideal variety with determinate growth habit and higher yield.

Many legumes such as common bean (*Phaseolus vulgaris*), soybean (*Glycine max*) and mung bean (*Vigna radiata*) were originally indeterminate in growth but eventually developed determinacy after years of domestication or breeding efforts (Kwak et al., 2012; Tian et al., 2010). Dwarfed and bushy winged bean mutants have been produced through mutation using gamma rays on mature dried seeds (Klu, 1996). There is a need to develop new varieties not only with determinate growth habit but with a good balance of high yield and nutritional qualities.

In the present study, we used morpho-physiological traits to characterize 24 contrasting winged bean accessions. We evaluated the effects of flowering time, levels of branching and photosynthetic rate and yield. The study also developed a large expressed sequence dataset and identified simple sequence repeats (SSRs). These markers will be used in a genetic diversity analysis of winged bean germplasm. Combined, morpho-physiological and genomic data will serve as an important platform to develop genetic tools that can be used in winged bean improvement programmes.

Materials and methods

Morpho-physiological analysis

Twenty four winged bean accessions were used in this study as described in Table 1. The experiment was conducted at the Lady Bird Farm, Broga, Semenyih, Malaysia (Latitude: 20 57′°N, Longitude: 1010 50′6°: altitude, 45 meters) using a completely randomized design with two experimental units of five subsamples for each accession. Each plant was given a spacing of 1 m x 0.67 m. Border plants were grown at the edges to reduce competitive variation. Irrigation was automated twice per day. The plants were supported on the raffia string tied up vertically to the wire mesh 2 m above ground. Plants were pruned and maintained at the wire mesh level from week 10 onwards. Harvesting was done 20 weeks after planting.

Table 1. Origin and source of accessions used in the study.

Accession	Country of origin	Source	Seed colour	Climate
M2	Malaysia	Local planters	Brown	Tropical
M3	Malaysia	Local planters	Brown	Tropical
M4	Malaysia	Local planters	Brown	Tropical
M6	Malaysia	Local planters	Brown	Tropical
M7	Malaysia	MARDI	Brown	Tropical
M8	Malaysia	MARDI	Brown	Tropical
T5	Nigeria	IITA	Red-black	Tropical
T9	Dr. T.N.Kahn (1973)	IITA	Brown	-
T10	Papua New Guinea	IITA	Brown	Tropical
T12	Liberia	IITA	Brown	Tropical
T14	Indonesia	IITA	Brown	Tropical
T15	Indonesia	IITA	Brown	Tropical
T16	Indonesia	IITA	Brown	Tropical
T17	Indonesia	IITA	Brown	Tropical
T18	Dr. D Nangju (1976)	IITA	Brown	-
T19	Nigeria	IITA	Red-black	Tropical
T22	Papua New Guinea	IITA	Brown	Tropical
T26	Nigeria	IITA	Brown	Tropical
T31	Indonesia	IITA	Brown	Tropical
T33	Unknown	IITA	Brown	-
T51	Bangladesh	IITA	Dark brown	Tropical
T53	Bangladesh	IITA	Dark brown	Tropical
S319	Sri lanka	Research partner	Red-black	Tropical (coastal region)
S271	Sri lanka	Research partner	Cream	Tropical (coastal region)

Morphological parameters measured included: days to 50% flowering, seed yield, pod yield and harvest index. The formulae used to calculate harvest index was grain yield/(grain + straw yield) (Fageria et al., 2011). Data were analysed using statistical software Genstat 12th edition (VSN International,2010). One-way ANOVA analysis was used for statistical tests between accessions and standard deviation was used to show variation within accessions. At week seven, photosynthetic rate was measured using a LI-6400XT-leaf chamber fluorometer (LI-COR, USA) at intervals of ten seconds (up to nine points) on four random subsamples (three leaves for each sub-sample). The settings used were 390 µmol reference cell CO_2, 1000 µmol m^{-2} s^{-1} PARin, 400 μmol s^{-1} flow rate, 60%-70% relative humidity and 30°C leaf temperature. Using similar settings, photosynthetic rate was measured once on every four random subsamples at 400, 300, 200, 100, 50, 400, 400, 600 and 800 μmol reference cell CO_2 at week 8.

RNA isolation and sequencing

A total of six Malaysian accessions (two from the Malaysian Agricultural Research and Development Institute and four obtained from local planters) were used. RNA was isolated separately from leaf, root and reproductive tissues (pod, bud and flower). Specific tissue samples of each accession and of approximately the same weight were pooled and used for RNA extraction and transcriptome sequencing. RNA was isolated using Trizol extraction (Life Technologies). Different barcoded libraries were established from leaf, root and reproductive tissues of pooled accessions. Equimolar concentrations of barcoded libraries were mixed and subjected to paired-end MiSeq sequencing (Illumina, 2013). MisaScript (Maia *et al*., 2008) was used to identify SSR loci.

Results and discussion

Morphological characterization

Days to 50% flowering and the mean values of pod yield, seed yield and harvest indices are shown in Table 2. All morphological traits were significantly different between ($P<0.001$) accessions and this would suggest the potential for improvement through selection of superior breeding material.

Pod and seed yields were highest in S319 and lowest in M4. Pod yields ranged from 103.88 kg/ha to 2987.66 kg/ha while seed yields ranged from 34.86 kg/ha to 1421.66 kg/ha, respectively (Table 2). A study conducted by Khan et al. (1976) reported winged bean seed yield of up to 720 kg/ha at 1 x 1m spacing. They projected the potential of increase in grain yield per unit area to as high as 1946 kg/ha if the spacing were to be reduced to 0.61 x 0.61 m as used in an earlier study by Pospisil et al., (1971). In this experiment, the seed yield could double up to 2559.84 kg/ha if similar spacing is used, although competition between plants may play a greater role. Similarly, pod yield would also increase up to 5378.47 kg/ha. Rahman (1998) successfully improved winged bean grain yield to as high as 6260 kg/ha using the ratooning technique. Harvest index clearly reflects the distribution ratio of dry matter between vegetative and reproductive parts (Fageria et al., 2011). The highest harvest index of S319 suggests that this accession partitions a relatively higher proportion of dry matter into seed yield compared with the other accessions.

Even though diversity in the available genetic material would benefit future breeding programmes, the lack of uniformity would jeopardise consistency and ease of production. One of the main reason farmers choose to grow major crops is because of the presence of high-yielding cultivars with promising productivity (Smartt & Haq, 2008). The standard deviation of S319 was lowest (±0.05) among the top five yielding accessions (S319, T51, T53, T9 and T10) and would suggest that individual plants within S319 were more uniform in yield production and have a greater potential to form stable lines.

Table 2. Days to 50% flowering and mean values ± standard deviation of pod yield, seed yield and harvest index of winged bean germplasm.

Accession	Days to 50% flowering	Pod yield per unit area (kg/ha)	Seed yield per unit area (kg/ha)	Harvest index
M2	79	399.46±30.59	183.13±14.64	0.08±0.07
M3	116	915.66±67.36	438.55±32.76	0.10±0.09
M4	NA	103.88±25.89	34.86±8.688	0.01±0.02
M6	118	304.82±29.15	133.76±13.62	0.04±0.04
M7	118	365.31±33.51	170.65±15.85	0.05±0.05
M8	118	709.78±89.53	276.64±36.29	0.05±0.06
T5	120	317.61±33.26	119.17±12.80	0.03±0.04
T9	62	1714.34±97.06	824.32±47.68	0.21±0.14
T10	62	1807.06±149.10	738.07±61.34	0.15±0.11
T12	117	321.11±32.27	126.04±13.53	0.04±0.05
T14	124	733.00±94.29	286.10±37.86	0.08±0.10
T15	126	590.78±77.46	249.72±33.66	0.05±0.08
T16	NA	128.76±17.70	46.62±6.53	0.02±0.03
T17	96	715.79±63.45	278.95±25.13	0.09±0.09
T18	62	1061.40±88.60	445.25±40.08	0.11±0.01
T19	NA	488.42±121.70	197.55±49.24	0.02±0.07
T22	NA	316.79±45.39	122.78±17.76	0.03±0.06
T26	89	1022.46±71.51	400.68±28.42	0.10±0.08
T31	117	444.27±71.92	183.28±31.89	0.04±0.08
T33	NA	258.88±57.12	118.23±26.91	0.04±0.11
T51	58	2724.54±98.37	1235.40±42.49	0.23±0.09
T53	93	2065.42±140.40	858.31±59.40	0.17±0.12
S319	51	2987.06±67.59	1421.66±39.22	0.34±0.05
S271	112	505.34±35.82	194.62±14.53	0.07±0.06
One-way ANOVA	<0.001	<0.001	<0.001	<0.001

Correlation between days to 50% flowering and harvest index

On average, the number of days to achieve 50% flowering was 97 days (Table 2). S319 was the earliest accession to achieve 50% flowering. In contrast, M4, T16, T19, T22 and T33 did not achieve 50% flowering and exhibited a relatively low yield. Further analysis based on a simple linear regression showed an inverse correlation between days to 50% flowering and harvest index (Figure 1). Thus, early flowering in this case is closely associated with high yield. This is contrary to the study by Hardings et al. (1978) who reported that winged bean seed yield was not correlated with flowering time but depended on the days to maturity. This is undeniable because late flowering genotypes can also produce high yield but would be expected to have longer growing durations. In most cases planters would prefer varieties with short life cycles to maximize profit.

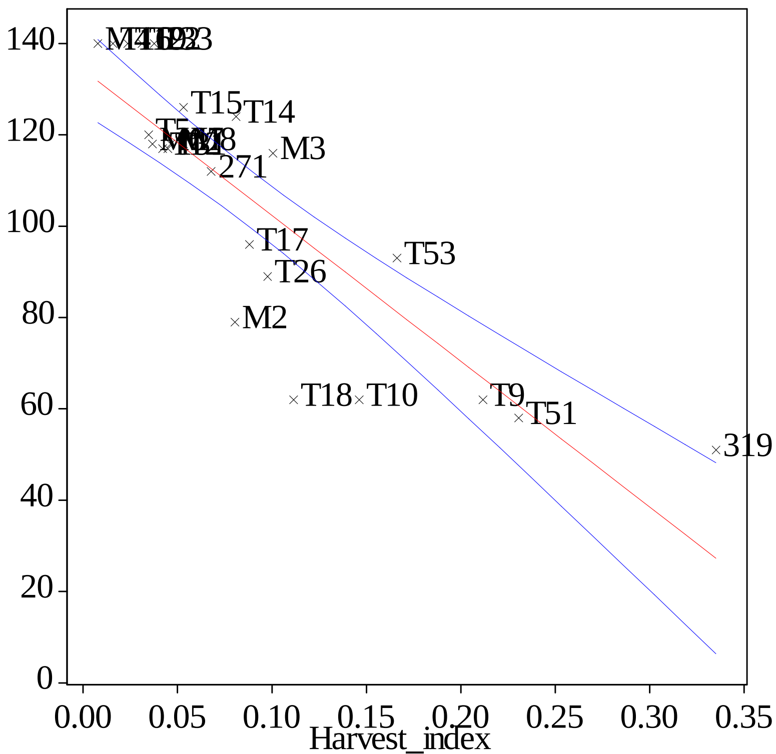

Figure 1. Simple linear regression between 50% flowering time and harvest index with 95% confidence limits.

Physiological characterization

Plant yield is controlled by the levels of light and efficiency of light capture, photosynthesis and dry matter partitioning into grain (Long et al., 2006). Recently, Evans and Caemmerer (2011) mentioned that once a crop has fully adapted to light interception and partitioning of biomass to grain, further yield increment would require enhanced-photosynthesis. This study examined photosynthetic rate across the germplasm under a constant light level. The highest photosynthetic rate of T17 (Figure 2) would suggest that this accession has the most efficient photosynthetic system followed by M3, T16, M4 and T19. However, these accessions appeared to show only moderate harvest indices from 0.01 to 0.1 that could be due to reduced light interception efficiency and partitioning of biomass to grain. In the future, environmental CO_2 concentration is projected to increase alongside global warming. Increasing atmospheric CO_2 is closely associated with the an increase in photosynthesis and subsequently yield (Liu et al., 2012; Long et al., 2006). Figure 3 showed an increase in photosynthetic rate at elevated CO_2 concentration and projects the potential of winged bean for future climates.

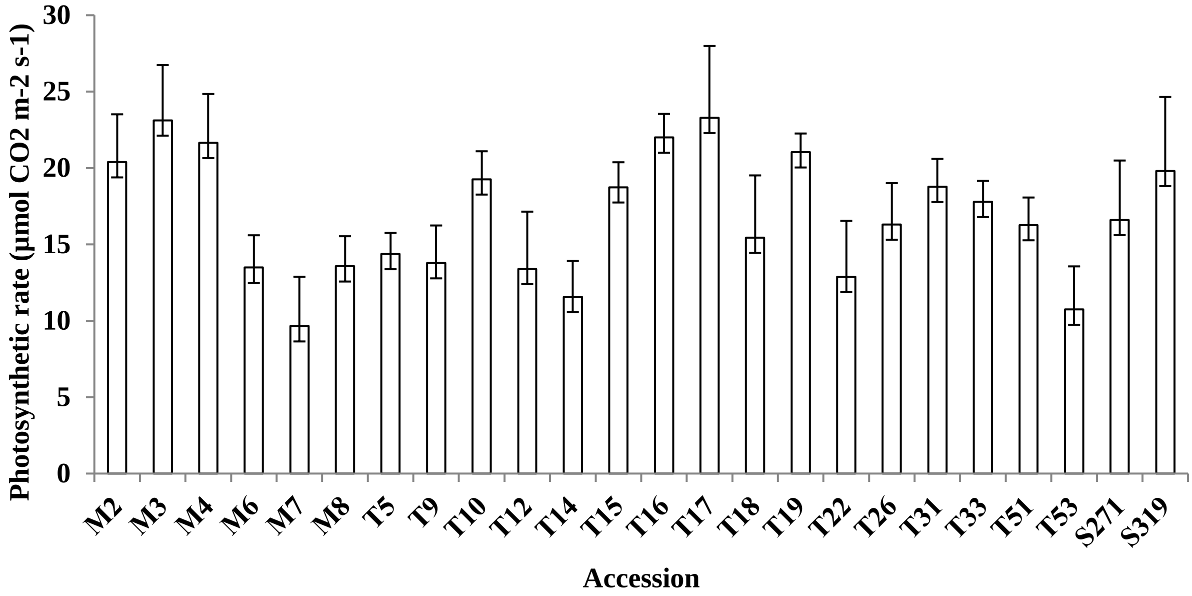

Figure 2. Means of photosynthetic rate with standard deviation bars at 390 µmol reference cell CO_2, 1000 µmol m^{-2} s^{-1} PARin, 400 µmol s^{-1} flow rate, 60%-70% relative humidity and 30°C leaf temperature.

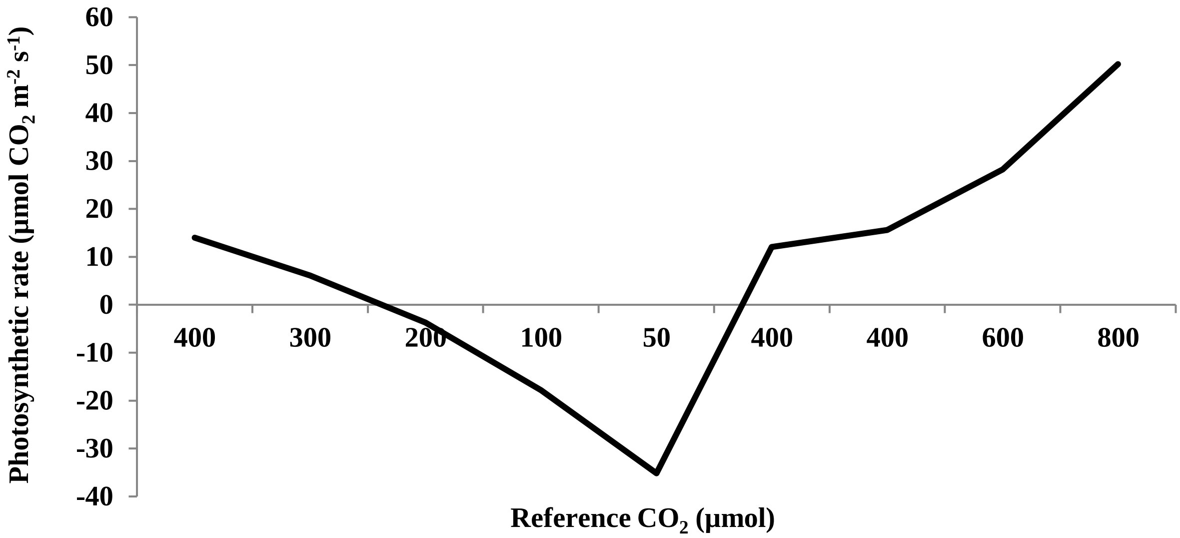

Figure 3. The trend of photosynthetic rate at varying CO_2 concentration. Measurement was adjusted to 1000 PARin, 60-70% chamber humidity and 30ºC leaf temperature.

Genetic analysis

Paired end Mi-Seq sequencing yielded 3.29 Gb, 3.97 Gb and 3.67 Gb of raw sequence data for libraries of leaf, root and reproductive tissues, respectively. A total of 3857 potential SSR sequence loci were detected with motifs ranging from monomer to hexamer repeats. Excluding monomer repeats (2103 loci), trimers (786 loci) and dimers (738 loci) are the most abundant in the gene pool, similarly to the other major crops (*Sorghum bicolor*, *Zea mays* and *Oryza sativa*) and model legumes (*Glycine max*, *Medicago truncatula* and *Lotus japonicas*) (Jayashree et al., 2006).

Conclusion

Based on morpho-physiological evaluations, significant variation between accessions suggests that there is an opportunity for future winged bean improvement programmes through selection of improved material. A number of accessions showed superior characters particularly the accession S319 that has the highest harvest index, consistency in yield production and had the earliest days to 50% flowering and could be used as a parent in future breeding programmes. The correlation studies showed that flowering time was inversely correlated with yield. A set of potential SSR primers were designed for genetic diversity analysis. Combined phenotypic and genomic data will serve as an important platform in winged bean breeding programmes to overcome current production constraints and increase its productivity. Development of improved varieties of winged bean will enhance the crop's popularity and elevate its importance for food and nutritional security.

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References

Cernusak, L.A., Winter, K., Martinez, C., Correa, E., Aranda, J., Garcia, M., Jaramillo, C. & Turner, B. 2011. Responses of legume versus non-legume tropical tree seedlings to elevate CO_2 concentration. *The American Society of Plant Biologists* 157(1): 372-385

Duguma, B., Kang, B.T. & Okali, D.U.U. 1988. Effect of pruning intensities of three woody leguminous species grown in alley cropping with maize and cowpea on an alfisol. *Agroforestry Systems* 6: 19-35

Evans J.R., Caemmerer S. 2011. Enhancing photosynthesis. Plant Physiology 155:19.

Fageria N.K., Moreira A., Coelho A.M. 2011. Yield and yield components of upland rice as influenced by nitrogen sources. Journal of Plant Nutrition 34:361-370.

FAO. 2013. Biodiversity: Plants. Retrieved November 11, 2013 from http://www.fao.org/biodiversity/components/plants/en/

Harder D.K., Smartt J. 1992. Further evidence on the origin of the cultivated winged bean, *Psophocarpus tetragonolobus* (L.) DC. (Fabaceae): Chromosome numbers and the presence of a host-specific fungus. Economic Botany 46:187-191.

Harding J., Martin F.W., Kleiman R. 1978. Seed protein and oil yields of winged bean, *Psophocarpus tetragonolobus*, in Puerto Rico. Tropical Agriculture 55:307-314.

Illumina. 2013. Systems/ MiSeq benchtop sequencer. Retrieved September 11, 2013 from http://www.illumina.com/systems/miseq.ilmn

Jayashree B., Punna R., Prasad P., Bantte K., Hash C.T., Subhash C., Hoisington D.A., Varshney R.K. 2006. A database of simple sequence repeats fom cereal and legume expressed sequence tags mined *in silico*: survey and evaluation. In Silico Biology 6: 607-620.

Khan T.N. 1976. Papua New Guinea: A centre of genetic diversity in winged bean (*Psophocarpus tetragonologus* (L.) DC.). Euphytica 25:693-706.

Khor H.T., Tan N.H., Wong K.C. 1982. The protein, trypsin inhibitor and lipid of the winged bean (*Psophocarpus tetragonolobus* (L.) DC.) seeds. Journal Science Food Agriculture 33:996-1000.

Kwak M., Toro O., Debouck D.G, Gepts P. 2012. Multiple origins of the determinate growth habit in domesticated common bean (*Phaseolus vulgaris*). Annals of Botany 110:1573-1580.

Klu G.Y.P. 1996. Efforts to accelerate domestication of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) by means of induced mutations and tissue culture. Thesis. Wageningen Agricultural University.

Long S.P., Zhu X.G., Naidu S.L, Ort DR. 2006. Can improvement in photosynthesis increase crop yields? Plant, Cell and Environment 29:315-330.

Mnembuka B.V., Eggum B.O. 1995. Comparative nutritive value of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) and other legumes grown in Tanzania. Plant Foods for Human Nutrition 47:333-339.

National Academy Press. 1981. *The winged bean: A high-protein crop for the tropics*, 2nd edn. Washington DC.

Pospisil F., Karikari S.K., Mensah E.B. 1971. Investigations of winged bean in Ghana. World Crops 23:260-264.

Rahman M.M. 1998. Physiological responses of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) to support systems and ratooning. Thesis abstract, UPM. Retrieved October 10, 2013 from http://psasir.upm.edu.my/10416/1/FP_1998_6_A.pdf

Smartt J., Haq N. 2008. New crops and uses: their role in a rapidly changing world, 1st edn. Centre for Underutilized Crops, UK.

Stephenson R.A. 1981. The winged bean flyer. International Documentation Center for the Winged Bean Vol 3, Number 2. pp. 1-66. Published by International Documentation Center for the Winged Bean, Agricultural Information Bank for Asia, College, Laguna 3720, Philippines.

Tian Z., Wang X., Lee R., Li Y., Specht J.E., Nelson R.L., McClean P.E., Qiu L., Ma J. 2010. Artificial selection for determinate growth habit in soybean. Proceedings of the National Academy of Sciences 7:8563-8568.

VSN International. 2010. GenStat for Windows 12th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk

Yoneyama T., Fujita K., Yoshida T., Matsumoto T., Kambayashi I., Yazaki J. 1986. Variation in natural abundance of 15N among plant parts and in 15N/14N fractionation during N2 fixation in legume-rhizobia symbiotic system. Plant Cell Physiology 27:791-79.

Ethnobotanic study and agro-morphological evaluation of varieties of the minor crop, Kersting's groundnut (*Macrotyloma geocarpum*) in Benin

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Abstract
Kersting's groundnut (*Macrotyloma geocarpum* - local name: "Doyi") is a grain legume with high nutritional and economic value widely consumed in Benin. In order to document indigenous knowledge on production, diversity and use of this species, 30 villages from the south and centre of Benin were surveyed. In each village, 10 to 15 households (374 farmers in total) were randomly interviewed using participatory research appraisal tools and techniques. This study revealed that the production of Kersting's groundnut is particularly concentrated in the departments of Zou and Collines, but is fast declining. Ten reasons were identified to explain the production decrease; the most important were the high cost of production (40.74% of responses), susceptibility of local varieties to high soil moisture (25.58% of responses) and complexity of cultural practices (25.24% of responses). Only three local varieties were recorded differing by the seed colour (white, red and black). The white seed variety was the most widely cultivated due to the colour after cooking. Gender role analysis indicated that women (especially young women) are more involved in *M. geocarpum* production. Phenotypical analysis of the different accessions revealed only three groups which differed in leaflets width, yield and date of 50% plant-flowering. Agronomic evaluation indicated a significant difference between grain yields of the three local varieties with average 1062±93 kg/ha, 1197±77 kg/ha and 1548±102 kg/ha for the white, red and black seed varieties, respectively. Results from the current study will contribute to define appropriate conservation strategies and also to implement adequate breeding programmes. These will help to improve and promote local varieties.

Key words: Agro-morphological evaluation, Benin, indigenous knowledge, Kersting's groundnut, varietal diversity

Introduction
Kersting's groundnut (*Macrotyloma geocarpum* Harms) is a grain legume from the Fabaceae family which produces pods in the ground. It is a West African indigenous crop notably from Nigeria, Mali, Burkina Faso, Niger, Togo and Benin (Obasi and Agbatse, 2003) where it is widely consumed. From a nutritional point of view, *M. geocarpum* is grown for its edible seeds which are rich in protein (25%) and contain 42% of essential amino acids (mainly leucine, lysine, phenylalanine and valine) and 60 to 70% of carbohydrates (Chikwendu, 2007). It is also a good source of mineral salts, iron, zinc, phosphorus, calcium, magnesium and potassium (Ajayi and

Oyetayo, 2009). It can be used as complementary food formulation for children (Chikwendu, 2007), and to combat malnutrition (Dansi et al., 2012). Its seeds constitute a useful source of income: one kg of seed costs two to six US dollars. Despite its importance, this crop is cultivated on a small scale and identified as an under-utilized indigenous legume in Benin (Dansi et al., 2012). It is gradually disappearing from traditional food production (Aderanti, 2001). In Benin, according to the MAEP (2011), production of Kersting's groundnut decreased from 2358 tons in 2005 to 1050 tons in 2010. Moreover, few scientific studies are carried out on this crop. To improve our knowledge on production constraints, uses and variability of this plant, the following objectives were addressed: (1) to identify the production areas and importance of *M. geocarpum* in southern and central Benin; (2) to explore indigenous knowledge related to production, diversity and use of this "neglected and underutilized" crop; (3) to investigate the major production constraints of this legume; (4) to undertake participatory evaluation of local varieties for agronomic traits; and (5) to assess morphological variation in Kersting's groundnut landraces from southern and central Benin for development and research programmes.

Materials and methods

Study area and sites selection

Our study was conducted in the south and centre of Benin which is situated in humid agro-ecological zones characterized by a subequatorial climate with two rainy seasons and two dry seasons. Annual mean temperature ranges from 26°C to 28°C and annual rainfall varies between 800 to 1400 mm in southern regions (Yabi and Afouda, 2012) and between 800 to 1200 mm in central Benin (Adam and Boko, 1993). The dominant ethnic groups are: Adja, Cotafon, Holly, Ouémènou, Pédah, Saxwè, Tori, Watchi, Xwla, Yoruba, Fon, Mahi, Idaasha, Fè and Tchabè (INSAE, 2002). Vegetation types are semi-deciduous forests or woodlands and savannah woodlands (Akoègninou et al., 2006). A total of 30 villages was surveyed (Table 1; Figure 1) and were selected after an exploratory study through agricultural research institutions, local and urban market visits, discussion with farmers' associations and sellers and with the agricultural extension services, Centre d'Agriculture Régional pour le Développement Rural (CARDER).

Data collection and analysis

From each village, 12 producers on average were randomly selected in different districts using the method of Ayoola et al. (2011). In total, 374 people including 72 females were interviewed through participatory research appraisal tools and techniques (Amujoyegbe et al., 2010; Sesay et al., 2013). Data collection included: socio-demographic characteristics; farmers' perception on Kersting's cultivation; farming systems; production zones and constraints; causes of decline production; importance and uses of *M. geocarpum*; farmers' preference and varietal selection criteria; seed management, and gender role in crop production.

Twenty two Kersting's groundnut accessions were collected from southern and central Benin for agro-morphological characterization. Seeds were sown in random complete blocks with three replications per accession. Accessions were sown in single rows of 3 m spaced 0.30 m (within rows) and 0.30 m (among rows) between plants according to Bampuori (2007) and farmers' perceptions. A total of 15 traits (quantitative and qualitative) were recorded (Table 3**)** using 12 plants per accession following the protocol of IPGRI et al. (2000).

Survey data were analysed using descriptive statistics. Analysis of variance, Student and Newman-Keuls test, computation of Pearson coefficients of correlation etc. have been used to classify and order variation observed from quantitative traits using the STATISCA software package (Statistica, 2005). The main morphological groups of accessions were identified using a Principal Component Analysis (PCA) performed with the same software.

Table 8. List of administrative locations and socio-cultural groups of villages surveyed

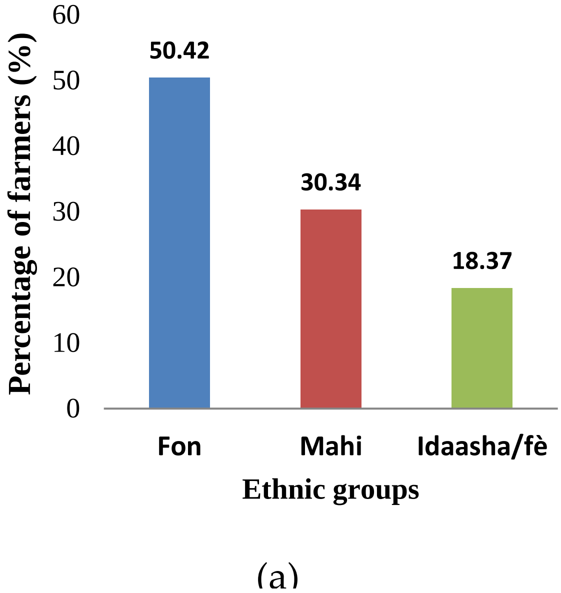
Villages	Districts	Departments	Socio-cultural groups
Adakplamè	Kétou	Plateau	Mahi, Yorouba
Agbondjèdo	Savè	Collines	Fon
Agonhohoun	Djidja	Zou	Fon
Agonlin Kpahou	Kétou	Plateau	Fon, Mahi
Agoua	Bantè	Collines	Fè
Ahokponhoué	Savè	Collines	Tchabè, Fon
Aklankpa	Glazoué	Collines	Mahi
Amakpa	Djadja	Zou	Fon
Banamè	Zagnanando	Zou	Mahi
Bêtêkoukou	Dassa-Zounmè	Collines	Idaasha
Daguè-Daguè	Savè	Collines	Tchabè
Fita	Dassa-Zounmè	Collines	Idaasha, Mahi
Gobada	Savalou	Collines	Mahi
Hoko	Glazoué	Collines	Mahi
Kitikpli	Savalou	Collines	Fon, Fè
Konou	Glazoué	Collines	Mahi
Kossokanmè	Djidja	Zou	Fon
Kpadji	Savalou	Collines	Fon
Kpakpamè	Zakpota	Zou	Fon
Kpakpkakanmè	Djidja	Zou	Fon
Lahotan	Savalou	Collines	Mahi
Mahou	Dassa-Zounmè	Collines	Idaasha, Mahi
Mougnon	Djidja	Zou	Fon
Sovlègni	Djidja	Zou	Fon
Sowignandji	Glazoué	Collines	Mahi
Tan	Zagnanando	Zou	Mahi
Tangbé	Zakpota	Zou	Fon
Tanvè	Agbangnizoun	Zou	Fon
Za-Adikogon	Zakpota	Zou	Fon
Zounzonmè	Abomey	Zou	Fon

Figure 1. Map showing geographical location of villages surveyed

Results

Socio-demographic characteristics

Eighty one per cent of respondents were male against 19% female during an average of 10 years of surveys. Respectively 50.42%, 30.34%, 18.37% of farmers speak Fon, Mahi and Idaasha/Fè (Figure 2). The area under cultivation was low varying from 100 m^2 to 4 ha per household (average: 0.48 ha). Farmers' ages varied from 20 to 64 years (average: 40 years old).



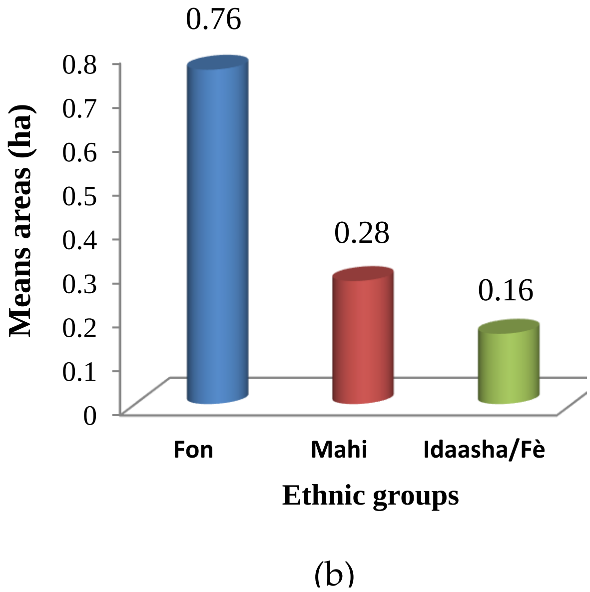


Figure 2. Producers interviewed (a) ethnic groups and (b) the area under cultivation per ethnic group

Reasons of production decrease

Farmers interviewed (15.38%) noted that the production of Kersting's groundnut was increasing while 55.13% reported that it was reducing. A total of ten reasons grouped in four categories justify the decrease of production (Table 2). The major reasons were high costs of inputs, high labour required, susceptibility to high soil moisture, difficulty of harvest and difficult seed de-hulling.

Table 2: Reasons for decreasing cultivation of *M. geocarpum*

Categories	Reasons	Percentage of responses (%)	Rank
Economic (55.98%)	High production costs	40.74	1
	High labour required	10.77	3
	Lack of good market for seed supply	2.69	6
	High costs of seeds	1.68	8
Agronomic (29.28%)	Susceptibility to high soil moisture	25.58	2
	Inadaptability to all types of soil	2.69	7
	Difficult post-harvest storage	1.01	9
Technological (14.47%)	Difficulty of harvest	8.08	4
	Difficulty of seed de-hulling	6.39	5
Cultural (0.37%)	Traditional consideration (myth, taboo)	0.37	10

Cultural practices and knowledge of pests

Kersting's groundnut was usually planted between June and August on mounds without other crops. The few farmers (ca. 2%) who have intercropped this plant with cassava or maize harvested low-yields. No fertilizers or pesticides were used. The majority of producers (88.48%) weeded their field at least twice (four and ten weeks after sowing), and harvesting was done in the dry season around November and January. Yellowing and wilting leaves and petioles indicated plant maturity. Pods harvested later were vulnerable to weevils. Some pests and diseases were observed on plants of which the most important was early yellowing of leaves and petioles (Table 3 and Figure 3). In general, this attack had no great effect on the yield of this crop.

Lepidoptera caterpillars

Kersting's groundnut diseases

Figure 3. Attacks observed on *Macrotyloma geocarpum* in the field

Local varieties cultivated and uses

Three local varieties differing by seed tegument colour were recorded (Figure 4). The white seed was the most cultivated and favoured for consumption. The black and red varieties are neglected because of the colour after cooking.

Figure **4.** Local varieties cultivated in south and centre of Benin

According to the households surveyed, Kersting's groundnut was produced for either home consumption (22.65% of respondents) or market (72.64% of respondents). Seed is usually used in the preparation of delicious foods for special days (New Year, funeral ceremonies, anniversaries, etc.). Regarding medical uses, only a few producers (4.71%) from the ethnic group Fon reported that the cooking water from the black seed variety is used to treat diarrhea, stomach troubles, ulcers and cough.

Seeds management and gender role in crop production

Mainly plastic buckets and maize bags were used to store the seeds of this crop (Figures 5a and 5b). Farmers noted that seeds were very susceptible to storage insect attacks (beetles etc.). To minimize pest effects, producers used many products (Figure 6) especially ash, 'sofa grain' (a black solid insecticide), warm sand, liquid cotton insecticide (endosulfan), canfo and small pepper fruit. Storage periods varied from 5 to 12 months, depending on products used. Thus, sofa grain, endosulfan, small chili pepper fruit and cotton insecticide powder have been identified as the most efficient. Their storage duration ranged from 8 to 12 months. Several of these are chemicals which are dangerous to human health.

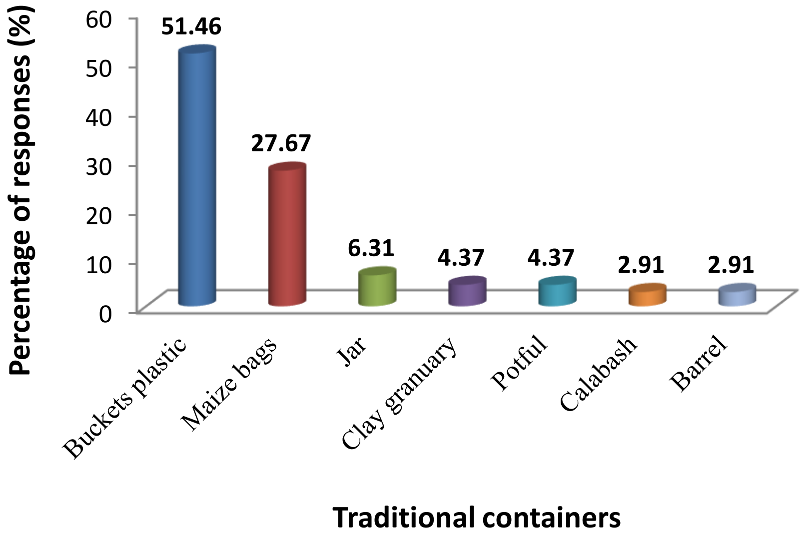


Figure 5a. Seed conservation methods

Figure 5b. Some traditional structures for seed storage

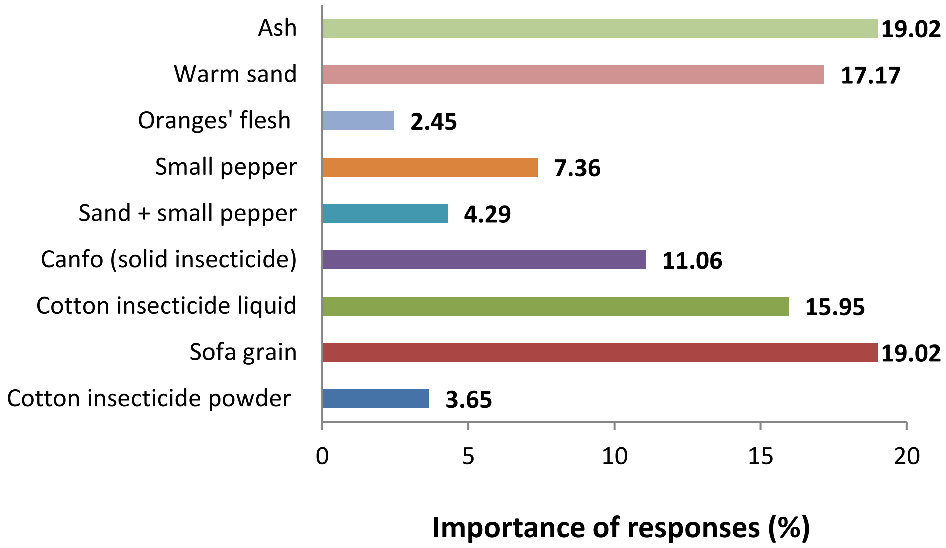


Figure 6. Products used for seeds conservation

Concerning gender participation, both females and males were knowledgeable about crop production and management. Land preparation was predominantly a male activity while processing and seed commercialization were women's activities. Sowing, weeding, harvest, seeds storage were activities done equally by men and women.

Phenotypic diversity and agronomical evaluation of *M. geocarpum*

Qualitative traits distinguished two classes of Kersting's groundnut. The first one grouped white seed accessions while the second included red and black seeds accessions (Figure 7).

In general, coefficients of variation were low for most (80%) of the studied quantitative traits (CV<20%; Table 3). Correlations between quantitative traits revealed negative correlations between the date of 50% plant-flowering and yield parameters (number of pods per plant, seed weight per plant and grain yield). High correlations (r≥90) were however noted between yield parameters (Table 4).

White seed accessions **Red and black seed accessions**

Figure 7. Classification of Kersting's groundnut based on qualitative variables

Table 3. Analysis of 15 quantitative variables measured between accessions

N°	Characters	Code	Minimum	Maximum	Means	SD	CV (%)
1	Plant height (cm)	PHT	31.30	40.33	35.22	2.06	5.86
2	Leaflets length (cm)	LEL	6.23	7.38	6.79	0.28	4.25
3	Leaflets width (cm)	LEW	4.31	5.39	4.80	0.23	4.97
4	Petiole length (cm)	PEL	14.16	26.36	20.27	3.32	16.38
5	Diameter of plant (cm)	DIP	23.40	86.00	67.57	13.39	19.82
6	Number of pods per plant	NPP	49.00	342.00	131.71	61.64	46.80
7	Seed length (mm)	SEL	3.50	6.67	4.78	0.71	14.97
8	Seed width (mm)	SEW	1.62	3.15	2.53	0.40	15.83
9	Yield per plant (g)	YPP	6.17	38.40	15.83	7.04	44.52
10	100 seeds weight (g)	HSW	10.70	14.71	13.05	1.03	7.90
11	Grain yield (kg/ha)	GRY	617.00	3840.00	1588.46	699.86	44.06
12	Days to 50% lifting (d)	DTL	5.00	6.66	5.46	0.49	9.00
13	Days to 50 % flowering (d)	DTF	61.00	64.00	62.71	0.88	1.42
14	Days to first fructification (d)	DFF	112.00	120.00	117.53	2.03	1.73
15	Days to maturity (d)	DTM	144.00	150.00	148.68	2.36	1.59

In total, three groups G1, G2 and G3 of Kersting's groundnut were identified following the two first axes of principal component analysis (representing 42.20% of the total variance; Figure 8). The multiple comparison of means of the different groups for each morphological trait revealed significant differences ($0.001<P<0.05$) for only five parameters such as leaflets width (LEW), number of pods per plant (NPP), seed weight (SEW), grain yield per hectare (GRY) and date of 50% flowering (DTF). Characteristics of each group were identified and highlighted **(**Table 5**).** The group G1 with 16 accessions was characterized by very wide leaflets (4.86±0.24 cm). The second agro-morphological group (six accessions) yielded a high value (224.17±64.36 pods per plant; 26.66±6.25 g of seeds per plant and 2693.33±626.95 kg/ha) and G3 included 10 accessions flowering later (63.18±0.87 days).

Table 4. Correlations between quantitative agro-morphological traits measured for accessions

	PHT	LEL	LEW	PEL	DIP	NPP	SEL	SEW	YPP	HSW	GRY	DTL	DTF	DFF	DTM
PHT	1														
LEL	0.50*	1													
LEW	0.39*	0.75*	1												
PEL	-0.26	-0.14	-0.11	1											
DIP	0.27	0.31	0.22	0.04	1										
NPP	-0.15	0.26	0.14	0.31	0.23	1									
SEL	0.12	-0.03	0.05	-0.05	-0.05	0.03	1								
SEW	0.09	0.11	0.27	0.17	0.03	0.27	0.72*	1							
YPP	-0.12	0.32	0.25	0.37*	0.26	0.94*	-0.09	0.21	1						
HSW	0.08	0.26	0.14	0.12	0.09	0.15	0.19	0.27	0.28	1					
GRY	-0.12	0.31	0.25	0.42*	0.27	0.90*	-0.15	0.15	0.97*	0.26	1				
DTL	0.05	0.18	0.22	-0.02	0.28	-0.18	-0.09	-0.01	-0.24	-0.1	-0.19	1			
DTF	-0.09	-0.22	-0.13	-0.3	0.12	-0.45*	0.02	-0.23	-0.4*	0.26	-0.39*	0.24	1		
DFF	0.13	-0.04	-0.01	-0.05	0.12	-0.17	0.25	-0.02	-0.13	-0.02	-0.20	-0.10	0.17	1	
DTM	-0.24	0.09	0.06	0.06	-0.02	0.27	0.29	0.12	0.31	0.08	0.28	-0.06	-0.09	0.38*	1

Table 5. Comparison of means of the different groups of Kersting's groundnut performances (indicated in bold)

Traits	Group 1	Group 2	Group 3	F
PHT	35.27±1.70	33.50±1.76	34.45±2.33	1.834 ns
LEL	6.78±0.32	6.88±0.16	6.61±0.26	2.018 ns
LEW	**4.86±0.24b**	4.72±0.17ab	4.60±0.16a	5.320*
PEL	19.00±3.20	22.00±3.46	19.64±2.80	1.998 ns
DIP	64.67±16.03	74.67±9.43	67.18±10.49	1.210 ns
NPP	127.4±28.59b	**224.17±64.36c**	87.18±35.02a	23.951***
SEL	4.70±0.67	4.65±0.33	4.92±0.92	0.393 ns
SEW	2.49±0.35	2.68±0.27	2.46±0.52	0.611 ns
YPP	15.53±2.56b	**26.66±6.25c**	9.46±2.86a	45.157 ***
HSW	12.33±1.23	13.17±0.75	12.45±0.93	1.355 ns
GRY	1610.8±231.42a	**2693.33±626.95ab**	390.09±323.52b	4.506 *
DTL	5.44±0.41	5.21±0.40	5.59±0.59	1.170 ns
DTF	62.67±0.82ab	62.00±0.63a	**63.18±0.87b**	4.203 *
DFF	117.67±1.49	116.17±2.40	118.09±2.30	1.910 ns
DTM	149.33±1.79	149.00±2.44	147.63±2.80	1.790 ns

* P<0.05; ** P<0.01; *** P<0.001; ns = not significant

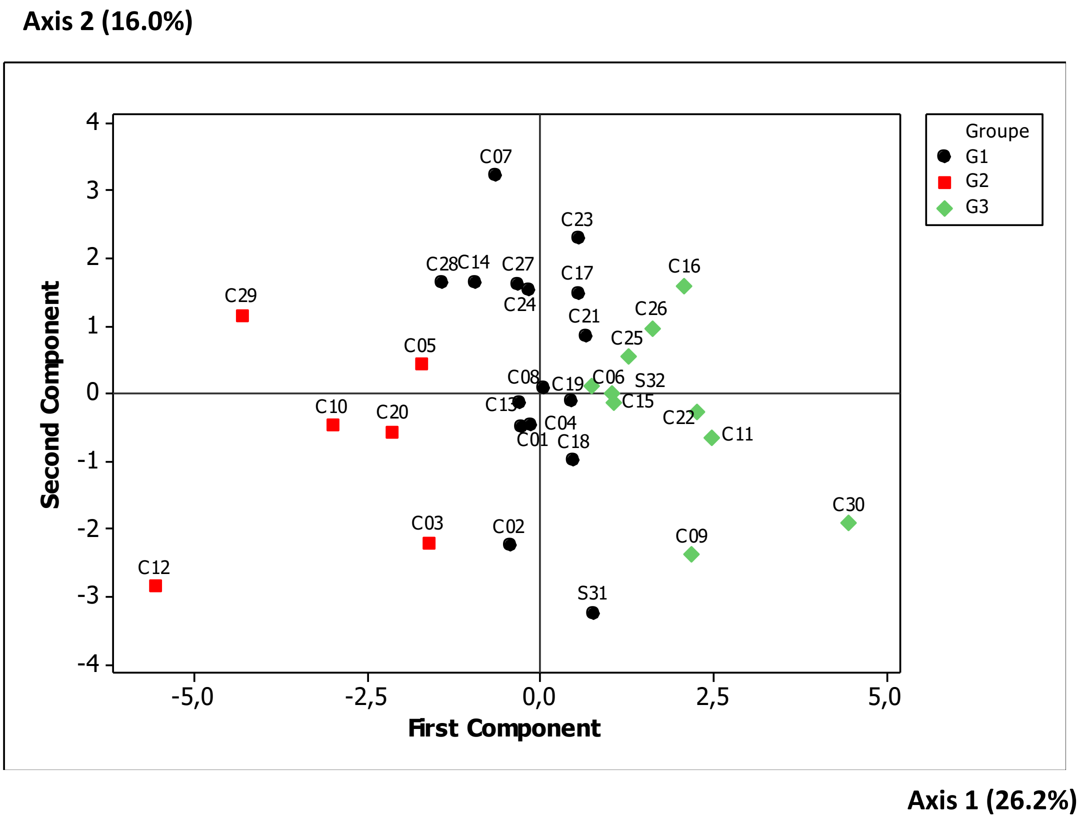


Figure 8. Principal component analysis showing the different grouping of the accessions

For agronomical variation, a significant difference was observed among white seed accessions (P = 0.001) and between local varieties (P = 0.015). Means of 1062±93 kg/ha, 1197±77 kg/ha and 1548±102 kg/ha were obtained respectively for white, red and black seeds varieties. The black-seed variety was the best yielder.

Discussion

Current state of production of the crop

Both young and old farmers were involved in production of this legume because its cultivation requires much physical investment. In contrast, Bampuori (2007) in Ghana and Amujoyegbe et al. (2010) in Nigeria reported that Kersting's groundnut was cultivated by elderly farmers. Besides, its commercialization can bring a high income to resolve urgent problems (children's education, funeral ceremonies, etc). This can contribute to preservation of the crop. Women play vital roles in food production, processing and marketing while men are significantly involved in its production systems (Ayoola et al., 2011; Ogato, 2011). They were more empowered by *M. geocarpum*, a high priority national and regional neglected and underutilized crop (Dansi et al., 2012). According to Bampuori (2007), Kersting's groundnut is produced in small areas. According to Amujoyegbe et al. (2010), the crop was gradually abandoned by many people mainly because of its high production costs, the sensitivity of plants to high soil moisture and its high labour requirement. As noted on cowpea by Baco et al. (2008), seed storage was very susceptible to insects. This contributed also to the production decrease. Plant breeders must create or introduce varieties which are tolerant to storage insects in local agriculture. Ash, sand, small pepper and oranges' peel were natural and safe products which can be recommended to protect seeds during storage.

Agro-morphological variation in Kersting's groundnut accessions

Traditionally, only three varieties differed by seed colour and which were cultivated in rural communities. This low local diversity was also observed on this crop in Ghana (Bayorbor et al., 2010) and is in contrast to other pulses such as Bambara groundnut (Ouédraogo et al., 2008; Bonny and Dje, 2011) and cowpea (Gbaguidi et al., 2013) which are very diverse. The white seed variety was the most popular and consumed in the majority of households. Red and black seeds were disappearing. Each variety can be used in plant breeding programmes and varietal selection programs (Ghalmi et al., 2010). It is therefore urgent to define the best strategies and policies to preserve rare varieties and create improved varieties (by crossing). Food uses will be the best incentive for promotion of these varieties.

Principal Component Analysis of quantitative traits in this study showed three clusters, compared to two in Ghana (Bayorbor et al., 2010). The low coefficients of variation obtained for most characters reveal the low variability. This can depend on the mode of seed distribution (self-production and purchase; Bennett-Lartey et al., 2002). Descriptors help breeders in varietal genotype and genetic purity identification, and play an important role in plant breeding through phenotypical characteristics evaluation (Bayorbor et al., 2010). However as related by Bayorbor et al. (2010), a high agronomical difference was noted between accessions collected. This ranged between 617 and 3840 kg/ha. Among local varieties, black seeds of Kersting's groundnut yielded highest. Similar results were found in Ghana (Bampuori, 2007). Agronomic characterization constitutes the first step to appreciate genetic diversity (Bayorbor, 2010) and therefore yield per plant is an essential character on which breeding decisions are based (Witcombe et al., 2001; Ouédraogo et al., 2008). Negative correlation observed between flowering date and yield revealed that early maturity accessions showed a high yield. According to Bonny and Dje (2011), correlations are a primordial tool for choosing characters which may be included in plant selection programmes. The molecular characterization of accessions will help to better understand the genetic structure.

Conclusion and perspectives

The ethnobotanical study in south and central Benin revealed that Kersting's groundnut production is decreasing mainly for reasons of high cost of production, plant susceptibility to high soil moisture and high labour required. The number of varieties cultivated was very low and also disappearing in local production. Apart from the white seed variety which is widely consumed, no strategies exist to conserve the other varieties. In order to better appreciate the genetic structure of the plant, molecular characterization of the accessions is necessary. The best strategies and policies should be urgently developed to conserve rare varieties. Diversity within *M. geocaprum* must be strengthened by creating and/or introducing improved varieties adapted to climate conditions. Biochemical analysis (nutritional value and toxins) of local cultivars could help to promote Kersting's groundnut for food utilization.

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References

Adam S., Boko M. 1993. Le Bénin. Les éditions du Flamboyant/EDICEF, p 96.

Aderanti F.A. 2001. Morphological characterization of Kersting ground bean (*Macrotyloma geocarpum* (Harms) Marechal and Baudet) B. Agric. Thesis, Department of Plant Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Akoègninou A., van der Burg W.J., van der Maesen L.J.G. 2006. Flore analytique du Bénin, Backhuys Publishers, Leiden, the Netherlands.

Ajayi O.B., Oyetayo F.L. 2009. Potential of *Kerstingiella geocarpa* as a health food. Journal of Medicinal Food 12:184-7.

Amujoyegbe B.J., Obisesan I.O., Ajayi A.O., Aderanti F.A. 2010. Disappearance of Kersting's groundnut (*Macrotyloma geocarpum* (Harms) Marechal and Baudet) in south-western Nigeria: an indicator of genetic erosion. Bioversity International FAO. 152: 45-50.

Ayoola J.B., Dangbegnon C., Daudu C.K., Mando A., Kudi T.M., Amapu I.Y., Adeosun J.O., Ezui K.S. 2011. Socio-economic Factors Influencing Rice Production among Male and Female Farmers in Northern Guinea Savannah, Nigeria: Lessons for Promoting Gender Equity in Action Research. Agriculture and Biology Journal of North America 2:1010–1014.

Baco M.N., Ahanchedé A., Bello S., Dansi A., Vodouhè R., Biaou G., Lescure J-P. 2008. Évaluation des pratiques de gestion de la diversité du niébé (Vigna unguiculata) : une tentative méthodologique expérimentée au Bénin. Cahiers Agricultures 17:183-188.

Bampuori A.H. 2007. Effect of Traditional Farming Practices on the Yield of Indigenous Kersting's Groundnut (Harms) Crop in the Upper West Region of Ghana. Journal of Developments in Sustainable Agriculture 2:128-144.

Bayorbor T.B., Dzomeku I.K., Avornyo V.K., Opoku-Agyeman M.O. 2010. Morphological variation in Kersting's groundnut (*Kerstigiella geocarpa* Harms) landraces from northern Ghana. Agriculture and Biology Journal of North America. ISSN Print: 2151-7517, ISSN Online: 2151-7525.

Bennett-lartey S.O., Ayenor G.S., Markwei C.M., Asante I.K., Abbiw D.K., Boateng S.K., Anchirinah V.M., Ekpe P. 2002. Contribution of home gardens to *in situ* conservation of plant genetic resources in farming systems in Ghana. In: Watson JW, Eyzaguirre PB, editors. Proceedings of the Second International Home Gardens Workshop: 17-19 July 2001, Witzenhausen, Federal Republic of Germany. International Plant Genetic Resources Institute, Rome.

Bonny S.B., Dje Y. 2011. Variabilité morphologique et agronomique des variétés traditionnelles de voandzou [*Vigna subterranea* (l.) verdc. (fabaceae)] de Cote d'Ivoire. Journal of Applied Biosciences 41:2820-2835.

Chikwendu N.J. 2007. Chemical composition of four varieties of groundbean (*Kerstingiella geocarpa*). Journal of Agriculture, Food, Environment and Extension 6:73-84.

Dansi A., Vodouhè R., Azokpota P., Yedomonhan H., Assogba P., Adjatin A., Loko Y.L., Dossou-Aminon I., Akpagana K. 2012. Diversity of the Neglected and Underutilized Crop species of importance in Benin. The Scientific World Journal. Volume 2012, Article ID 932947, 19 pages.

Gbaguidi A.A., Dansi A., Loko L.Y., Dansi M., Sanni A. 2013. Diversity and agronomic performances of the cowpea (*Vigna unguiculata* Walp.) landraces in Southern Benin. International Research Journal of Agricultural Science and Soil Science 3:121-133.

Ghalmi N., Malice M., Jacquemin J-M., Ounane S-M., Mekliche L., Baudoin J.P. 2010. Morphological and molecular diversity within Algerian cowpea (*Vigna unguiculata* (L.) Walp.) landraces. Genetic Resources and Crop Evolution 57:371–386.

IPGRI, IITA, BAMNET. 2000. Descripteurs du pois Bambara (*Vigna subterranea*). Institut International des Ressources Phytogénétiques, Rome, Italie; Institut International d'Agriculture Tropicale, Ibadan, Nigeria; Réseau International de pois Bambara, Allemagne.

INSAE. 2002. Enquête sur les ménages au Bénin. Voir rapport d'activité (2005-2006).

MAEP. 2011. Données sur l'évolution des principales cultures du Bénin.

Obasi M.O., Agbatse A. 2003. Evaluation of nutritive value and some functional properties of Kerstings groundnut seeds for optimum utilization as a food and feed resource. East African Agricultural and Forestry Journal 68:173-181.

Ogato G.S. 2011. Gendered Knowledge and innovation in Crop Production and Management Practices: Case Study of Three Rural Communities in Ambo District, Ethiopia. Journal of Research in Peace, Gender and Development 1:216-228.

Ouédraogo M., Ouédraogo J.T., Tigneré J.B., Balma D., Dabire C.B., Konaté G. 2008. Characterization and evaluation of accessions of Bambara groundnut (Vigna subterranea (L.) Verdcourt) from Burkina Faso. Sciences and Nature 5:191-197.

Sesay L., Norman P.E., Massaquoi A., Kobba F., Allieu A.P., Gboku M.L., Fomba S.N. 2013. Assessment of farmers' indigenous knowledge and selection criteria of yam in Sierra Leone. Sky Journal of Agricultural Research. 2:1-6.

Statistica, 2005. Statistica for Windows, version 7.1. StatSoft Inc.

Witcombe J.C.R., Joshi K.D., Rana R.B., Vick D.S. 2001. Increasing Genetic Diversity by Participatory Variety Selection In High Potential Production Systems In Nepal And India. Euphytica 122:575-588.

Yabi I., Afouda F. 2012. Extreme rainfall years in Benin (West Africa). Quaternary International 262:39-43.

Morphological and use-value-based management of enset, *Ensete ventricosum* (Welw.) Cheesman diversity and distribution in Ethiopia: perspectives for on-farm conservation of crop genetic resources

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Abstract

Enset, *Ensete ventricosum* (Welw.) Cheesman is a crop that is important for food security for more than 20% of Ethiopia's population. The objective of this work was to assess the importance of morphological and use-value related characterization for enset diversity and distribution management. A total of 280 farm households in seven zones was surveyed through individual household interviews. The frequency distribution of phenotypic similarity and other attributes revealed the presence of some variation among enset clones. The observed traits showed low to high levels of diversity among enset clones with a Shannon-Weaver diversity index (H′) value of 0.154 to 0.732 for bulla (extracted starch) quality and midrib colour, respectively. Kocho (fermented starch) yield was significantly correlated with bulla quality and plant vigour. Based on their morphological and use value a total of 218 enset clones was recorded in the surveyed areas. The number of clones maintained on individual farms ranged from 2 to 26 (mean of 8.9 ± 0.94). Hadiya with 59 clones had the highest richness and Sidama with 30 clones had the lowest richness; mean richness being 39.4 ± 10.1 clones per zone. Clone abundance was highest at Sidama (12.8) and lowest for GamoGofa (8.5). Mean abundance was 9.64 ± 2.05 farms per clone. Knowledge of farmers' practices is currently used to validate agronomic innovations and inform the setting up of a network of genotype collections managed by farmers. Diversifying selection, and back-up *ex situ* conservations are key mechanisms responsible for the high diversity observed. Strategies of conservation of genetic resources should take these dynamic processes into account.

Key words: enset, indigenous classification, management, morphology, use value

Introduction

Enset mainly grows at elevations ranging from 1200 to 3100 meters above sea level (masl), but scattered plants can also be found at lower altitudes (Haile et al., 1996). Optimal plant growth is observed at altitudes between 2000 and 2750 masl (Diro and Tabogie, 1994).

The major food types obtained from enset are kocho, bulla and amicho (Brandt et al., 1997). Kocho is fermented starch obtained from decorticated (scraped) leaf sheaths and grated corms. Bulla is a liquid which is obtained when leaf sheaths and corms are pulverized, the liquid containing starch being squeezed out from scraped leaf sheathes and grated corm and the resultant starch allowed to concentrate into white powder. Amicho is boiled enset corm/rhizome pieces that are prepared and consumed in a similar manner to other root and tuber crops (Brandt et al., 1997).

Farmers characterize and select enset clones based on morphological and agronomic traits and use value from different enset growing areas of Ethiopia. Shigeta (1990) studied the local enset taxonomy of the Ari people, while Alemu and Sandford (1996) attempted to establish a field guide to characterize enset clones in North Omo using morphological characteristics. In addition, Negash et al. (2002) and Tsegaye (2002) stated that vernacular names of enset clones are generally consistent, distinguishing different enset clones linguistically, phenotypically and in terms of utilization. Farmer clone selection is based on quality and quantity of food products, maturation period, disease and drought tolerance, forage, medicinal value, ease of scraping for extraction of starch, quality of corm and productivity (Tsegaye, 2002).

Reports of landrace diversity in enset are numerous. Alemu and Sandford (1991) reported names of 99 enset clones in the North Omo area, while Shigeta (1990) listed 78 vernacular names of cultivated enset clones in the Ari region of southern Ethiopia. In addition, Negash (2001) reported that farmers maintain and enrich the diversity of enset, and select or classify clones for various uses. Tesfaye (2002) indicated that enset landraces are not evenly distributed across the region mainly due to altitude variations. Tsegaye (2002) reported that numerous enset clones were identified in each region and the variations in the number of clones were attributed to a combination of socio-cultural and agro-ecological factors. Furthermore, Birmeta (2004) reported that the observed genetic diversity in cultivated enset in a particular area appears to be related to the extent of enset cultivation and the culture and distribution pattern of the different ethnic groups.

These earlier enset diversity studies were limited to one or a few ethnic groups or a specific and limited enset growing region. Therefore, the present study was conducted to assess and document the skills with which farmers recognize, classify, select and manage enset diversity in seven major enset production areas in southern Ethiopia.

Materials and methods

Description of the research area

Enset clone selection by farmers and their cultural practices were studied in the Southern Nations, Nationalities and Peoples Regional State (SNNPRS). Seven provinces (Dawro, Gamo Gofa, Gurage, Hadiya, Kembata, Sidama and Wolaita) were selected to carry out the present study and one district was selected in each province. The selection was based on the prominence of enset cultivation and information about enset distribution obtained from the Departments of Agriculture of the respective zones. Based on enset diversity, two farmer associations were selected in each district. From each association, 20 households were selected randomly. Data were collected, using a structured questionnaire, through individual interviews with household heads and household members responsible for enset field management.

Data collection and analysis

Farmers' classification

The farmers' classifications of enset were assessed during the survey by asking respondents to describe a clone's distinguishing features, selection criteria and attributes that are important in their decision to maintain it. Frequency distributions and the number of phenotypic classes distinguished by farmers (Table 1) were used to calculate the Shannon-Weaver diversity index (*H'*) for each character (Hennink and Zeven, 1991).

The index is defined as: $H = \frac{-\sum p_i \ln p_i}{\ln n}$ where p_i is the proportion of the total number of individuals (genotypes) in the i^{th} class and *n* is the number of phenotypic classes.

Correlation between traits

From the total of 218 enset clones correlation analysis was carried out between 11 traits across 165 enset clones which are well known by the farmers of each location using the Pearson's correlation coefficient (r).

Enset diversity and distribution

Simpson's Index of Diversity $(1\text{-}D) = 1 - \sum (ni/N)^2$ where, n_i = the frequency of the i^{th} clone, frequency being the number of farms in which the clone is found in the district, and N = the total number of farms surveyed in the district.

Shannon–Weaver diversity index **(H')** = - $\Sigma\ pi\ ln\ pi$ (Magurran, 1988). Where pi, the proportional abundance of the i^{th} clone = $(\frac{ni}{N})$. . E = H'/lnS, where H' is Shannon index and S refers to the number of clones described in each zone.

Both of them were calculated for all the provinces. Pearson's correlation coefficient was used to compare diversity and distribution values at different sites.

Results and discussion

Morphological and use-value characterization

Farmers in the study area use a combination of similar criteria to classify enset clones (Table 1). Three morphological characters (midrib colour, petiole colour, and leaf colour), growth attributes (vigour, maturity), kocho quality, disease resistance and use value (bulla quality, amicho use, medicinal value and fibre quality) were the major criteria used by farmers. These major criteria were used for data collection and characterization.

Polymorphism was observed for all assessed traits, with H' values ranging from 0.154 for bulla quality to 0. 0.732 for midrib colour (Table 1). However, the overall mean H' value (0.399) of all the traits indicated a low level of phenotypic diversity amongst the studied clones. In general, however, separate H' values of each trait revealed the existence of a considerable level of diversity of enset.

Enset bacterial wilt, caused by *Xanthomonas campestris* pv. *musacearum,* is the most important biotic constraint to enset cultivation (Brandt et al., 1997). Thirteen clones were identified by farmers as tolerant to enset bacterial wilt (Table 2). The kocho yield of these disease-tolerant clones was generally low when compared with other enset clones evaluated: only four ('Alenticho', 'Dirbo', 'Hawe' and 'Mesmesa') gave kocho yields above the average of 9.9 t ha^{-1} yr^{-1} previously reported in a study of 240 enset clones (Yemataw, 2010).

Farmers also listed 14 other enset clones that have been used for medicinal purposes (Table 3) even if they display lower kocho yield. These enset clones have an average cycle duration/maturity period and plant height, but in general have low kocho yield (8.6 t ha^{-1} yr^{-1}). Nevertheless, the squeezed kocho yield of 'Chamia', 'Gishera', 'Guariye', 'Senkutie' and 'Tuzuma' was found to be greater than the mean of all 240 enset clones studied by Yemataw (2010).

In Wolaita, GamoGoffa and Dawro zones, in addition to the above classification, farmers recognize two major categories of enset clones: 'male' and 'female' enset (Table 4). Designation of the clones as 'male' and 'female' has no reference to the reproductive biology of the clones. Besides, this classification takes into account a certain degree of ecological adaptation. The men prefer male enset clones which are late maturing, disease resistant and have less amicho quality. In contrast female farmers prefer female enset clones, since they mature early, and can be consumed earlier. In the current study we can see that both 'male' and 'female' plants are maintained. This vernacular distinction of enset gender is used by different ethnic and cultural groups in North Omo and Kefa-Sheka (Alemu and Sandford, 1991; Negash et al., 2002).

Correlation between traits

From the total of 218 enset clones correlation analysis was carried out between 11 traits across 165 enset clones which are well known by the farmers of each location. A correlation analysis carried out on 11 traits across 165 clones revealed few significant correlations (Table 5). Kocho yield was positively and significantly correlated with bulla quality and plant vigour. However, it was negatively correlated with corm usage and medicinal value. Corm usage had a significantly positive correlation with medicinal value and a negative correlation with plant vigour and maturity time (Table 5). Fibre quality had a significantly positive correlation with plant vigour. Based on the results of this study there is a possibility of selecting enset clones for further improvement using desirable agronomic traits. During a survey conducted in 2009 farmers also mentioned that clones that are good for amicho have a medicinal value but low kocho yield (Yemataw 2010). Tsegaye (2002) stated that plant height, kocho yield and bulla quality had a positive relationship. In order to improve kocho, bulla and fibre yield, traits like plant height should be considered.

Enset clone richness

The number of clones cultivated on individual farms ranged from 2 to 26 (mean of 8.94 ± 0.94; Table 6). The average number of clones per farm ranged between 10.25 for Kembata to 7.53 for Wolaita. Dawro with 9.48, Sidama with 9.47 and Gurage with 8.95 clones per farm had high farm level richness (Table 6). This is because they have many farms with 11-15 clones, while other zones such as Kembata have few such clones, although the total number of clones in the zone was the highest (Table 6).

Diversity indices for the seven zones studied were computed from the numbers of clones present on the 40 farms within the province (Table 6). Although provinces differed in richness, they were similar in diversity. The Simpson's 1-D ranged between 0.971 (Sidama) to

0.977 (Wolaita), H′ ranged between 3.577 for Sidama to 3.671 for Wolaita, while evenness also had a very narrow range: 0.97 for Gamo Gofa to 0.99 for Wolaita (Table 8). All these values indicate the high enset diversity in these seven provinces.

Based on the total number of different clones recorded (richness of the province) and the number of enset clones per farm, Hadiya was the richest province with a total of 59 clones (Table 6). The lowest richness was found in Sidama province with 30 clones. In previous studies, comparable results were reported by Tsegaye (2002), who described morphologically diverse enset clones from three provinces (52 clones from Sidama, 55 clones from Wolaita and 59 clones from Hadiya). Moreover, Birmeta (2004) described 111 enset clones from nine growing areas of Ethiopia.

Distribution and abundance of clones

There was also a considerable difference among the clones with respect to their distribution across the zones covered by this study. Out of the 218 clones, 178 (81.65%) were observed in only one zone. Twenty nine (13.3%) of the clones were present in two zones. Eight clones (3.7%) were present in three zones. Two clones (0.9%) were present in four of the seven zones and only one clone (Gena) was present in five of the seven zones (Table 7). Household characteristics, distance from one location to another and ethnic preferences in few locations for few numbers of clones brings high clonal diversity, while for higher numbers of clones that do not fulfill the selection criteria of each ethnic group brings clonal paucity. The abundance of cultivars in the region is generally uneven because some cultivars, particularly those having merits of better kocho yield and quality have a wider distribution within and between the sites.

The abundance of clones across sites within a zone and the distribution of clones across the seven zones were generally uneven, because of a limited number of widespread and dominant clones. The other clones had a rather limited abundance and distribution. The hierarchical nature of the spatial distribution of enset clones where a small number of highly abundant clones which are also grown throughout the region and a much larger number of moderately common and rare ones has been documented for enset (Tesfaye, 2002), as well as several other crops including cassava (Boster, 1985) and yam (Tamiru, 2006).

Conclusion and recommendations

Knowledge of farmers' practices is useful to select the right agronomic innovations in the areas under consideration. A small number of highly abundant clones are grown throughout the region, while a much larger number of moderately common and rare clones characterize the distribution-abundance pattern. Farmers may retain their preferred cultivars for many years, often claiming they received no external inputs of seed to these lots. Most planting material exchange is local, though a proportion extends beyond the local group of villages (Farmers' Association), reflecting relationships among neighbours and kin in most cases. Widely distributed cultivars have probably been cultivated for long periods during which time farmers developed a preference for those with the most favourable attributes.

The widespread distribution of some clones challenges the view that traditional farming systems are isolated and closed, with limited exchange of germplasm. Highland regions/provinces like Dawro, Gurage and Kembata have a high concentration of diverse and unique enset landraces and should be given priority in efforts aimed at collection and *in* *situ* germplasm conservation. The spread of modern agricultural techniques for enset cultvation in Ethiopia might lead to disappearance of some of the mechanisms generating diversity in traditional agro-ecosystems. Therefore on-farm conservation of enset diversity should be taken into account. It is also recommended to enable policy and institutional framework supporting *in situ* conservation of agro-biodiversity and wild crop relatives; to establish entrepreneurship, strong and fair partnerships between producers, dealers, consumers and other stakeholders in the production to consumption chain; to support a participatory integrated learning approach by all partners, and; to establish *in situ* gene banks and on farm conservation sites to enhance and ensure the long term availability and conservation of enset germplasm.

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References

Alemu K., Sandford S. 1991. Enset in North Omo region. Farmer's research technical pamphlet, No.1. Farm Africa, Addis Ababa, Ethiopia.

Alemu K., Sandford S. 1996. Enset clone. In: Abate T, Hiebsch C, Brandt S, Geberemariam S (eds) Enset-based Sustainable Agriculture in Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia, pp 138-148.

Birmeta G. 2004. Genetic variability and biotechnological studies for the conservation and improvement of *Ensete ventricosum*. Doctoral thesis, Swedish University of Agricultural Sciences, Alnarp. 91 pp.

Boster J. 1985. Selection for perceptual distinctiveness: evidence from Aguarina cultivars of *Manihot esculenta*. Economic Botany 39:310-325.

Brandt S., Spring A., Hiebsch C., McCabe S., Tabogie E., Diro M., Welde-Michael G., Yentiso G., Shigeta M., Tesfaye S. 1997. "The 'Tree Against Hunger": Enset-based Agricultural Systems in Ethiopia. American Association for the Advancement of Science, Washington, DC, USA, pp. 56.

Diro M., Tabogie E. 1994. Preliminary investigation on enset culture. In: Herath E and Dessalegn L (eds.). Proceedings of the second national horticultural workshop of Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia, pp. 120-131.

Haile B., Diro M., Tabogie E. 1996. Agronomy Research on Enset. In: Abate T, Hiebsch C, Brandt S and Geberemariam S (eds.) Enset-based Sustainable Agriculture in Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia, pp. 235-241.

Hennink S., Zeven A.C. 1991. The interpretation of Nei and Shannon-Weaver within population variation indices. Euphytica 51:235-240.

Magurran A. 1988. Ecological diversity and its measurement. Croom Helm, London. 125pp.

Negash A. 2001. Diversity and conservation of enset (*Enset ventricosum* Welw. Cheesman) and its relation to household food and livelihood security in South-western Ethiopia. PhD dissertation, Wageningen University, The Netherlands. 247pp.

Negash A., Tsegaye A., Van Treuren R., Visser B. 2002. AFLP analysis of *Ensete* clonal diversity in south and southwestern Ethiopia for conservation. Crop Science 42:1105-1111.

Shigeta M. 1990. Folk *in-situ* conservation of ensete (*Ensete ventricosum* (Welw) Cheesman): towards the interpretation of indigenous agricultural science of the Ari, southwestern Ethiopia. African Study Monographs 10:93-107.

Tamiru M. 2006. Assessing diversity in yam (*Dioscorea spp.)* from Ethiopia based on morphology, AFLP marker and tuber quality, and farmers' management of landraces. Ph.D. thesis, George-August University. Germany. pp. 13-39.

Tesfaye B. 2002 . Studies on landrace diversity, *in vivo* and *in vitro* regeneration of enset (*Enset ventricosum* Welw.). PhD dissertation, Humboldt University, Berlin, Germany, 129 pp.

Tsegaye A. 2002. On indigenous production, genetic diversity and crop ecology of enset (*Enset ventricosum* (Welw.) Cheesman). PhD dissertation, Wageningen University, The Netherlands. 197 pp.

Yemataw Z. 2010. Variability study and indigenous classification method of enset (*Ensete ventricosum* (Welw.) Cheesman) clones in southern Ethiopia. M.Sc. thesis. Hawassa University, Hawassa, Ethiopia, 130 pp.

Table 1. Farmers' criteria for classification of enset clones in, Dawro, Gamo Gofa, Gurage, Hadiya, Kembata, Sidama and Wolaita provinces and frequency distribution and Shannon-Weaver Diversity indices (H') of eleven traits for 165 enset clones.

Trait	**Descriptor state**	**Morphological and use value descriptor coding**	**Freq**	**H'**
Plant vigour	Poor (<4m)	1	22	0.445
	Medium (4-6m)	2	41	
	High (>6m)	3	38	
Maturity (cycle duration)	Early (<4 years)	1	33	0.446
	Intermediate (4 -5 years)	2	43	
	Late (> 6 years)	3	24	
Kocho yield	Low (<9.9 t ha^{-1} yr^{-1})	1	9	0.385
	Medium (9.9 to 20 t ha^{-1} yr^{-1})	2	53	
	High (> 20 t ha^{-1} yr^{-1})	3	38	
Bulla quality	Not good	1	12	0.154
	Good	2	88	
Corm use	Not used	1	58	0.283
	Used	2	42	
Fibre quality	Low	1	23	0.430
	Medium	2	51	
	High	3	26	
Medicinal value	Not used	1	88	0.154
	Used	2	12	
Disease response	Susceptible	1	79	0.27
	Intermediate	2	8	
	Tolerant	3	12	
Petiole colour	Green	1	46	0.594
	Green yellow	2	1	
	Pink purple	3	4	
	Red	4	29	
	Red purple	5	11	
	Purple	6	5	
	Brown	7	4	
	Black	8	1	
Midrib colour	Green	1	36	0.732
	Green yellow	2	1	
	Red	3	17	
	Red purple	4	16	
	Pink	5	13	
	Pink purple	6	10	
	Purple brown	7	4	
	Black	8	1	
	Ivory	9	1	
Leaf colour (upper surface)	Light green	1	61	0.387
	Medium green	2	24	
	Green	3	15	
		Overall mean		0.39

Table 2. Mean value of agronomic traits of enset clones identified by farmers as *Xanthomonas* wilt tolerant at the time of harvest

Clone name	MT (years)	PH (m)	PSH (m)	PSC (m)	LN	USQKB (ton $ha^{-1} yr^{-1}$)	FSQKB (ton $ha^{-1} yr^{-1}$)
Abatmerza	4.8	6.8	2.23	1.4	9.5	12.09	8.29
Agina	4.6	3.7	1.4	0.93	10	7.64	4.75
Alenticho	3.8	7	2.05	1.56	10.5	15.12	10.57
Bedadia	4.8	4.8	1.63	1.01	9.5	13.85	8.66
Bota-meziya	4.8	4.8	1.64	1.14	7.5	7.71	5.21
Buzzare	4.4	5.4	1.83	1	10.5	12.78	9.68
Dirbo	2.6	5.3	1.5	1.2	10.5	22.61	14.8
Hawe	3.7	6.7	2.15	1.15	11.5	18.82	13.6
Jegeda	5	4.7	1.5	1	7.5	8.43	5.61
Kekere	4.6	4.4	1.45	1	8	10.24	6.57
Kucharkia	4.6	4.7	1.53	1.05	9.5	7.52	5.16
Mariya	4.2	5.2	1.7	1.18	12.5	14.16	9.27
Mesmesa	3.8	6.8	1.95	1.13	10.5	16.55	10.79
Mean	4.3	5.4	1.74	1.13	9.8	12.89	8.689

MT = maturity time (cycle duration); PH = plant height; PSH = pseudostem height; PSC = pseudostem circumference; LN = leaf number; USQKB = fermented unsqueezed kocho yield; FSQKB = fermented squeezed kocho yield.

Table 3. Mean value of agronomic traits of enset clones for medicinal purposes by enset farmers.

Clone name	MT (years)	PH (m)	PSH (m)	PSC (m)	USQKB (ton $ha^{-1} yr^{-1}$)	FSQKB (ton $ha^{-1} yr^{-1}$)
Adinona	4.82	4.43	1.54	0.89	4.21	2.7
Aeluwa	4.84	5.22	1.65	1.26	12.69	7.8
Argema	4.91	4.8	1.5	0.9	12.79	8.16
Astara	3.18	5	1.6	0.97	14.03	8.38
Bedadia	6.63	4.21	1.25	1.08	5.21	4.55
Chamia	4.97	5.05	1.78	1.32	18.63	11.05
Gishera	3.19	5.98	1.73	1.25	21.89	14.39
Guarye	3.71	5.8	1.8	1.23	19.35	12.38
Hargamo	2.87	5.35	1.55	0.98	9.69	4.96
Jegeda	5.04	4.7	1.5	1	8.43	5.61
Kekere	4.64	4.36	1.45	1	10.24	6.57
Ored	4.62	6.23	1.97	1.46	13.34	9.6
Senkutie	3.85	6.01	1.98	1.18	16.47	10.79
Tuzuma	3.5	6.05	1.95	1.18	21.42	13.27
Mean	4.34	5.23	1.66	1.12	13.45	8.6

MT = maturity time (cycle duration); PH = plant height; PSH = pseudostem height; PSC = pseudostem circumference; USQKB = fermented unsqueezed kocho yield; FSQKB = fermented squeezed kocho yield.

Table 4. Characteristics of 'male' and 'female' enset clones in Southern Ethiopia.

Characteristics	**Category**	
	Male Enset	**Female Enset**
Plant vigour	Vigorous	Less vigorous
Disease reaction	Tolerant	Susceptible
Kocho quality	Less quality	More quality
Maturity	Late maturing	Early maturing
Amicho palatability	Non-edible	Edible and tasty
Fibre quality	High strength	Low strength

Table 5. Pearson's correlation coefficient (r) among different characteristics of 165 enset clones

Parameters	**MC**	**PC**	**LC**	**KY**	**BU**	**CU**	**FQ**	**MV**	**PV**	**MT**	**DR**
MC (midrib colour)	1										
PC (petiole colour)	0.349**	1									
LC (leaf colour)	-0.064	-0.094	1								
KY (kocho yield)	0.008	0.022	0.08	1							
BU (bulla use)	0.124	0.109	0.045	0.322**	1						
CU (corm use)	-0.061	0.056	-0.037	-0.0606	-0.0606	1					
FQ (fibre quality)	-0.435	0.103	0.027	0.229	0.308**	0.033	1				
MV(medicinal value)	0.0005	-0.062	-0.069	-0.084	0.0241	0.212**	0.143	1			
PV (plant vigour)	0.001	0.123	0.021	0.392**	0.02006**	-0.21	0.3237**	-0.004	1		
MT (maturity time)	0.084	-7.00E-04	-0.113	0.0215	-0.048	-0.185	-0.145	0.023	0.11	1	
DR(disease response)	-0.039	-0.082	0.082	0.018	0.0422	-0.101	0.144	0.067	0.15	0.13	1

*Significant at probability level P<0.05; **significant at probability level P<0.01

Table 6. Enset clone diversity in the seven provinces, Southern Ethiopia, expressed as richness, Simpson (1-D) and Shannon (H') diversity indices, and evenness

Provinces	Richness (%)	Mean richness /farm	Minimum richness	Maximum richness	No. of unique landraces	1-D	H'	Evenness
Sidama	30 (10.8*)	9.47	3	18	24	0.971	3.577	0.97
Wolaita	39 (14.02)	7.53	4	19	22	0.977	3.671	0.995
GamoGoffa	34 (12.23)	8.98	3	17	23	0.972	3.586	0.972
Kembata	43 (15.5)	10.25	4	10	24	0.975	3.636	0.986
Hadiya	59 (21.2)	7.95	2	26	33	0.974	3.606	0.977
Dawro	42 (15.1)	9.48	3	15	29	0.974	3.606	0.978
Gurage	31 (11.15)	8.95	2	24	23	0.975	3.631	0.984
Mean ± Standard error	39.7 ± 3.75	8.94 ± 0.94						

*Calculated on the basis of the 278 clones described throughout the study area

Table 7. Distribution of enset clones across the seven provinces

Number of provinces	**Number of enset clones (%)**
One	178 (81.65)
Two	29 (13.3)
Three	8 (3.7)
Four	2 (0.9)
Five	1 (0.46)
Six	0
Seven	0
Total	218

Effect of drying methods and variety on functional properties of trifoliate yam (*Dioscorea dumentorum*) flour

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Abstract

The effect of drying method and variety on the functional properties of trifoliate yam (*Dioscorea dumetorum*) flour was investigated in this study. Flour was produced from yellow and white varieties of trifoliate yam using sun drying, solar dryer, oven (40^{0}C), and cabinet (40^{0}C) dryers, respectively. Some functional properties of the flour were determined using standard laboratory procedures. The pH, bulk density, dispersibility, water absorption index, oil absorption capacity and emulsion capacity ranged from 5.77 to 6.65, 0.66 to 0.76 g/ml, 16.67 to 50.33%, 135.47 to 189.87%, 118.33 to 136.67%, and 43.00 to 50.67%, respectively. The effect of variety and drying methods on these parameters was significant ($P<0.05$) except for water-binding capacity and foaming capacity. There were also significant differences in the effect of drying methods and variety on the particle size distribution ($P< 0.05$) of the flours. The peak, trough, breakdown, final and setback viscosities (in cP) ranged from 1066.5 to 2258.5, 684.5 to 1276.5, 382.0 to 1087.0, 1385.0 to 2207.5, and from 615.5 to 931.0, respectively. Time to attain peak viscosity and pasting temperature ranged from 4.8 to 5.1 min and 68.5° to 88.9°C, respectively. The effect of drying method and variety were significant ($P<0.05$) on the pasting profile of trifoliate yam flour except for setback viscosity, peak time and pasting temperature. The study showed that variety and drying method significantly affected the functional properties of trifoliate yam flour. The data on functional properties of the flour reported will serve as useful baseline information for breeders and commercial utilization of trifoliate yam flour for food and non-food purposes.

Introduction

Yam (*Dioscorea spp*.) is not only a staple food and income generating crop in West Africa, it also has much social value. In some West African communities, the size of a yam farm and volume of output (quantity of tubers) are used to assess the wealth of the owner/farmer. Some yam varieties are widely known and well exploited for food, while many other varieties are exploited as food only in a few rural communities in Nigeria.

Trifoliate yam (*Dioscorea dumetorum*) is known by various names including three-leaved yam, bitter yam and cluster yam. The tuber skin is coarse; one plant usually produces a cluster of tubers. Trifoliate yam is high-yielding compared to other yam species. *D. dumetorum* starch granules are smaller, more soluble and more digestible than those of other yam species. Its starch is as digestible as corn starch (Delpeuch and Favier, 1980) and is made up of tiny polygonal or spherical granules (less than 10 mm) with type A X-ray diffraction structure, similar to that of cereals (Afoakwa and Sefa-Dedeh, 2001). The tubers are rich in protein

(9.6%), and reasonably balanced in essential amino acids (chemical score of 0.94) compared to white yam. It is also rich in vitamins and minerals.

Despite the agronomic and nutritional advantages of trifoliate yam, it is one of the numerous tropical tubers that are yet to be fully exploited and is fast being driven to extinction. A major limitation to its use is the tuber hardening which begins a few hours after harvest thus becoming hardened and hard to chew even after long hours of cooking. Earlier studies (Afoakwa and Sefa-Dedeh, 2002) reported that the chemical compositions of the two main cultivars (white and yellow) consumed are similar, but observations (Afoakwa, 1999) are that the white cultivars harden relatively quicker than the yellow cultivars. Generally, the tubers are left in the soil and harvested as needed for food and often boiled before selling in the market.

The tubers of trifoliate yam, when properly processed, can be used in the production of yam flakes, instant flour for the bakery sector or starch in diverse pharmaceutical preparations. The goal of this study was to find convenient means of adding value to trifoliate yam by processing into flour for use as industrial raw materials.

Materials

Source of trifoliate yam

Freshly harvested yellow and white flesh trifoliate yam (*Dioscorea dumentorum)* was purchased from Kuto market in Abeokuta, Ogun State, Nigeria.

Processing of trifoliate flour

Trifoliate yam tubers were washed with clean water to remove adhering soil and other undesirable material, peeled, sliced, drained and dried using sun (48-72 h), solar dryer (48-72 h), oven dryer (40˚C, 48 h), and cabinet dryer (40˚C, 48 h). Then the dried trifoliate yam chips were milled using a laboratory hammer mill and sieved through 0.250 mm mesh laboratory sieves. The resulting flour was packaged in polyethylene bags and stored at 4°C until used for laboratory analysis.

Analytical determinations

Particle size distributions of the flour samples were determined using the AOAC (2000) method. The bulk density was determined by the method of Wang and Kinssela (1976), swelling power and solubility index using the Takashi and Sieb (1988) method, dispersibility according to Kulkarni et al. (1991) and water absorption index was determined using the modified method of (Ruales et al., 1993). Water binding capacity was carried out using the Medcalf and Gillies (1965) method, and whole emulsification capacity was determined by the method of Padmashree et al. (1987). Foaming capacity was determined by the method of Nwosu et al. (2010), oil absorption capacity by the method of Sosulki (1962), and pasting properties by Rapid Visco Analyser (RVA).

Statistical analysis

All data obtained were subjected to a two way analysis of variance (MANOVA) using the SPSS version 17.0 package. Significance of treatment was tested at the 5% probability level using Duncan’s Multiple Range Test.

Results and discussion

Functional properties of trifoliate yam flour

Table 1 shows the effect of drying method and variety on the functional properties of trifoliate yam flour. The functional properties are those parameters that determine the application and use of food material for various food products. The bulk density of the yellow variety ranged from 0.66 g/ml (cabinet dried) to 0.73 g/ml (oven dried) while that of the white variety ranged from 0.67 g/ml (cabinet dried) to 0.76 g/ml (sun dried). The bulk density of the trifoliate yam flour samples was significantly affected by the drying method and variety ($P< 0.05$). The bulk density is an important parameter that determines the ease of packaging and transportation of particulate foods (Shittu et al., 2007). The bulk density of the flours is comparable to values reported by Shittu et al. (2007) for high quality cassava flour. The pH of the yellow variety was considerably higher with values ranging from 6.05 (oven dried) to 6.65 (solar dried) compared to the white variety with values ranging from 5.77 (sun dried) to 5.90 (solar dried). The pH values were significantly affected by drying method and variety ($P< 0.05$).

The dispersibility of the yellow variety ranged from 19.67% (cabinet dried) to 50.33% (solar dried) while that of the white variety ranged from 16.67% (cabinet dried) to 42.67% (solar dried). Dispersibility is a measure of the degree to which flour or flour blends reconstitute in water and the higher the dispersibility, the better the flour reconstitutes in water (Adebowale et al., 2005). The higher dispersibility value exhibited by flour from the yellow variety compared to the white variety is indicative of their ability to reconstitute more easily in water.

The water absorption index of the yellow variety ranged from 135.47% (solar dried) to 167.20% (oven dried) while the white variety ranged from 147.20% (oven dried) to 189.87% (cabinet dried). The water absorption index of the yellow trifoliate yam flour samples was significantly different ($P< 0.05$) from that of the white variety. The white variety had a higher water absorption index compared to the yellow variety. This agrees with the finding of (Akinwande et al., 2008) that the white variety would require more water during reconstitution than the yellow variety. The high water absorption index has been attributed to loose association of starch polymers in the native granule (Ekwu et al., 2005). The water binding capacity and foaming capacity of trifoliate yam flour were not significantly affected by drying method and tuber variety ($P>0.05$).

Table 1. Effect of drying method and variety on some functional properties of trifoliate yam flour

Drying method	Variety	BD	Disp	WAI	WBC	FC	OAC	pH	EC
sun drying	Yellow	0.68	48.67	153.87	11.47	10.00	125.00	6.60	50.67
	White	0.76	39.67	157.07	8.27	12.00	119.00	5.77	47.33
solar dryer	Yellow	0.69	50.33	135.47	8.27	11.33	129.67	6.65	45.33
	White	0.71	42.67	154.93	11.47	12.67	119.67	5.90	44.00
cabinet dryer	Yellow	0.66	19.67	144.53	7.20	8.67	125.00	6.14	45.33
	White	0.67	16.67	189.87	10.40	10.67	136.67	5.85	45.00
oven dryer	Yellow	0.73	27.67	167.20	12.53	13.33	120.33	6.05	43.00
	White	0.71	21.67	147.20	10.93	12.00	118.33	5.79	43.33
P of drying method		*	*	*	ns	ns	*	*	*
P of variety		*	*	*	ns	ns	ns	*	*
P of drying method ×		*	*	*	ns	ns	*	*	*

Values are means of three replicates

* Significantly different (P<0.05)

ns Not significantly different (P>0.05)

BD = Bulk density; Disp = Dispersibility; WAI = Water absorption index; WBC = Water binding capacity; FC= Foaming capacity; OAC = Oil absorption capacity; EC = Emulsification capacity

The effect of variety and drying methods on oil absorption capacity was significantly different (P< 0.05). Values ranged from 120.33% (oven dried) to 129.67% (solar dried) for the yellow variety and the white variety ranged from 118.33% (oven dried) to 136.67% (cabinet dried). The oil absorption capacity allows the physical entanglement of oil and the binding of fat to the polar chain of protein (Wang and Kinsella, 1976). The emulsification capacity was significantly different (P< 0.05) with values ranging from 43.00% (oven dried) to 50.67% (sun dried) of the yellow variety and the white variety 43.33% (oven dried) to 47.33% (sun dried).

Particle size distribution of trifoliate yam flour

The results of the effect of drying method and variety on the particle size distribution of trifoliate yam flour are presented in Table 2. The particle size distribution was significantly affected by variety and drying method (P<0.05). The 250 μm sieve retained a low percentage of oven dried (0.28%) and cabinet dried (0.53%) flours for the yellow variety and for the flour of the white variety, the corresponding figures were 0.48% (cabinet dried) and 0.48% (oven dried). The samples retained in the sieve size 180 μm ranged from 8.68% (oven dried) to 25.30% (sun dried) for the yellow variety and 10.85% (oven dried) to 23.41% (sun dried) for the white variety and are significantly different (P<0.05). Large amounts of particles were retained as the samples passed through the sieve size 106 μm which ranged from 28.21% (oven dried) to 57.65% (solar dried) for the yellow variety and 32.21% (sun dried) to 55.99% (oven dried) for the white variety and are significantly different (P<0.05). The samples retained in sieve size 90 μm ranged from 19.69% (oven dried) to 21.90% (cabinet dried) for the yellow variety and 17.87% (oven dried) to 22.85% (sun dried) for the white variety and are significantly different (P<0.05). Particle size has been correlated with the swelling power of the trifoliate yam flour. The variation in the particle size distribution is due to the milling procedure of the dried slices during processing (Oduro et al., 2000).

Table 2. Effect of drying method and variety on particle size distribution of trifoliate yam flour

Drying method	Variety	Coarse		Medium	Fine	
		250 µm	180 µm	106 µm	90 µm	Base
sun drying	Yellow	0.40	25.30	55.69	12.55	6.03
	White	0.68	23.41	32.21	22.85	20.85
solar dryer	Yellow	0.53	17.03	57.65	18.51	6.28
	White	0.76	11.41	50.35	19.94	17.54
cabinet dryer	Yellow	0.46	7.52	49.58	21.90	20.54
	White	0.48	11.07	54.74	18.01	15.36
oven dryer	Yellow	0.28	8.68	28.21	19.69	43.14
	White	0.68	10.85	55.99	17.87	14.62
P of drying method		*	*	*	*	*
P of variety		*	*	ns	*	*
P of drying method × variety		*	*	*	*	*

Values are means of three replicates

* Significantly different (P< 0.05),

ns Not significantly different (P>0.05)

Pasting properties of trifoliate yam flour

The effect of drying method and variety on the pasting properties of trifoliate yam flour is shown in Table 3. The peak, trough, breakdown, final and setback viscosities ranged from 1066.5 to 2258.5 cP, 684.5 to 1276.5 cP, 382.0 to 1087.0 cP, 1385.0 to 2207.5 cP, and from 615.5 to 931.0 cP, respectively. Time to attain peak viscosity and pasting temperature ranged from 4.8 to 5.1 min and 68.5° to 88.9°C, respectively. The effect of drying method and variety were significant (P<0.05) on the pasting profile of trifoliate yam flour except for setback viscosity, peak time and pasting temperature. When starch or starch-based foods are heated in water beyond a critical temperature, the granules absorb a large amount of water and swell to many times their original size. Beyond a critical temperature, this is characteristic of a certain starches, undergoing an irreversible process known as gelatinization. When the temperature rises above the gelatinization temperature, the starch granules begin to swell and viscosity increases on shearing (Adebowale et al., 2005).

Table 3. Effect of drying method and variety on pasting properties of trifoliate yam flour

Drying	Variety	Peak	Trough	Break-	Final	Setback	Peak	pasting
Sun drying	Yellow	1997.5	1276.5	721.0	2207.5	931.0	4.9	86.5
	White	1502.5	945.5	557.0	1736.0	790.5	4.8	85.6
solar dryer	Yellow	1601.0	969.0	632.0	1809.5	840.5	4.9	87.4
	White	1455.5	770.5	685.0	1426.0	655.5	4.9	86.5
cabinet	Yellow	1792.0	1113.5	678.5	2007.5	894.0	5.0	87.7
	White	1066.5	684.5	382.0	1385.0	700.5	5.0	87.7
oven dryer	Yellow	1560.0	1127.0	433.0	2052.0	925.0	5.1	88.9
	White	2258.5	1171.5	1087.0	1912.5	741.0	4.8	68.5
P of drying method		*	*	ns	*	*	ns	ns
P of variety		*		ns	*	*	*	ns
P of drying method ×		*	*	*	*	ns	ns	ns

Values are means of three replicates

* Significantly different ($P<0.05$),

ns Not significantly different ($P>0.05$)

Conclusion

The study showed that most of the functional properties of trifoliate yam flour were significantly influenced by tuber variety and drying methods. The data on functional properties of the flour reported will serve as useful baseline information for breeders and commercial utilization of trifoliate yam flour for food and non-food purposes.

References

Adebowale A.A., Sanni L.O., Awonorin S.O. 2005. Effect of texture modifiers on the physicochemical and sensory properties of dried fufu. Food Science and Technology International 11:373-382.

Afoakwa E.O. 1999. Biochemical and quality changes in *Dioscorea dumetorum* tubers after harvest. MPhil thesis, Department of Nutrition and Food Science, University of Ghana, Legon-Accra.

Afoakwa E.O, Sefa-Dedeh S. 2001. Chemical composition and quality changes in trifoliate yam *Dioscorea dumetorum* tubers after harvest. Food Chemistry 75:85–91.

Afoakwa E.O, Sefa-Dedeh S. 2002. Textural and microstructural changes associated with post-harvest hardening of trifoliate yam *Dioscorea dumetorum* tubers. Food Chemistry 77: 279-284.

Akinwade B.A., Abiodun O.A., Adeyemi I.A., Akanbi C.T. 2008. Effect of steaming methods and time on the physical and chemical properties of flour from yam tubers. Nigerian Food Journal 26:97-105.

AOAC 2000. Official Methods of Analysis, Association of Official Analytical Chemists International, Washington, DC.

Delpeuch F., Favier J.C. 1980. Caracteristiques des amidons de plantes alimentaires tropicales: action de l'amylase, gonflement et solubilite. Annales de technologie agricole 27:809–826.

Ekwu F.C., Ozo N.O., Ikegwu O.J. 2005. Quality of fufu flour from white yam varieties (*Dioscorea* spp). Nigerian Food Journal 23:107-113.

Kulkarni K.O., Kulkarni D.N., Ingle U.M. 1991. Sorghum malt based weaning food formulation preparation, functional properties and Nutritive Value. Food and Nutrition Bulletin 13:322-327.

Medcalf D.G., Gillies K.A. 1965. Determination of starch damage by rate of iodine absorption. Cereal Chemistry 42:546-547.

Nwosu J.N., Ogueke C.C., Owuamanam C.I., Iwouno J.O. 2010. Functional properties and proximate composition of asparagus bean (*Vigna sesquipedalis)* as influenced by malting. Journal of American Science 6:376-382.

Oduro L., Ellis W.O., Dziedzoave N.T., Nimakoyeboah K. 2000. Quality of gari from selected processing zones in Ghana. Food Control 11:297-303.

Padmashree T.S., Vijayalakshmi L., Puttaraj S. 1987. Effect of traditional processing on the functional properties of cowpea (*Vigna cajan*) flour. Journal of Food Science andTechnology 24: 221-226.

Ruales J., Valencia S, Nair B. 1993. Effect of processing on the physiochemical characteristics of guinea flour (*Chenopodium guinea* Wild). Starch 46:13-19.

Shittu T.A., Sanni L.O., Awonorin S.O., Maziya-Dixon B., Dixon A. 2007. Use of multivariate techniques in studying the flour making properties of some CMD resistant cassava clones. Food Chemistry 101:1606–1615.

Sosulki F.W. 1962. The centrifuge method for determining flour adsorption in hard red spring wheats. Cereal Chemistry 39:344-350.

Takashi S., Seib P.A. 1988. Paste and gel properties of prime corn and wheat starches with and without native lipids. Cereal Chemistry 65:474.

Wang J.C., Kinsella J.E. 1976. Functional properties of novel proteins; alfalfa leaf protein. Journal of Food Science 41:286–289.

Physiological responses of a Bambara groundnut segregating population to mild drought stress

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Abstract

The effect of mild drought stress on a Bambara groundnut F_5 segregating population derived from the cross between single genotypes derived from the DipC and Tiga Nicuru landraces was evaluated in controlled-environment glasshouses at the FutureCrop Glasshouses, University of Nottingham, UK, in 2012. Sixty-five F_5 lines had mild drought stress imposed from 50 days after sowing (DAS) until 92 DAS when an average 50% decline in stomatal conductance was observed in the water-stressed plot. Drought stress reduced stomatal conductance significantly, with variation observed in the segregating population, but without a significant change in leaf carbon (Delta C^{13}) isotope analysis at harvest. The applied drought stress also did not significantly influence plant phenology as measured by estimated days to podding or morphology and growth parameters, including pod weight per plant, seed number per plant and seed dry weight. Nevertheless, internode length, peduncle length, shoot dry weight and pod number per plant were significantly higher in the water-stressed plot than in the irrigated plot. In addition, 100-seed weight and harvest index were significantly reduced by 8% and 15.6%, on average, by drought. Higher stomatal density was observed in plants under drought conditions than irrigated conditions ($P<0.01$). This showed a negative correlation with 100-seed weight and harvest index ($r= -0.40$; $r= -0.42$) at a significance level of $P<0.01$. The response of Bambara groundnut to a short period of water deficit might provide an insight into the early defence mechanisms of this drought- tolerant species when plants become stressed. The variation observed among the individual lines could also identify candidate lines that have greater tolerance under drought stress for future breeding programmes in Bambara groundnut.

Keywords: Bambara groundnut, water stress, stomatal conductance, stomatal density, yield

Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc) is an indigenous African legume that produces protein-rich (16-25%) and nutritious seeds (Brough and Azam-Ali, 1992). It is mainly grown by subsistence and small-scale farmers in the semi-arid regions of sub-Saharan Africa where rainfall is low. Bambara groundnut has long been recognized as a drought-tolerant crop as it can survive and produce higher seed yield than many other legume crops under drought conditions, although a comprehensive set of comparisons between legume species is still needed. Landrace differences in Bambara groundnut in response to drought have been reported (Mwale et al., 2007), providing the potential to select and breed higher yielding landraces and/or cultivars under water stress. In order to investigate the species' genetic diversity for drought tolerance, the exploration of the mechanisms underlying the response of Bambara groundnut to drought is essential.

Various responses are displayed in crop plants when they are stressed and they can be categorized into three groups: escape, avoidance and tolerance. Bambara groundnut, for instance, was shown to have a shortened vegetative period, to flower earlier, have a reduced reproductive stage and also mature earlier in response to water stress (Mabhaudhi et al., 2013). Landraces from Jozini, South Africa, such as 'Red' and 'Brown' landraces had an earlier maturity date (mean: 122.75 DAP, $P<0.01$) when the plants were stressed at 30% of the crop water requirement (ETa) as compared to 100% ETa (mean: 128 DAP, $P<0.01$; Mabhaudhi et al., 2013). The change in leaf orientation, which is known as paraheliotropic movement, was observed in drought-stressed Bambara groundnut landraces such as AS-17 (Stadler, 2009). In addition, Collinson et al. (1997) suggested that Bambara groundnut maintains plant water status over the drought period through stomatal regulation of water loss, osmotic adjustment and a reduction in leaf area.

In this study, mild drought stress was applied to a Bambara groundnut F_5 segregating population at the early flowering stage in order to investigate the immediate response of Bambara groundnut to water stress and the effects of mild drought on final yields. Here we are interested in how the crop deals with the early stages of drought stress, when the changes in gene expression are likely to reflect initial protective mechanisms, rather than extreme stress, where gene expression may represent plants in a terminal state beyond recovery. The parental genotypes were derived from landraces from Botswana (DipC) − where the mean rainfall is 450 mm with temperature ranges from 15°C-18°C (Burgess, 2006), and Mali (Tiga Nicuru) − where the mean rainfall is 440 mm with temperatures of 16°C-39°C (Pedercini et al., 2012). As the segregating populations consist of lines which may show genetic variation, potential candidates that have higher yielding characteristics and also greater tolerance under drought stress could be selected for future breeding programmes.

Materials and methods

Experimental site and plant material

The drought experiment was conducted in controlled-environment glasshouses at the FutureCrop Glasshouses, Sutton Bonington Campus, University of Nottingham, UK in 2012. The F_5 segregating population is derived from a cross between single genotype DipC (maternal) X Tiga Nicuru (paternal) lines. Plant material, consisting of two parental lines and 65 F_5 individual lines were planted in both water-stressed and irrigated plots.

Experimental design and crop management

There were two independent soil pits (5 m x 5 m x 1 m) in the glasshouses. One pit was used as the water-stressed plot and the other as the irrigated plot. The experiment was arranged in a randomized block design with three blocks for each soil pit. Each line had three replicates, one in each block, and each replicate was represented by a single plant. All lines were randomly allocated within a single block. Three seeds were sown per replicate in each soil pit, a total of 9 seeds per line, at a depth of 3-4 cm and spacing of 25 cm x 25 cm between each replicate, giving 20 plants per row. Twenty days after sowing (DAS) the plants were thinned to one plant per hole. Photoperiod was 12 hours, day temperature 28°C, with 23°C at night.

Trickle irrigation using PVC micro-porous tubing placed besides each row irrigated the plants at 0600 hrs and 1800 hrs for 20 minutes each time. Soil moisture content was monitored using a PR2 probe. After 100% flowering was observed across all the lines at 50 DAS, the irrigation system was terminated for six weeks until 92 DAS, until a 50% reduction in stomatal conductance was observed. The control (irrigated) plot was continued with the earlier regime of watering.

Soil moisture measurement

Three evenly spaced PR2 profile tubes (Delta-T devices, UK) were inserted into each soil pit across the diagonal from the irrigation source towards the end of the trickle tape. Three PR2 readings, which were displayed in units of %Vol (volumetric water content as a percentage), were taken twice a week at 1000 h starting from 16 DAS until 133 DAS at soil depths of 300 mm, 400 mm, 600 mm and 1000 mm.

Morpho-physiological traits (drought-related trait) measurement

A range of morphological and physiological traits was measured on both plots based on the Bambara groundnut descriptor list (IPGRI, 2000). These were days to emergence, days to flowering, estimated days to podding, internode length, peduncle length, pod number per plant, pod weight per plant, seed number per plant, seed weight per plant, 100-seed weight, shoot dry weight and harvest index. In addition, drought-related traits including stomatal conductance, leaf carbon (Delta C^{13}) isotope analysis and stomatal density were also measured. Methods for measuring stomatal conductance were modified from Vurayai et al. (2011). Due to time constraints, seven measurements were carried out on the water-stressed plot and only four on the irrigated plot during the course of the experiment.

Stomatal conductance (mmols/m^2s): The reading of stomatal conductance (g_s) on only the abaxial side of the leaf was undertaken using an AP4 leaf porometer (Delta-T devices, UK) as readings of g_s on the adaxial side of the leaf were very low, in agreement with Jorgensen et al., (2011). The middle leaflet of three fully expanded leaves, for each plant replicate, was measured between 0800 hrs and 1200 hrs. Measurements were conducted weekly, starting from 49 DAS until 107 DAS.

Leaf carbon (Delta C^{13}) isotope analysis: Seed samples collected from both parental lines (three replicates) and 65 individual lines (one replicate) were freeze-dried using a Benchtop Freeze Dryer LSBC50 (MechaTech Systems, UK) for a week. These samples were then milled into a fine powder using an Ultra Centrifugal Mill ZM200 (Retsch, Germany). The carbon (Delta C^{13}) isotope analysis was performed at the Mylnefield Research Services Ltd, Dundee, Scotland. Based on their recommended protocol, approximately 0.2-0.3 mg of milled samples was encapsulated in the tin capsules that were provided. The value of the discrimination (Δ) for ^{13}C was calculated based on Farquhar et al. (1989), the final equation for CID being:

$$\Delta = 1000 \text{ X } (-0.008 - \delta 13C(‰)/1000)/(1+ \delta 13C(‰)/1000)$$

Stomatal density: One leaf from each replicate for both parental lines and 65 individual lines was harvested. The abaxial side of the leaf was painted using nail polish and a thin film was mounted on a glass slide after peeling from the leaf. A drop of water was then added on top of the thin film. Counts of stomata were performed after the images were captured using a Leica BF200 compound microscope with Leica LAS EZ software (Leica Microsystems, Switzerland) at a magnification of 40x. Three counts per impression were made with a square area of 0.8071 mm^2 per impression. Therefore, stomatal density = count of stomata/0.8071 mm^2

Statistical analysis

Data for all traits were subjected to analysis of variance (ANOVA) using Genstat 15th edition (VSN International, 2012) to determine whether statistical differences existed between lines for a given trait, to investigate the population distributions through descriptive statistics and the correlation relationship between the traits. Non-normal distributed traits were also transformed using a square root function after conducting an Anderson-Darling normality test.

Results

Soil moisture

Across all depths, the reduction in soil moisture content based on PR2 readings in the water-stressed plot was 52.7% compared to the irrigated plot which was 9.5%, from 50 DAS to 92 DAS. Soil moisture was lost rapidly at a rate of 1.95% per day at a soil depth of 400 mm, followed by 1.65% per day at a soil depth of 600 mm (Figure 1). At 1000 mm, water-stressed plots showed relatively constant soil moisture content and losses only became apparent at 86 DAS. In contrast, no significant changes occurred in the irrigated plot.

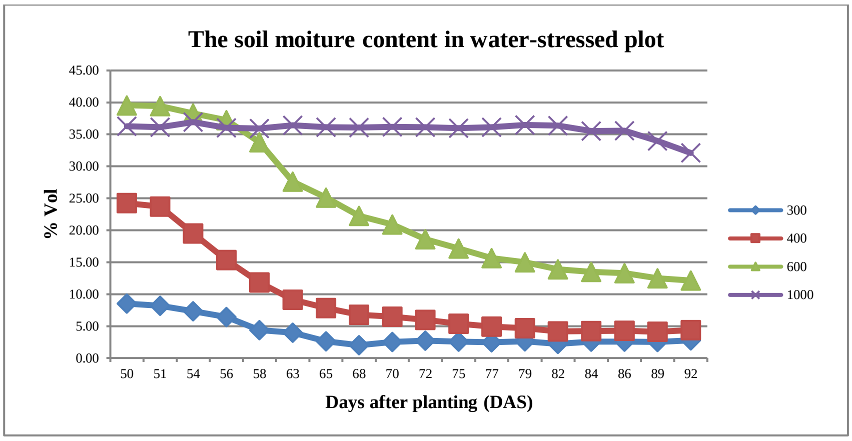

Figure 1. Soil moisture content based on a PR2 reading (%vol) in the water-stressed plot throughout the treatment from 50 DAS (days after sowing) to 92 DAS.

Stomatal conductance (g_s)

Throughout the drought stress period, grand mean values for stomatal conductance (g_s) declined gradually in the water-stressed plot from 540 mmol m^2 s^{-1} to 220 mmol m^2 s^{-1} (Figure 2). Drought treatment was applied at 50 DAS; g_s before treatment (49 DAS) was measured and served as a baseline for g_s over the drought period. Although there are some missing data due to the priority given to the water-stressed plot, consistently high values are observed in the irrigated plot (500 mmol m^2 s^{-1} – 600 mmol m^2 s^{-1}). The sudden increase in g_s at 107 DAS in the water-stressed plot was a result of the water recovery treatment applied at 92 DAS. ANOVA showed significant differences among the lines ($F_{(64,130)}$=16.27, P<0.01), as well as between the treatments ($F_{(1,130)}$=2259.59, P<0.01). Some lines are shown to have high g_s

under both drought and irrigation conditions, for example, L101 (D: 274.1 mmol m^2 s^{-1}; IR: 581.1 mmol m^2 s^{-1}), L89 (D: 269.3 mmol m^2 s^{-1}; IR: 584.4 mmol m^2 s^{-1}) and L94 (D: 261.8 mmol m^2 s^{-1}; IR: 617.8 mmol m^2 s^{-1}) at 84 DAS. However, L5 (D: 166.1 mmol m^2 s^{-1}; IR: 432.8 mmol m^2 s^{-1}), L7 (D: 185.9 mmol m^2 s^{-1}; IR: 519.4 mmol m^2 s^{-1}) and L37 (D: 193.6 mmol m^2 s^{-1}; IR: 524.2 mmol m^2 s^{-1}) were lines that showed low g_s at 84 DAS.

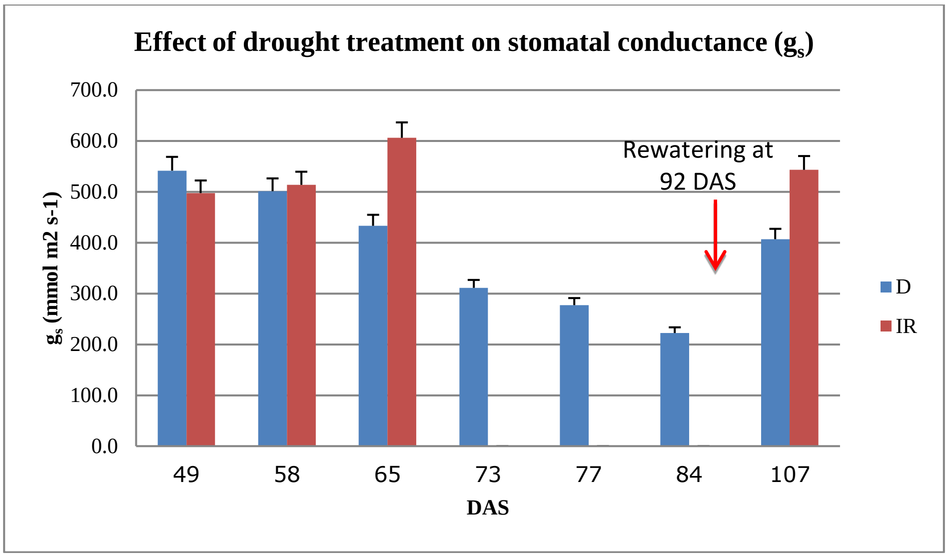

Figure 2. The effect of the drought treatment on stomatal conductance (g_s) in the water-stressed and irrigated plot between 49 DAS (days after sowing) to 107 DAS. Data points represent mean value ± standard error, n=65. Arrow: rewatering of plants at 92 DAS.

Leaf carbon (Delta C^{13}) isotope analysis

Significant differences between the two parental lines for leaf carbon isotope analysis (δC^{13}; $F_{(1,6)}$=21.33, P<0.01) were found. Table 1 showed that lower δC^{13} was associated with higher yield as observed in DipC, compared to Tiga Nicuru. However, there was no obvious effect of drought treatment on δC^{13} as no significant difference was observed between treatments for two parental lines. Although no ANOVA analysis was carried out in the segregating population due to the lack of replicates, the population exhibited variation for δC^{13} and, based on the use of this surrogate measure, water use efficiency was expected to have variation due to genotypic differences derived from two parental lines.

Table 1. The δC^{13} value of DipC and Tiga Nicuru under drought and irrigated conditions.

Sample	Treatment	Average δC^{13}	Average yield (g/plant)
DipC	Drought	17.85	33.0
DipC	Irrigation	17.77	31.6
Tiga Nicuru	Drought	19.65	8.6
Tiga Nicuru	Irrigation	19.73	7.5

Stomatal density

Stomatal density is significantly different among the individual lines ($F_{(64,258)}$= 4.08, P<0.01) and also between the treatments ($F_{(1,258)}$=22.55, P<0.01). Higher stomatal density was observed in the water-stressed plot (mean: 11.64 pores cm^{-2}) compared to the irrigated plot (mean: 10.07 pores cm^{-2}). Among the segregating population, some lines obtained high stomatal density under drought conditions such as L37 (D: 14 pores cm^{-2}; IR: 12 pores cm^{-2}), L94 (D: 13 pores cm^{-2}; IR: 11 pores cm^{-2}) and L7 (D: 11 pores cm^{-2}; IR: 12 pores cm^{-2}) whereas there were lines showing low stomatal density, L112 (D: 6 pores cm^{-2}; IR: 8 pores cm^{-2}), L101 (D: 7 pores cm^{-2}; IR: 7 pores cm^{-2}) and L5 (D: 7 pores cm^{-2}; IR: 9 pores cm^{-2}). Stomatal density was discovered to have a moderate and negative relationship with 100-seed weight and harvest index (r= -0.40, P<0.01; r= -0.42, P<0.01).

Morpho-physiological traits measurement

The result shows that most of the traits are normally distributed, except for days to emergence, internode length (irrigated), pod weight per plant (irrigated) and seed weight per plant (irrigated) (Table 2). As the F_5 is a segregating population, genetic variability between lines would be expected. For each trait, several lines were better or worse than parental lines in the drought treatment, suggesting possible transgressive segregation is being observed in the population. For instance, the population had a maximum internode length of 4.15-5.29 cm (L64) and minimum 0.52-0.71 cm (L103) while DipC had an internode length ranging from 1.54 -2.22 cm (population mean: 2.48; population s.d.: 1.00) and Tiga Nicuru 1.57-3.04 cm (population mean: 2.48; population s.d.: 1.00) (Table 2). Major traits that are significantly different between the two parents and segregate in the F_5 population are peduncle length, pod number per plant, 100-seed weight and harvest index.

Drought stress did not significantly influence estimated days to podding, pod weight per plant, seed number per plant and seed weight per plant. Nevertheless, a significant increase of internode length ($F_{(1,258)}$= 27.45, P<0.01), peduncle length ($F_{(1,258)}$= 33.09, P<0.01) and shoot dry weight ($F_{(1,258)}$= 8.56, P<0.01) is observed between lines in the water-stressed plot, which is suspected to be the result of rapid plant growth when the water stress is relieved. Although pod number per plant was higher in the water-stressed plot (P<0.05), a significant reduction in 100-seed weight and harvest index ($F_{(1,258)}$= 19.4, P<0.01; $F_{(1,258)}$= 12.87, P<0.01) by 8% and 15.6%, respectively, occurred between lines, implying that mild drought may negatively influence yield accumulating processes in Bambara groundnut. Given that ANOVA analysis showed significant differences ($F_{(64,258)}$= 7.66, P<0.01) among the lines for 100-seed weight, lines such as L89 (D: 81.89 g; IR: 89.42 g), L5 (D: 72.46 g; IR: 70.79 g) and L101 (D: 69.42 g; IR: 64.19 g) was observed to produce high 100-seed weight whereas L41 (D: 24.69 g; IR: 37.33 g), L45 (D: 27.22 g; IR: 28.77 g) and L37 (D: 33.53 g; IR: 26.67 g) had low 100-seed weight in the water-stressed plot (population mean and s.d.: 49.24 and 12.02) and irrigated plot (population mean and s.d.: 53.55 and 12.53), respectively.

Table 2. Descriptive statistics analysis for morphological and physiological traits measured in F_5 segregation population under both drought and irrigated conditions.

Traits	Treatment	Mean	SD	Min	Max	Skewness	Kurtosis	Normality	DipC		Tiga Nicuru	
									Min	Max	Min	Max
Days to emergence	-	7.374	0.611	6.458	9.833	1.302	2.807	1.2766**	7.0	8.0	6.0	6.5
Days to flowering	-	31.84	2.536	27.33	41.17	0.806	1.397	0.4968ns	28.0	33.0	28.5	35.0
Estimated days to podding	Drought	57.35	3.45	49.67	64.33	-0.1660	-0.4110	0.283ns	55.0	61.0	50.0	56.0
	Irrigation	57.31	3.24	50.33	63.67	-0.3920	-0.3290	0.7341ns	53.0	58.0	51.0	54.0
Internode length (cm)	Drought	2.48	1.00	0.71	5.29	0.4690	-0.1370	0.3558ns	1.74	2.22	2.54	3.04
	Irrigation	2.21	0.92	0.52	4.15	0.3260	-0.9180	0.8567*	1.54	2.04	1.57	2.82
Peduncle length (cm)	Drought	3.50	1.48	0.60	7.28	0.1200	-0.6530	0.3779ns	2.54	3.06	3.54	4.6
	Irrigation	3.12	1.48	0.57	6.15	0.1750	-0.9880	0.591ns	1.65	2.38	1.945	3.56
Pod. No/plant	Drought	53.40	25.45	7.50	126.70	0.5030	0.0890	0.4647ns	59.0	73.0	20.0	32.0
	Irrigation	46.79	23.76	3.00	105.70	0.4180	-0.2450	0.3895ns	44.0	106.0	21.0	23.0
Pod weight (g/plant)	Drought	36.01	19.12	4.36	83.09	0.4780	-0.1290	0.4893ns	39.21	49.64	11.32	14.36
	Irrigation	38.25	22.65	1.98	85.51	0.3330	-1.0590	1.1301**	28.41	76.83	10.77	11.26
Seed. No/plant	Drought	53.47	26.60	6.50	129.30	0.5010	-0.0613	0.44ns	58.0	72.0	26.0	28.0
	Irrigation	48.28	26.35	3.00	116.70	0.4990	-0.4690	0.6893ns	38.0	105.0	15.0	16.0
Seed weight (g/plant)	Drought	26.47	13.96	1.95	62.36	0.4540	-0.1220	0.4681ns	28.0	39.4.	8.57	8.59
	Irrigation	27.12	16.24	1.28	57.72	0.3350	-1.1490	1.3672**	23.14	61.79	6.81	8.24
100-seed weight (g)	Drought	49.24	12.02	24.48	81.89	0.4230	0.1040	0.6622ns	52.83	58.8	37.34	44.15
	Irrigation	53.55	12.53	26.67	89.42	0.3680	-0.1650	0.6843ns	58.85	60.89	45.4	51.5
Shoot dry weight (g/plant)	Drought	50.62	16.83	17.03	100.20	0.6180	0.5990	0.5969ns	44.75	51.36	26.23	32.96
	Irrigation	45.88	17.41	14.93	92.30	0.5840	0.1670	0.6102ns	48.47	105.26	27.31	29.39
Harvest index	Drought	0.65	0.23	0.19	1.23	-0.1040	-0.3100	0.5175ns	0.81	1.11	0.43	0.44
	Irrigation	0.77	0.31	0.10	1.65	0.0259	-0.1540	0.3979ns	0.73	1.00	0.38	0.39

Discussion

A rapid reduction in g_s when mild drought is applied implies that the regulation of stomata closure for water loss is one of the early events in the response of Bambara groundnut to drought. A rapid decline in g_s between 65 DAS to 72 DAS (15.23 mmol m^2 s^{-1} per day), followed by a relatively slow and steady decline between 72 DAS and 84 DAS (8.07 mmol m^2 s^{-1} per day) was observed in the water-stressed plot (Figure 2). Collinson et al. (1997) stated that stress-induced stomata closure is believed to be accompanied by osmotic adjustment. Once the decline of g_s reaches a threshold value due to drought stress, g_s shows little or no change as the plants are thought to keep the stomata opened for carbon uptake while maintaining their plant water status by osmotic adjustment. Collinson et al. (1997) also observed a relatively unchanged g_s value (0.13 cm s^{-1}-0.25 cm s^{-1}) at lower leaf potentials in Bambara groundnut and thus suggested that this is a common response to drought contributed by osmotic adjustment to maintain turgor in the plant.

Drought stress reduced stomatal conductance significantly with trait variation observed in the segregating population but did not reveal significant differences between lines for δC^{13} analysis. For δC^{13} analysis, the lower the value of δC^{13}, the higher the water use efficiency and thus the final yield (Ebdon and Kopp, 2004), although the direction of the relationship with respect to yield can be influenced by the severity of the drought. For example, a positive correlation between δC^{13} and yield was identified in barley and wheat in Mediterranean irrigated conditions, whereas in some areas such as where crop growth is reliant on stored soil water, a negative correlation is associated with higher grain yield (Araus et al., 2007). No significant difference was observed between the treatments for parental lines, indicating that mild drought does not significantly bias carbon fixation during the drought period. δC^{13} implies that there is no significant impact of the drought on water use efficiency.

In addition, stomatal density was found to be significantly influenced by drought stress. However, instead of stomatal effects, reduced leaf area seems to be the factor that caused higher stomatal density in plants that were stressed. Although total leaf area per plant was not determined, leaf area of the same leaf used for stomatal counts - a total of three leaves per line - was measured. ANOVA showed that smaller leaf areas were obtained in the water-stressed plot (mean: 18.92 cm^2) than in the irrigated plot (mean: 22.25 cm^2; $P<0.01$). This result is consistent with previous reports of a negative correlation between leaf area and stomatal density in *Leymus chinensis* under moderate drought (Xu and Zhou, 2008). The moderate negative relationship between stomatal density and 100-seed weight as well as harvest index observed in the present study is also comparable with the result presented by Meng et al. (1999) in which the net photosynthetic rate is significantly negatively correlated with stomatal density in rice. Thus, in addition to stomatal closure, a reduction in leaf area is also an early response to drought stress, allowing plants to reduce water loss and thus potentially decreasing carbon uptake and fixation, leading to limitations in photosynthetic assimilation (Xu and Zhou, 2008). It is worth noting that soil water deficit alone was measured in the experimental conditions. As both soil pits were in the same glasshouse, it is likely that vapour pressure deficit reflects the combined effects of water-stressed and irrigated plots and the humidity within the glasshouse did not decrease below 30%. This is also likely to have mitigated the effects of the drought treatment.

The effect of the water stress could only be observed after 1-2 weeks after treatment, which is at the early pod-filling stage. Therefore, the plants in the water-stressed plot are believed to have been well-established before the stress took effect, resulting in a better crop performance overall that was not significantly different from plants in the irrigated plot. Nevertheless, the pod-filling stage is affected by water stress, despite more pods per plant being observed in the water-stressed plot; 100-seed weight and harvest index were reduced significantly ($F_{(1,258)}$= 19.4, P<0.01; $F_{(1,258)}$= 12.87, P<0.01) by 8% and 15.6% respectively in the segregating population. Combining the responses of Bambara groundnut plants to mild drought stress, there are some lines in the segregating population that have both drought tolerance and high seed weight characters. For example, L89 and L94 had a high g_s and moderate stomatal density (moderate leaf area) while L5 showed a low g_s and low stomatal density (large leaf area) with high 100-seed weight. However, different responses were shown by L101 and L112 which both showed a high g_s and low stomatal density (large leaf area) with high yield. Although no significant difference between treatments for 100-seed weight and harvest index was observed in parental lines, segregation in offspring lines allows high-yielding lines to become potential candidates in future programmes for maintenance of yield under mild drought.

The reduction in final yield was possibly the combined result of stomatal closure and reduced leaf area which could reduce water loss, but also limit photosynthesis capacity, and hence carbon deposition in the seeds. Stomatal conductance is able to provide some indication of transpiration rates, but the relationship is not direct as transpiration will be affected by the combined result of the increase of vapour pressure gradient due to increases in leaf temperature under high irradiance and continued irrigation of one soil pit in the same glasshouse as well as stomatal closure (Collinson et al., 1997). Unlike other legumes such as pea, chickpea and mungbean, Mwale et al. (2007) found that Bambara groundnut did not carry out a redistribution of dry matter during the pod filling stage as Bambara groundnut probably lacks important vegetative structures to store carbohydrates before redistributing to the pods (Mwale et al., 2007). Therefore, a cause of decreased seed yield in Bambara groundnut plants could be lower photosynthetic levels of plants during the pod filling stage due to mild drought stress (Mwale et al., 2007).

Conclusion

Under glasshouse conditions, the responses of a Bambara groundnut F_5 segregating population to mild drought imposed at the early flowering stage were studied. Stomatal conductance, δC^{13}, 100-seed weight, harvest index and stomatal density could be potential criteria for breeding selection for drought tolerance. However, the relationship between the impact of the drought and final yield is not straightforward. Several measurements such as total leaf area, number of leaves, transpiration rate and photosynthetic level in plants would need to be carried out in order to establish a clearer relationship. As DipC is different from Tiga Nicuru in terms of plant morpho-physiology, variation is expected to be observed among the segregating population. Potential candidates that have higher yielding characteristics and perform better than parental lines under drought stress could be selected for future breeding programmes.

References

Araus J.L., Bort J., Tambussi E.A. 2007. Water use efficiency in C3 cereals under Mediterranean conditions: a review of some physiological aspects. In : Lamaddalena N., Shatanawi M., Todorovic M., Bogliotti C., Albrizio R. (eds). Water use efficiency and water productivity: WASAMED project. Bari: CIHEAM pp. 189-203 (Options Méditerranéennes: Série B. Etudes et Recherches; n. 57)

Brough S.H. and Azam-Ali S.N. 1992. The effect of soil moisture on the proximate composition of Bambara groundnut (*Vigna subterranea* (L.) Verdc). Journal of Science of Food and Agriculture 60: 197-203

Burgess J. 2006. Country Pasture/ Forage Resource Profiles – Botswana. FAO. Rome.

Collinson S.T., Clawson E.J., Azam-Ali S.N., Black C.R. 1997. Effects of soil moisture deficits on water relations of Bambara groundnut (*Vigna subterranea* L. Verdc.). Journal of Experimental Botany 48: 877-884

Ebdon J.S. and Kopp K.L. 2004. Relationships between water use efficiency, carbon isotope discrimination, and turf performance in genotypes of Kentucky bluegrass during drought. Crop Science 44: 1754-1762

Farquhar G.D., Ehleringer J.R., Hubick T. 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40: 503-537

IPGRI, 2000. *Descriptors for Bambara groundnut (Vigna subterranea)*. International Plant Genetic Resources Institute, Rome, Italy; International Institute of Tropical Agriculture, Ibadan, Nigeria; The International Bambara Groundnut Network, Germany.

Jorgensen S.T., Ntundu W.H., Ouedraogo M., Christiansen J.L., Liu F. 2011. Effect of a short and severe intermittent drought on transpiration, seed yield, yield components, and harvest index in four landraces of Bambara groundnut. International Journal of Plant Production 5: 25-36

Mabhaudhi T., Modi A.T., Beletse Y.G. 2013. Growth, phenological and yield responses of a Bambara groundnut (Vigna subterranea L. Verdc) landrace to imposed water stress: II. Rain shelter conditions. Water SA 39: 191-198

Meng L., Li L., Chen W., Xu Z., Liu L. 1999. Effect of water stress and temperature on leaf size and number of epidermal cells in grain sorghum. Crop Science 14: 751-755

Mwale S.S., Azam-Ali S.N., Massawe F.J. 2007. Growth and development of Bambara groundnut (*Vigna subterranea*) in response to soil moisture 1. Dry matter and yield. European Journal of Agronomy 26: 345-353

Pedercini M., Kanamaru H. and Derwisch S. 2012. Potential impacts of climate change on food security in Mali. Natural Resources Management and Environment Department, FAO, Rome.

Stadler F. 2009. Analysis of differential gene expression under water-deficit stress and genetic diversity in Bambara groundnut (*Vigna subterranea* (L.) Verdc.) using novel high-throughput technologies. PhD Thesis. Technical University of Munich, Germany.

Xu Z.H. and Zhou G.S. 2008. Reponses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal of Experimental Botany 59: 3317-3325

Vurayai R., Emongor V., Moseki B. 2011. Physiological responses of Bambara groundnut (*Vigna subterranea* (L.) Verdc) to short periods of water stress during different development stages. Asian Journal of Agricultural Sciences 3: 37-43

VSN International. 2012. Genstat for Windows 15th Edition. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk

Evaluation and identification of suitable finger millet genotypes for higher productivity in combating climate change

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Abstract

Finger millet (*Eleusine coracana* L. Gaertn.) is a staple food crop in the majority of drought prone areas in several East African and South Asian countries. Finger millet is hardy in nature and resilient to adverse climatic factors. The grains are rich in nutrients and the crop yields valuable fodder. Based on this, an investigation was carried out in the Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during 2011-2012 to develop high-yielding genotypes with best nutritional qualities and high yield potential even under changing climatic conditions using phenotypic selection methods. Initially, 100 finger millet genotypes were evaluated for different productivity and quality traits. Among these, 24 finger millet genotypes were screened based on their yield characters. Selection was based primarily on agronomic traits such as days to maturity, plant height, number of productive tillers per plant, numbers of fingers per earhead, finger length, 1000 grain weight and grain yield per plant. This study will serve as an initiative for utilization of variability and allows the selection of genotypes for different agro-climatic situations. Hence, even under changing climatic and other environmental conditions, direct selection based on yield and yield-contributing traits with high nutrients will help people affected by malnutrition.

Key words: Finger millet, climate change, nutritional quality, selection and variability.

Introduction

Among the millets of the world, finger millet (*Eleusine coracana* L. Gaertn.) ranks fourth after pearl millet, foxtail millet and proso millet. Almost the entire production is confined to Africa and Asia. India alone produces between 40 and 45 per cent of the total world production, and most of the rest of finger millet is produced in Central Africa. In India, the states of Karnataka, Tamil Nadu and Andhra Pradesh produce most of the finger millet crop, with Karnataka and Tamil Nadu producing about 61 per cent of the total crop. However, finger millet is also grown, to a more limited extent, in the Western Ghats of southwest India and in the foothills of the Himalayas and this production extends along the hills of southern Asia as far east as China. Finger millet grain is one of the most nutritious cereals with respect to protein, minerals (calcium and iron) and amino acids. It has 8-10 times more calcium than wheat or rice. Finger millet carbohydrates have the unique property of slower digestibility and can be regarded as a 'long sustenance 'food.

Finger millet is one of the hardiest crops grown under the dry cultivation conditions in its regions of adaptation. It has a wide range of adaptation to soil, but grows especially well in lateritic soils being mainly grown on red, light red, light black or grayish loams and sandy loams. In some areas it may be cultivated on well drained alluvial soil as well. In addition, it is reputed to tolerate a certain degree of alkalinity in the soil. Rain-fed finger millet is often rotated with pulses, oilseeds or mixtures of these.

Aside from its uses as a cereal grain and/or fodder, finger millet is often grown because of its high reproduction − ranging between 200 and 500 fold in terms of seed. Thus a little seed goes a long way in reproducing the crop. A second very valuable attribute of this cereal is its grain-keeping quality. The grain is highly resistant to storage insect pests, even without any special care or attention (Ayyangar and Rao, 1932). For starting any crop improvement work, information about the genetic variability available in the population is a prerequisite. The presence of high variability in the genotypes of this crop offers much scope for its improvement (Poehlman, 1987). Estimation of genetic parameters in the context of trait characterization is an essential component in developing high yielding varieties. Hence, an attempt was made to estimate the extent of variation of yield-contributing traits in 100 finger millet genotypes by studying genetic parameters such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance, which may contribute to formulation of suitable selection indices for improvement in this crop.

Materials and methods

The experimental material consisted of 100 finger millet genotypes maintained at the Small Millets Section of the Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore which is situated at about 11°N latitude and 77°E longitude at an altitude of 427 m above MSL and the average annual rainfall is around 700 mm. For evaluation and characterization, these genotypes were grown in a randomized complete block design with three replications. Observations were recorded from five randomly selected competitive plants in each accession for 13 quantitative characters − plant height, flag leaf sheath length, flag leaf sheath width, flag leaf blade length, flag leaf blade width, productive tillers, finger number, finger length, finger width, days to maturity, thousand grain weight and single plant grain yield according to the descriptors for *Eleusine coracana* except days to 50% flowering; days to 50% flowering was noted on a single row basis. Phenotypic and genotypic variances were estimated according to the formula given by Lush (1940). Phenotypic and genotypic coefficients of variability were computed according to the method suggested by Burton (1952). Heritability in a broad sense was calculated by the formula given by Allard (1960). The range of heritability was categorized as suggested by Johnson et al. (1955). Genetic advance was expressed as the percentage of the mean by using the formula suggested by Johnson et al. (1955). Traits were classified as having high, moderate or low genetic advance according to the method suggested by Johnson et al. (1955). Nutritional analysis was carried out through atomic absorption spectrometry (AAS) using an ECIL AAS (Perkin Elmer) using the protocol described by Zarcinas et al. (1987).

Results and discussion

Analysis of variance was carried out for 13 characters in 100 genotypes. Highly significant mean sums of squares due to genotypes and a wide range of variability were observed among the genotypes for all the 13 characters studied. The estimates of mean, range, phenotypic coefficient of variation, genotypic coefficient of variation, heritability in a broad sense and genetic advance as percentage of mean are presented in Table 1.

The magnitude of phenotypic coefficient of variation (PCV) was higher than that of the genotypic coefficient of variation (GCV) for all characters under study. It indicated that apparent variation is not only due to genotypes but also due to the influence of environment. However the narrow differences between PCV and GCV indicated little influence of environment on the expression of these characters and variability was due to genetic components only. This implied phenotypic variability to be a reliable measure of genotypic variability. Similar reports were earlier reported by Kebere et al. (2006). For all 13 traits, the phenotypic coefficient of variation and genotypic coefficient of variation ranged from 6.11 to 25.40% and 5.94 to 24.08% in both cases for finger width and single plant grain yield, respectively. Genotypic coefficient of variation followed a similar trend to that of phenotypic coefficients of variation. The coefficient of variation at phenotypic and genotypic levels was high for single plant grain yield indicating that this character is more variable in the genotypes. There is a great scope for improvement of this character by direct selection among the genotypes. Similar reports were earlier reported by Ulaganathan and Nirmalakumari (2013), Dagnachew et al. (2012) and Kebere et al. (2006) for single plant grain yield.

Table 1. Estimates of variability, heritability and genetic advance as per cent of mean for 13 characters in 100 finger millet genotypes

Characters	**Range**		**Mean**	**PCV (%)**	**GCV (%)**	**h^2(BS) (%)**	**GAM**
	Min.	**Max.**					
Days to 50 per cent flowering	58.40	80.18	68.99	9.50	9.21	93.8	3.57
Days to maturity	61.22	106.01	85.14	12.46	11.38	83.4	21.41
Plant height (cm)	5.59	12.83	9.21	18.48	17.43	89.0	14.18
Productive tillers	8.11	14.17	10.62	14.61	12.86	77.4	12.48
Flag leaf sheath length (cm)	0.75	1.22	0.97	14.56	12.54	74.1	43.45
Flag leaf sheath width (cm)	20.18	43.28	31.11	18.31	16.19	78.2	8.38
Flag leaf blade length (cm)	0.81	1.32	1.04	12.78	11.23	77.1	38.78
Flag leaf blade width (cm)	6.86	13.41	9.44	13.14	12.34	88.1	12.93
Finger number	7.00	12.45	9.13	13.61	12.41	83.2	13.69
Finger length (cm)	7.30	12.36	9.70	8.50	6.57	60.8	9.22
Finger width (mm)	89.76	114.36	100.99	6.11	5.94	94.4	2.29
Thousand grain weight (g)	1.61	3.29	2.38	15.39	14.52	88.9	25.99
Single plant grain yield (g)	15.12	45.39	30.24	25.40	24.08	89.9	9.01

PCV: phenotypic coefficient of variation; GCV: genotypic coefficient of variation; h^2(BS): heritability in a broad sense; GAM: genetic advance as per cent of mean

In the present study, the estimates of heritability were found to be high for all the characters under study indicating that these characters were less influenced by environmental conditions and selection would be effective on the basis of phenotype alone with equal probability of success and it ranged from 60.8 (finger length) to 94.4% (finger width). Similarly high heritability for all the characters studied were reported by Ulaganathan and Nirmalakumari (2013), Ganapathi et al. (2011), for plant height (Dagnachew et al., 2012), for days to 50% flowering (Dhagate et al., 1972), and for thousand grain weight. Heritability which is the heritable portion of phenotypic variance is a good index of transmission of characters from parents to offspring (Falconer, 1981). The estimates of heritability help the plant breeder in the selection of elite genotypes from divergent populations. But heritability itself does not provide any indication of the amount of genetic progress that would result in selecting best individuals; rather it depends upon the amount of genetic advance. Genetic advance as percentage of mean ranged from 3.57 (days to 50% flowering) to 43.45 (flag leaf sheath length). High genetic advance as percentage of means was observed for days to maturity, flag leaf sheath length, flag leaf blade length and thousand grain weight. High genetic advance indicated that these characters are governed by additive genes and selection will be rewarding for improvement of these traits. High heritability coupled with high genetic advance was observed for days to maturity, flag leaf sheath length, flag leaf blade length and thousand grain weight. This indicated that the heritability is due to additive gene effects and can be improved by simple selection.

Conclusion

Genetic variability present in the population is mainly used for varietal improvement in future breeding programmes. High coefficients of variation were observed for single plant grain yield indicating that this character is more variable in the germplasm. There is great scope for improvement of these characters by direct selection among the genotypes. Estimates of heritability were high for all the characters and high genetic advance as percentage of mean was observed for days to maturity, flag leaf sheath length, flag leaf blade length and thousand grain weight. High heritability coupled with high genetic advance was observed for days to maturity, flag leaf sheath length, flag leaf blade length and thousand grain weight suggested that these characters may be successfully used as selection criteria in improving grain yields.

Reference

Allard,R.W. 1960. Principles of plant breeding. John Wiley and Sons Inc., U.S.A.

Ayyangar G. N. R. and U. A. Rao (1932). Inheritance of characters in ragi, *Eleusine coracana* Gaertn., Part VI. Earhead shapes. Indian Journal of Africultural Science 2: 254-65.

Burton G.W. 1952. Quantitative inheritance in grasses. In: Proc. of the 6th International Grassland Congress, pp. 277-283.

Lule D., Tesfaye K., Fetene M., De Villiers S.. 2012. Inheritance and Association of Quantitative Traits in Finger Millet (*Eleusine coracana* Subsp. *Coracana*) Landraces Collected from Eastern and South Eastern Africa. International Journal of Genetics 2(2): 12-21.

Dhagate N.K., Patidar G.L., Shrivastava P.S., Joshi R.C. 1972. Correlation and genetic variability study in ragi [*Eleusine coracana* (L.) Gaertn]. Jawahallal Nehru Krishi Vishwa Vidyalaya Research Journal 6:121-124.

Falconer D.S. 1981. Introduction to Quantitative Genetics. 2nd ed. Longman, London.

Ganapathy S., Nirmalakumari A., Muthiah A.R. 2011. Genetic Variability and Interrealtionship Analyses for Economic Traits in Finger Millet Germplasm. World Journal of Agricultural Sciences 7 (2): 185-188.

Johnson H.W., Robinson H.F., Comstock R.E. 1955. Estimates of genetic and environmental variability in soybean. Agronomy Journal 47: 314-318.

Kebere Bezaweletaw, Prapa Sripichitt, Wasana Wongyai, Vipa Hongtrakul. 2006. Genetic Variation, Heritability and Path-Analysis in Ethiopian Finger Millet [*Eleusine coracana* (L.) Gaertn] Landraces. Kasetsart J. (Nat. Sci.) 40: 322 – 334.

Lush J.L. 1940. Intra – sire correlation and regression of offspring on dams as a method of estimating heritability of characters. In: Proc. of American Society of Animal Production 33: 293 – 301.

Poehlman J. M. (1987). Breeding field crops. 3rd ed. AVI Publishing Company, Inc. West Port, CT. Pp. 187-213.

Ulaganathan V., Nirmalakumari A. (2013). Phenotypic selection of elite genotypes based on genetic analysis in finger millet [*Eleusine coracana* (l.) Gaertn] germplasm. Plant Archives 13: 1003-1008

Zarcinas B. A., Cartwright B., Spouncer L. R. 1987. Nitric acid digestion and multi element analysis of plant material by inductively coupled plasma spectrometry. Comm. Soil Sci. Pl. Analysis 18: 36-40.

Evaluation of sorghum genotypes to enhance production in Makueni County, Kenya

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Abstract

Sorghum is one of the most important cereal crops in the semi-arid tropics. It is a globally cultivated cereal, unique due to its tolerance to drought, water lodging, salinity, alkali, infertile soil and high temperatures. Sorghum, which originates from Africa, can thrive under conditions and locations where other cereal plants cannot survive due to lack of water, and thus can be used to adapt to the effects of increased drought due to climate change. However, despite its importance, sorghum potential has remained unexploited in Kenya due to low productivity, lack of diversified uses, inadequate product promotion, poor market linkages, an unfavourable policy environment and low adoption of improved varieties. A study was carried out to evaluate 15 sorghum genotypes suitable for Makueni county in Kiboko and Kampi ya mawe locations. The results showed that, there were significant differences ($P \leq 0.01$) in the panicle weight and panicle length of the genotypes. A statistically significant positive correlation was observed between the panicle length and the panicle weight ($r = 0.741$, $P<0.001$). The yield of the genotypes varied between the two sites and the genotypes, with the hybrids showing higher yields than the older varieties. The variety Gadam had the lowest yield, which correlated to the short panicle lengths. A farmers' field day was held for interaction with researchers, extension officers, and sorghum merchants. The farmers reported that their main challenges in producing sorghum were damage from birds, poor yields and poor rainfall. Despite the ready market for the Gadam variety, few farmers were producing it. There is a need to create awareness of this market. These results constitute important information in the enhancing of production of sorghum with new varieties and hybrids and marketing.

Introduction

Sorghum is one of the most important cereal crops in the semi-arid tropics. It is a globally cultivated cereal, unique due to its tolerance to drought, water lodging, salinity, alkali, infertile soil and high temperatures (Takuji and Baltazar, 2009; Ritter et al., 2007). The crop performs well in areas between 500 m and 1700 m above sea level, with seasonal rainfall of 300 mm and above. Sorghum is used not only for human food, but also for fodder and feed for animals, building material, fencing, or for brooms. There is high demand for sorghum mainly in the brewing industry to replace barley, yet the amount produced by farmers is too low to satisfy the market demand.

Sorghum, which originates from Africa (Mann et al., 1983), can thrive under conditions and locations where other cereal plants cannot survive due to lack of water. Sorghum yields in Africa are low with an average of 0.949 t ha^{-1}in 2011 (FAOSTAT, 2011). Most small-scale farmers who plant landraces and crop varieties in sub-Saharan Africa use on-farm produced and saved seed the quality of which is usually poor (Muliokela, 1999; Kimani, 1998; Wobil, 1998; Ministry of Home Affairs and National Planning, 2006. Sorghum hybrids could be used if their potential were demonstrated (Leo, 2005). In Kenya, sorghum is the second most important cereal crop in semi-arid eastern Kenya after maize. Over the years, Kenya has witnessed prolonged episodes of drought that have resulted in some instances of total crop failure. This situation is becoming worse with the effects of global warming witnessed in the country in terms of increased temperatures and reduction of arable land. The arid and semi-arid lands constitute about 84% of Kenya's land mass and sorghum is one of the most suitable cereal crops in these agro-ecoogical zones due to its tolerance to drought and high temperatures. It is grown principally in the often drought-prone marginal agricultural areas of Eastern, Nyanza and Coast Provinces. As an indigenous Kenyan crop, sorghum could provide food security and become a suitable alternative in eastern Kenya where maize crop failure is common (Jaetzold et al., 2006; Ministry of Agriculture, 2003). The production of sorghum in the eastern province of Kenya is low due to constraints such as lack of income to purchase fertilizer and chemicals, seed of inadequate quality, susceptibility to pests and diseases resulting in low yields (Muui et al., 2013).

To address the challenge of using better varieties, this study set out to assess 15 genotypes of sorghum that included local varieties and hybrids, for their agronomic performance and yield. A farmers' field day was held for farmers to interact with the researchers, extension officers, and sorghum merchants.

Materials and methods

Fifteen sorghum genotypes (Table 1) were planted in two sites at Kampi ya mawe (37°40'E, 01 ° 51S) and Kiboko (situated at about 37.8°E and 2.3°S) both in Makueni county, in the eastern part of Kenya. Kiboko (Makueni) received erratic rainfall (average rainfall of 446.28 mm per annum while Kampi ya mawe received average rainfall of 432.05 mm per annum for the years 2009-2012. The experiment was laid out in 3 m x 4 m plot sizes and in a randomized complete block design, replicated three times. Makueni is classified as a Lower Midland (LM) zone (Jaetzold et al., 2006). Seeding was done by hand and weeds controlled manually.

Data were collected on the panicle length and weight, days to flowering, plant height and yield. Analysis of variance and correlations were carried out using Genstat data analysis software. Treatment means were compared using protected Fisher's least significant difference (LSD) test at P=0.05.

Results

The trial sites received rainfall of about 252.2 mm in Kiboko and 213.0 mm in Kiboko during the period October 2011 to February 2012. The Kiboko site was also irrigated during the sorghum growth season.

The means of the panicle weights ranged from 67.6 g to 157.4 g (Table 1) with the analysis of variance showing a significant difference ($P \leq 0.01$) in the panicle weight of the varieties (Table 2). However, there were no significant differences ($P \geq 0.05$) in the site by genotype interaction (Table 2). The mean panicle length of the 15 varieties ranged from 21.01 cm to 33.38 cm with very significant differences ($P \leq 0.01$) in the genotypes and the two sites, but no significant differences ($P \geq 0.05$) in the genotype by site interaction (Table 2). Among the genotypes, the

Gadam variety had the shortest panicle length and the lowest weight of the panicles. The hybrids P9537 x Chokwe and the P9535A x Chokwe had the highest panicle weights and also had longer panicles (Table 1). Most of the hybrids exhibited higher panicle lengths and weights than the local varieties Seredo, Serena, KARI Mtama 1 and Gadam (Table 1).

Table 1. Means separated using the Fischer's least significant difference test

	Sorghum variety/ hybrid	Panicle length (cm)	Panicle weight (g)	Plant height (cm)	Yield (kg/ha)
1	P9531A X ICSR 92074	32.6a	125.9bc	144.2cd	2847a
2	P9535A X CHOKWE	34.51a	135.6ab	145.2cd	2764a
3	P9507A X KAT 1369 X MAKUENI LOCAL	29.62bc	120.4bcd	179.2a	2472ab
4	P9535A X PIRIRA 1	29.86b	132.8ab	136.7de	2403ab
5	P9537 X CHOKWE	33.38a	157.4a	147.7c	2403ab
6	TXARG/K567A X SEREDO	29.49bcd	112.8bcd	172ab	2472ab
7	TXARG/KS67A X NL 9623	26.05ef	102.6cdef	117.2gh	2417ab
8	P9537A X FPR (168 X G570)	27.39de	102.6cdef	163.7b	2361bc
9	P9537A X KUYUMA	29.21bcd	108.7bcde	125.5fg	1993bcd
10	SEREDO	25.56ef	81.8efg	131.2ef	2035bcd
11	Gadam	21.01h	67.6g	99.5i	1715cd
12	ICSV 111	22.89gh	111.2bcd	175.6a	1528d
13	KARI Mtama 1	22.41h	91.5defg	138.9cde	1507d
14	P9508A X ICSR 91005	27.54cde	120.2bcd	109.4hi	1681d
15	SERENA	24.99fg	74.3fg	119.3gh	1507d
	Mean	27.77	109.70	140.36	2140
	Lsd	3.03	41.10	14.77	961.7

Means within a column followed by the same letter are not significantly different (P>0.05)

Table 2. Analysis of variance of mean squares for panicle length, panicle weight, plant height and yield of the 15 sorghum genotypes

Source	d.f.	Panicle length (cm)	Panicle weight (g)	Plant Height (cm)	Yield (kg)
Rep	2	5.38	711.2	348.48	313,947
Genotype	14	97.37**	3,523.9**	3,564.57**	1,274,645**
Site	1	318.441**	118,955.6**	71,982.03**	20,984,742**
Site x Genotype	14	3.391	860.8	498.24**	722,175
Error	58	3.438	632.3	81.69	346,195
C.V.		6.7	22.9	6.4	27.5

** P≤0.001

The mean plant height ranged from 99.5- 179.2 cm. The heights varied among the varieties and the hybrids, with most hybrids exhibiting longer panicles. The Gadam variety was the shortest (mean height, 99.5 cm) while the hybrid P9507A x KAT 1369 x Makueni local, was the tallest (mean height, 179.2 cm). KARI Mtama 1 and Seredo had no significant differences, while Serena was shorter than these two (Table 1). Hybrid P9508A x ICSR 91005, also had a short mean plant height, and lower mean panicle weight, unlike hybrid TXARG/KS67A x NL 9623, which had a similar height, but higher yields. Two of the old varieties that farmers prefer, Serena and Seredo, showed low mean yields as compared to the hybrids which had higher yields. The genotypes were not significantly different in the two sites for the parameters tested (Table 2). However, the patterns for the yields of the genotypes differed in the two sites (Figure 1). Hybrid P9537 x Chokwe showed better yields under rain-fed conditions than in irrigated conditions. Also hybrid TXARG/KS67A xNL 9623, Gadam and P9535A x Pirira 1 showed no significant differences for the mean yields between the two sites (Figure 1).

A statistically significant positive correlation was observed between the panicle length and the panicle weight (r = 0.741, P<0.001) of the 15 sorghum genotypes. The positive correlation between the length and weight of the panicles indicates that the yields of the longer panicles are higher than those of the shorter ones, suggesting that one can predict the yield of the genotypes from the length of the panicles.

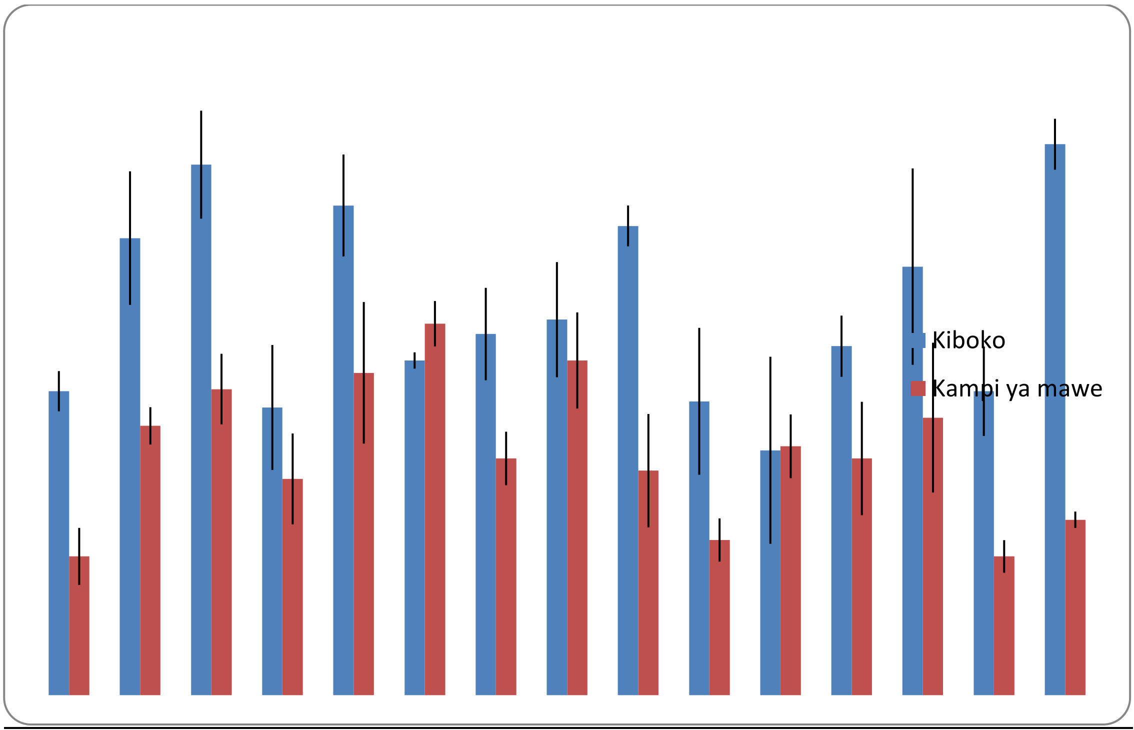

Figure 1: Mean yield (kg/ha) of the 15 genotypes in the two sites

Discussion

Sorghum, a drought-resistant crop, is very important in addressing food security (Sally et al., 2007), and has a strong adaptive advantage. Sorghum has a high yield potential of more than 4t/ha. The production of sorghum may be effectively increased by the use of improved production technologies and creating awareness among farmers on the importance of the crop and increasing the production area. A survey carried out in the Makueni area showed that only 5% of the farmers do not plant sorghum in (Muui et al., 2013). The crop is grown on very small plots, ≤0.05 ha, either as few stands within another crop or strips along the farm edge.

The majority of the genotypes showed similarities in the two sites for the parameters tested. However, variations in this study were observed in the panicle height, weight and yield within a site. This was similar to studies carried out by Muturi et al. (2012).

In the Kampi ya mawe site, the mean yield patterns of the genotypes were different from that at the Kiboko site. This is because, the Kiboko site was irrigated unlike the Kampi ya mawe, which experienced low rainfall. Thus the Kiboko site had more water available for production of the sorghum. The low rainfall can be used to select the genotypes more adaptable to drought, which also had higher yields. The genotypes include P9535A x Chokwe, P9537 x Chokwe and TXARG/KS67A x NL 9623.

Farmers, merchants and agricultural extension staff in these regions participated in a field day at the irrigated Kiboko site. Also, farmers compared the new improved sorghum genotypes for livestock feed and grain yield, and also for plant height in the case of Gadam, which would make harvesting easier. During discussions after the tour of the field, farmers expressed their concerns regarding production of sorghum. However, from data collected during the field day, farmers have continued to plant the old varieties which this study has shown exhibit lower yields than the hybrids and newer varieties. The farmers reported that their main challenges for sorghum production were the damage caused by birds and the high labour cost for bird scaring, poor yields and poor rainfall. Despite the ready market for the Gadam variety, few farmers were producing it. The extension officers and researchers acknowledged the need for awareness for this market and sorghum production as a commercial entity. These results constitute important information in the enhancing of production of sorghum with new varieties and hybrids and marketing.

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References

Food and Agricultural Organization of the United Nations, FAOSTAT database. (FAOSTAT 2011) URL: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor accessed on 13th February 2013

Jaetzold R., Schmidt H., Hornetz B.,Shisanya C. 2006. Farm Management Handbook of Kenya, Vol. II/C1. Ministry of Agriculture, Kenya and German Agency Technical Cooperation team (CTZ)

Kimani P.M. 1998. Research and Teaching of Seed Science and Technology in University of Nairobi. In: Ochuodho JO, Mathenge PW, Rheenen H, and EO Auma (Eds). Seed Production and Certification. Proceedings of a workshop held in Eldoret, Kenya.

Leo T. M. 2005. Evaluation of the heterotic potential of sorghum [*Sorghum bicolor* (l.) Moench] adapted to the Southern Africa region. Master of Science Thesis. Texas A&M University

Mann J. A., Kimber C. T., Miller F. R. 1983. The origin of early cultivation of Sorghum in Africa. Texas AAT University Bulletin,

Ministry of Agriculture. 2003. The Annual Report, Crop Development Division, Kenya 2003.

Ministry of Home Affairs and National Planning. 2006. Government of Kenya Bomet District Development Plan (2003-2008), Ministry of Home Affairs and National Planning. Nairobi, Kenya, 2006. Nairobi, Kenya.

Muliokela S.W. 1999. The Challenge of Seed Production and Supply in Africa Seed Science and Technology 27:811-824.

Muturi P.W., Rubaihayo P., Kyamanywa S., Mgonja M., Sharma H.C. 2012. Performance of selected grain sorghum genotypes for improved food security and livelihoods. Proceedings of The Third RUFORUM Biennial Meeting 24 - 28 September 2012, Entebbe, Uganda 257-260.

Muui C.W., Muasya R.M., Kirubi D.T. 2013. Baseline Survey on Factors Affecting Sorghum Production and use in Eastern Kenya. African Journal of food, Agriculture, Nutrition and Development 13:7339-7357.

Ritter K.B., McIntyre C.L., Godwin I.D., Jordan D.R., Chapman S.C. 2007. An assessment of the genetic relationship between sweet and grain sorghums, within *Sorghum bicolor* ssp. bicolor (L.) Moench, using AFLP markers. Euphytica 157: 161-176.

Sally L.D., Frances M.S., Robert J.H., Giovanni C., Liz I. 2007. Domestication to crop improvement: Genetic resources for sorghum and *saccharum* (Andropogoneae). Annals of Botany 100 (5):975-989.

Takuji S., Baltazar, A.A. 2009. Sorghum genome: drought tolerance in genes. Nature 457: 547-548

Wobil J. 1998 Seed Security Issues in Southern Africa. Proceedings of International Workshop on Seed Security for Food Security. FAO, Rome and Academia dei Georgofili, Florence, Italy 217-226.

Methodology mapping for resilient production systems: approaches and results from surveys in Bolivia, India, and Nepal

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Abstract
Traditional and novel management strategies for agricultural biodiversity will be central in the adaptation of smallholder farmer systems to climate change. As many neglected and underutilized species (NUS) of crops and trees are tolerant of harsh conditions linked to climate change, these species could be integral in strengthening resilience of farming systems. Relative to their importance, very little is known about how farmers are using agricultural biodiversity and NUS in climate change adaptation. We thus investigated this topic in a survey of 2118 smallholder farmers in Bolivia, Nepal, and India. Many farmers in all locations perceived increasing temperatures and shifts in precipitation, leading to lower yields and food insecurity. Planting new crops or varieties was a common coping strategy in all three locations. In Bolivia, farmers faced with intensified crop pests and disease, planted disease-resistant varieties, such as blight-resistant potato *doble H*. Quinoa, and native *luki* potatoes were also recognized for their hardiness in Bolivia. In India and Nepal, farmers faced with increased drought planted early-maturing varieties, such as spring rice 1442. Many also identified minor millets, which are tolerant of marginal and dry soils, as resistant to climate change. Planting trees was another common coping strategy in all three countries. Trees can shelter crops from heat and desiccation, as well as diversify production to protect against crop failure. Given the importance of agricultural biodiversity in farmer adaptation strategies, actions to halt the erosion of agricultural biodiversity and to effectively deploy this resource will be important in strengthening food security under climate change. Stress-tolerant NUS (e.g. millets, quinoa) deserve more attention in climate change adaptation strategies.

Key Words: Climate change, adaptation, NUS, agricultural biodiversity, farmer perceptions

Introduction
Strengthening food security for a growing population in conditions of climate change is a pressing global issue (Wheeler and von Braun, 2013). Rising temperatures and shifts in precipitation could mean crops growing locally become maladapted, while greater incidence and severity of extreme events such as drought and flooding will seriously challenge crop production (Easterling et al., 2007; Lane and Jarvis, 2007). Shifts in pest and pollinator interactions, soil fertility, and other ecosystem functions will further impact crop performance (Jarvis et al., 2010). Substantial declines in crop yields and stability are projected to result from these impacts in many parts of the world, but especially in developing countries, where existing hunger could be strongly exacerbated (Wheeler and von Braun, 2013).

In areas expected to be most affected by climate change, much of the population is reliant on the production of smallholder farms (Nwanze, 2011). Climate change is expected to compound the existing vulnerability of these producers, who are strongly reliant on their farm production, have few available resources and face numerous other pressures, including poor market integration, population growth, insecure land tenure, and erosion of traditional knowledge (Morton, 2007; Mijatovic et al., 2012). The resilience and adaptability of these farming systems depend on farmers' assets, knowledge, social networks, the political environment, and perceptions of risk (Nyanga et al., 2011).

Smallholder farmers typically manage diverse production systems that involve intercropping several different crops and varieties, collecting wild resources, and raising livestock (Morton, 2007). Agricultural biodiversity and associated traditional practices will play a vital role in climate change adaptation (Mijatovik et al., 2012). The practice of crop diversification mitigates risk by leveraging the insurance effect of diversity (Di Falco and Perrings, 2003). Meanwhile, smallholder farmers' traditional use and development of locally adapted crops and varieties through dynamic seed selection and exchange practices facilitates adaptation to shifts in the abiotic and ecological community context (Wood and Lenne, 1997).

While traditional practice holds promise for adaptation, the speed and magnitude of the shift in conditions could require drastic adjustments in cropping systems, planting schedules, locations, and soil and water management practices. Farmers may need to increase production or introduce crops that are better suited to prevailing conditions (Kurukulasuriya and Mendelsohn, 2008). The cultivation of more stress-tolerant crops and varieties is expected to be crucial in sustaining production as the weather becomes harsher under climate change (Mijatovik et al., 2012). In this sense, the neglected and underutilized species (NUS), which include a vast diversity of crops and trees cultivated in traditional production systems that are not well exploited in global markets, could play a strong role (Padulosi and Hoeschle-Zeledon, 2004). Many of these crops are tolerant of marginal conditions and produce nutritious food products, and so could be integral in diversification strategies for climate change adaptation (Nangula et al., 2010; Bala Ravi et al., 2010).

Considering its importance, the role of agricultural biodiversity in the resilience and adaptation of smallholder farming systems to climate change has not been adequately recognized. A recent review brought together examples highlighting the role of agricultural biodiversity in climate change resilience at crop/variety, farm, and landscape levels (Mijatovik et al., 2012). Still, very little is known about how farmers are making use of agricultural biodiversity and specifically, NUS crops and trees to cope and adapt to climate change. This study by Bioversity International in partnership with the Foundation for the Promotion and Research of Andean Products (PROINPA), Local Initiatives for Bioverdiversity Research and Development (LI-BIRD), the M.S. Swaminathan Research Foundation (MSSRF), and the Platform for Agrobiodiversity Research (PAR) addressed these issues through a survey of smallholder farmers in Bolivia, Nepal, and India.

Materials and Methods

The survey was carried out in late 2012 and early 2013. Farmers were interviewed in two departments in Bolivia (Cochabamba and La Paz), two districts in India (Namakkal and

Nainital), and four districts in Nepal (Bara, Dolakha, Kaski, and Jumla). The survey questions used for the study have been published previously (see Padulosi et al., 2012; pp.188-197). Some survey questions were modified or the sequence in which they were asked was shifted in the different locations to suit the local language and context.

In total, 2118 farmers were interviewed. We sought to include a strong representation of women in the sample so the sex-ratio of respondents was effectively 50-50 in all countries (Table 1). The farmers interviewed were younger in India compared to Nepal and Bolivia, with more under 40 years of age and fewer over 60. In Nepal and India, it was most common for farmers to have no formal education, whereas in Bolivia, farmers were most-commonly educated to primary level.

Numerous farms were larger than 10 ha in Bolivia whereas, essentially no farm in Nepal or India was so large (Table 1). These larger farms were included in this preliminary analysis, as they could be considered smallholders under definitions that emphasize reliance on farm production (Berdegué and Fuentealba, 2011).

Table 1**.** Survey participants and their farm profiles.

	Bolivia (% of N=234)	**Nepal** (% of N=1171)	**India** (% of N=713)
Women	52	47	50
Age			
Under 40	22	39	48
40 to 59	43	40	40
Over 60	35	22	12
Education			
None	12	52	42
Primary	60	16	25
Secondary	24	25	20
Intermediate	1	5	11
University	0	3	1
Other	3	0	0.1
Farm size			
< 1 ha	17	76	81
1 to 10 ha	44	24	19
> 10 ha	36	0.2	0
Livestock	94	90	85
Irrigation system	23	74	49
Tractor	0.4	11	24
Vehicle	11	0	19
Hire farm labour	29	52	39

Results

The grand majority (88%) of farmers interviewed had noted a change in the weather in the last 20 years (Figure 1). In India especially, effectively all farmers interviewed had noted changes in the weather. Higher temperatures and shifts in the timing of precipitation were observed by the majority of farmers across all three sites, with late rains commonly noted in Bolivia and India. In the south Asian sites, many farmers noted a reduced amount of rainfall, leading to drought or lower water availability (i.e. springs drying up, lower water levels). In

Bolivia, heightened pest and disease pressure was a highly noted impact. As a result of these changes, many farmers in Bolivia and India reported yield declines and in Nepal many suffered food insecurity.

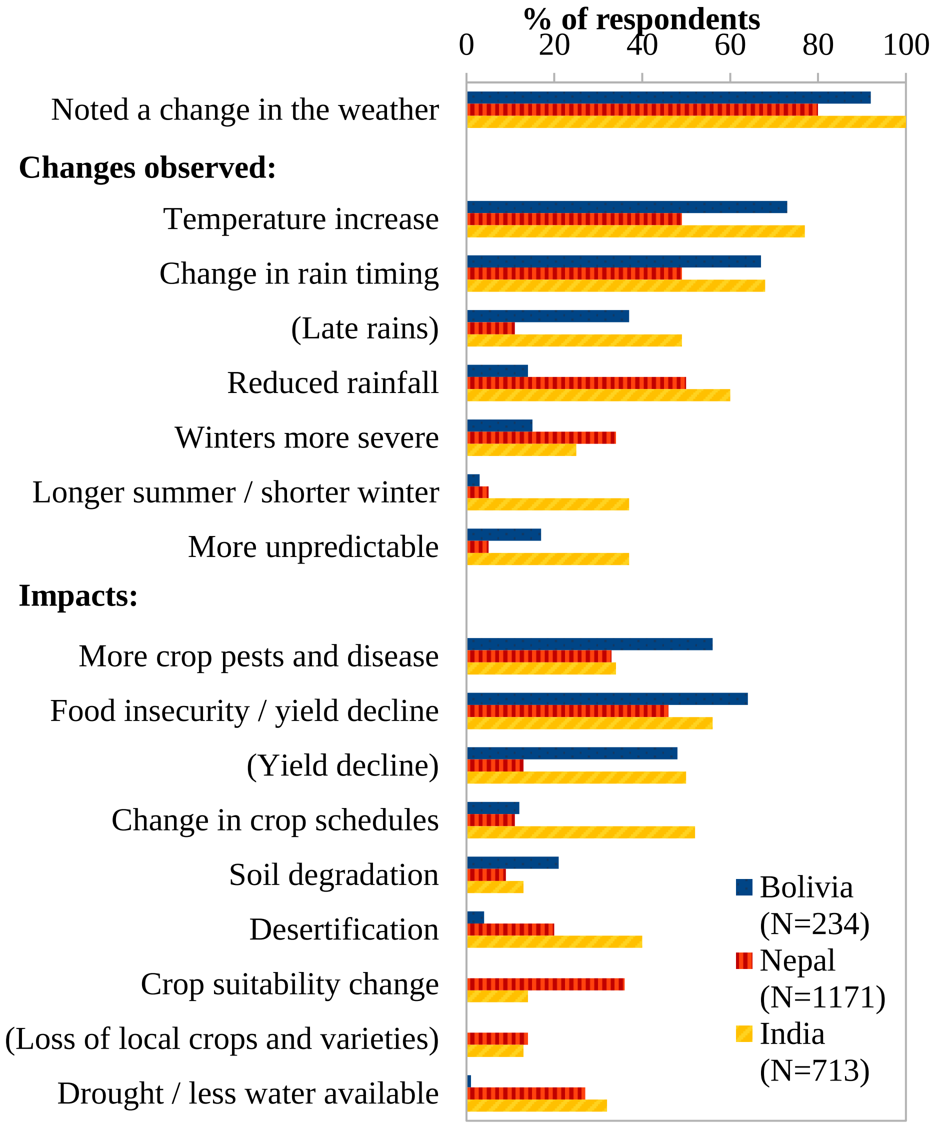

Figure 1. Perceptions of climate change and its impacts by smallholder farmers in Bolivia, Nepal, and India.

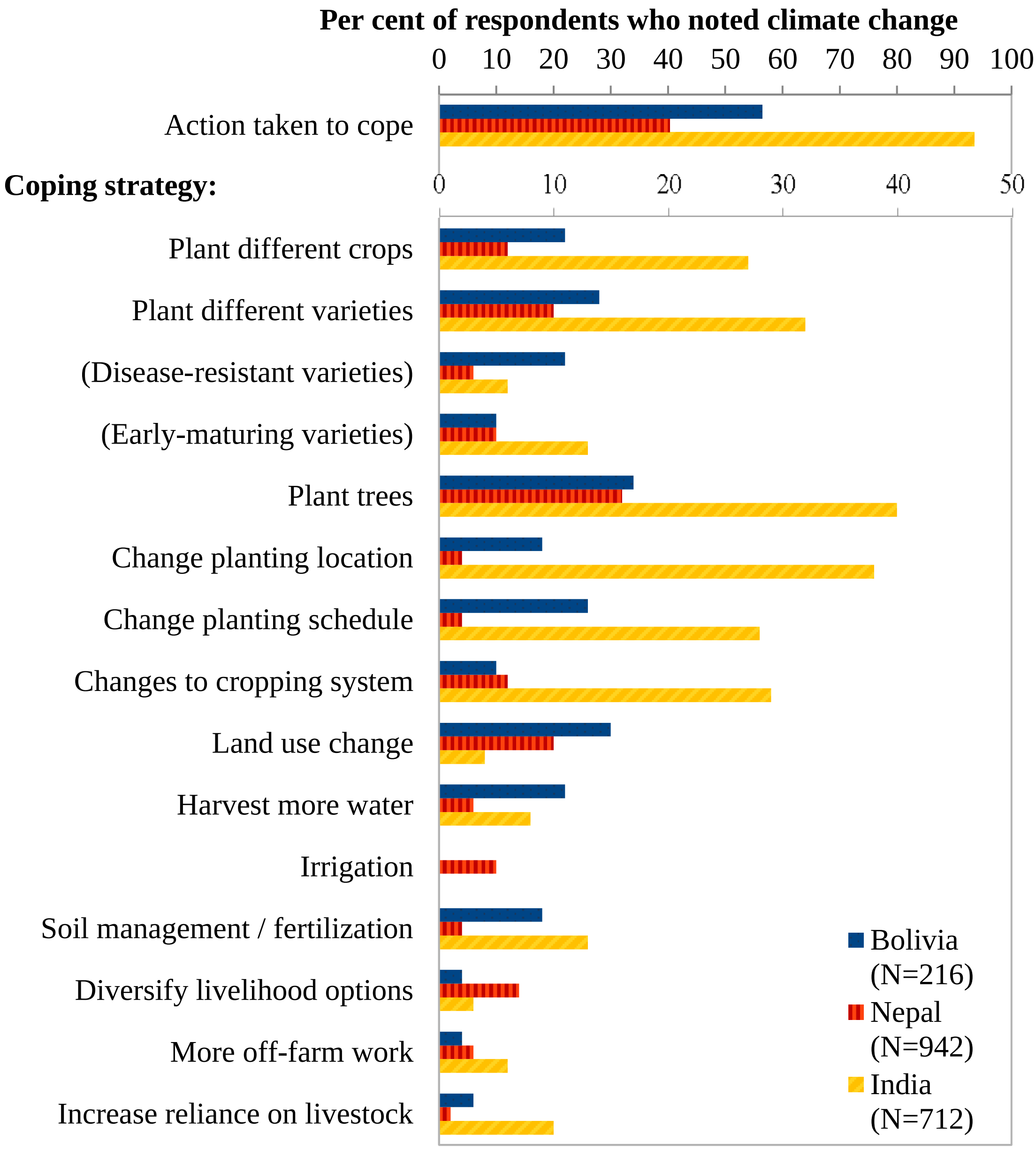

Figure 2. Farmers taking action to cope with perceived climate change and their most common coping strategies. Note the scale change for the coping strategies.

In India, over 90% of farmers who noted a change in the weather reported taking action to cope with the impacts (Figure 2). Fewer farmers in Bolivia and Nepal took action to cope. Farmers reported taking a broad range of coping actions. Planting different crops species or varieties was common across all sites. In Bolivia, many farmers planted disease-resistant varieties and in India, early-maturing varieties. Planting trees and changing land use or cropping systems were other common coping actions taken in all three countries. In Bolivia and India, many farmers modified cropping locations and schedules.

Farmers considered several NUS crops to be resistant to the challenges they faced with climate change (Table 5). Quinoa was considered resistant by over a quarter of farmers who noted climate change in Bolivia. In India and Nepal, minor millets including finger millet, Italian foxtail millet, and barnyard millet were popularly considered resistant, with finger millet the top-listed resistant crop in the Indian sites. In Namakkal, however, many farmers considered millets to be susceptible to climate change (data not shown).

Traditional and modern varieties of dominant crops such as potato, wheat, rice and maize were also considered by many farmers to be resistant to the challenges faced with climate change. In Bolivia, about three quarters of farmers who noted climate change considered potato to be resistant but unfortunately, very few farmers indicated specific varieties. Where varieties were noted, the most commonly mentioned were native *luki* potatoes and introduced variety *doble H* (also known as *runa toralapa*; Uzeda, 2005). The resistant varieties of rice and wheat noted in Nepal were both modern bred and were only listed by farmers in the Terai (Joshi et al., 2012; Ghimire et al., 2012).

Table 2. Most common crops farmers listed as resistant to climate change.

	Bolivia (% of N=216)	**Nepal** (% of N=942)	**India** (% of N=712)
Potato	76	3	1
Variety *luki*	13		
Variety *doble H*	8		
Wheat		21	8
Variety *NL297*		12	
Rice		20	6
Variety *1442*		5	
Maize		8	36
Barley	11	8	0
Quinoa	28		
Minor Millets			
Finger Millet		8	45
Italian Foxtail Millet			8
Barnyard Millet			6
Kodo Millet			4
Red gram (pigeon pea)		1	8
Peas		6	0.4
Taro		5	3

Table 3. The percent of farmers who took action to cope with perceived changes in the weather depending on whether they were informed about climate change or not.

	Bolivia	**Nepal**	**India**
Informed: Took action	72%	45%	90%
Not informed: Took action	48%	36%	89%

A good proportion of farmers in Bolivia and Nepal (34% in both sites) had been provided information on climate change but it was still a minority. Only 12% of farmers in India had been informed. Farmers had various sources of information, including the radio and NGOs. In Bolivia and Nepal, farmers who were informed about climate change were more likely to take action to cope with perceived impacts (Table 3).

Discussion

Farmers in all three countries noted increasing temperatures and shifts in precipitation over the last two decades that have lead to lower yields and food insecurity, among other impacts. These results are consistent with data that demonstrate rising global temperatures, shifts in precipitation patterns, and their projected impacts for food security (Wheeler and von Braun, 2013). Strong perception of climate change by farmers has also been documented in a similar survey by Oxfam Novib et al. (2013) in Peru, Vietnam and Zimbabwe.

Results presented here reflect the most common observations. There was strong agreement among farmers about some of the effects of climate change but their observations conflicted in other cases. Divergence could result from different microclimates within the landscape or farmers' different sensitivities. A finer geographic-scale analysis of climate change effects will be developed, which may resolve some of these conflicts. In any case, the focus of this study was not to judge the merit of farmer observations, rather to understand how their perceptions and responses would relate to their adaptive capacity and resilience.

Many farmers reported taking action to cope with the problems they encountered with climate change. It is unclear from the survey whether these actions were made proactively in anticipation of greater change to come. Rather, it is more likely these actions were reactive adjustments of practice to deal with circumstances that hindered production. Here, the distinction between strategies for "coping" and "adapting" is not emphasized because coping strategies can become means of adaptation (Morton, 2007).

The spatial and temporal deployment of agricultural biodiversity was central in farmer adaptation strategies. In this sense, a notable coping action in all three locations was planting new crops or varieties. The specific crops and varieties farmers planted are not conclusively known from the survey, but those recognized as resistant to climate change were likely among the seeds introduced. For instance, spring rice variety 1442 that was recognized as resistant to climate change in Bara, Nepal may have been among the fast-maturing varieties farmers planted to cope with drought (Kafle et al., 2012). Minor millet species that were considered resistant to climate change in India and Nepal may also have been planted to cope with drought, as they are tolerant of arid soils (Padulosi et al., 2009). In Bolivia, modern blight-resistant potato variety *doble H* was likely one of the disease-resistant varieties farmers introduced to deal with heightened pest and disease pressure. Farmers in Bolivia also recognized several crops as resistant that are more generally appreciated for their hardiness, including frost-resistant *luki* potatoes and quinoa, which is tolerant of dry and low-input conditions (Galewey, 2003; Giuliani, 2013). Stress-tolerant crops and varieties such as these are expected to be crucial in climate change adaptation, as extreme conditions occur more frequently (Mitajovik et al., 2013).

The crops considered to be resistant to climate change included both NUS and major crop species. Development and use of climate-hardy varieties of major staple crops will play an essential role in adapting food production to global climate change. However, we note that these crops are already gaining strong attention in research and development efforts, unlike the NUS crops that farmers also recognize to be resistant to climate change. NUS crops are also typically accessible to poor farmers and embedded in local cultural traditions, and this gives them greater potential to enhance socio-ecological resilience as compared to introduced or modern crops and varieties. We thus argue that more attention should be paid to hardy NUS crops in climate change adaptation strategies. Quinoa and minor millets, for instance, could be highly strategic in strengthening food security under climate change, as they are both resistant to harsh environmental conditions and provide highly nutritious grains (Saleh et al., 2013; Padulosi et al., 2009).

Planting trees was another common coping strategy across all three sites. This action has several effects that can alleviate the impact of climate change on farm production. Trees shelter crops from heat and desiccation by providing shade, protect the soil from wind erosion, and can diversify farm production to protect against crop failure (Rao et al., 2007). In Bolivia, planting trees is a strategy recommended by NGOs (e.g. CARE − Cooperative for Assistance and Relief Everywhere) working in the region to mitigate soil erosion and climate change impacts. In the Kolli Hills (in Namakkal), tribal farm families are engaged in a project to establish integrated *Wadi Farms* that consist of food and cash crops, fruit and timber trees, and fodder grasses. Such diversification strengthens the capacities of farm families to meet the challenges of vagaries of the weather. While the identity of the trees the farmers planted is not known definitively from the survey, this strategy could include NUS. In one of the sites we surveyed in Nepal (Jumla), farmers have been supported by LI-BIRD and The Development Fund of Norway in planting the dhatelo tree (*Princepia utilis*) on barren slopes. The farmers appreciate this species for its fruit, oil and evergreen quality but it has been in decline in the area. The restoration of the dhatelo population would recover the benefits of the tree for local community use and also the option to gain income through sale of the oil in high value international markets. Similar benefits from planting trees have been promoted in Africa through conversion to agroforestry with the multi-purpose and nutritious moringa tree that is gaining attention as a global superfood (Amaglo, 2013).

Diversification by planting trees fits with tradition in these systems of maintaining high diversity for risk mitigation. Maintaining a broad portfolio of crops was surprisingly not mentioned by farmers as a strategy to cope with climate change. However, as diversification is standard practice in these production systems, it may be that the farmers did not consider this a specific “coping strategy”. In Nepal, farmers interviewed planted on average three cereals, three legumes, nine vegetables, four spices, 3-4 fruit species and typically more than one variety within each crop. In Bolivia, the farmers maintained an average of seven varieties of potato and a few other crops, including native tubers (isaño, oca, and papalisa) and andean grains (quinoa and cañihua). In India, the farmers practised mixed cropping, relay cropping and crop rotation of small millets and pulses. This diversity provides alternative sources of food or income, strengthening resilience to climatic stress.

While many farmers took action to cope with climate change, many others who observed changes in the climate did not report taking specific action to cope. Information had a positive effect on farmers' likelihood of taking action in Nepal and Bolivia. In India, the fact that very few farmers were informed about climate change may underlie the absence of this effect. Our results corroborate research that identifies access to information as a major barrier to climate change adaptation (Deressa et al., 2008). However, the nature and quality of information is critical. Further study should investigate the availability of information concerning the role of traditional crops in climate change adaptation, which may be weakly available. An interesting observation is that farmers reported taking a wide range of coping strategies that were often not being practised by their neighbours. Improving information access and sharing within and between communities regarding climate change coping options could improve adaptive capacity in these locations. We also note that access to information often differs between gender and social groups. Further analysis will be performed to see if divergence between gender exists in these sites that may lead to different vulnerabilities, coping strategies, and support required.

Conclusions

This study revealed that smallholder farmers in Bolivia, Nepal and India strongly perceive climate change and that the management of agricultural biodiversity to be central in their adaptation strategies. The strong recognition of NUS crops as resistant to climate change conditions points to their potential role in adaptation. The resistant crops (both NUS and dominant) recognized by the farmers could be leveraged to strengthen resilience through diversification of production systems or replacement of crops that no longer perform well via (re-)introduction or increasing the area allocated to their production.

Adding to the challenge of adapting to climate change is that agricultural biodiversity fundamental to climate change adaptation is increasingly being lost due to a suite of interacting pressures, including development of global markets, agricultural technology, and shifts in cultural norms (Padulosi et al., 2012). In order to secure food security for humankind today and the future, we must promote the conservation of agricultural biodiversity and its effective deployment to cope with climate change.

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References

Amaglo N.K. 2013. Moringa as a Climate Change Mitigation Strategy? Aotearoa Independent Media Centre. Online resource accessed February 15, 2013 at http://www.indymedia.org.nz/articles/777.

Bala Ravi S., Hrideek T.K., Kumar A.T.K., Prabhakaran T.R., Bhag Mal, Padulosi S. 2010. Mobilizing neglected and underutilized crops to strengthen food security and alleviate poverty in India. Indian Journal of Plant Genetic Resources 1(23):110-116.

Berdegué J.A., Fuentealba R. 2011. Latin America: The State of Smallholders in Agriculture. Conference on New Directions for Smallholder Agriculture 24-25 January, 2011. IFAD. Rome, Italy.

Deressa T., Hassan R.M., Yesuf M., Ringler C. 2008. Analysing the determinants of farmers' choice of adaptation methods and perceptions of climate change in the Nile basin of Ethiopia. IFPRI Discussion Paper 00798.

Di Falco S., Perrings C. 2003. Crop genetic diversity, productivity and stability of agroecosystems: a theoretical and empirical investigation. Scottish Journal of Political Economy 50(2):207-216.

Easterling W., Aggrawal P., Batima P., Brander K., Erda L., Howden M., Kirilenko A., Morton J., Soussana J-F., Schmidhuber J., Tubiello, F. 2007. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Parry M.L., Canziani O.F., Palutikof, J.P., van der Linden, P.J., Hanson, C.E., Eds). Cambridge University Press, UK, 273-313.

Galeway N.W. 2003. The potential of quinoa as a multi-purpose crop for agricultural diversification: a review. Industrial Crops and Products 1(2-4):101-106.

Ghimire S., Dhungana S.M., Krishna V., Teufel N., Sherchan D.P. 2012. Biophysical and socio-economic characterization of cereal production systems. CIMMYT. Mexico, DF.

Giuliani A. 2013. Biodiversity and underutilized species in the context of global challenges. Biodiversity of Andean Grains: Balancing Market Potential and Sustainable Livelihoods (Guiliani, A., Hintermann, F., Rojas, W., Padulosi, S., Eds). Bioversity International, Rome, Italy.Jarvis A., Ramirez J., Anderson B., Leibing C., Aggarwal P. 2010. Scenarios of climate change within the context of agriculture. Climate Change and Crop Production (Reynolds, M. P., Ed.). CAB International, London, UK.

Joshi K.D., Devkota K.P., Harris D., Khanal N.P., Paudyal B., Sapkota A., Witcombe J.R. 2012. Participatory research approaches rapidly improve household food security in Nepal and identify policy changes required for institutionalisation. Field Crops Research 131(13):40-48.

Kafle B., Paudel M.N., Ghimire R.C. 2012. Assessing current status of early rice varieties in river basin area of Nepal: a concern to diffusion. International Journal of Agriculture Research and Review 2(2):59-61.

Kurukulasuriya, P., Mendelsohn, R. 2008. Crop switching as a strategy for adapting to climate change. AfJARE 2(1):105-126.

Lane A., Jarvis A. 2007. Changes in climate will modify the geography of crop suitability: agricultural biodiversity can help with the adaptation. SAT eJournal 4(1):1-12.

Mijatovic D., Van Oudenhoven F., Eyzaguirre, P., Hodgkin, T. 2012. The role of agricultural biodiversity in strengthening resilience to climate change: towards an analytical framework. International Journal of Agricultural Sustainability 11(2):95-107.

Morton J.F. 2007. The impact of climate change on smallholder and subsistence agriculture. PNAS 104(50):19680-19685.

Nangula P., Uusikua A., Oelofsea K.G., Duodub M.J., Besterc M.F. 2010. Nutritional value of leafy vegetables of Sub-Saharan Africa and their potential contribution to human health: a review. Journal of Food Composition and Analysis 23(6):499–509.

Nyanga P.H., Johnsen F.H., Aune J.B., Kalinda T.H. 2011. Smallholder farmers' perceptions of climate change and conservation agriculture: evidence from Zambia. Journal of Sustainable Development 4(4):73-85.

Nwanze K. 2011. Smallholders can feed the world. IFAD. Rome, Italy.Oxfam Novib, ANDES, CTDT, SEARICE, CGN-WUR. 2013. Building on farmers' perceptions and traditional knowledge. Biodiversity management for climate change adaptation strategies. Oxfam Novib. The Hague, The Netherlands.

Padulosi S., Hoeschle-Zeledon I. 2004. Underutilized plant species: what are they? Leisa Magazine March:5-6.

Padulosi S., Bhag Mal, Bala Ravi, Gowda J., Gowda K.T.K, Shanthakumar G. Yenagi N., Dutta M. 2009. Food security and climate change: role of plant genetic resources of minor millets. Indian Journal of Plant Genetic Resources 22(1):1-16.

Padulosi S. Bergamini N., Lawrence T., Eds. 2012. On-farm conservation of neglected and underutilized species: status, trends and novel approaches to cope with climate change: Proceedings of the International Conference Friedrichsdorf, Frankfurt, 14-16 June, 2011. Bioversity International, Rome, Italy.

Rao K.P.C., Verchot L.V., Laarman J. 2007. Adaptation to climate change through sustainable management and development of agroforestry systems. SAT e-journal 4(1):1-30.

Saleh A.S.M., Zhang Q., Chen J., Shen Q. 2013. Chen millet grains: nutritional quality, processing, and potential health benefits. Comprehensive Reviews in Food Science and Food Safety 12(3):281-295.

Uzeda V.H.A.C., 2005. The arabesque of local knowledge: potatoes, farmers and technicians in highland Tiraque, Cochabamba, Bolivia. Ph.D. Thesis, Wageningen University, The Netherlands.

Wheeler T., von Braun J. 2013. Climate change impacts on global food security. Science 341:508-513.

Wood D., Lenne J. M. 1997. The conservation of agrobiodiversity on-farm: questioning the emerging paradigm. Biodiversity and Conservation 6:109-129.

Neglected and underutilized crops for sustainable agriculture in marginal areas

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Abstract

Some neglected and underutilized crops are known to be more resilient and better adapted than staple crops to grow in marginal environments constrained by water scarcity, poor soils and other such yield-limiting factors. Studies conducted in the United Arab Emirates (UAE) showed that underutilized crops such as mustard greens, quinoa, guar, safflower and saltwort are of value in providing cost-effective and long-term solutions to sustain agricultural production in areas stressed with diminishing quality and quantity of irrigation water, nutrient-poor soils and high temperatures. Among the crops studied, were quinoa and mustard which are nutritionally rich and can thus play a crucial role in combating vitamin and micronutrient deficiencies frequently experienced by inhabitants of marginal environments, and safflower, cluster bean and saltwort are multi-purpose crops that could increase farm income through diversifying products and creating new agro-industries. Research to improve the productivity and value of these crops, and to encourage them to be more widely cultivated, would effectively contribute to food, nutritional and income security of the smallholder farmers in marginal environments.

Introduction

Agricultural productivity is particularly sensitive to environmental stresses, especially temperature extremes and water scarcity. In addition to these, land degradation due to soil nutrient depletion and salinity is becoming another major global issue in recent years, impacting the growth and yield potential of most of the commonly cultivated commodity crops. Globally, the productivity of some lands has already declined by 50% and in Africa yield reduction due to land degradation is estimated to range from 2 to 40%, with a mean loss of 8.2% across the continent (Eswaran et al., 2001). Among the various factors that contribute to land degradation, soil salinity (accumulation of soluble salts of sodium, magnesium and calcium in soil to the extent that soil fertility is severely reduced), exacts major economic and environmental costs. Ghassemi at al. (1995) estimated the total soil area degraded by human-induced salinization (because of poor soil and water management) to be 76.3 Mha, with Africa accounting for nearly 5.9 Mha of this total (Squires and Glenn, 2004). Ghassemi et al. (1995) also estimated the global income loss due to salinity to be about US$ 11.4 billion per year in irrigated and US$ 1.2 billion per year in non-irrigated areas.

Agriculture in general is sensitive to climate change and Africa is especially vulnerable because it is largely dependent on rainfed agriculture (Gbetibouo and Ringler, 2009). Projections of climate change indicate that the area suitable for agriculture, the length of growing seasons and yield potential along the margins of semi-arid and arid areas might decrease, potentially exacerbating malnutrition and food insecurity (Boko et al., 2007). To sustain agricultural productivity in stressed environments, one possible strategy is to look for crops with trait values that currently exceed the equivalent trait in major crops, especially where these are evident in hostile environments where salinity, drought and/or heat stress restrict productivity (Mayes et al., 2011). In this context, neglected and underutilized species

(NUS) offer major opportunities because of their comparative advantage over the staple food crops in terms of tolerance to harsh growing conditions. In addition to their crucial role in food production, the multiple uses of many NUS offer greater opportunities to raise income of local people through product diversification.

There are many NUS, but under the overarching goals of food security, poverty elimination and environmental sustainability, it is important to select appropriate species on the basis of their capacity to best address such challenges. However, NUS have largely been neglected by research and studies are needed to improve yields and quality of produce through identification of high yielding genotypes, better cultivation, management, harvesting and post-harvesting practices, value chains and markets. Bringing the benefits of NUS to the poor in marginal environments therefore requires further research focus on species, especially those that have the highest potential value in terms of food and nutrition security, particularly for farmers in marginal areas. This paper summarizes the work undertaken at the Dubai based International Center for Biosaline Agricultutre (ICBA) in the United Arab Emirates (UAE) to identify crops and varieties that have good potential for marginal environments, especially those stressed by salinity, poorly developed soils and high temperatures, typical of the arid and hyper-arid areas worldwide. A wide range of NUS was examined for yield potential under saline and arid conditions and results of evaluation of crops such as mustard greens (*Brassica juncea* (L.) Czern.), quinoa (*Chenopodium quinoa* Willd.), guar (*Cyamopsis tetragonoloba* (L.) Taub.), safflower (*Carthamus tinctorius* L.), saltwort (*Salicornia bigelovii* Torr.), which are perceived to be of value for African agriculture are discussed in this paper.

Materials and methods

The crops were selected mainly on the basis of their reported salinity and drought tolerance or their ability to grow under harsh environments. The number of accessions studied varied with the crop. All the accessions were initially evaluated for local adaptation and yield potential at ICBA research station (25°05′49″N and 55°23′25″E) between the years 2006 and 2010. The soils at ICBA experimental fields are sandy in texture (sand 98%, silt 1%, and clay 1%), calcareous (50–60% $CaCO_3$ equivalents), porous (45% porosity) and moderately alkaline (pH 8.22) with very low organic matter (<1%). The average daytime temperature is 25°C during winter (November-March) and 37°C in summer (April-October). The rainfall is scant, averaging 94 mm and mostly occurring during the winter months in the form of showers and thunderstorms.

Prior to planting, the soil fertility of the experimental site was improved by incorporating farmyard manure at the rate of 40 t ha^{-1}. The sowing time for mustard greens, quinoa and salicornia was mid October to early November. Safflower was sown in early January and guar in late February. Each accession was sown in plots consisting of four rows of 2.5 m, spaced 50 cm apart. The distance between plants within each row and between two accessions was maintained at 25 cm and 1 m, respectively. The plants were irrigated with low-salinity water of about 2-3 dS m^{-1} using the drip-irrigation system. Standard agronomic data such as plant height, days to 50% flowering and seed weight were recorded from five randomly selected plants from the two middle rows within each plot. All crops were harvested at full maturity and dried at 25°C under forced ventilation before manual extraction of the seed.

Based on the results of preliminary evaluations over 2-3 seasons, the few best-performing accessions were identified in each crop, which were then subjected to a more detailed study of the agronomic performance and/or salinity tolerance in field trials laid out using a randomized block design with three replicates. Salinity tolerance studies involved application of irrigation water with an electrical conductivity (EC_w) 5, 10 and 15 dS m^{-1} obtained by mixing saline ground water with fresh water. In all cases, the seeds were germinated with fresh water and salinity treatment was generally initiated after two weeks of growth, starting with low salinity and gradually increasing to the maximum over a period of 2-4 weeks. In the case of quinoa, the performance of three selected genotypes was further studied by growing in a salt-affected farm located near Ghayathi (23°42′ 49.9′′N and 52° 54′ 10.6′′E) in the Western Region of Abu Dhabi, UAE. The data on plant growth, seed and yield, etc. were subjected to analysis of variance using GenStat ver. DE 10.3.

Results and discussion

Mustard greens

Twelve accessions, selected for their leafiness from an initial set of 100 accessions showed significant differences for the number of leaves and leaf area when evaluated during the cropping season 2009-2010 (Table 1). Of these, six accessions namely, ATC 90333, 93322, 93424, 93471, 93569, 94294, when studied for salinity tolerance during the year 2010-2011, had an average green biomass of 3.0 kgm^{-2} at 5 dSm^{-1} after 45 days of growth (before flowering) ,which decreased to 2.8 kgm^{-2} (i.e., by 33%) and to 1.5 kgm^{2} (i.e., by 50%) when salinity was increased to 10 dSm^{-1} and 15 dSm^{-1}, respectively (Figure 1). In terms of relative tolerance to salinity, two accessions (ATC 93471 and ATC 93569) were found to be outstanding while ATC 93424 was highly sensitive. Previous reports on salinity tolerance of mustard by Shannon et al. (2000) show that late salinization reduces yields by 50% (C_{50}) at 15 dSm^{-1}, while at a lower, but early salinity (10 dSm^{-1}), yields are reduced by the same amount.

The water requirement for mustard under the UAE conditions was estimated to be around 250 mm, which is very similar to lettuce and spinach, but leaf mustard has the advantage of being more salt-tolerant than these commonly grown leafy greens, and hence of great potential for marginal areas of Africa. Mustard greens are high in vitamins K, A and C and in iron. The fresh leaves are also a very good source of folic acid and an excellent source of several essential minerals, including calcium, iron, magnesium, potassium, zinc, selenium and manganese (Schippers and Mnzava, 2007). In recent years, leaf mustard has been the direct focus of some health-oriented research studies, since it ranks high among vegetables for total glucosinolate content, which has cancer-preventive properties (Mateljan, 2006). Besides its cultivation for the tender green leaves, mustard seeds are a source of edible oil and also used as a condiment.

Table 1. Characteristics of 12 mustard accessions evaluated for leafiness under the UAE growing conditions.

ID	Plant spread (cm)	No. of leaves	Leaf area (cm^2)	Leaf dry weight (g)
ATC 90333	30.6	13.6	348	0.47
ATC 93245	24.6	9.5	317	0.40
ATC 93322	29.0	15.7	355	0.38
ATC 93337	30.5	10.4	255	0.57
ATC 93424	27.4	12.8	452	0.73
ATC 93446	28.1	16.5	343	0.39
ATC 93470	33.3	9.3	328	0.50
ATC 93471	29.5	10.4	388	0.30
ATC 93472	37.9	6.9	424	0.53
ATC 93474	31.6	8.5	352	0.40
ATC 93569	32.7	10.9	395	0.57
ATC 94303	33.1	6.3	251	0.37
LSD (5%)	n.s.	4.3	125	n.s.

n.s.= not significant

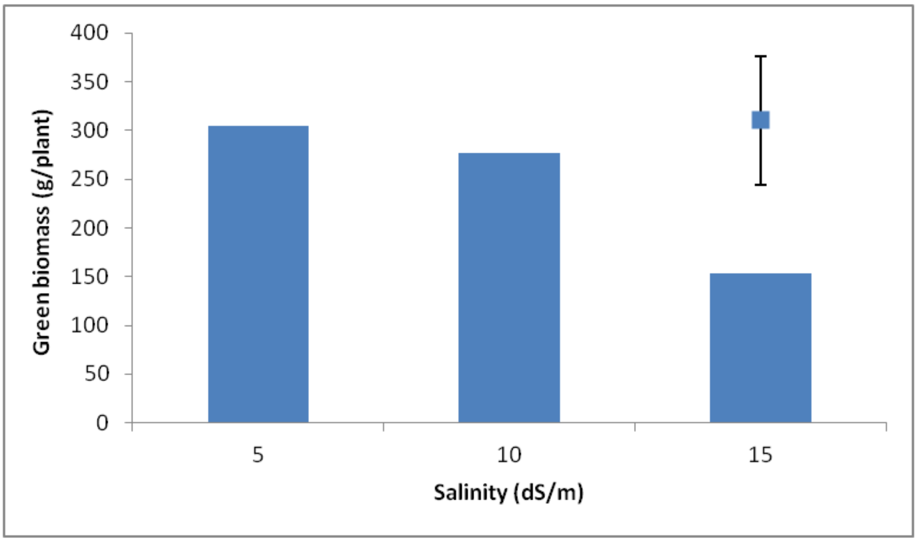

Figure 1. Mean green biomass yields of six mustard greens accessions (ATC 90333, ATC 93322, ATC 93424, ATC 93471, ATC 93569 and ATC 94294). The error bar is the Least Significant Difference (LSD) (P=0.05).

Quinoa

From an initial grow-out of 60 quinoa accessions, 20 were selected for superior agronomic performance under the UAE growing conditions, which were further evaluated for their growth performance and yield potential over two consecutive cropping seasons (winter 2007-2008 and 2008-2009). Five top-ranking accessions (Ames 13742, Ames 13749, Ames 13757, Ames 13761, NSL 106399), selected on the basis of average yield obtained over the two seasons, when further studied for their yield performance in a replicated field trial during the growing season, 2009-2010 produced an average grain yield of 456.6 gm^{-2} and average dry matter yield of 1464 gm^{-2}, both being higher than the averages reported from the traditional growing areas in the Andes (Rao and Shahid, 2012). The study showed that quinoa can perform well under ecologically extreme desert conditions and holds great promise as a food, feed and forage crop for diversification of the agricultural production systems in salt-affected areas.

The performance of selected cultivars of quinoa was also studied in the model farms in the western region of Abu Dhabi, where the salinity of irrigation water was in the range of 15 dSm^{-1}, and the data obtained of biomass and seed yield corroborated the earlier findings that quinoa holds promise as a multipurpose crop for salt-affected areas (Figures 2 and 3). Thus, the mean seed yield of three accessions was found to be about 750 gm^{-2}, which was on a par with the highest yields reported from the non-saline traditional quinoa growing areas. The green biomass yield was also high - the mean of the three cultivars being 4.3 kgm^{-2}, indicating the potential of quinoa as an alternative forage crop for saline areas. Earlier research in Peru showed also that quinoa's salt tolerance is very high, being able to grow and be productive in salt concentrations close to sea water (Jacobsen et al., 2003; Koyro et al., 2008). Another attractive feature of quinoa is its low water requirement, estimated as 150-250 mm per growing season for optimum yields (Martinez et al., 2009). Recent studies in the South Sinai desert also demonstrated the value of quinoa as a crop suitable for arid climates (Shams, 2011).

Quinoa seeds are generally used to make flour, soup and for breakfast cereals. The seeds are also used for brewing beer and for animal feed. Quinoa seeds are highly nutritious with outstanding protein quality and high amounts of a range of vitamins and minerals (Schlick and Bubenheim, 1996). Its high levels of salinity tolerance, low-water demand and exceptionally high nutritional quality place quinoa in an enviable position as an alternative crop for marginal environments.

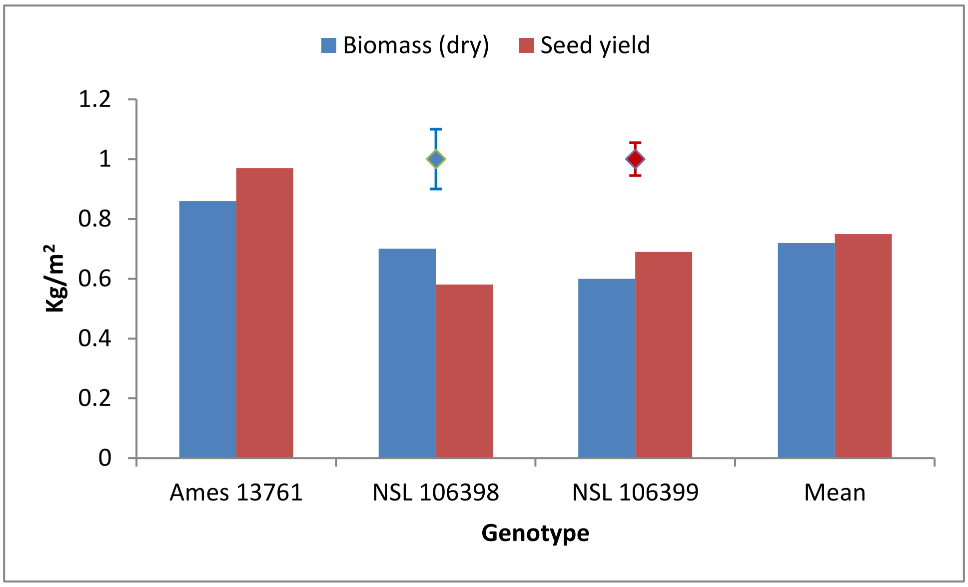

Figure 2. The mean biomass and seed yields of three quinoa accessions (Ames 13761, NSL 106399, NSL 106399) grown in a salt-affected farm in the UAE. The error bars are Least Significant Differences (LSD) (P=0.05)

Figure 3. Quinoa growing with saline irrigation water (EC_w 15 dSm^{-1}) in the UAE.

Guar

The ten top-performing guar (also known cluster bean) accessions selected from an initial set of 100 accessions, when evaluated for their performance in the UAE showed the average number of pods per plant to vary between 164.4 and 113.4, with a mean of 149.4 over accessions. Seed yield varied between 2.5 tha^{-1} and 1.4 tha^{-1}, the overall mean being 2.2 tha^{-1}. Both the pod and seed yields were higher than the average yields reported from the traditional growing areas in Asia, indicating the potential of guar as a multipurpose crop for marginal areas (Rao and Shahid, 2011).

The results from the study on the effect of salinity showed significant effects on growth and yield (Table 2). Compared to the control, the mean plant height declined by 12% at 5 dSm^{-1} and 57% at 10 dSm^{-1}. Similarly, increase in salinity to 5 dSm^{-1} and 10 dSm^{-1} reduced the mean number of pods per plant by 41% and 58% of the control, respectively. Seed yield per plant was 15% less at 5 dSm^{-1} and 24% at 10 dSm^{-1}, in comparison with the control treatment. Francois et al. (1990) reported that guar is more salt tolerant than many other grain legumes, with a high salinity threshold of 8 dSm^{-1}. However, results from the present studies do not confirm this. Since the trial was conducted during summer under desert conditions, the combination of salinity and heat stress would have probably contributed to significant reductions in yields even at a low salinity (5 dSm^{-1}). Besides genotypic variation in salinity tolerance, it is also possible that the number of accessions evaluated by us is too small to identify genotypes with significantly high levels of stress tolerance.

Table 2. Effect of salinity on growth and yield of guar. The values represent the means of five accessions namely: PI 158123, PI 158129 PI 263877 PI 323083 PI 426643.

	Irrigation water salinity			
Trait	Control	5 dS/m	10 dS/m	LSD (5%)
Plat height (cm)	104.0	91.2	44.4	10.9
Plant width (cm)	58.0	55.0	34.0	8.4
No. of Branches	12.8	11.3	9.3	n.s.
Pods/plant (number)	181.6	107.5	74.4	41.8
Seed yield /plant (g)	35.6	20.5	11.6	9.8

n.s.= not significant

Guar is known for its drought tolerance and grows without irrigation even in areas with as little as 250 mm of annual rainfall (Undersander et al., 1991). The water requirement for guar in the UAE was estimated to be 354 mm, similar to other vegetables such as cucumber and cabbage. However, the higher salt tolerance of guar and its multiple uses as a vegetable for human consumption, forage for cattle and source of industrial gum make guar superior to other vegetable crops (Rao and Shahid, 2011).

Safflower

An assessment of phenotypic diversity for quantitative and qualitative traits in 631 accessions originating from 11 countries in three regions (Central Asia, Southwest Asia and Africa) in the UAE suggested that adaptation of the species to the wide spatial and temporal variation in the Middle East resulted in a multitude of ecotypes and in an enormous amount of local variation (Jaradat and Shahid, 2006). A multivariate selection criterion for high biological and seed yield, long rosette period and no or few spines identified five accessions (PI 251262, PI 251985, PI 237550, PI 251285) that can be introduced into subsistence farming systems as a multipurpose crop under saline agriculture. More recently, evaluation of 52 genotypes in field plot trials over two seasons using irrigation water salinities corresponding to electrical conductivities of 10 and 15 dSm^{-1} showed that salinity reduced biological and grain yields and the flower number by 50, 75 and 25%, respectively (Fraj M.B., personal communication). The results from this study show that safflower is moderately salt-tolerant and cultivation on salt-affected land can prove beneficial to farmers. Another main advantage of safflower over other agricultural crops is its drought tolerance and the ability to adapt to hot and dry climates. Safflower has an extensive root system capable of extracting subsoil water at greater depths than other crops (Oelke et al., 1992).

Safflower, although mainly grown for its high quality oil, is an annual multipurpose crop. The tender leaves and shoots, which are rich in vitamin A, iron, phosphorus, and calcium are used as a pot herb and salad. Safflower herbage is valuable as green fodder. Historically, safflower was grown for the red and yellow dyes obtained from the petals, which are excellent for dyeing silk, linen and cotton (Oyen and Umali, 2007). The introduction of cheap synthetic dyes resulted in disuse of safflower dyes, but the growing demand for vegetable dyes internationally, not only for dyeing cloth but also for food colouring, is likely to be a significant market niche for exploitation.

Saltwort

In a detailed evaluation carried out of nine lines of saltwort in sandy soils with sea water in the UAE, plant height varied from 38.5 to 75.4 cm, dry weight from 36.1 g to 69.0 g and the seed weight from 0.8 g to 6.5 g/plant (Jaradat and Shahid, 2012). At the rate of 20 000 plants/ha, the biomass yield obtained was close to the average yields of 1.7 kgm^{-2} reported from Mexico by Glenn et al. (1991). The study, in addition to demonstrating that saltwort can be grown successfully in arid regions using seawater for irrigation, has identified genotypes with favourable combinations of traits that can be used by farmers in small-scale vegetable production, in large-scale biomass and oilseed production, or for reclamation of saline lands.

Saltwort is a speciality vegetable crop cultivated for its succulent photosynthetic shoots tips. The seeds can germinate directly with seawater and it has a high oil (30%) and protein (35%) content, and low concentration of salt (<3%). The oil quality is comparable to that of major oilseed crops with a high content of linoleic (75%) and linolenic (omega-3) fatty acids. Despite its potential as a crop with multiple uses, saltwort improvement has received very little attention.

Summary

Water scarcity and salinity are major constraints to agricultural production globally including Africa. Furthermore, climate change projections for the region indicate considerable negative impact on farm-level productivity due to the area's high dependency on climate-sensitive agriculture. NUS, being more resilient and better adapted to grow in marginal environments than current staple crops, offer cost-effective and viable solutions to sustain farm productivity. NUS identified in this paper offer robust alternatives to sustain agricultural productivity. NUS such as mustard greens and quinoa are also nutritionally rich and have a real potential to contribute to combating vitamin and micronutrient deficiencies, particularly in rural communities dependent on agriculture. However, there are major gaps in our knowledge and capacity to make the best use of these NUS because agricultural research has so far paid little attention to these species. Research to increase the value of these crops and encourage them to be more widely cultivated would broaden the resource base and increase the livelihood options especially for smallholder farmers in marginal areas. The growing demand from consumers for diversity and novelty in foods is creating new market niches for NUS such as mustard and quinoa. Designer foods with balanced amino acid and micronutrient profiles can be developed using appropriate blends of major crops and NUS to the meet demand and encourage farmers to grow these crops.

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References

Boko M., Niang I., Nyong A., Vogel C., Githeko A., Medany M., Osman-Elasha B., Tabo R., Yanda P. 2007. Africa. Climate Change 2007: Impacts, Adaptation and Vulnerability. In: Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden P.J., Hanson, C.E. (eds), Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge UK, pp. 433-467.

Eswaran H., Lal R., Reich P.F. 2001. Land degradation: an overview. In: Bridges, E.M., I.D. Hannam, L.R. Oldeman, F.W.T. Pening de Vries, S.J. Scherr, and S. Sompatpanit (eds), *Responses to Land Degradation*. Proceedings of the Second International Conference on Land Degradation and Desertification, Khon Kaen, Thailand. Oxford Press, New Delhi, India.

Francois L.E., Donovon T.J., Maas E.V. 1990. Salinity effects on emergence, vegetative growth and seed yield of guar. Agronomy Journal 82: 587-592.

Gbetibouo G.A., Ringler C. 2009. Mapping South African Farming Sector vulnerability to Climate Change and Variability: A Subnational Assessment. IFPRI Discussion Paper 00885, International Food Policy research Institute, Washington D.C. USA. http://www.ifpri.org/sites/default/files/publications/ifpridp00885.pdf

Ghassemi F., Jakeman A.J., Nix H.A. 1995. Salinization of Land and Water Resources. University of New South Wales Press, Ltd., Canberra, Australia.

Glenn, E.P., O'Leary J.W., Watson M.C., Thompson T.L., Kuehl, R.O. 1991. *Salicornia bigelovii* Torn: An oilseed halophyte for seawater irrigation. Science 25: 1065-1067.

Jacobsen S. E., Mujica A., Jensen C.R. 2003. The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse abiotic factors. Food Reviews International 19: 167-177.

Jaradat A.A., Shahid M. 2006. Patterns of phenotypic variation in a germplasm collection of carthamus tinctoria L. from the Middel East. Genetic Resources and Crop Evolution 53: 225-244.

Jaradat A.A., Shahid M. 2012. The dwarf saltwort (*Salicornia bigelovii* Torr.): Evaluation of breeding populations. ISRN Agron. doi:10.5402/2012/151537

Koyro H.W., Lieth H., Eisa S.S. 2008. Salt tolerance of *Chenopodium quinoa* Willd. In: H. Leith, M.G. Surce and B. Herzog (eds.), Mangroves and Halophytes: Restoration and Utilisation. Springer, Dordrecht, p.133-145.

Mateljan G. 2006. Mustard greens. In: World's Healthiest Foods: Essential Guide for the Healthiest Way of Eating. The George Mateljan Foundation, California. www.whfoods.com/genpage.php?tname=foodspice&dbid=93

Martinez E. A., Veas E., Jorquera C., Martin S. R., Jara P. 2009. Re-introduction of quinoa into arid Chile: cultivation of two lowland races under extremely low irrigation. Journal of Agronomy & Crop Science 195:1-10.

Mayes S., F. J. Massawe F.J., Alderson P. G., Roberts J. A., Azam-Ali S. N., Hermann M. 2011. The potential for underutilized crops to improve security of food production. *Journal of* Experimental Botany doi: 10.1093/jxb/err396.

Oelke E.A., Oplinger E.S., Teynor T.M., Putnam D.H., Doll J.D., Kelling K.A., Durgan B.R., Noetzel, D.M. 1992. Safflower. Alternative Crops Field Manual. Universities of Wisconsin Cooperative Extension Service, University of Minnesota Center for Alternative Plant and Animal Products & Minnesota Extension Service. http://www.hort.purdue.edu/newcrop/afcm/saffl ower.html

Oyen L.P.A., Umali B.E. 2007. *Carthamus tinctorius* L. In: H.A.M. van der Vossen, Mkamilo, G.S. (eds.). PROTA (Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale)14: Vegetable oils/Oléagineux. PROTA, Wageningen.

Rao N.K. and Shahid M. 2011. Potential of cowpea [*Vigna unguiculata* (L.) Walp.] and guar [*Cyamopsis tetragonoloba* (L.) Taub.] as alternative forage legumes for the United Arab Emirates. Emirates Journal of Food and Agriculture 23: 110-119.

Rao N.K., Shahid M. 2012. Quinoa- A promising new crop for the Arabian Peninsula. American-Eurasian Journal of Agricultural & Environmental Sciences 12: 1350-1355.

Schlick G., Bubenheim D. L. 1996. Quinoa: Candidate crop for NASA's Controlled Ecological Life Support Systems. In: Janick, J. (ed), Progress in new crops, ASHS Press, Arlington, pp. 632-640.

Schippers R.R., Mnzava N.A. 2007. *Brassica juncea* (L.) Czern. In: van der Vossen H.A.M., Mkamilo G.S. (eds), PROTA (Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale), Wageningen. http://www.prota4u.org/search.asp

Shams A., 2011. Combat degradation in rain-fed areas by introducing new drought tolerant crops in Egypt. *International Journal of Water Resources and Arid Environments* 1: 318-325.

Shannon M.C., Grieve C.M., Lesch S.M., Draper J.H. 2000. Analysis of salt tolerance in nine leafy vegetables irrigated with saline drainage water. Journal of American Society of Horticultural Science 125: 658-664.

Squires V.R., Glenn E.P. 2004. Salination, Desertification, and Soil erosion, In: V. R. Squires V.R. (ed), The Role of Food, Agriculture, Forestry and Fisheries in Human Nutrition, Encyclopedia of Life Support Systems (EOLSS), UNESCO-EOLSS Publishers, Oxford, UK.

Undersander D.J., Putnam D.H., Kaminski A.R., Kelling K. A., Doll J.D., Oplinger E.S., Gunsolus J.L. 1991. Guar. In: *Alternative Field Crop Manual.* University of Wisconsin Cooperative Extension Service, University of Minnesota Extension Service, Center for Alternative Plant & Animal Products. http://www.hort.purdue.edu/newcrop/afcm/cowpea.html

Anthelmintic effects of a diet containing a traditional plant *Viscum verrucosum* on faecal egg count and eosinophils of naturally infected Tswana goats

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Abstract
Twenty (one-year old) female goats, naturally infected by internal parasites, were acclimatized to individual pens and fed for three weeks and then blocked for live weight and faecal egg count and randomly allocated to four treatments: a standard diet (crude protein = 12%; n = 5; liveweight = 24.1 kg; faecal egg count = 2640 epg), a high protein diet (crude protein = 16%; n = 5; liveweight = 25.7 kg; faecal egg count = 2630 epg), a *Viscum verrucosum* plant diet; crude protein = 12%; n = 5; liveweight = 26 kg; FEC = 2720 epg) and a standard diet dosed with a commercial drug, valbazen (crude protein = 12%; n = 5; liveweight = 25 kg; FEC = 2800 epg). Faecal samples were collected weekly while blood samples were collected from the jugular vein, at the beginning of the experiment and at 21, 35 and 49 days. Live weight was measured at the beginning and end of the study. There was a highly significant (P<0.001) treatment and treatment x time interaction on faecal egg count whereby *Viscum verrocosum* and valbazen animals excreted fewer eggs on days 28, 35, 42, 49 and 56. Eosinophil counts were significantly lower (P<0.001) on days 35 and 49 in goats fed *V.verrocosum* and in those dosed with valbazen. There was no significant difference in liveweight daily gain between the treatments (high protein, 21.4; crude protein, 44.6; valbazen, 39.2, and valbazen, 53.6 g/d; P>0.05) indicating that protein supply had not been improved. It however, also shows that at the inclusion rate used in the current study, *V. verrucosum* was not detrimental to growth. The *V. verrucosum* based diet was as effective as the chemical, valbazen, in reducing faecal egg count, indicating that the use of natural flora may be a beneficial option for low-resource farmers who cannot afford to purchase drugs to control internal parasites. Eosinophils were reduced in *V.verrucosum* and valbazen goats, suggesting that the lack of eosinophils is associated with reduced faecal egg count or worm burden. In contrast, the high eosinophil counts found in the high protein and crude protein goats may point to the preservation of parasites as a mechanism to facilitate a future response to re-infection.

Keywords: Eosinophil, faecal egg count, internal parasites, valbazen, *Viscum verrucosum*

Introduction

Parasites are one of the major problems of goat production in Botswana. Crafting new technologies in controlling internal parasites in small stock requires concerted efforts in research and the use of locally available resources in order to make goat production a viable economic venture. Indigenous plants, such as *Viscum verrucosum* have been found to reduce faecal egg count in goats (Madibela and Jansen, 2003; Madibela et al., 2010; Moncho et al., 2012). Haematological tests have been widely used for the diagnosis of various animal diseases and so it is of interest to investigate the effects of the *Viscum verrucosum* plant on some blood parameters of Tswana goats. However, no literature is available on haematological parameters of Tswana goats infected by nematode parasites and fed diets containing traditional medicinal plants.

Helminths are major problems in goat production in Botswana. According to a study by Segwagwe and Ramabu (1999) major causes of mortalities (43.9%) in goats were attributed to helminthiasis/coccidiosis. The experience of the National Veterinary Laboratory (NVL) showed that a higher number of cases of internal parasites is due to helminthiasis (NVL Annual Report, 1994). Internal parasites cause economic and production losses (Coop and Holmes, 1996) as well as through the cost of control measures through the frequent purchase of anthelmintic remedies. The main concern regarding the use of drugs in recent times is parasite drug resistance and drug residues in livestock products (meat and milk; Ramaphane et al., 1999). When producers continuously deworm their flocks with the same antihelminthics, fewer and fewer parasites are killed with every treatment, and genetic selection for resistant parasites occurs (Hartwig, 2000). To overcome the problem of drug resistance it has been proposed that biological control may be the way for sustainable parasite control. According to Butter et al. (2001) there is a need for sustainable and practical approaches to parasite control to be introduced in the farming systems. These authors (Butter et al., 2001) proposed that one such possibility could be the exploitation of forage species capable of reducing worm infection levels solely, or in conjunction with limited use of drugs. It has been reported that feeding forage browse containing condensed tannins reduces faecal egg count in sheep (Butter et al., 2001) and in goats (Osoro et al., 2007).

Crafting new technologies in eradicating and/or controlling goat parasites requires intense research and harnessing locally available resources in order to make goat production a viable practice. Parasitic plants, such as *Viscum verrucosum*, have been fed to small stock by resource-limited farmers and this indigenous knowledge needs to be harnessed to reduce the frequency of use of antihelminthic drugs and to increase effectiveness of these drugs (Madibela and Jansen, 2003). Haematological tests have been widely used for the diagnosis of various animal diseases. The main objective of this study was to evaluate the effectiveness of the *Viscum verrucosum* plant as an antihelminthic medicine with the consequence of affecting blood parameters, reduction of faecal egg count and improving live weight gain through the following: faecal egg count, packed cell volume (PCV), total white cells (TWC), and haemoglobin (HB) and live weight.

Materials and methods

Twenty (1-year old) female goats that were naturally infected by internal parasites were acclimatized to individual pens and feeding for three weeks and blocked for live weight (LW) and initial faecal egg count (FEC) and randomly allocated into four treatments: Standard diet (SD; CP = 12%; n = 5; LW = 24.1 kg; FEC = 2640 epg, high protein (HP; CP =

16%; n = 5; LW = 25.7 kg; FEC = 2630 epg), *Viscum verrucosum* diet (VV; CP = 12%; n = 5; LW = 26 kg; FEC = 2720 epg); and high standard diet and dosed with a commercial drug, valbazen (SDD; CP = 12%; n = 5; LW = 25 kg; FEC = 2800 epg).

Faecal samples were collected weekly while blood samples were collected from the jugular vein at the beginning of the experiment and at 21, 35 and 49 days into a test tube containing EDTA anticoagulant. Live weight was measured at the beginning and end of the study. Data for faecal egg count (FEC) was analysed using the Proc Mixed Procedures of SAS (2002-2008) for repeated measures. Before analysis FEC was log transformed as $\log_{10}$(FEC +1). Diet effects on blood parameters and LW daily gain was tested using the general linear model procedure of SAS (2002-2008).

Results and discussion

There was a highly significant ($P<0.001$) treatment as well as treatment x time effects on faecal egg count (FEC) (Figure 1 and Table 2), whereby SDD and VV animals had lower FEC (1670.09 ± 0.096 and 1839.77±0.096 epg, respectively) than HP and SD goats (2909.72±0.096 and 2772.32±0.096 epg, respectively). VV and SDD animals excreted fewer eggs on day 28, 35, 42, 49 and 56. This corroborates earlier studies (Madibela and Jansen, 2003; Moncho et al., 2012) that *V. verrucosum* plants as diets or aqueous extract orally dosed is able to reduce FEC in goats. Eosinophil counts for all the treatments were similar at 0 to 21 days but were significantly ($P<0.001$) low in goats fed VV or dosed with a drug during later days (Table 1).

Contrary to the long standing paradigm of eosinophil toxicity in nematode infection (Fabre et al., 2009) the present study may suggest that maintaining a high count of circulating eosinophils promotes survival of parasites (Fabre et al., 2009; Gebreselassie et al., 2012) in order to preserve antigen stimulus for a Th2 response that prevents future infection (Gebreselassie et al., 2012). There was no significant difference in LW daily gain between the treatments (HP; 21.4, SD; 44.6, SDD; 39.2 and VV; 53.6g/d; $P>0.05$; Table 2) indicating that protein supply was not being improved. However, it also shows that at this inclusion rate *V. verrucosum* was not detrimental to growth.

Conclusion and implications

Viscum verrucosum was found to be as effective as valbazen in reducing FEC. Eosinophils were reduced in VV and SDD goats, suggesting that the lack of eosinophils is associated with reduced FEC or worm burden. In contrast high eosinophils, as was the case in HP and SD goats, may indicate preservation of parasites as a cue for a future response to reinfection. However there is a need to test more neglected plants for efficacy in control of nematode parasites. This will result in conserving both indigenous knowledge and plants for sustainable control of internal worms and increased livestock productivity. The use of natural flora in the control of internal parasites is a novel way of preventing parasite resistance to drugs. In addition, smallholder small stock farmers would benefit from less expensive use of indigenous plants to control nematode parasites.

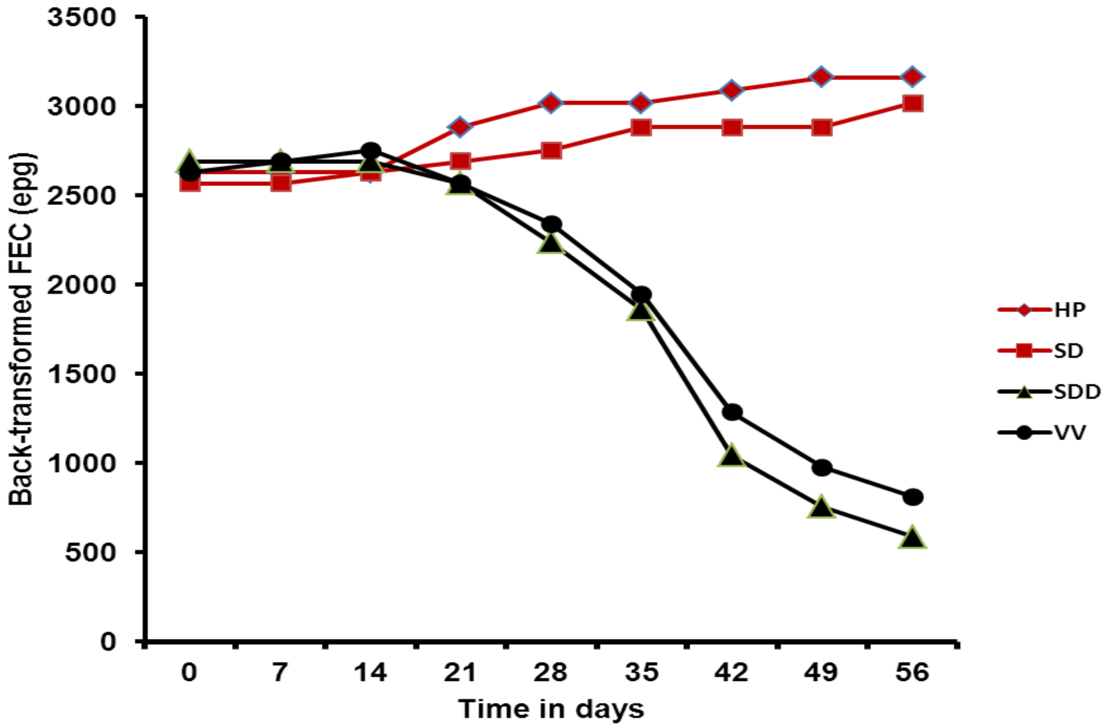

Figure 1. Back-transformed FEC (epg) of goats fed different diets or dose with drug

Table 2. Neutrophils, eosinophils, basophils and lympocytes of goats fed different diets and infected by nematode parasites

	Neutrophils				Eosinophils				Basophils				Lymphocytes			
	0	21	35	49	0	21	35	49	0	21	35	49	0	21	35	49
HP	45.4	42.8	46.2	45.4	8.0	8.0	8.6	8.0	9.2	9.2	9.4	8.8	11.0	10.8	8.0	9.0
SD	51.0	45.4	45.8	43.8	10.0	10.0	10.0	9.2	6.4	7.0	8.8	8,2	14.0	14.6	10.6	12.8
SDD	53.6	47.6	47.2	46.4	9.4	7.2	3.6	0.8	8.2	8.6	9.0	7.8	12.0	13.2	10.2	12.2
VV	50.6	41.4	45.8	46.8	8.8	7.2	3.2	1.4	7.0	6.4	8.4	7.0	13.0	10.6	9.0	9.2
SL	NS	NS	NS	NS	NS	NS	***	***	NS	*	NS	NS	NS	NS	NS	0.098

NS = Not significant; * = P<0.05; *** = P<0.001

Table 2. Mean back-transformed FEC (epg) and daily gain (g/day) of naturally infected female Tswana goats fed a different diet

Parameter	n	Feacal egg counts (epg)	Live weight gain (g/day)
HHP	5	2906.368[a]	21.4
SD	5	2771.682[a]	44.6
SDD	5	1671.630[b]	39.2
VV	5	1838.077[b]	53.6
SE		0.039	13.4
SL(Trt)#		*	NS
SL(Trt x Time)		***	NS

#NS = Not significant; *= P<0.05; **= P<0.01; ***= P<0.001; Trt= Treatment; SL = significance level

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References

Butter N.L., Dawson J.M., Wakelin D., Buttery P. J. 2001. Effect of dietary condensed tannins on gastrointestinal nematodes. Journal of Agricultural Science (Cambridge)137: 461-469

Coop R.L., Holmes P.H. 1996. Nutrition and parasite infection. *International Journal of Parasitology*. 26:951-962

Fabre V., Beiting D. P., Bliss S.K., Gebreselassie N.G., Gagliardo L.F., Lee N.A., Lee J.J., Appleton J.A. 2009. Eosinophil deficiency compromises parasite survival in chronic nematode infection. The Journal of Immunology 182:1577-1583

Gebreselassie N.G., Moorhead A.R., Fabre V., Gagliardo L.F., Lee N.A., Lee J.J., Appleton J.A. 2012. Eosinophils preserve parasitic nematode larvae by regulating local immunity. *The* Journal of Immunology 188:417-425

Hartwig N., 2000. Control of Internal Parasites of Sheep. MSc Dissertation. Iowa State University, USA.

Madibela O.R. and Jansen K. 2003. The use of indigenous parasitic plant (*Viscum verrucosum*) in reducing faecal egg count in female Tswana goats. Livestock Research for Rural Development 15 (9). http://www.lrrd.org/lrrd15/9/madi159.htm

Madibela O.R., Ramabu S., Machete J. 2010. The effects of parasitic plant (*Viscum verrocosum*) on live weight and faecal egg count in female Tswana goats. Paper presented at 5th All Africa Conference on Animal Agriculture 25th – 28th October 2010, Addis Ababa, Ethiopia. http://www.slideshare.net/esapethiopia/7-ricky-the-effects-of-viscum

Moncho T., Madibela O.R., Machete J.B., Bonang V., Motlhanka D.M.T. 2012. Effect of crude extracts of *V. verrucosum* treatment on nematode parasite faecal egg count in female Tswana goats. In: Tusiime, G., Majaliwa, J. G. M., Nampala, P. and Adipala, E. Third RUFORUM Biennial Regional Conference, 24-28 Sept 2012, Entebbe, Uganda.

National Veterinary Laboratory 1994. Annual Reports. Ministry of Agriculture, Department of Animal Health. Botswana Government Printers, Gaborone.

Osoro K., Benito-Pena A., Frutos P.C., Garcia U., Ortega-Mora L.M., Celaya R., Ferre I. 2007. The effect of heather supplementation on gastrointestinal nematode infections and performance in Cashmere and local Celtiberic goats on pasture. *Small Ruminant Research* 67:184–191.

Ramaphane M., Senyatso E.K., Makore J.,Gaolebogwe, J., 1999. A survey on small stock parasite control in communal areas of Botswana. In: Aganga, A.A., Chabo, G., Kgosimore. M., Letso, M. and Omphile U.J. (Eds). Enhancing sheep and goat production in Botswana. Proceeding of the sheep and goat workshop, Botswana College of Agriculture, Gaborone, Botswana, April 19-23, 1999.

SAS Institute Inc. 2003-2008. SAS User guide. Statistics. Version 9.2. SAS Institute Inc., Cary, NC, USA.

Segwagwe B.V.E., Ramabu S.S. 1999. Causes of mortalities in sheep and goats in Botswana. In: Aganga, A.A.A., Chabo, R.G., Kgosimore, M., Letso, M. and Omphile, U.J. (eds). Enhancing sheep and goat production in Botswana. Proceeding of the sheep and goat workshop, Botswana College of Agriculture, Gaborone, Botswana, April 19-23, 1999

Simple sequence repeats (SSR) polymorphisms and its relationship with phytochemical composition in Nigerian sesame *(Sesamum indicum* L) cultivars

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Abstract

Genetic characterization of 30 accessions of Nigerian sesame (*Sesamum indicum* L.) and association with phytochemical composition was investigated using simple sequence repeats. High genetic variability was found among Nigerian sesame genotypes with the repeat motifs (TC_{12}-TC_{25}) generated from two highly informative primer pairs. Based on length of repeats, 30 accessions were divided into six main groups. Two cultivars did not belong to any group when an unweighted pair-group method with arithmetic average (UPGMA) cluster analysis was applied. The average allele number per SSR locus was 4.0 whereas genetic distances among the accessions ranged from 0 to 0.20. Phytochemical concentrations including tannins, flavonoids, saponins, glycosides and alkaloids varied in different accessions with no clear association to SSR length except for one SSR (TC_{12}) in accession Ciano 27 which had a possible association with a low flavonoid concentration. This research genetically characterizes Nigerian sesame in relation to phytochemicals that may be useful in nutrition and health.

Key words: Sesame, genetic characterization, SSR-markers, phytochemicals.

Introduction

Sesame seeds are the main product from the tropical annual crop *Sesamum indicum.* Sesame is grown in many parts of the world on over 5 million hectares, of which 70% is cultivated in Asia and 26% in Africa (Kinman and Martin, 1999). Nigeria is a major producer of sesame contributing over 60% of the overall global sesame export market (Bruce, 1953; Laurentin and Karlovsky, 2006).

Sesame meal is an excellent feed for poultry and livestock (Oplinger et al., 1997). Many recipes contain sesame seeds as an ingredient (Home Cooking, 1998). Furthermore, *Sesamum indicum* has medicinal properties used in traditional Chinese and Japanese medicine in their Ayurvedic preparations (Smith and Salerno, 2001). Phytochemicals that inhibit various hormone and metabolic pathways associated with the development of cancer have also been identified (Osagie et al., 1986).

Saponins and glycosides are further compounds of interest, often referred to as natural detergents because of their foamy nature when mixed with water (Seigler, 1998). They posses both beneficial and deleterious properties depending on the concentration in the seed (Oakenful and Sidhu, 1989). Flavonoids, another group of sesame phytochemicals, exert multiple biological effects including antibacterial, antiviral, antitoxic and anti-inflammatory activities (Cook and Samman, 1996). Many of these alleged effects have been linked to known functions as strong antioxidants, free radical scavengers and metal chelators

(Nakayama et al., 1993. Alkaloids and tannins present in sesame seeds can boost the immune system thereby helping the body to fight infection (Musa et al., 2000).

Several DNA-based marker systems are currently available permitting the accurate assessment of the degree of diversity at the DNA level within a germplasm collection of any crop species (Baumung et al., 2004). The SSR (short sequence repeat) or microsatellite method of assaying polymorphisms involves utilizing the high degree of length variation resulting from certain repeating nucleotide sequences (simple sequence repeats) found in most genomes. SSRs can detect a high degree of genetic polymorphism and have been previously successfully applied for comparative analysis and mapping of mammalian and plant genomes (Powell et al., 1996).

SSR loci are further commonly used for individual genotype identification, population diversity estimations, differentiation of populations and genetic relationships (Takundua et al., 2010; Ubi, 2008). SSRs have recently been used to study both genetic diversity and the phylogenetic relationship in sesame (Dixit et al., 2005; Cho et al., 2011; Vinod and Sharma, 2011; Gebremichael and Parzies, 2011; Wang et al., 2012). However, Dixit and his colleagues (2005) identified only 10 out of 50 SSR markers developed from a sesame DNA library being polymorphic in 16 sesame accessions and these SSRs have also been tested in our study.

To the best of our knowledge, a report on sesame germplasm characterization applying SSR marker technology to find any association with content of phytochemicals has yet not been reported. The particular aim of this study was therefore to determine the phytochemical composition of sesame seeds and to apply known sesame SSRs to identify any association between concentrations of phytochemicals and SSRs present in Nigerian sesame accessions.

Materials and methods

Plant material

Thirty different accessions of sesame were obtained from the National Cereals Research Institute (NCRI) Badeggi, Niger State, Nigeria. Plants were grown from seeds in the field at the crossing block of the Biotechnology Research and Development Centre of Ebonyi State University, Abakaliki, Nigeria and also in a temperature-controlled greenhouse at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Field/greenhouse plant growth

All plants were grown in the field during the rainy season in the years 2008 and 2009. The experiment was conducted in Nigeria on a clay-loam soil at an altitude of 1300–1500 m above sea level and an average daily temperature of 29.8^{o}C. Annual precipitation ranged from 1650 to 2000 mm, distributed in two rainy seasons. The field layout was a randomized complete block design with three replications. Plot size was 1.5 m by 1.0 m and seeds were sown at a spacing of 0.25 and 0.50 m within and between rows, respectively. Diammonium phosphate (DAP) fertilizer was applied at a rate of 50 kg/ha during planting. Experimental plots were weeded twice and diseases and pests controlled by spraying Fusilade and Treflan. Seeds were harvested at maturity and used for phytochemical analysis.

Sesame seeds were also raised in the greenhouse of FABI using a mixture of vermiculite and polystyrene growth media (temperature: 25-37°C; light: 12 hours photoperiod; pH of 5-8; water treatment of 50-70 cm^3). Plant growth was maintained using Hoagland's nutrient solution for fertilization.

Phytochemical screening

Titrimetric method of Harbone was used for quantitative determination of tannins and glycosides (Harbone, 1983), while a spectrophotometric method of the Association of Analytical Chemists was applied for the determination of saponins, alkaloids and flavonoids (Association of analytical chemists, 1989; Odebiyi and Sofowora, 2007).

DNA Isolation

DNA was extracted from leaves based on a previously described protocol (Vos et al., 2007). The Zymo Research plant/seed DNA KIT™ D6020 was used for DNA extraction. The eluted DNA was finally filtered through a Zymo-spinTM IV-HRC spin filter (C1010-50) and collected in a clean 1.5 ml micro-centrifuge tube and centrifuged for 1 min at 8000 g. DNA concentration and purity was determined using the nanodrop technique (ND-1000 Spectrophotometer). The integrity and concentration of the DNA was confirmed by separation of DNA on a 1.5% agarose gel while DNA bands were visualized under UV light after ethidium bromide staining.

PCR analysis of SSRs

The Polymerase Chain Reactions (PCR) for SSR detection was carried out in a PTC-100 thermocycler (MJ Research). Each PCR (20 µl) containing 20 ng of genomic DNA, 2 µl of 10xPCR buffer, 1.2 µl of 25 mM $MgCl_2$, 1.0 µl of 10 mM dNTP, 0.5 µl of 25 mM of forward and reverse primer and 0.2 µl of 5 units *Taq* polymerase (NeoTherm). PCR profile was one cycle at 94°C for 3 min (pre-heating) followed by 35 cycles at 94°C for 30 sec (denaturing), 55–60°C for 45 sec (annealing), 72°C for 30 sec with a final extension at 72°C for 10 min and stored at 4°C. Amplified products were resolved on a 2% agarose gel, visualized under UV light after ethidium bromide staining, and then photographed.

Two SSRs were selected from the literature (Dixit et al., 2005), and used on DNA samples of sesame. PCR-derived SSR products were purified using the QIA quick purification kit and a direct sequencing PCR reaction (10 µl) was performed using 2 µl big dye, 4 µl DNA sample, 1 µl forward or reverse primer, 1 µl big dye buffer and 2 µl of water. The sequencing reaction (PCR) consisted of 35 cycles at 96°C for 10 sec, 57°C for 5 sec, 60°C for 4 min, 72°C for 10 min. Product fragments were cleaned with a Millipore sephacryl S-500 spin column and transferred into a 0.5 ml tube and dried in a vacuum drier at 60°C for 25 min. SSR alleles were resolved on an ABI Prism 3010 DNA sequencer (Applied Biosystems, UK) with GENESCAN 3.7 software.

Molecular data analysis

Repeat numbers were physically counted from DNA sequences. Number of alleles per primer, polymorphism information content (PIC) and gene diversity were determined as previously reported (Weir, 1990), Gene diversity values based on allele frequencies were calculated for each SSR's locus with unbiased statistics (Nei et al., 2007). A dendrogram was constructed by the UPGMA clustering method from the length of SSR sequences with the CLC Bio-sequence analysis software version 6.4 to demonstrate any genetic relationship among sesame accessions at the selected SSR loci.

Results

The number of alleles per SSR locus was 4.0 and the fragment size varied from 179 bp to 282 bp. Expected heterozygosities (H_E) and polymorphism information contents (PICs) applying the POPGENE version 1.32 for analysis (Yeh and Boyle,1997), ranged from 0.70 to 0.77 and 0.66 to 0.74, respectively (Table 1). This indicated a high informative nature of SSRs. Genetic distances among the accessions ranged from 0.00 to 0.20. A dendrogram of the tested 30 Nigerian sesame cultivars was constructed from two SSRs using bootstrapping and the Unweighted Paired Group Method using Arithmetic Average (UPGMA) on the basis of their phytochemicals. Application of the method distinguished the tested accessions into six main clusters and into two cultivars not belonging to any cluster (Figure 1).

In order to correlate the pytochemical content with a specific SSR, the content of specific phytochemicals was evaluated. Phytochemicals determined in sesame seeds were tannins (1.72±0.35 mg/100g), flavonoids (1.14±0.37 mg/100g), saponins (2.40±0.66 mg/100g), cynogenic glycosides (0.42±0.14 mg/100g) and alkaloids (5.89±0.58 mg/100g) (Table 2). Significant variability was found in these phytochemicals among the tested cultivars ($P<0.001$;Table 3). The most abundant phytochemicals in sesame seeds were alkaloids (5.85±0.58) and the least abundant were cyanogenic glycosides (0.42±0.14). Significantly negative correlation ($r = -0.2859$, $P<0.0242$) was found between saponins and flavonoids, while a positive correlation ($r = 0.30391$, $P<0.0163$) was observed between saponins and cyanogenic glycosides (Table 4).

The study further revealed that accessions formed clusters and sub-clusters in relation to the concentration of phytochemicals. The single cultivar “Ciano” in Group I, with a TC_{12} sequence repeats was the lowest in flavonoids but high in cyanogenic glycosides. In contrast, “Yobe machina”, at the opposite end of the dendrogram (Figure 1) comprising Group VIII (TC_{15}), contains a relatively low concentration of tannins and flavonoids, but has higher concentrations of saponins, glycoside cyanogens and alkaloids (Table 2). Group II consisted of five accessions (TC_{20} sequence repeat) all low in tannins and glycoside cyanogens, but high in saponins, and either high or low in flavonoids or alkaloids.

However, one of the Group II sub-clusters containing the “Yobe gadaka white” and “Pachequeno” cultivars had similar phytochemical concentrations. Cultivars in Group III (TC_{19}) slightly differed in their phytochemical concetrations with flavonoids, saponins and alkaloids. Group IV consisted of seven accessions, with a TC_{22} polymorphic repeat sequence. They all had high alkaloid concetrations but varied slightly in the amounts of all other phytochemicals tested. Groups V and VI are seemingly heterogeneous groups of accessions with no consistent association of an SSR to phytochemicals. However, a sub-cluster in Group V consisting of two accessions, “Otobi” and “Domu” (TC_{21}), was consistent in its phytochemical concentration. Group VII cultivars, consisting of six cultivars with TC_{18} and TC_{17} sequence repeats, greatly differed in their phytochemical concentration. A sub-cluster in this group, made up of two accessions “Yobe gadaka brown” and “69-882” with a TC_{17} polymorphic sequence repeat, was almost identical in phytochemical concentrations and consistent with its phylogenetic grouping (Figure 1).

Discussion

Our data indicate that genetic diversity within the characterized Nigerian sesame accessions and SSR repeat motifs (TC_{12}-TC_{25}) could be generated from two highly informative primer pairs (Genebank accession no AY838916, AY838922 repeats motifs; TC,21, TC,15 and TC,20).

The expected heterozygosities (H_E) and polymorphism information contents (PICs) ranged from 0.70 to 0.77 and 0.66 to 0.74, respectively, and number of alleles per SSR locus was 4.0. This indicates genetic variability in accessions rendering these SSRs useful for detection of diversity within Nigerian sesame accessions. This result also confirms previous work with these SSRs by Dixit et al. (2005). However, the overall degree of polymorphism, with four alleles per primer pair in the tested Nigerian accessions is rather low. This seems to be inherent in cultivated sesame due to the domestication process and the inherent self-pollination mechanism.

Variability was found in phytochemical concentrations among the tested Nigerian accession and the difference in concentrations of these phytochemicals among the genotypes might be attributed to genetic differences among members of the diverse Nigerian germplasm collection (Jimoh and Oladiji, 2005). We could, however, roughly cluster tested accessions into six main clusters, with two accessions not belonging to any cluster, regarding phytochemical concentrations present and association with a specific SSR.

The most abundant phytochemicals in sesame seeds were alkaloids and the least available were cyanogenic glycosides. In particular, the single accession "Ciano 27" in Group I, with a TC_{12}, was the lowest in flavonoids but high in cyanogens glycosides. In contrast, "Yobe machina", at the opposite end of a dendrogram comprising Group VIII (TC_{15}), contained a relatively low concentration of tannins and flavonoids, but had higher concentrations of saponins, glycoside cyanogens and alkaloids. The possibility exists that the identified SSR TC_{12} might be associated with low flavonoid concentration in sesame. However, a detailed study has to be carried in the future proving that this SSR is indeed directly associated with a low flavonoid content in sesame. We are currently screening a greater number of sesame accessions to confirm that the identified TC_{12} SSR is associated with the flavonoid trait and that all low flavonoid plants have a TC_{12} SSR.

In many sesame gene-banks, particularly in Africa, only a fraction of the conserved germplasm has actually been characterized in greater detail especially for their nutritional and medicinal value and genetic diversity. This study was therefore a first important step to the characterization of a nutritional and medicinal trait in the Nigerian sesame accessions and possible association with genetic diversity monitored by SSR marker technology. In general, sesame shows a low level of polymorphism since all sesame varieties are derived from the one cultivated sesame species *Sesamum indicum* L. Low level polymorphism has been clearly demonstrated by application of universal markers, including SSRs, and this current lack of useful molecular markers has limited any genetic research.

This study has investigated possible associations between phytochemical and genetic diversity in Nigerian sesame accessions for selection and exploitation in plant breeding programmes and health care. Results indicate that accessions cluster on the basis of their phytochemical composition and genetic diversity explored by SSR markers and in particular one SSR was associated with low flavonoid content.

References

Association of analytical chemists. 1989. Method of determining antinutrients using spectrophotometer. Laboratory Science 21:8-10.

Baumung R., Simianer H., Hoffmann, I. 2004. Genetic diversity studies in farm animals - a survey. Journal of Animal Breeding 121:361–373.

Bruce E.A. 1953. Notes on African pedaliaceae. Kew Bulletin 69:417-429.

Cho Y.L., Park J.H., Lee C.W., Ra W.H., Chung J.W., Lee, J.R., Ma K.H., Lee S.Y., Lee K. S., Lee M.C., Park Y.J. 2011. Evaluation of the genetic diversity and population structure of sesame (*Sesamum indicum* L.) using microsatellite markers. Genes Genomics 33:187–195.

Cook N.S., Samman, S. 1996. Flavonoids – Chemistry, metabolism, cadioprotective effect and dietary sources. Journal of Nutritional Biochemistry 7: 66-*76.*

Dixit A., Jin M. H., Chung J.W., Yu J.W., Chung H.K., Ma K.H,., Park Y.J., Cho E.G. 2005. Development of polymorphic microsatellite markers in sesame (*Sesamum indicum* L.*).* Molecular Ecology Notes 5:736–738.

Gebremichael D.E., Parzies H.K. 2011. Genetic variability among landraces of sesame in Ethiopia. African Crop Science Journal, Vol. 19, No. 1, pp. 1 – 13.

Home Cooking, 1998. Sesame seeds home cooking.about.com/library/weekly/aa060898.htm. International Trade Center. Market development, market brief on sesame seeds. World Overview 3-10.

Jimoh F.O., Oladiji A.T. 2005. Preliminary studies on Piliostigma thonningii seeds: proximate analysis, minerals composition and phytochemical screening. African Journal of Biotechnology 4:1439-1442.

Kinman M.L., Martin J.A. 1999. Present status of sesame breeding in the United States. Agronomy Journal 46:24–27.

Laurentin H., Karlovsky P. 2006. Genetic relationship and diversity in sesame (*Sesamum indicum* L.) germplasm collection using amplified fragments length polymorphisms (AFLP). BioMed Central Genetics 7:10-22.

Musa K.Y., Ahmend A., Ibrahim H., Arowosiaye G., Olanitola O. S. 2000. Phytochemical and antimicrobial studies of leaves of Acalypha racemosa. Nigerian Journal of Natural Products and Medicine 4:67-69.

Nakayama N.G., Lindsey M.L., Michael L.H. 1993. Inhibition of the infectivity of influenza virus by tea polyphenolds. Antiviral Research 21: 289-299.

Nei M., Tamura K., Dudley J., Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24:1596-1599.

Oakenful D., Sidhu G. S. (eds). 1989. Saponins in toxicants of plant origin. (Checke R.R. ed) Acad Press N.Y, 78-113.

Odebiyi A., Sofowora A.E. 2007. Phytochemical screening of Nigerian medicinal plants. Part III Iioydia 41: 243-246.

Oplinger E.S., Putnam D.H., Kaminski A.R., Hanson C.V., Oelke E.A., Schulte,E.E., Doll J.D. (eds). 1997. Sesame: New approaches for crop improvement. In: Janick, J. and Simon, J.E. (eds.). Advances in new crops. Timber Press, Portland, 1971–1980.

Osagie U.A., Okoye W., Oluwayose B.O.,Dawudu, O.A. 1986. Chemical quality parameters and fatty acid composition of oils of some underexploited tropical Seeds. Nigerian Journal of Applied Science 4:151-162.

Powell W., Machay G.C., Provan, J. 1996. Polymorphism revealed by simple sequence repeats, Trends in Plant Science 1:215-222.

Seigler D.S. 1998. Plants with saponins and cardiac glycosides. www.lifwe.vinc.edu/plantbio/363/saponinslides 6-12.

Smith D.E., Salerno J.W. 2001. Selective growth inhibition of a human malignant melanoma cell line by sesame oil invitro. J. Prost. Leuk. Ess. Fatty Acids 46:145–150.

Takundua M., Chimwamurombe P.M, Kunert K., Cullis C.A. 2010. Isolation and characterization of microsatellite repeats in Marama bean (*Tylosema esculentum*) African Journal of Agricultural Research 5:561-566.

Ubi B.E. 2008. Genetic markers and their utility in plant breeding. Journal of Agriculture, Biotechnology and Ecology, Monograph Series 1:1-45.

Vinod K., Sharma S. N. 2011. Comparative potential of phenotypic, ISSR and SSR markers for characterization of sesame (*Sesamum indicum* L.) varieties from India. Journal of Crop Science and Biotechnology 14:163–171.

Vos P., Hogers R., Bleeker,M., Reijans M., Vandelee T., Homes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M. 2007. Development of polymorphic microsatellites markers in sesame (S*esamum indicum L.*). Molecular Ecology 5:736-739.

Wang L., Yanxin Z., Xiaoqiong Q., Yuan G., Xiurong Z. 2012. Development and characterization of 59 polymorphic cDNA-SSR markers for the edible oil crop *Sesamum indicum* (Pedaliaceae). American Journal of Botany e394–e398.

Weir B. (eds). 1990. Genetic data analysis: Methods for discrete population. Sinauer Associates Inc., Sunderland, MA.

Yeh F.C., Boyle T.J.B. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany 129:157-159.

Table 1. Characteristics and sequence information of two SSR-markers used in study.

Locus	GenBank Accession no	Primer Sequence	Repeat motifs	*T*a (°C)	*N*a	Alleles Range (bp)	H_O	H_E	PIC
GBssr-sa-123	AY838916	F: 5-GCAAACACATGCATCCCT-3 R: 5-GCCCTGATGATAAAGCCA-3	(TC)21, (TC)15	61	4	272–282	0.00	0.70	0.66
GBssr-sa-184	AY838922	F: 5-TCTTGCAATGGGGATCAG-3 R5-GAACTATAGATAATCACTTGGAA-3	(TC)20	55	4	179–193	0.20	0.77	0.74

Ta, annealing temperature; Na, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphism information content.

Table 2. Genetic diversity and phytochemical concentration (mg/100g seed fresh weight) in sesame cultivar seeds.

Genotypes	Repeats	Tannins	Flavonoids	Saponins	Cyanogens	Alkaloids
Group I						
Ciano 27	TC12	1.68 L	0.28 L	1.26 L	0.59 H	4.75 L
Group II Kwander	TC20	1.69 L	1.91 H	1.93 H	0.21 L	4.24 L
Incriben0.2m	TC20	1.30 L	2.01 H	2.83 H	0.28 L	6.13 H
Pachequeno	TC20	1.81 L	0.81 L	3.76 H	0.39 L	5.84 H
43-9-1	TC20	1.29 L	1.30 H	2.79 H	0.24 L	5.60 L
Yobe gadaka white	TC20	1.25 L	0.81 L	3.77 H	0.44 L	7.09 H
Group III						
Ciano 16	TC19	2.32 H	0.79 L	2.78 H	0.30 L	4.18 L
69-1-1	TC19	1.61 L	1.20 L	2.78 H	0.80 H	5.37 L
Group IV						
Adaukiari	TC22	1.68 L	2.02 H	2.38 H	0.32 L	5.86 H
Chimkwale	TC22	2.20 H	1.62 H	1.57 L	0.18 L	6.87 H
Chimkwale yellow	TC22	1.10 L	1.76 H	2.06 L	0.21 L	6.14 H
E.8	TC22	1.27 L	0.91 L	1.19 L	0.21 L	6.19 H
Eva	TC22	1.27 L	0.97 L	2.38 H	0.78 H	4.11 L
Zuru	TC22	2.51 H	0.77 L	2.21 L	0.31 L	6.27 H
34-4-1	TC22	2.07 H	1.12 H	3.04 H	0.60 H	5.71 H
Group V						
Alaide	TC21	2.42 H	1.24 L	2.04 L	0.76 H	6.06 H
Cameronu white	TC21	2.50 H	1.77 H	2.83 H	0.46 L	5.18 L
Domu	TC21	1.39 L	0.84 L	2.70 H	0.44 L	6.74 H
Otobi	TC21	1.62 L	0.76 L	3.68 H	0.28 L	6.47 H
Yorri	TC21	2.20 H	0.78 L	3.00 H	0.46 L	6.87 H
Group VI						
ABBS	TC25	2.20 H	0.75 L	3.79 H	0.60 H	5.18 L
Incriben0.3L	TC25	1.35 L	0.81 L	2.04 L	0.46 L	6.44 H
Cross 95	TC25	2.59 H	0.82 L	2.21 L	0.31 L	4.82 L
Group VII						
Jigawa	TC18	1.26 L	2.16 H	0.90 L	0.60 H	6.68 H
Kachia	TC18	1.10 L	1.90 H	1.24 L	0.32 L	5.84 H
Incriben 0.1m	TC18	2.99 H	0.78 L	1.24 L	0.17 L	6.16 H
NCRI (Iwo)	TC18	1.82 L	0.79 L	2.08 L	0.72 H	5.57 L
Yobe gadaka Brown	TC17	1.61 L	0.58 L	3.68 H	0.24 L	6.05 H
69-882	TC17	1.69 L	0.91 L	3.12 H	0.31 L	6.33 H

Group VIII						
Yobe machine	TC15	1.24 L	0.83 L	3.8 H	0.72 H	6.86 H
Mean ± SE	_	1.72± 0.35	1.14±0.37	2.40± 0.66	0.42± 0.14	5.89± 0.58
LSD	_	0.37	0.06	0.05	0.03	0.04
P – Values	-	<.0001	<.0001	<.0001	<.0001	<.0001

NB: Where the mean difference is higher than the LSD values then the cultivar is significantly different from each other in the mean phytochemical content. Similarly, where P<0.05 then it is statistically different in phytochemical composition among genotypes. (1) Tannins; low (L) concentration = 1.10-2.03 mg/100g; high (H) concentration = 2.04-2.99 mg/100g (2) Flavonoids; low (L) = 0.28-1.23 mg/100g; high = (H) 1.24-2.16 mg/100g (3) Saponins; low = 0.90-2.35 mg/100g; high = 2.36-3.8 mg/100g (4) Glycosides; low = 0.17-0.54 mg/100g; high = 0.55-0.80 mg/100g (5) Alkaloids; low = 4.11-5.61 mg/100g; high = 5.62-7.09 mg/100g.

Table 3. Descriptive statistics of phytochemicals in sesame cultivars (mg/100g seed fresh weight).

Phytochemicals	N	Mean	Std Dev	Sum	Minimum	Maximum
Tannins	60	1.72	0.35	106.84	1.10	2.99
Flavonoids	60	1.14	0.37	70.69	0.28	2.16
Saponins	60	2.40	0.66	148.99	0.90	3.80
Glycoside cyanogens	60	0.41	0.14	25.97	0.17	0.80
Alkaloids	60	5.88	0.58	365.00	4.11	7.09

N - Number of variables: alkaloids showed the highest value of 7.09 mg/100 g, followed by saponins 3.8 mg/100 g, while glycoside cyanogens had the lowest value of 0.17 mg/100 g followed by flavonoids 0.28 mg/100 g.

Table 4. Correlation matrix among phytochemical composition of sesame seeds.

Factor	Tannins	Flavonoids	Saponins	Glycoside cyanogens	Alkaloids
Tannins	1.00000				
Flavonoids	-0.08688 0.5019	1.00000			
Saponins	-0.02033 0.8753	-0.28597** 0.0242	1.00000		
Glycoside cyanogens	-0.22773 0.0750	-0.18250 0.1557	0.30391* 0.0163	1.00000	
Alkaloids	-0.19512 0.1286	-0.00883 0.9457	0.12762 0.3229	-0.09952 0.4416	1.00000

*Positively correlated and statistically significant at P<0.05

** Negatively correlated and statistically significant at P<0.05

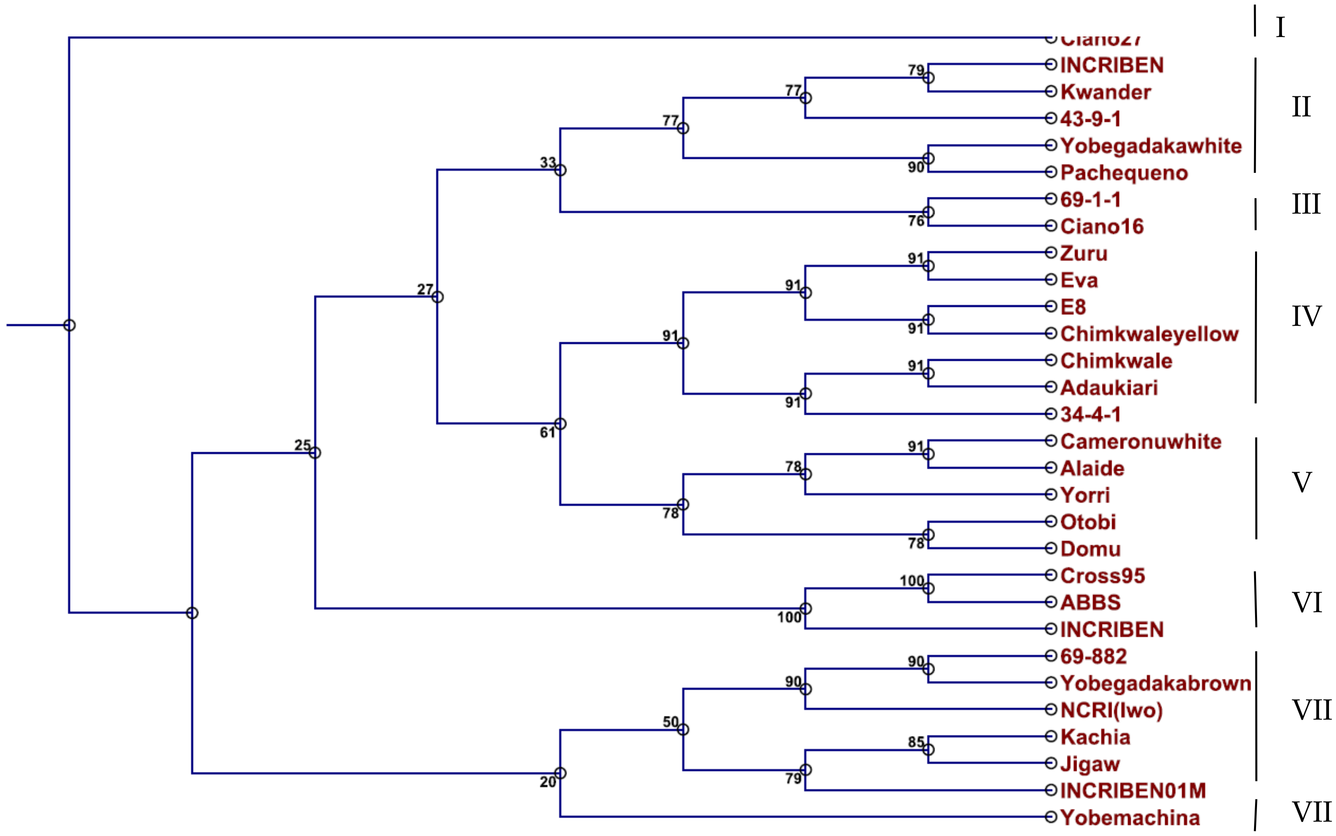

Figure 1. Dendrogram based on two SSR data of 30 Nigerian sesame cultivars using bootstrapping and UPGMA cluster analysis.

Evaluation of the nutrient and health potential of wild and domesticated trifoliate yam (*Dioscorea dumetorum)* in Nigeria

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Abstract
The bitterness, toxicity and long cooking time associated with wild *Dioscorea dumetorum* have led to its neglect and near extinction. Another cultivar which cooks faster and with little or no toxicity was instead developed and domesticated. The objective of this work was to assess the nutrient, anti-nutrient and phytochemical components of the wild and domesticated *D. dumetorum* cuvltivars. Proximate composition was conducted using the standard methods of the Association for Official and Analytical Chemists (AOAC). Gas chromatography/mass spectrophotometry was used to identify the phytochemical components. The results illustrated that there were differences in the nutrient and phytochemical composition of the cultivars investigated. Wild *D. dumetorum* had higher crude protein (11.41%), ash (3.41%), saponin (3.76%) and alkaloid (3.23%) contents than the domesticated cultivar. Thirteen compounds were identified in wild *D. dumetorum* while eleven were identified in the domesticated cultivar. Fatty acids such as cis-oleic acid, lauric acid, myristic acid and palmitic acid were identified in the two *D. dumetorum* cultivars. Phenolic compounds such as 3,5-Di-t-butylphenol and 3-Decanone-5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) which have been reported to have potential antioxidant activity were identified in the wild cultivar. A compound, decahydro {1,7}naphthyridine, was identified for the first time in wild *D. dumetorum*. This work established the nutritional and phytochemical contents of these underutilized cultivars of yam and recommended the increased utilization of wild *D. dumetorum* as a means of ensuring food security.

Keywords: trifoliate yam, phytochemical composition, wild, domesticated.

Introduction
Nigeria is one of the African countries that is endowed with varieties of crops that are required for sustainable food security. Unfortunately, one of the major causes of food insecurity experienced in many African countries and Nigeria in particular is the underutilization of some potential food security crops in the continent (Saka et al., 2004). Amongst the underutilized crops with high food potential in Nigeria is trifoliate yam (*Dioscorea dumetorum* Pax). Trifoliate yam belongs to the genus *Dioscorea* and family Dioscoreaceae (Bai and Ekanayake, 1998). *Dioscorea* is the largest genus of the family Dioscoreaceae, containing between three and six hundred species (Vernier, 1998). *Dioscorea dumetorum* has not been as widely studied as other species. It grows readily on various soils, the yield being 3-7 times that of other widely grown yam species (Treche and Guion, 1980). Nutritionally, *D. dumetorum* is superior to the commonly consumed yams, having higher protein and mineral contents (Baquar and Oke, 1976, 1977; Treche and Guion, 1980). Alozie et al. (2009) reported that the wild variety of *D. dumetorum* had a significantly higher protein content (11.37 g/100g) and quality than the edible variety (7.0 g/100g). The amino acid profile of *D. dumetorum* has been reported to be quite balanced in essential amino acids with slight

deficiency in sulphur-containing amino acids and lysine as the most limiting (Alozie et al., 2009). A feeding diet based on *D. dumetorum* results in higher apparent protein digestibility, net protein retention and net protein utilization than a *D. rotundata*-based diet (Mbome et al., 1995; Lape and Treche, 1994). *Dioscorea dumetorum* consists of the wild cultivar locally called *Ighu* and the hybrid which is locally called *ona*. The wild cultivar is referred to as bitter yam because of the bitterness of the tubers which is caused by the presence of a toxic alkaloid, dihydrodioscorine. Hence the wild type of *D. dumetorum* needs to be detoxified by submerging in running water and thorough processing before being consumed. Cultivars of *D. dumetorum* have been developed which can be consumed without being detoxified because they have a negligible quantity of the bitter alkaloid (Degras, 1993). Trifoliate yam is mainly cultivated for household consumption in Nigeria and the excess is sold in the local market as boiled, unpeeled yams.

Some of the factors militating against increased trifoliate yam production in Nigeria are: long cooking times associated with the tuber, severe hardening which develops after harvest, high concentration of anti-nutrient factors leading to bitterness and toxicity and lack of diversified utilization of the crop. *D. dumetorum* is regarded as an underutilized crop partly due to the lack of detailed information on its compositional analysis. This study investigates the nutritional and health potentials and opportunities in *D. dumetorum*. This will be useful for potential uses of the tuber in the food industry, animal feed industry and cosmetic or pharmaceutical industries. Additionally, increased research on *D. dumetorum* could add to the likelihood of exploitation of the species as an economic plant and bring about further work on its cultivation.

Materials and methods

Two cultivars of *D. dumetorum* (the wild and the cultivated cultivars) were obtained from the yam programme of the National Root Crops Research Institute, Umudike. The tubers were washed peeled, washed and chipped with a chipping machine (locally fabricated). The chips were then air dried. Dried samples were ground in a Mill (Panasonic, MX- J220P) to pass a 1 mm sieve. Proximate composition was determined with the AOAC (1990) method while the anti-nutritional factors such as the alkaloid, saponin, phenol and tannin contents were determined by the method of Obadoni and Ochuko (2001). Compounds present in the ethanol extract of *D. dumetorum* by GC-MS were analysed using a GC-MS-QP2010 PLUS Shimadzu, Japan linked to an Elite 5 MS Column with a length of 20 m and internal diameter of 0.18 µ. The temperature was programmed from 200^{o}C to 300^{o}C at a rate of 4^{o}C min^{-1} with 10 minutes hold. The injector was at 200°C. The carrier gas was helium with a constant flow at 1 ml/min. The mass method used was Electron Ionization with an ionization voltage of (EI+) 70 eV for m/z value 50 to 300 with a scan time of 0.3 sec and interscan delay of 0.1 sec.

The interpretation of mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and discussion

Proximate composition

Figure 1 shows the comparative assessment of the nutritional qualities of *D. dumetorum* and *D. alata*. The crude protein contents of the *Dioscorea* species were 9.12%, 11.41% and 10.62%

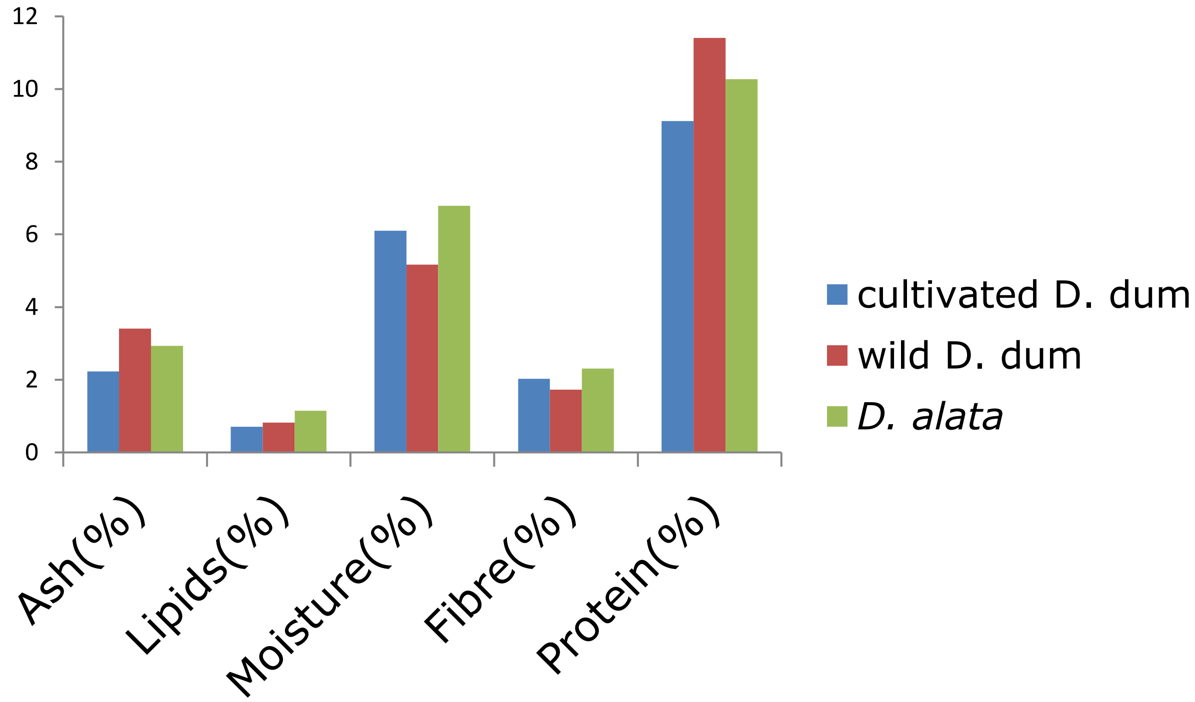
for cultivated *D. dumetorum*, wild *D. dumetorum* and *D. alata* respectively. The protein contents of the *D. dumetorum* cultivars were higher than those reported for other tropical roots including cassava, sweetpotato and taro (FAO, 1972; Onwume, 1978; Bradbury, 1988). The higher protein content of these yams highlights their nutritional superiority as a staple food. Yam proteins are reported to have a better amino acid balance compared to other tropical roots (Splittstoesser et al., 1973). Wild *D. dumetorum* had the highest crude protein content. This emphasizes the advantage of the wild and neglected variety over the cultivated ones.

Fats are very necessary to the structure and biological functions of cells and are used as an alternative energy source. The crude fat content of the *Dioscorea* species ranged from 0.705% to 1.145%. Low lipid contents have been reported for most yams (Osagie, 1992). *D. alata* had a higher lipid content than the *D. dumetorum* cultivars.

The crude fibre content of cultivated *D. dumetorum*, wild *D. dumetorum* and *D. alata* was 2.03%, 1.73% and 2.40% respectively. There is current evidence that dietary fibre may have a direct effect upon some human biochemical and physiological processes. Fibre increases the water-holding capacity and bulk of the stool since hemicellulose and cellulose absorb water and swell. Fibre also lowers the blood low density lipoprotein (LDL) cholesterol levels and improves the cholesterol ratio. Low dietary fibre intake is reportedly associated with several disorders of the human body including diverticulosis and cancer of the colon, constipation, ischaemic heart disease, diabetes and other diseases of the gastro-intestinal tract.

The carbohydrate contents of the yam varieties were quite high; 79.71%, 77.46% and 76.56% for the cultivated *D. dumetorum*, wild *D. dumetorum* and *D. alata* varieties. The high carbohydrate content observed indicates that these yams are good sources of energy and hence reliable food security crops.

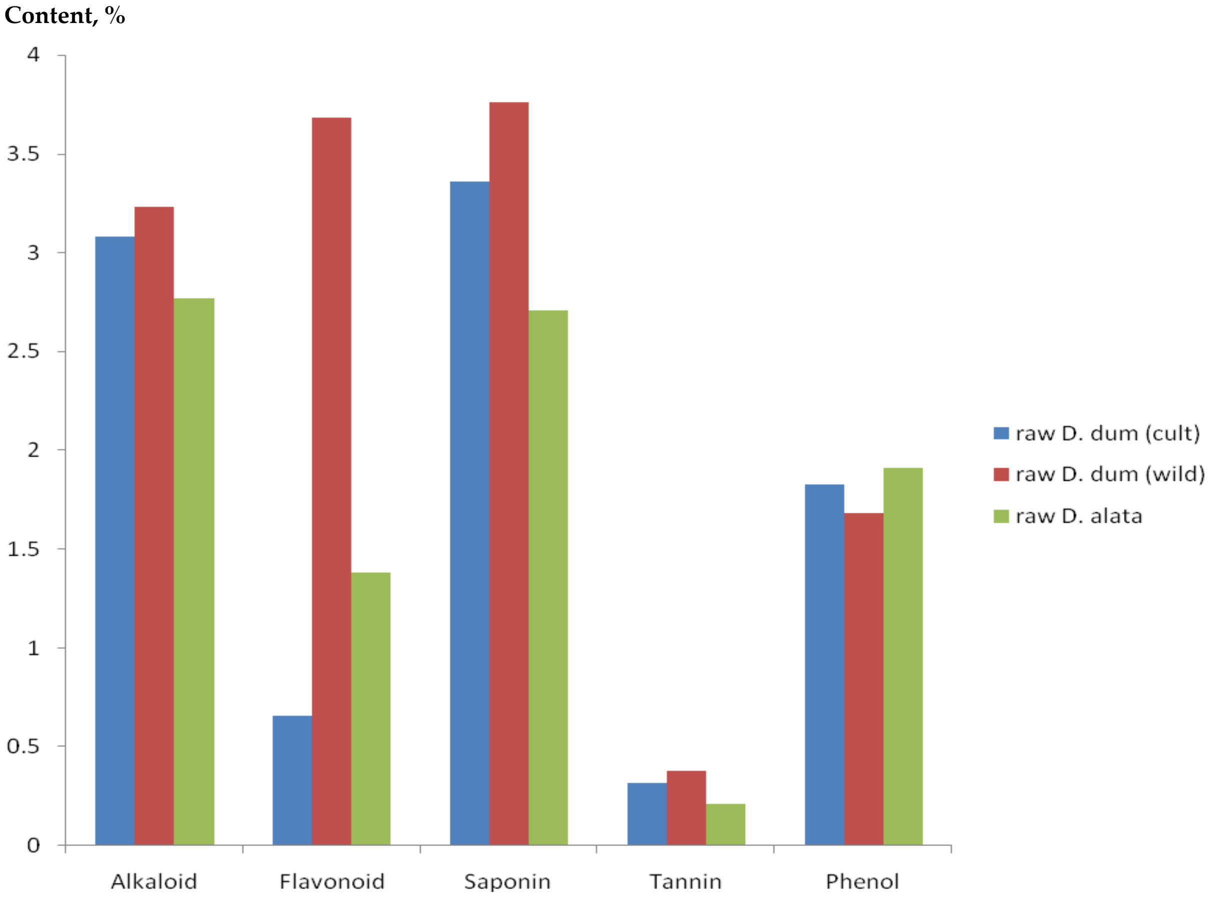
Figure 1. Comparative assessment of the nutritional qualities of *Dioscorea dumetorum* and *D. alata* on a dry weight basis.



Anti-nutrient factors

As shown in Figure 2, wild *D. dumetorum* had the highest saponin content (3.76%) of the *Dioscorea* species investigated while cultivated *D. dumetorum* had the least saponin content (3.36%). The alkaloid content of the wild *D. dumetorum* (3.23%) was also higher than that of the cultivated *D. dumetorum* (3.08%) and *D. alata*. This agrees with the work of Eka, (1998) who reported that alkaloids, including the toxic ones are found more in the wild and bitter varieties of yam. This high alkaloid content of the wild *D. dumetorum* implies that this variety of yam must be thoroughly processed before consumption. Flavonoid content was higher in wild *D. dumetorum* (3.75%), while cultivated *D. dumetorum* (0.68%) and *D. alata* (1.38%) had a lower concentration of flavonoids. Cultivated *D. dumetorum* contained 1.83% phenols while the wild *D. dumetorum* had 1.68% concentration of phenol. Phenolic compounds inhibit the activity of digestive as well as hydrolytic enzymes such as amylase, trypsin, chymotrypsin and lipase (Salunkhe, 1982). Phenolics have been suggested to exhibit health-related functional properties such as anticarcinogenic, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activities (Shetty, 1997).

Figure 2. Anti-nutrient factors of *Dioscorea dumetorum* cultivars and *D. alata.*



Identification of compounds

Table 1 shows the compounds identified in the two cultivars of *D. dumetorum*. GC-MS studies on the ethanol extracts of the tubers resulted in the identification of 13 compounds in cultivated *D. dumetorum* and 10 compounds in the wild *D. dumetorum*. The compounds identified in the tubers included fatty acids and their esters, phenols, sterols, aldehydes and ketones, hydrocarbons and amines.

The main fatty acids identified in the cultivated *D. dumetorum* were oleic acid and palmitic acid. Others include lauric acid, n-pentadecylic acid and 1-tridecane carboxylic. This agrees with the work of Alozie et al. (2010) on the fatty acid composition of *D. dumetorum*. Lauric acid is a saturated fatty acid with a 12-carbon atom chain and has been proven to have antimicrobial properties (Bartolotta et al., 2000; Ouattara et al., 2000; Hoffman et al., 2001; Dawson et al., 2002). N-hexadecanoic acid (palmitic acid) has antioxidant and antimicrobial activities as well as larvicidal effects. It helps relieve atherosclerosis (Bodoprost and Rosemeyer, 2007). Palmitic acid was the most abundant saturated fatty acid in cultivated *D. dumetorum* (21.82%), a similar observation having been made by Alozie et al. (2010) and Muzac-Tucker et al. (1993). Palmitic acid was also identified in the wild *D. dumetorum* (10.83%). Oleic acid is a mono-unsaturated omega-9-fatty acid found in various animal and vegetable sources, and being unsaturated it is considered as a healthy source of fat in the diet. Oleic acid may help boost memory (Valeria et al., 2001). Oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with an increased risk of breast cancer (Valeria et al., 2001). Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil (Terés et al., 2008). It has been reported to have antiinflammatory**, a**ntiandrogenic, cancer preventive, dermatitigenic, hypocholesterolemic, properties and it is reported to have 5-alpha reductase inhibitor, anemiagenic, and insect repellent activities (Spiller, 1996).

3,5, di-t-butyl phenol was identified in the three *Dioscorea* species investigated. The wild *D. dumetorum* had a higher concentration of 3,5, di-t-butyl phenol (1.47%) than the cultivated *D. dumetorum* (1.18%). 3,5-Di-t-butyl phenol is a lipophylic (fat soluble) organic compound, chemically a derivative of phenol, and it is important because of its antimicrobial, antioxidant, anti inflammatory and analgesic properties. Another phenolic compound identified in the wild *D. dumetorum* was 3-decanone-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) at a concentration of 3.38%. This phenol was absent in cultivated *D. dumetorum.* Phenols have been reported to have antioxidant effects. The high concentration of phenolic compounds identified in wild *D. dumetorum* suggests that wild *D. dumetorum* may have higher antioxidant activity than cultivated *D. dumetorum*. Phenols have been identified in potatoes (Petersen et al., 1999), cocoa (Rodriguez-Campos et al., 2012) and *D. alata* (Ozo et al., 1984).

Vanillyl acetone which is an aromatic aldehyde was identified in the wild *D. dumetorum.* Vanillyl acetone has been reported to act as an antioxidant; it plays a significant role in lipid oxidation by inhibiting oxidation of phospholipid liposomes in the presence of iron (III) and ascorbate to prevent heart attacks. Studies show that a typically applied extract containing vanillyl acetone may help to prevent some skin cancers (Preetha et al., 2008). It may be useful in nutritional programmes for arthritis and for fibromyalgia (Preetha et al., 2008).

17-(1,5-dimethyl-hexyl-10-13-dimethyl-1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetra-decahydro-/H-cyclo-penta[a] phenanthren-3-ol which is a phytosterol was identified in the two *Dioscorea dumetorum* cultivars investigated. Plant sterols possess valuable physiological activities; they are biogenetic precursors of many hormones and oviposition stimulants of some insects (Harborne and Williams, 2000). 10-methyl-17-(5-methylhexyl) hexadecahydro-1H-cyclopenta[a] phenanthren-3-ol is one of several phytosterols (plant sterols) with chemical structures similar to that of cholesterol. Alone and in combination with similar phytosterols,17-(1,5-Dimethylhexyl-10-13-dimethyl 2, 3, 4 ,5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-/ H-cyclo-penta[a]phenanthren-3-ol reduces blood levels of cholesterol, and is sometimes used in treating hypercholesterolemia. 17-(1,5-Dimethylhexyl-10-13-dimethyl-2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetra decahydro-/H-cyclo-penta[a]phenanthren-3-ol inhibits cholesterol absorption in the intestine (Matsuoka et al., 2008). Phytosterols and phytostanols both inhibit the uptake of dietary and biliary cholesterol, decreasing the levels of LDL and serum total cholesterol.

9,12-octadecadien-1-ol was identified in both the cultivated and wild *D. dumetorum.* Four esters were identified in cultivated *D. dumetorum* while none was identified in the wild *D. dumetorum*.

A high concentration of an alkaloid decahydro{1,7} naphthyridine was identified in wild *D. dumetorum*. This suggests why wild *D. dumetorum* must be thoroughly processed before consumption.

Conclusions and recommendations

The results obtained from this study showed that *D. dumetorum* has a high potential to contribute to food security and health in Nigeria. Programmes aimed at educating people on the potential value of these crops will help to improve people's perception of these. There should be more public and private sector investment on research and development targeted at conserving and improving the quality of these crops. More effort should be targeted at adding value to the crop so that it can compete in the market.

Acknowledgement

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Table 1. Phytochemical components of *Dioscorea dumetorum*

Compounds	Ret. Time	*D. dum* (cult)	*D. dum* (wild)
Fatty acids			
Lauric acid	25.77	0.56	nd
Myristic acid	29.91	2.25	nd
n-pentadecylic acid	31.35	5.59	nd
Palmitic acid	32.59	21.82	10.83
cis-oleic acid	22.67	10.95	nd
Phenolics			
3.5-Di-t-butyl phenol	23.63	1.18	1.47
3-Decanone.5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)	35.59	nd	3.38
Sterols			
17-(1,5-Dimethyl hexyl-10,13-dimethyl 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17 Tetradecahydro-/H-cyclo-penta[a]phenanthren-3-ol	44.55	5.63	4.47
Aldehydes and ketones			
vanillyl acetone	27.075	nd	0.64
Alcohols			
9.12-octadecadien-1-ol	34.46	33.52	19.81
Hydrocarbons			
(1-methyl-2-piperidinyl)methane	32.11	nd	19.16
Prntadec-1-ene	26.87	nd	0.7
Esters			
2-Hydroxy-1-(hydroxymethyl)ethyl ester	38.85	6.15	nd
Glycerol-1-monolinoleate	38.85	6.39	nd
Palmitic acid beta monoglyceride	37.46	6.07	nd
Methyl(13E,16E)-octadecadienoate	34.29	0.53	nd
Amines			
Oleic acid amide	36.23	1.12	1.92
Alkaloid			
Decahydro{1,7}naphthyridine	30.66	nd	21.09

nd = not detected

References

Alozie Y., Akpanabiatu M.I., Eyong E.U., Umoh I.B., Alozie G. 2009. Amino acid composition of *Dioscorea dumetorum* varieties. Pakistan Journal of Nutrition 8(2): 103-105.

Alozie Y.E., Lawal O.O., Umoh I.B., Akpanabiatu M.I. 2010. Fatty acid composition of *Dioscorea dumentorum* (Px) varieties. African Journal of Food Agriculture Nutrition and Development 10: 2956-2566.

A.O.A.C. (Association of Official Analytical Chemists) 1990. Official methods of analysis (13th ed.) Washington, DC: Association of Official Analytical Chemists.

Bai K.V., Ekanayake I.J. 1998. Taxonomy, Morphology and Floral Biology. In: Food Yams. Advances in Research. (Orkwor, G., Asiedu, R. and Ekanayake, I.J., editors) IITA/NRCRI, Nigeria co-publication. International Institute of Tropical Agriculture, Ibadan and National Root Crops Research Institute, Umudike, pp13-37.

Baquar S. R., Oke O. L. 1976. Protein in Nigerian yams (Dioscorea spp.). Nutrient Reports International 14: 237–248.

Baquar S.R., Oke O.1. 1977. Mineral constituents of Nigerian yams. Nutrition Reports International 15: 265-272.

Bradbury J.H. 1988. The chemical composition of tropical root crops. ASEAN Food J. 4:3-13.

Bartolotta S. García C. C., Candurra N. A., Damonte E. B. 2000. Effect of fatty acids on arenavirus replication: inhibition of virus production by lauric acid. Archives of Virology 146: 777–790.

Bodoprost J., Rosemeyer H. 2007. Analysis of phenacylester derivatives of fatty acids from human skin surface sebum by reversed-phase HPTLC: chromatographic mobility as a function of physicochemical properties. International Journal of Molecular Sciences 8: 1111–1124.

Dawson P.L., Carl G.D., Acton J.C., Han I.Y. 2002. "Effect of lauric acid and nisin-impregnated soy-based films on the growth of *Listeria monocytogenes* on turkey bologna". Poultry Science 81 (5): 721–726.

Degrass L. 1993 The Yam: A Tropical Root Crop. The Macmillan Press Ltd, London, England.

Eka O.U 1998. Root and Tuber Crops, In nutritional quality of plant foods. Ossagie A, Eka OA (eds) Postharvest res. Unit Publ. Univ. Benin.

FAO. 1972. Food Composition Table for use in Asia. FAO, Rome.

Harborne J.B., Williams, C.A. 2000. Advances in flavonoid research since 1992. Phytochemistry 55:481–504.

Hoffman K.L., Han I.Y., Dawson P.L. 2001. Antimicrobial effects of corn zein films impregnated with nisin, lauric acid, and EDTA. Journal of Food Protection 64 (6): 885–9.

Lape M. I., Treche, S. 1994. Nutritional quality of yam (*Dioscorea dumentorum* and *D. rotundata*) flours for growing rats. Journal of the Science of Food and Agriculture 66: 447-455.

Matsuoka K., Nakazawa T., Nakamura A., Honda C., Endo K., Tsukada, M. 2008. Study of Thermodynamic Parameters for Solubilization of Plant Sterol and Stanol in Bile Salt Micelles. Chemistry and Physics of Lipids 154: 87-93.

Mbome I.L., Agbor E.T., Treche, S. 1995. Digestibility and metabolism of flour from two yam species (*D. dumetorum* and *D. rotundata*) in school age children. Ecology of Food and Nutrition 34: 217-225.

Muzac-Tucker I., Asemota H.N., Ahmad M.H. 1993. Biochemical composition and storage of Jamaica Yams (*Dioscorea* spp.). Journal of the Science of Food and Agriculture 62: 219-224.

Obadoni B.O., Ochuko P.O. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences 8: 203-208.

Onwueme I.C. 1978. The tropical tuber crops: Yams, cassava, sweet potato and cocoyams. Chichester: John Wiley & Sons.

Osagie A.U. 1992. The Yam in Storage. Post Harvest Research Unit, University of Benin, Nigeria pp. 25-32.

Ouattara B., Simard R.E., Piett G., Bégin A.,Holley, R.A. 2000. "Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan". International Journal of Food Microbiology 62 (1-2): 139–48.

Ozo O. N., Caygill J. C.,Coursey, D. G. 1984. Phenolics of five yam (Dioscorea) species. Phytochemistry 23(2), 329–331.

Petersen M.A., Poll L., Larsen, L.M. (1999). Identification of compounds contributing to boiled potato off-flavour (POF). Lebensmittel-Wissenschaft and Technologie 32(1):32-40.

Preetha A., Ajaikumar B.K., Clitra S., Kuzhuvelil B.H., Sheeja T.T., Oiki S.L., Bokyang S., Bharat B.A. 2008. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical Research 25(9) ; 2097-2116.

Rodriguez-Campos J., Escalona-Buendía H.B., Contreras-Ramos S.M., Orozco-Avila I., Jaramillo-Flores E., Lugo-Cervantes, E. 2012. Effect of fermentation time and drying temperature on volatile compounds in cocoa. Food Chemistry 132 ; 277–288.

Saka J.O., Ajibade S.R., Adeniyan O.N., Olowoyo R.B.J., Ogunbodede B.A. 2004. Survey of underutilized grain legume production systems in the Southwest agricultural zone of Nigeria. Journal of Agriculture and Food Information 6: 93-108.

Splittstoesser W.E., Martin F.W., Rhodes A.M. 1973. The amino acid composition of five species of yams (*Dioscorea*). Jornal of the American Society for Horticultural Science 98: 563-567.

Salunkhe D.K. 1982. Legumes in human nutrition: current status and future research needs. Current Science 51: 387-394.

Shetty K. 1997. Biotechnology to harness the benefits of dietary phenolics focus on Lamiaceae. Asia Pacific Journal of Clinical Nutrition 6: 162-171.

Spiller G.A. 1996. CRC Handbook on Lipids in Human Nutrition. CRC Press Boca Raton, FL. 233pp.

Treche S., Guion P. 1980. The nutritional potential of some tropical tuber crops in the Cameroons: influence of maturity of harvest. L'Agronomie Tropicale 34(2): 127-137

Terés S., Barceló-Coblijn G., Benet M., Alvarez R., Bressani R., Halver J. E., Escribá, P.V. 2008. Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proceedings of the National Academy of Sciences of the United States of America 105 (37): 13811.

Vernier P. 1998. Yam chips production in West Africa. The news letter of post harvest Africa. International institute of tropical agriculture. No. 2.

Valeria Pala., Vittorio K., Paola M., Véronique C., Elio R., Andrea M., Mitra S., Sabina S., Franco B. 2001. Erythrocyte Membrane Fatty Acids and Subsequent Breast Cancer: a Prospective Italian Study. Journal of the National Cancer Institute 93 (14): 1088.

Velvet bean (*Mucuna pruriens*) in monogastric animal nutrition: effect of some local processing methods.

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Abstract

Velvet bean (*Mucuna pruriens*) plants are widely used as a cover crop in many tropical countries. It yields heavily in seeds that are little known and used by humans or used for animals. Available data on its nutritional value indicate that the seed is high in protein but contains toxic substances which limit its use in monogastric animal feeds. We examined the effect of some local processing methods (cooking, toasting, soaking in water or calcium hydroxide solution treatment prior to cooking and, cracking the seeds prior to soaking and cooking in water or maize-cob-ash solution) of velvet bean on the performances of broilers, laying hens and pigs in different experiments. The proximate compositions of velvet bean subjected to the various processes varied considerably depending on the type of the process. Whole velvet bean cooked for 1 h in water allowed for 10% inclusion in broiler diets while velvet bean soaked for 48 h in water or calcium hydroxide selection before cooking for 1 h allowed for 20%. Cracked velvet bean soaked in water before cooking in water improved the performance of broilers or laying hens at 20% inclusion and pigs at 40% while cooking in maize cob ash solution improved broiler performance at the 30% inclusion level. This paper discusses various methods by which the nutritive value of velvet bean can be improved for monogastric animals and calls for its development as an economic crop in order to alleviate the current pressure on soybean and groundnut meals.

Key words: Velvet bean, processing methods, proximate composition, monogastric, performance

Introduction

Velvet bean (*Mucuna pruriens*) is an annual, or perennial, herbaceous, vigorous climbing leguminous vine. It is an important cover crop (or green manure crop) in many parts of the world, especially among subsistence farmers (Buckles, 1995). It improves soil fertility (Berhe, 2001) and is efficient in the control of weeds (Akobundu and Udensi, 1995; Carsky et al., 1998).

Velvet bean can tolerate a wide range of well drained soils, and has excellent germination and vigorous initial growth characteristics that makes its establishment relatively easy. It takes about 180-270 days to reach maturity with the pods containing three to five black seeds that are relatively large – about 1.2 to 1.5 cm long and 0.9 to 1.1 cm broad, three times the size of the soybean. Its yields range from 10 to 35 tons green material/ha and 250 to 3300 kg seeds/ha depending on the cultivation conditions (Ecocrop, 2011).

Like other beans, velvet bean contains important quantities of proteins, vitamins and minerals necessary for the diet of humans and many animals (Del Carmen et al., 1999; Udedibie and Carlini, 1998). The crude protein content of dried seeds ranges from 21% to 32%. It has a good essential amino acid profile with a methionine and lysine content of 1.0 g and 7.1 g per 16 gN. Relative to protein content, the amount of essential amino acids in velvet bean is comparable in most cases to those in soybean meal (Del Carmen et al., 1999).

Unfortunately, the beans have been neglected for a long time in all respects, be it research and production or from consumption and nutrition standpoints. The bean can be said to be 'underutilized' since little has been done to explore ways to stimulate its production, processing, marketing or use. For many years velvet bean seeds have been considered useless in poultry rations because of poor growth (Olaboro et al., 1991, Emenalom and Udedibie, 1998) and poor egg production (Harms et al., 1961) encountered with their use.

Previous studies have shown that the bean contains antitrypsin factors, tannins and cyanides (Houghton and Shari, 1994; Udedibie and Carlini, 1998), analgesic, antipyretic and anti-inflammatory factors (Lauk et al., 1993), and others (Olaboro et al., 1991). L-dopa, a potentially neurotoxic agent is found in relatively large amount in the velvet bean (Bell and Janzen, 1971).However studies have shown that anti-nutritional factors in legumes can be eliminated or inactivated, to a large extent, by appropriate heating and processing during food preparation (Udedibie and Carlini, 1998).

This paper therefore discusses the various methods of processing velvet beans developed so far at our station with the aim of developing it as a possible protein and energy feed for monogastric animal industries in tropical countries.

Processing methods and feeding trials.

Velvet bean seeds were subjected to different processing methods and used in different feeding trials as follows:

1. **Raw**: whole raw velvet bean seeds were ground into meal using a hammer mill.
2. **Cooking**: whole velvet bean seeds were cooked for 60 minutes in water, taking the period of cooking as starting from boiling, then sun dried and ground into meal.
3. **Toasting**: raw velvet beans were ground into meal and toasted by having the meal thinly spread on a pan seated over a tripod stand with firewood as the source of heat. The meal was stirred from time to time in batches to maintain uniform heating. The toasting was considered adequate when the meal changed from whitish to light brown and became crispy to the touch.

Feeding trial

Addition of 10% raw velvet bean, toasted velvet bean and cooked velvet bean and 20% cooked velvet bean in broiler finisher diets fed at 5-8 weeks of age caused a decline in growth and by the end of the trials the birds weighed 63.2, 71.3, 82.7 and 66.0% of the controls, respectively (Table. 1). Birds fed toasted and 20% cooked velvet bean had a significantly lower feed intake than the control. With raw velvet bean there was clear depression in growth rate and marked deterioration in feed conversion values but not feed intakes. Weights of heart and gizzard were heavier in velvet bean diet groups than the control. Liver weight decreased only with the 10% cooked velvet bean diet. A decrease in organ weights would be expected as the birds grow more slowly, but the changes in liver and gizzard weights did not follow this expectation. The increases in weight may represent a greater burden of digestion and metabolism in these organs in the processing of velvet bean.

Table 1**.** Performance and organ weights of finisher broilers fed raw, cooked and toasted velvet bean

	Control	10%Raw velvet bean	10% Cooked velvet bean	20%Cooked velvet bean	10%Toasted velvet bean
Feed Intake (gm/d)	88.95[a]	89.39[a]	82.13[a]	68.34[b]	73.64[b]
Growth Rate (gm/d)	48.19[a]	30.46[b]	39.85[a]	31.82[b]	34.39[b]
Feed Conv.	1.85[a]	2.96[b]	2.08[a]	2.16[a]	2.14[a]
Heart	0.45	0.54	0.51	0.56	0.54
Liver	1.81[a]	2.07[b]	1.74[a]	2.06[b]	1.85[a]
Gizzard	4.31[a]	5.06[b]	4.57[a]	5.01[b]	5.18[b]

Adapted from: Emenalom and Udedibie (1998). Means followed by different letters P<0.05.

4. **Soaking in water prior to cooking:** Following the finding of Udedibie and Carlini (1998) and Wanjekeche (2001) that presoaking in water greatly reduces cooking time to normal softness, velvet bean seeds were soaked in water for 48 h, rinsed with fresh water, cooked for 60 minutes, sun dried and ground into meal.

Feeding trial

Adding water-soaked and cooked Nigerian or Brazilian velvet bean in broiler finisher diets at 20% and 30% levels each, caused 12%, 10% and 28%, 19% reductions in growth rate (Table. 2). There was a marked deterioration in feed conversion values only in birds fed 30% velvet bean. At the 20% dietary level, the birds compared favourably with those on the control diet.

Table 2**.** Performance of broilers fed soaked and cooked Nigerian and Brazilian velvet bean (5-9 weeks)

Measurements	Control	20% NMS	30% NMS	20%BMS	30%BMS
Initial body wt (g)	662.8	648.8	660.2	658.9	658.4
Final body wt (kg)	1.89[a]	1.72[a]	1.54[b]	1.76[a]	1.65[b]
Avg. daily gain (g)	43.8[a]	38.3[a]	31.4[b]	39.3[a]	35.4[ab]
Feed intake (g/d)	166.4[a]	151.1[ab]	160.4[a]	144.4[b]	170.0[a]
Feed gain ratio	3.60[a]	3.95[a]	5.10[b]	3.67[a]	4.82[b]

Adapted from: Udedibie et al. (2001). Means followed by different letters P<0.05; NMS – Nigerian *Mucuna* seed; BMS – Brazilian *Mucuna* seed

5. **Cracking prior to soaking and cooking**: whole velvet bean seeds were cracked into 2-4 parts per seed, soaked in water for 48 h, cooked for 60 minutes, sun dried and ground into meal.

Feeding trials with broilers, laying hens and pigs.

Adding 20-30% cracked-soaked and cooked *Mucuna* to broiler rations fed 7 to 38 days caused a decline in weight gain (Table 3), and by the 28th day of age broilers fed 20%, 25% and 30% velvet bean weighed 87.4%, 89.6% and 83.3% of the controls, respectively. Feed intake values were unchanged while feed conversion values deteriorated only with 30% velvet bean.

Table 3. Performance of broilers fed cracked-soaked and cooked velvet bean (1-5 weeks)

Parameters	Control (0.0%)	CSCM (20.0%)	CSCM (25.0%)	CSCM (30.0%)
Average initial weight (g)	291.67	289.59	291.67	300.00
Average final weight (g)	954.17	868.75	885.42	862.50
Average weight gain (g)	662.50	579.17	593.75	562.50
Growth rate (g/b/d)	31.55	27.57	28.27	26.29
Average total feed intake (kg)	1.74	1.70	1.78	1.82
Feed conversion ratio (g feed/g gain)	2.63[b]	2.94[ab]	2.99[ab]	3.24[a]

Adapted from: Emenalom et al. (2005a). Means followed by different letters P<0.05; CSCM: cracked-soaked and cooked *Mucuna.*

Adding 20-30% cracked-soaked and cooked *Mucuna* in broiler finisher diets fed at 28-42 days of age caused a decline in weight gain (Table 4) and by 42 days of age broilers fed 20%, 25% and 30% velvet bean weighed 85.0%, 85.0% and 61.1% of controls, respectively. Feed intake values also declined but were not significantly lower than the control. There was a marked deterioration in feed conversion values except at 30% velvet bean levels.

Table 4. Performance of finisher broiler fed cracked and soaked and cooked velvet bean seed meal diets.

Parameters	0%	20%CSCM	25%CSCM	30%CSCM
Average initial weight (kg)	0.97	0.97	0.98	0.97
Average final weight (kg)	2.91[a]	2.62[b]	2.62[b]	2.61[a]
Average weight gain (kg)	1.93[a]	1.64[a]	1.64[a]	1.18[b]
Daily weight gain (g/b/d)	55.17[a]	46.90[a]	46.80[a]	33.75[b]
Feed intake (kg)	5.37	4.84	5.04	4.79
Protein ratio	1.79[a]	1.71[a]	1.64[a]	1.26[a]
Feed conversion ratio	2.78[b]	2.95[b]	3.08[b]	4.06[a]

Adapted from: Emenalom et al. (2008a). Means followed by different letters P<0.05; CSCM: cracked-soaked and cooked *Mucuna.*

Adding 20-30% of the processed velvet bean in the diet of laying hens caused a progressive declined in hen-day egg production (Table 5) and by the end of the trial, laying hens fed 20%, 25% and 30% velvet bean produced 88.1%, 82.1% and 77.5% of the control levels, respectively. Feed intake values increased significantly with 25% and 30% velvet bean. Birds on velvet bean diets produced significantly heavier and bigger sized eggs than the control. Feed conversion ratio (kg feed/kg egg) was better in the velvet bean group than the control.

Adding 15% raw, and 20%, 30% and 40% cracked-soaked and cooked *Mucuna* to pig rations caused a significant decline in weight gain only with 15% raw velvet bean (Table 6) and by the end of the experiment, pigs fed the different diets weighed 72.6%, 92.5%, 94.1% and 95.2 % of the controls, respectively. Feed intake values were comparable among the treatments while feed conversion value deteriorated only with raw velvet bean. Mortality occurred only in pigs fed raw velvet bean diet. Organ weights (liver, heart and kidney) were significantly lower with raw velvet bean than with the other treatment groups. Carcass yield was significantly better in pigs fed 20% velvet bean diets than the other velvet bean diet groups when compared with the control.

Table 5. Effect of cracked-soaked and cooked velvet bean on the performance and egg quality characteristics of laying hens.

Parameters	Control (0.0%)	CSCM (20%)	CSCM (25%)	CSCM (30%)
Hen-day production (%)	77.80[a]	68.53[a]	63.86b	60.32[b]
Av.egg weights (g)	61.31[b]	65.22[ab]	66.65[a]	68.19[a]
Feed intake (g/b/d)	112.68[b]	116.05[a]	115.81[a]	112.66[b]
Kg feed/kg egg	1.84[a]	1.78[a]	1.74a[b]	1.65[b]
Haugh unit (HU)	78.03	80.69	78.18	79.93
Shell Thickness (mm)	0.39	0.36	0.38	0.36
Yolk index	0.39	0.40	0.41	0.40
Albumen index	0.05	0.05	0.05	0.05
Horizontal circum. (cm)	13.93[b]	14.17[ab]	14.33[a]	14.07[b]
Oblong circum. (cm)	16.16a[b]	16.56[a]	16.63[a]	15.93[b]

Adapted from: Emenalom et al. (2008b). Means followed by different letters $P<0.05$; CSCM: cracked-soaked and cooked *Mucuna.*

Table 6. Performance, carcass and organ weights of pigs fed raw and, cracked-soaked and cooked velvet bean

Parameters	Control (0%)	RM` (15%)	CSCM (20%)	CSCM (30%)	CSCM (40%)
Initial live weight (kg)	17.00	17.25	17.50	17.25	17.25
Final live weight (kg)	35.50[a]	28.50[b]	31.83[a]	31.83[a]	32.00[a]
Weight gain (kg)	15.50[a]	11.25[b]	14.33[a]	14.58[a]	14.75[a]
Growth rate (g/d)	369[a]	268[a]	341[a]	341[a]	351[a]
Feed intake (kg) (Dry wt.)	36.9	36.0	36.9	36.9	36.9
Feed conv. ratio	2.38[b]	3.20[a]	2.58[b]	2.53[b]	2.50[b]
Mortality (%)	0.00	0.50	0.00	0.00	0.00
Carcass weight (kg)	25.00[a]	24.00[a]	25.00[a]	20.00[b]	21.00[ab]
Dressed percentage (%)	65.79[a]	60.00[b]	65.79[a]	58.82[b]	60.00[b]
Liver (%)	5.55[b]	5.75[b]	5.68[b]	6.50[a]	5.71[b]
Heart (%)	1.87[a]	0.95[c]	1.74[ab]	1.38[abc]	1.23[bc]
Kidney (%)	1.18[ab]	0.95[c]	1.05[bc]	1.29[a]	0.97[c]
Lungs (%)	3.11[ab]	2.20[b]	3.82[a]	4.32[a]	2.46[b]

Adapted from: Emenalom et al. (2004). Means followed by different letters $P<0.05$; CSCM: cracked-soaked and cooked *Mucuna.*

6. **Soaking in $Ca(OH)_2$ solution**: following the finding of Ruiz Sesma (1999) that 24h soaking in water containing 4% $Ca(OH)_2$ improved the nutritive value of velvet bean, whole velvet bean seeds were subjected to three different processing methods: 1) velvet bean was soaked in water for 48 h, cooked for 60 minutes, sun dried and ground into meal; 2) velvet bean was processed as in 1, but the soaking was done in water containing 3% $Ca(OH)_2$ and 3) velvet bean was soaked in water containing 3% $Ca(OH)_2$, rinsed with fresh water, sun dried and ground into meal.

Feeding trials with broilers

Adding 20% $Ca(OH)_2$-soaked, $Ca(OH)_2$-soaked and cooked, and water-soaked and cooked velvet bean to starter broiler rations fed at 7-28 days of age caused a decline in weight gain

(Table 7) and by the 28th day of age, broilers fed the diets weighed 44.0%, 86.1% and 82.0% of controls, respectively. Feed intake values were not significantly different when compared with the control. Feed conversion values deteriorated only with $Ca(OH)_2$-soaked velvet bean. Birds fed 20% $Ca(OH)_2$-soaked and cooked velvet bean had a slightly better performance than those fed water-soaked and cooked velvet bean, indicating a positive effect of the alkaline on the seed.

Adding 20% and 30% $Ca(OH)_2$-soaked and cooked velvet bean in the diet of finisher broilers caused a progressive decline in weight gain (Table 8) and by the end of the experiment the birds weights were 97.0% and 80.0% of the controls respectively. Feed intake values increased significantly only with 20% velvet bean, while feed conversion ratio deteriorated with 30% velvet bean when compared with the control. Organ weight (heart, liver lungs and gizzard) increased with velvet bean diets in spite of the lower weights of the birds.

Table 7. Performance of broilers fed $Ca(OH)_2$-soaked, $Ca(OH)_2$-soaked and cooked , and water-soaked and cooked velvet bean (1-5 weeks)

Parameters	Control	CS 20%	CSC 20%	WSC 20%
Initial body wt (g)	101.0	102.0	101.0	102.0
Final body wt (g)	775.0^a	398.0^b	690.0^a	665.0^a
Average wt gain (g)	674.0^a	296.0^b	580.0^a	553.0^a
Growth rate (g/d)	32.1^a	14.1^b	28.1^a	26.3^a
Feed intake (g/b/d)	56.2^{ab}	48.1^b	60.8^a	63.24^a
Feed conv. Ratio	1.75^a	3.41^a	2.16^b	2.41^b

Adapted from: Emenalom (2004). Means followed by different letters $P<0.05$; CS: $Ca(OH)_2$-soaked; CSC: $Ca(OH)_2$-soaked and cooked; WSC: water-soaked and cooked.

Table 8. Performance and organ weight of broilers fed $Ca(OH)_2$-soaked and cooked velvet bean (5-9 weeks)

	Dietary levels of velvet bean meals (%)		
Parameters	0.0%	20.0%	30.0%
Initial weight (kg)	0.90	0.90	0.90
Body weight (kg)	2.71^a	2.68^{ab}	2.35^b
Weight gain (kg)	1.81^a	1.78^{ab}	1.45^b
Growth rate (g/d)	65.00^a	63.00^{ab}	52.00^b
Total feed intake (kg)	4.58^b	4.76^a	4.38^b
Feed conversion ratio	2.57^b	2.73^{ab}	3.02^a
Heart	0.36^b	0.43^a	0.42^a
Liver	1.45	1.67	1.57
Lungs	0.51	0.56	0.61
Gizzard	2.16^b	2.42^{ab}	2.77^a

Adapted from: Emenalom and Nwachukwu (2006). Means followed by different letters $P<0.05$

7. **Soaking in water prior to cooking in maize-cob-ash solution:** whole and cracked velvet bean seeds were differently soaked in water for 48 h, cooked for 60 minutes (starting from boiling) in maize-cob-ash solution (in 1:4 water dilutions), sun dried and ground into meal, respectively. The meals thus produced were incorporated into broiler diets at 25% and 30% levels each.

Feeding trial with broilers

Addition of 25% and 30% each of water-soaked whole or cracked velvet bean, cooked in maize-cob-ash solution in starter broiler diets fed 0-28 days of age improved weight gain (Table 9) and by the 28th day, the birds gained 95.9%, 84.4%, 105.9% and 95.4% of control weights, respectively. Feed intake values increased significantly only with 25% cracked velvet bean, while feed conversion values were similar among treatments. At 25% and 30% inclusion levels, birds fed cracked velvet bean diets had a slightly better performance than those on whole velvet bean diets.

Table 9. Performance of broilers fed water-soaked whole or cracked velvet bean cooked in maize cob ash solution (0 - 4 weeks)

	Control	Whole velvet bean		Cracked velvet bean	
Parameters	0%	25%	30%	25%	30%
Av. Initial wt (g)	76.0	76.5	76.1	77.7	76.3
Av. final wt (g)	895^{ab}	862^{ab}	768^{b}	944^{a}	857^{ab}
Daily growth rate	39.0^{ab}	37.4^{b}	32.9^{c}	41.3^{a}	37.2^{b}
Av. daily feed intake	77.3^{b}	73.6^{b}	71.6^{b}	86.2^{a}	70.7^{b}
Feed conv. Ratio	1.98	1.99	2.22	2.14	1.94
Mortality	2	2	-	2	2

Adapted from: Emenalom et al. (2005b). Means followed by different letters P<0.05.

Addition of 25% and 30% levels each of water-soaked whole and water-soaked cracked velvet bean cooked in maize-cob-ash solution in finisher broiler diets fed at 29-42 days of age also improved weight gain (Table 10) and by the 42nd day, the birds gained 96.7%, 82.5%, 97.9% and 98.7% of control weights, respectively. Feed intake values increased significantly with 30% cracked velvet bean and decreased with 30% whole velvet bean when compared with the control. Birds fed with the 30% cracked velvet bean diet recorded the best feed conversion value that compared statistically with the control. Again, at 25% and 30% inclusion levels, birds fed cracked velvet bean diets had a slightly better performance than those on whole velvet bean diets. Indeed, water-soaked whole or cracked velvet bean cooked in maize-cob-ash solution appeared to be better than groundnut cake in broiler diets (Emenalom et al., 2009).

Table 10. Performance of broilers fed water-soaked whole or cracked velvet bean cooked in maize-cob-ash solution (5-8weeks)

Parameters	Control	25%Wvelvet bean	30%Wvelvet bean	25%Cvelvet bean	30%Cvelvet bean
Initial weights	431.3	435.5	433.4	456.3	448.0
Final body weights	1772.8^{b}	1731.7^{b}	1540.0^{a}	1770.0^{b}	1773.3^{b}
Weight gain	1341.5^{b}	1296.2^{b}	1106.6^{a}	1313.7^{b}	1325.4^{b}
Growth rate (g/d)	47.9^{b}	46.3b	39.5^{a}	46.9^{b}	47.3^{b}
Feed intake	3.38^{bc}	4.08^{a}	2.98^{c}	3.96^{ab}	4.31^{a}
Feed conv. Ratio	2.52^{b}	3.31^{a}	2.77^{ab}	3.02^{ab}	2.28^{ab}
Mortality (%)	2	3	-	2	-

Means followed by different letters P<0.05. Wvelvet bean: whole velvet bean; Cvelvet bean: cracked velvet bean.

Conclusion

Velvet bean (*Mucuna pruriens*) plants are widely used around the world as a soil improvement/cover crop. It produces prolific quantities of the beans or seeds that are little known and used as food or feed by humans or animals. Fortunately, the results of the various trials herein summarized show that processing methods can improve the nutritional and/or toxic characteristics of velvet bean, and that dietary levels of 20 to 40% can be attained in poultry and pigs, depending on the method of processing one chooses.

There is need therefore to promote the introduction of the velvet bean into the farming system of developing tropical countries in order to develop it as an economic crop. As an economic crop, it will obviously add to animal feed supply, and also help to reduce the current pressure on soybean, groundnut and maize as major feed ingredients in poultry and pig diets as there is little or no competition between humans and animals for velvet bean seeds.

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References

Akobundu I.O., Udensi U.E. 1995. Effect of *Mucuna* species and fertilizer levels on the control of Imperata (*Imperata cylindrical* (L). Abstract in Weed Science Society of Nigeria. 22nd Annual Conference, 6 – 10 Nov.1995. IITA, Ibadan, Nigeria.

Bell E. A., Janzen D.H. 1971. Medical and ecological consideration of L. Dopa and 5-HTP in seeds. Nature 229: 136-137.

Berhe T. 2001. *Mucuna*: Restoring the soil nutrients: In Feeding the future. Newsletter of the Sasakawa African Association. Issue 16:5.

Buckles D. 1995. Velvet Bean (*Mucuna pruriens*): A "new" plant with a history. Economic Botany 49(1): 1 3-25.

Carsky R. J., Tarawali S.A., Becker M., Chikoye D., Tian G., Sanginga N. 1998. *Mucuna* – herbaceous cover legume with potential for multiple uses. Resource and crop Management Research Monograph No.25 IITA. Ibadan, Nigeria.

Del Carmen J., Gernat A.G., Myhrman R., Carew L.B. 1999. Evaluation of raw and heated velvet beans (*Mucuna pruriens*) as feed ingredients for broilers. Poultry Science 78: 866-872.

Ecocrop, 2011. Ecocrop database. FAO http://ecocrop.fao.org/ecocrop/srv/en/home

Emenalom O.O., Udedibie A.B.I. 1998. Effect of dietary raw, cooked and toasted *Mucuna pruriens* seeds (velvet bean) on the performance of finisher broilers. Nigerian Journal of Animal Production 25 (2): 115 –119..

Emenalom O.O., Udedibie A.B.I., Esonu B. O., Etuk E.B., Emenike H.I. 2004. Evaluation of unprocessed and cracked, soaked and cooked velvet beans (*Mucuna pruriens*) as feed ingredients for pigs. Livestock Research for Rural Development 16(5)2004. http://www.cipav.org.co/1rrd/1rrd16/5/enem16033.htm

Emenalom O.O., Udedibie A.B.I., Esonu B.O., Etuk, E.B. 2005a. Evaluation of processed velvet bean (*Mucuna pruriens)* as a feed ingredient in starter diets for broiler chicks. The Journal of Poultry Science 42: 301 –307.

Emenalom O.O., Orji V.C., Ogbonna N.C. 2005b. Effect of velvet bean *(Mucuna pruriens*) seeds cooked in maize cob-ash solution on the performance of broiler starter chicks. Livestock research for rural development. 17(12) 2005. http://www.cipav.org.co/irrd/irrd17/12/emen/17135.htm

Emenalom O.O**.,** Udedibie A.B.I., Esonu B.O., Okeudo N.J. 2008a. Effect of cracked soaked and cooked velvet beans (*Mucuna pruriens*) on the performance and organ characteristics of finisher broilers. Nigerian Agricultural Journal 39(20): 131–136.

Emenalom O.O., Udedibie A.B.I., Okeudo N.J., Esonu B.O. 2008b. Evaluation of processed velvet beans (*Mucuna pruriens*) meals in the diet of laying hens. Bulletin of Animal Health and Production in Africa 56: 335-343.

Emenalo O.O., Nwakaobi J.C., Chima M. C., Maryann C. 2009. Growth, organ weights and blood composition of broilers fed diets with velvet bean (*Mucuna pruriens*). International Journal of Agriculture and Rural Development 12: 10-15. www.ajol.info/journals/ijard

Emenalom O.O., Udedibie A.B.I., EsonuB. O., Etuk E.B. 2005b. Cooking in local alkaline solution as a method for improving the nutritive value of velvet bean (*Mucuna pruriens*) for broilers. Proceedings of the 4th All Africa Conference on Animal Agriculture and the 31st Annual meeting of the Tanzania Society for Animal Production (TSAP) held at Arusha. Tanzania. 20-24 September, 2005.pp.239-245.

Emenalom O.O. 2004: Comparative performance of broiler chicks fed diets containing differently processed *Mucuna pruriens* seed meals. Nigerian Journal of Animal Production 31: 12 – 16.

Emenalom O.O., Nwachukwu I.C. 2006. Effect of calcium hydroxide soaked and cooked velvet beans *(Mucuna pruriens)* on the performance of finisher broilers. . Nigerian Journal of Animal Production 33(1): 53–57.

Harms R.H., Simpson C. F., Waldroup P.W.1961. Influence of feeding various levels of velvet beans to chicks and laying hens. Journal of Nutrition 75:127-131.

Houghton P.J., Skari K. P., 1994. The effect on blood clothing of some West African Plants used against snakebite. Journal of Ethnopharmacology 44:99-108.

National Research Council.1994. Nutrient Requirement Poultry.9 ed. rev. National Academy Press Washington, DC.

Olaboro G., Okot M.W., Mugerwa J.S., Latshawa J.D. 1991. Growth-depressing factors in velvet beans fed to broiler chicks. East African Agricultural and forestry Journal 57(2):103-110.

Ruiz Sesma B.1999 .Evaluation of velvet bean (*Stizolobium deeringianum*) without and with processing as an ingredient in the diet of pigs. M.Sc Thesis Universidad Autonoma de Yucatan, Facultda de Medicine Veternariay Zootecnia, Unidad de Posgrado e Investigacion

Udedibie A.B.I., Carlini C.R. 1998. Brazilian *Mucuna* seeds (velvet bean) lack haemagglutinating activity. Journal of Agricultural and Food Chemistry 166(4): 1450 – 1452.

Udedibie A.B.I., Emenalom O.O., Obikaonu H.O. 2001. Comparative evaluation of Nigerian and Brazilian *Mucuna pruriens* (velvet beans) as feed ingredients in broiler diets. International Journal of Agriculture and Food Development 2: 9-13.

Wanjekeche E. 2001. Evaluation of processing methods of *Mucuna* for food in Kenya: In *Mucuna* News. Published: CIACA. Forth issues, November 2001. P.2.

Effect of malting period on the chemical contents of mung bean '*Orarudi'* (*Vigna radiata)* flour

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Abstract
Flours were prepared from mung bean '*orarudi*' (*Vigna radiata*) seeds and malted for 0, 24, 48, 72 and 96 hours. Proximate composition, mineral, vitamin, and antinutrient contents of the flours were evaluated and compared with the unmalted flour. Results showed that unmalted flour contained 6.58, 30.77, 4.61, 1.95, 3.11 and 52.94 g/100g moisture, crude protein, crude fat, mineral ash, crude fibre and carbohydrate respectively. Malting significantly ($P<0.05$) increased the protein, ash, fibre and decreased the fat and carbohydrate contents of the mung bean flour. Mung bean flour malted for 72 hours showed the highest contents of all the minerals studied and vitamin B_1. Samples malted for 96 hours had the lowest contents of zinc, phytate, oxalate and carbohydrates.

Keywords: Malting, chemical composition, *Orarudi*, antinutrient, vitamins

Introduction
Increase in the demand for cheap and acceptable dietary protein for low income groups has motivated more studies on legume protein utilization. Legumes occupy an important position in the diets of people in Third World countries because they are rich sources of protein, calories, certain minerals and vitamins. El-Maki et al. (2007) noted that legumes are major contributors of protein and calories in most African diets but some of these legumes are unconventional and underexploited. Traditionally some of these unconventional legumes have been associated with bioactive compounds that can impact on human health but have not been fully explored and hence the need to study them. Among such unconventional legumes, is mung bean locally known as *orarudi*. Mung bean has high nutritional potential (Mensah and Olukoya, 2007). Structurally, mung bean consists of 12.1% seed coat, 2.3% embryo and 85.6% cotyledons (Singh et al.,1968). Like most legumes, mung bean is relatively high in protein, which makes up about 25% of the seed by weight. Consumption of mung bean like other legumes can fulfill the essential amino acid requirement of infants with the exception of sulphur-containing amino acids (Khalil, 2006). Mung bean protein is rich in lysine but deficient in methionine (Anderson, 2007).

There is a paucity of information on the use of mung bean in product formulation or value-added products in Nigeria. Furthermore, the presence of antinutritional factors and high viscosity may have constituted part of the limitation to the use of mung bean compared to other legumes. It is therefore important to develop strategies to process underutilized indigenous crops into flours of high nutritional quality that can be used in product development. One of the simplest traditional technologies adopted for improving the nutrient composition of plant foods is malting. Malting is controlled germination followed by controlled drying of the germinated kernels. Malting plays a significant role in promoting the development of hydrolytic enzymes which are not present in non-germinated grain. It improves both the nutritional and functional properties of legumes (Mensah and Tomkins, 2003) and allows preparation of low-bulk foods through elaboration of amylases resulting in reduced viscosity of the gelled germinated starch (Brandtzaeg et al., 1981; Kulkarni et al., 1991). Low bioavailability of nutrients, arising from the presence of antinutrients such as phytate, polyphenols, and oxalate, is another factor that limits the quality of predominantly plant-based diets. Research on the malting of legumes such as Bambara nut, and soybean among others has been undertaken to establish the optimum malting period (Maduko, 2002). However, there is a dearth of information in this regard concerning mung bean. Flours developed from cereals and legumes malted for different periods may perform differently in product development. Hence this study sought to determine the effect of malting periods on the chemical and antinutrient content of mung bean malt.

Materials and methods

Materials

Mung bean seeds were purchased from local retailers at Orba market in the Nsukka Local Government Area, Nigeria. Identification of the crop was done in the Crop Science Department, University of Nigeria, Nsukka.

Processing of mung bean seeds into flour

Four lots of mung bean seeds (200 g each) were weighed into porous bags (25 cm x 45 cm) in which they were malted at 30±0.2°C by a modification of the two-step wet-steep method of Etok-Akpan and Palmer (1990). The steeping schedule was based on the time for maximum water absorption characteristic of the mung bean seed. The seeds were steeped in water for 3 h, air rested for 90 min and re-steeped in fresh tap water for 3 h. One lot of mung bean seeds was wet-dehulled by abrasion in between palms, dried at 50°C in a Gallenkamp oven (Model 1H-150; Gallenkamp England) to a moisture content of 6.58%. The dried grains were milled, sieved through a 200 µm sieve, packed in polyethylene bags and stored at 4°C.

The second, third, fourth and fifth lots of 200 g seeds in malting bags were spread in a dark room to germinate for 24, 48, 72 and 96 h, respectively, during which they were, turned every 24 h. The samples were moistened on alternate days by dipping the malting bags containing the germinating grains in water for 30 sec. One lot (bag) was picked at interval of 24, 48, 72 and 96 h and the green malts were separated from the sprouts and hulls by abrasion between the palms followed by winnowing after drying at 50°C for 12 h. Subsequently each lot was milled using a Bentall attrition mill (Model 200 L090, E. H. Bentall U.K), sieved through a 200 µm sieve and stored in polyethylene bags until use.

Analytical methods

Determination of proximate and energy composition

Mung bean malt and flour was analysed for proximate composition (moisture, crude protein, fat, fibre and ash) using AACC (2000) methods. The nitrogen content was estimated by the Dumas method (AACC, 2000) on an EA 1110 CHNS-0 CE Instrument (CHNS-0 Elemental Analyzer, USA). Protein content was calculated using the formula, protein = nitrogen x 6.25. Carbohydrate was estimated by difference (100- % protein +% ash +% fat +%crude fibre +%moisture). Energy was determined using Atwater's conversion factors (4 x protein, 9 x fat and 4 x carbohydrates)

Determination of minerals and vitamin composition

Calcium, iron, zinc, phosphorous, potassium, sodium, magnesium and copper contents of the malt were determined using inductively coupled plasma atomic absorption spectrophotometry (AACC, 2000). The processed flours were analysed for vitamin A content according to the method described by Arroyave et al. (1982). Vitamin B1 was determined according to the methods of AOAC (2010).

Determination of antinutrients

Tannin, oxalate and phytate contents of the processed flours were determined according to the methods described by Buns (1971), Fassett (1973), and Latta and Eskin (1980), respectively.

Data analyses

Data obtained were subjected to statistical analysis using the one-way analysis of variance (ANOVA) (Steel and Torre, 1980). The mean separation was done by Duncan New Multiple Range Test using SPSS version 16.00 Software. Significance was accepted at P<0.05 levels.

Results

Proximate composition of flours.

Table 1 shows the proximate composition of the mung bean malted for different periods. The moisture content of unmalted mung bean was 6.58 g/100g and those for 24, 48, 72 and 96 h, malt were 9.19, 9.79, 10.10 and 11.4 g/100g respectively. The protein contents of unmalted mung bean was 30.77 g/100g while the protein contents of mung bean seed malted for 24, 48, 72 and 96 h were 32.54, 33.5, 34.47 and 31.4 g/100g respectively. The malted samples showed significantly (P<0.05) higher protein values than unmalted samples.

The fat content of the unmalted mung bean flour was 4.61 g/100 g while the values for the malted samples were 1.36, 1.16, 1.22 and 0.52 g/100 g for 24, 48, 72, and 96 h malting respectively.

The crude fibre contents of the 24, 48, 72, and 96 h malted bean flour were 3.44, 3.57, 3.63 and 3.49 g/100g, respectively. The crude fibre content of the mung bean was significantly (P<0.05) increased over the unmalted and the increases ranged from 10.61-16.72%.

The carbohydrate content of the unmalted sample (control) was 52.94 g/100g which was lower than the value 61.47 g/100g reported by Mubarak (2005) respectively.

Table 1. Proximate composition (g/100g) of changes in mung bean during malting

Malting period/h	Moisture	Protein	Crude Fat	Crude fibre	Ash	Carbohydrates
0	6.58^{e}±0.14	30.77^{e}±0.04	4.61^{a}±0.01	3.11^{d}±0.04	1.95^{e}±0.00	52.94^{a}±0.00
24	9.19^{d}±0.02	32.54^{c}±0.04	1.36^{b}±0.01	3.44^{c}±0.05	2.75^{d}±0.26	50.72^{b}±0.27
48	9.79^{c}±0.13	33.50^{b}±0.12	1.16^{d}±0.01	3.57^{b}±0.02	3.29^{c}±0.14	48.69^{d}±0.14
72	10.10^{b}±0.14	34.47^{a}±0.06	1.22^{c}±0.02	3.63^{a}±0.24	4.33^{a}±0.00	46.25^{e}±0.00
96	11.45^{a}±0.12	31.47^{d}±0.01	0.52^{e}±0.00	3.49^{c}±0.05	4.14^{b}±0.03	48.93^{c}±0.19

Values are means ±SD of triplicate determination. Means carrying different superscripts on the same column were significantly different (P<0.05).

Mineral and vitamin composition

Table 2 shows the mineral (calcium, zinc, copper, potassium, sodium, magnesium, iron and phosphorous) contents of the unmalted and malted samples. The Ca, Cu, K, P, and Mg contents increased progressively at significant levels (P<0.05) as the malting period was increased. In contrast, zinc, iron and sodium contents decreased for the first 48 h and then increased as malting period increased at 72 h. Samples malted for 72 h contained the highest value of iron, zinc and sodium. The unmalted sample had higher zinc content (5.16 mg/100g) compared to 24, 48, and 96 h malt (4.71, 4.60 and 3.82 mg/100g respectively) which increased by over 2.91% at 72 h of malting.

The vitamin A and vitamin B contents of the processed mung bean are also shown in Table 2. The samples showed low levels of vitamin A which ranged from 43.7-163.80 μgRE/100g. Vitamin A content of mung bean samples increased as the malting period increased. The 72 h and 96 h malts also had significantly (P<0.05) highest vitamin A content than that of the unmalted flour. Malting increased the vitamin B1 (thiamin) content of the mung bean flour from 4.6 mg/100g in the unmalted to 10.5 mg/100g in the 96 hour malt.

Table 2. Effect of malting on selected mineral and vitamin A and B contents of mung bean.

	Malting Periods /h				
Constituents	0	24	48	72	96
Calcium	$98.46^{c}\pm1.02$	$100.52^{c}\pm0.00$	$101.74^{bc}\pm0.01$	$122.00^{a}\pm0.08$	$102.98^{b}\pm0.45$
Zinc	$5.16^{b}\pm0.03$	$4.71^{c}\pm0.00$	$4.60^{d}\pm0.00$	$5.31^{a}\pm0.00$	$3.82^{e}\pm0.03$
Iron	$8.06^{b}\pm0.04$	$7.88^{b}\pm0.06$	$7.84^{b}\pm0.04$	$11.02^{a}\pm0.79$	$10.99^{a}\pm0.08$
Copper	$0.73^{d}\pm0.00$	$0.73^{d}\pm0.00$	$0.77^{c}\pm0.00$	$0.92^{a}\pm0.02$	$0.79^{b}\pm0.02$
Sodium	$5.47^{bc}\pm0.17$	$5.26^{cd}\pm0.02$	$5.11^{d}\pm0.02$	$7.07^{a}\pm0.19$	$5.63^{b}\pm0.4$
Potassium	$1147.80^{d}\pm1.05$	$1148.89^{d}\pm0.00$	$1270.21^{c}\pm2.84$	$1376.78^{a}\pm6.82$	$1304.98^{b}\pm0.69$
Phosphorus	$261.06^{d}\pm1.49$	$262.55^{d}\pm0.00$	$274.88^{c}\pm0.05$	$312.99^{a}\pm0.54$	$288.87^{b}\pm2.68$
Magnesium	$147.03^{c}\pm0.51$	$147.54^{c}\pm0.00$	$166.42^{b}\pm0.56$	$187.31^{a}\pm0.45$	$165.15^{b}\pm1.60$
Vitamin A (μgRE/100g)	$43.7^{a}\pm0.02$	$54.6^{b}\pm0.00$	$65.5^{e}\pm0.00$	$109.2^{c}\pm0.00$	163.8d±0.00
Vitamin B1(mg/100g)	$4.6^{a}\pm0.01$	$5.3^{b}\pm0.00$	$6.7^{c}\pm0.00$	$7.7^{d}\pm0.02$	$10.5^{e}\pm0.01$

Values are means ±SEM of triplicate determination. Means carrying different superscripts on the same row are significantly different (P<0.05)

Antinutrients

The tannin content was 475.50 mg/100g in unmalted samples and 384.50, 213.50, 220.00 and 231.00 mg/100g in the 24, 48, 72 and 96 h samples, respectively (Table 3). Tannin contents decreased as malting period increased with 19.18% and 57.40% decreases observed in 24 and 48 h-malted samples, respectively. However, a slight increase in tannin content (3.03% and 8.20%) was observed in the 72 and 96 h-malted samples respectively.

Generally there were significant decreases (P<0.05) in the phytate and oxalate contents of the samples as the malting period increased. Malting for 96 h reduced phytate and oxalate contents of the samples by 81.57% and 87.34%, respectively.

It was observed that the pH decreased as the malting period increased. Mung bean samples malted for 96 hours had the lowest pH (5.1) while out-of-steep flour had the highest pH (6.8).

Titratable acidity ranged from 0.057% to 0.143% and increased as the malting period increased. Unmalted flour had the least titratable acidity (0.057%). Samples malted for 96 h had the highest titratable acidity (0.143%; (Table 4).

Table 3. Changes in antinutrient contents (mg/100g) of Mung bean (*Orarudi*) during malting

Malting period/hr	Tannin	phytate	Oxalate
0	475.75^{a}±3.53	87.10^{a}±0.35	624.00^{a}±1.14
24	384.50^{b}±4.94	71.10^{b}±0.14	419.50^{b}±3.53
48	213.50^{e}±4.94	62.25^{c}±0.21	310.50^{c}±1.20
72	220.00^{d}±5.65	41.21^{d}±0.09	146.00^{d}±2.82
96	231.00^{c}±4.24	16.05^{e}±0.07	79.00^{e}±2.82

Values are means ±SD of triplicate determination. Means carrying different superscripts on the same column are significantly different (P<0.05)

Table 4. pH and titratable acidity of mung bean malt

Malting period/h	pH	Titratable acidity (%)
0	6.8	0.057
24	6.5	0.069
48	6.0	0.078
72	5.7	0.10
96	5.1	0.143

Discussion

The moisture content increased with increase in the malting period. Murwan et al. (2008) reported similar observations during the malting of two sorghum cultivars (feterita and tabat). The progressive increase in moisture content observed as the malting period increased was attributed to hydration of the seeds during steeping and germination. Oluwole et al. (2012) documented similar observations during malting of sorghum and maize grain and attributed the trend to prolonged addition of water. The protein content of unmalted mung bean was 30.77 g/100g which was in agreement with values (27.5 g/100g) reported by Mubarak (2005).

The increase in protein content with malting period could be due to breakdown of complex proteins into simpler forms, and reduction of the anti-nutritional factors such as phytate. Germination reduces the anti-nutritional factors such as phytates which form complexes with proteins, converts insoluble proteins to soluble components and increases the level of lysine (Nzelibe and Onyeniran, 2001). Kayembe and Rensburg (2013) also reported an increase in protein content in soybean seeds germinated for 2, 3, 4, 5, and 6 days and attributed the increase to utilization of carbohydrates as an energy source for developing sprouts.

Based on the results of this study, it could be concluded that mung bean is a rich source of protein. Consumption of 100 g of mung bean may, therefore, be capable of providing 27 g of protein which satisfies the recommended daily allowance of protein (13 g/day) for children.

This observation agrees with the report of Mubarak (2005) who noted a significant decrease in fat content of mung bean malted for 72 h. The decrease in fat content as the malting period increased could be due to the use of fat as an energy source during germination. The increased activities of the lypolytic enzymes during malting which hydrolyzed fats to fatty acids and glycerol may also explain the decrease. The low fat contents of all the samples showed that mung bean could not be classed among oil seeds and hence could form part of the diets of obese and hypertensive patients. The low fat content of the malt may also be beneficial to health since according to Antia et al. (2006), a diet providing 1-2% of its caloric of energy as fat is sufficient for human beings because excess fat consumption has been implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging. Oluwole et al. (2012) also reported a decrease in fat content with increasing malting period during malting of sorghum and maize grain. However, Kayembe and van Rensburg (2013) reported significant increases in fat content in soyabean germinated for 1-6 days.

The high fibre content of the malted mung bean could be of dietary importance if the flour is incorporated into foods. A similar increase in fibre content was observed by Muhammad et al. (2011) for malted barley. The ash content increased from 1.95 g/100g in the control to 4.33 g/100g at 72 h and then decreased to 4.14 g/100g for the 96 h malted flour. The higher ash content of the malted samples could be attributed to the reduction of the anti-nutritional factors which led to the released of the bound minerals. This result was, however, contrary to the findings of Oluwale et al. (2012) and Muhammad et al. (2011) who reported a decrease in ash content during malting of sorghum and barley. However, the result of this study agrees with the report of Obizoba and Atti (1991) on malted sorghum. Chikwendu (2003) reported an increase in the ash content of ground bean germinated for 72 h and attributed it to endogenous hydrolysis of complex organic compounds to release more nutrients.

The variation in the carbohydrate content observed in this study with those of previous researchers (Mubarak, 2005) could be due to processing method and varietal differences. Carbohydrate content decreased gradually with the malting period. The decrease could be due to increase in other components (protein, ash, fibre and moisture contents) of the flours since carbohydrate was determined by difference. Kirk-Uthmar (2007) also reported that malting affected carbohydrate molecules in the germinating grains through the action of the amylase enzymes produced during the malting process whereby the carbohydrates were reduced to maltodextrins and low molecular weight sugars. Mubarak (2005) has earlier reported that during germination, the carbohydrate content of mung bean seeds decreased and attributed the decrease to carbohydrate utilization as an energy source to start germination. Similar observations were also made by Kayembe and van Rensburg (2013) during germination of soybean.

The increase in the minerals with increase in malting period might be due to the decrease in antinutritional factors which led to the release of bound minerals from their complexes. The increase in calcium and iron contents during malting of beans was reported by Muhammad Rauf (1990). The decrease in zinc content at the initial stage could be due to the use of zinc in cell reproduction and tissue growth (Cascherz and Kirchoff, 2005). Zinc has been recognized as an essential nutrient and its deficiency has been reported to cause growth retardation and inadequate sexual development in humans (Wardlaw and Kessel, 2004). The values of zinc and iron observed in the 72 h malt can meet the RDA of 5 mg for zinc and 73.47% of RDA of iron for infants of 6-12 months (National Research Council, 1989). It is evident from the result that mung bean malt is a very good source of iron, zinc, magnesium and sodium. It is significant that at this time iron and zinc deficiencies are among the most serious forms of deficiencies that are of high public health concern. Iron deficiency is the single most common nutritional disorder world-wide and the main cause of anaemia in infancy, childhood and pregnancy. These minerals are important in maintaining normal body functions. Germination has been reported to increase vitamins B and C content of seeds (Nzelibe and Onyeniran, 2001).

The decrease in phytate contents could be attributed to the increased activity of phytase that progressively degraded phytic acid during malting. These results agree with the findings of Pawar and Machewad (2006) who also reported phytate reduction (28.9%) during malting of barley and attributed the reduction in phytic acid content to its degradation by phytase synthesized during the process. These results corroborate the observations of Akubor and Chukwu (1999) and Nzelibe and Onyeniran (2001). Levels of antinutrient reduction could account for high mineral content of the malt. pH gradually decreased as malting period increased while titratable acidity increased and this helps in preservation during storage due to high acid levels under which many organisms cannot survive, similar observations being made by Steinkraus (1996).

Conclusion

It is evident from this study that the malting period has a significant effect on the chemical composition of mung bean (*orarudi*) flour. Malting mung bean for 72 h resulted in the highest increase in all the mineral, protein, ash, crude fibre contents and vitamin B_1 contents and decreases in phytate, and oxalate contents. The highest reduction in antinutrient content occurred at after 96 h malting which did not differ significantly (P>0.05) from 72 h malting.

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References

Akubor P.I., Chukwu I.K. 1999. Proximate composition and selected functional properties of fermented and unfermented African oil bean (Pentaclethra macrophylla) seed flour. Plant Foods for Human Nutrition 54:277–278

Anderson P. 2007. Report on the nutritive value of Rice bean. European Commission 6th Framework Programme, Food security through Ricebean Research in India and Nepal (FOSRIN). Report 3. Nutronal qualities of rice bean, Bergen, Norway, Pp:1-9.

Antia B.S., Akpan E.J., Okon P.A., Umoren I.U. 2006. Nutritive and Anti-Nutritive Evaluation of Sweet Potatoes (Ipomoea batatas) Leaves. Pakistan Journal of Nutrition 5: 166-168.

AACC (2000): American Association of Cereal Chemists (AACC). 2000. AACC Approved Methods of Analysis. 10th ed. St. Paul, MN: American Association of Cereal Chemists.

AOAC (2010): Official Methods of Analysis 2010. Association of Analytical Chemists, 18th edition. Gaithersburg, Maryland, U.S.A.

Arroyave G., Chichester C.O, Hernando F., Glover J., Mejia L.A., Olson J.A. et al. 1982. Biochemical Methodology for the Assessment of Vitamin A Status. The Nutrition Foundation, Washington, DC, pp. 24–30

Brandtzaeg B, Malleshi N.G., Svanberg D., Desikachar H.S.R., Mellander O. 1981. Dietary bulk as a limiting factor for nutrient intake with special reference to the feeding of preschool children. III. Studies on malted flours from ragi, sorghum and green gram. Journal of Tropical Paediatrics, 27:184-9.

Buns R.B. 1971. Methods of Estimation of tannins in the grain sorghum. Agronomic Journal 63:517-519

Cascherz H., Kirchoff E. 2005. Trace elements in foods: zinc contents of raw foods- A comparison of data originating from different geographical regions of the world. Journal of food composition and analysis 19:283-420.

Chikwendu N.J. 2003. The effects of germination on the chemical composition and microbial quality of ground bean (*Kerstingiella geocarpa*) flours. Nigerian Journal of Nutritional Science, 24: 17-20.

El-Maki H.B., Abdel-Rahman S.M., Idris W.H., Hassan A.B., Babiker E.E., El-Tinay A.H. 2007. Content of antinutritional factors and HCl- extractability of mineral from white bean (phaseolus vulgaris). Cultivars: Influence of soaking and/ or cooking. Food Chemistry. 100: 342-362.

Etok-Akpan O.U., Palmer G.H. 1990. A simple diamylase procedure for estimation of α-amylase and diastatic activity. Journal of Institute of Brewing 96:89–91.

Fasset D.W. 1973. Oxalates, in Toxicants Occurring Naturally in Foods, ed. by National Research Council. National Academy of Science, Washington DC, Pp. 346–362.

Kayembe N.C., van Rensburg C.J. 2013. Germination as a processing technique for soybeans in small-scale farming. South-African Journal of Animal Science 43(2):167-173.

Khalil A.A. 2006. Nutritional improvement of Egyptian breed of mung bean by probiotic lactobacilli, African journal of Biotechnology 5: 206-212.

Kirk-Uthmar O. 2007. Food and Feed Technology. New York: Willey-Interscience. pp. 116-120

Kulkarni K.D, Kulkarni D.N., Ingle U.M. 1991. Sorghum malt based weaning food formulations: preparation, functional properties and nutritive value. Food and Nutrition Bulletin 13:322-7.

Latta M., Eskin M. 1980. A simple and rapid colorimetric method of phytate determination. Journal of Agricultural Food Chemistry 29:315–317.

Maduko C.O. 2002. Properties of maize-bambara groundnut complementary foods for infants. B. Sc. Thesis, Department of Food Science and Technology, University of Nigeria, Nsukka.

Mensah J.K., Olukoya R.T. 2007. Performance of mung beans (*Vigna mungo* (L) Hepper) grown in mid-western Nigeria. American-Eurasian Journal Agricultural and Environmental Science 2: 696-701.

Mensah P., Tomkins A. 2003. Household-level technologies to improve the availability and preparation of adequate and safe complementary foods. Food and Nutrition Bulletin 24:104-25.

Mubarak A.E. 2005. Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Journal of Food Chemistry 89:489-495

Muhammad Rauf K. 1990. Nutritive value of sprouted barley. Journal of Chemical Society of Pakistan 21: 406-408.

Muhammad A., Javed A. B.1, Faizullah K.1., Hamida A.I. 2011. Effect of soaking and malting on the selected nutrient profile of barle. Pakistan Journal of Biochemistry and Molecular Biology 44(1): 18-21

Murwan K. S. E ., Amna O. H. 2008. Effect of Malting on the Chemical Constituents, Anti-nutritional Factors, and Ash Composition of Two Sorghum Cultivars (Feterita and Tabat) Grown in Sudan. Research Journal of Agriculture and Biological Sciences 4(5): 500-504

National Research Council. 1989. Recommended Dietary Allowance 10th edn. National Academy Press. Washington D.C.

Nzelibe H.C., Oyeniran A.A. 2001. Phytate levels in different varieties of folio millet (finger millet, pearl millet and sorghum malts). Nigerian Journal of Biochemistry and Molecular Biology 16:855–875.

Obizoba I.C., Atti, J.V. 1991. Effect of soaking, sprouting, fermentation and cooking on nutrient composition and some anti-nutritional factors of sorghum (*Guinesia*) seeds. Plant Food for Human Nutrition 41:203–212

Oluwole O.B., Kosoko S.B., Owolabi S.O., Adeyoju A.O., Bankole A.O., Ozumba A.U., Elemo G. N. 2012. Development and production of high protein and energy density beverages from blends of maize (*Zea mays*), sorghum (*Sorghum bicolor*) and soybeans (*Glycine max*) for school aged children: effect of malting period on selected proximate parameters and sensory qualities of developed beverages. International Journal of Applied Science and Technology 2(7):285-292

Pawar V.D., Machewad G.M. 2006. Changes in availability of iron in barley during malting. Journal of Food Science and Technology 43: 28-30.

Singh S., Singh H.D., Sikka K.C. 1968. Distribution of nutrients in the anatomical parts of common Indian pulses. Cereal Chemistry 45:13

Steel R.G., Torrie J.A. 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd edn. McGraw-Hill International, Auckland pp. 5-102.

Steinkraus K.H. 1996. "Handbook of indigenous fermented foods". 2nd edn, Marcel Dekker, New York

Wardlaw G.M., Kessel M.W. 2004. Perspectives in Nutrition. 6th edn. McGraw Hill Publishers, New Delhi, Seoul, Toronto.

Ethnobotanical investigation of three leafy vegetables [*Alternanthera sessilis* (L.) DC., *Bidens pilosa* L., *Launaea taraxacifolia* Willd.] widely consumed in southern and central Benin

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Abstract

To document indigenous knowledge and farmers' know-how related to the leafy vegetables *Launaea taraxacifolia, Bidens pilosa and Alternanthera sessilis*, an ethnobotanical investigation using participatory approach research methods and tools, was conducted in 19 villages randomly selected across ethnic and agro-morphological zones of southern and central Benin. The geographical distribution of the three species was established and the southern area appeared suitable for an *in situ* conservation programme of genetic diversity of these leafy vegetables. Respectively, 11.11%, 55.56% and 90% of the respondents reported that *Launaea taraxacifolia Bidens pilosa and Alternanthera sessilis* are still harvested from the wild (level 0 of domestication) while 22.22% and 16.67% of respondents reported that *L. taraxacifolia* and *B. pilosa* are being cultivated (level 4 of domestication). Uprooting and cutting plant stems were the most common harvesting methods. The study revealed the existence of morphotypes resulting in the identification of different varieties of *L. araxacifolia* (three varieties), *B. pilosa* and *A. sessilis* (two). The frequency of consumption of each of the leafy vegetables and its consumption method varied according to the ethnic group. Regarding methods of preparation, sauce made from fresh leaves was reported only for *L. taraxacifolia* while pre-cooked leaves were otherwise used. The respondents also reported that these leafy vegetables possessed, in addition to their culinary value, several medicinal virtues. *L. taraxacifolia* was the most valued medicinally and is used for the prevention or healing of 21 diseases with 16 possible pharmacological functions. Further research is required on the biochemical and phytochemical characterization of the genetic diversity of these species as well as the effects of processing methods on their nutritional value.

Keywords: Benin, cultural practices, domestication, leafy vegetables, morphological diversity.

Introduction

Underutilized plants are those species which have a potential, not fully exploited for contributing to food security, health (nutritional/medicinal), income generation and environmental services and poverty alleviation (Ahmad and Javed, 2007). Because they have been for a long time neglected by scientific research, their production has remained traditional and their domestication process (Vodouhè et al., 2011) has hardly progressed.

In tropical countries in general and sub-Saharan Africa in particular, the interest of vegetable plants for food for rural communities is recognized (Andzouana and Mombouli, 2012). Traditional leafy vegetables (TLVs) are plants whose leaves (including immature green pod and flowers) are socially accepted, used and consumed by the local populations (Dansi et al., 2008). Moreover, traditional "African" vegetables are rich in micronutrients, antioxidants (Yang and Keding, 2009), and other health-related phytochemicals (Afari-sefa et al., 2012).

Traditional vegetables often provide higher amounts of provitamin A, vitamin C and several important minerals than common intensively bred crops both on a fresh weight basis and after preparation (Afari-sefa et al., 2012).

According to Adjatin et al. (2013a), many traditional leafy vegetables have long been known and reported to have some curative, regulative and stimulative properties besides food qualities and are used as nutraceuticals. Throughout the tropical world and particularly in West Africa, a large number of traditional leafy vegetables (TLVs) have been reported to play important roles in food security for people living in both rural and urban areas (Ukpong and Idiong, 2013; Adjatin et al., 2013b). They represent affordable but quality nutrition for a large proportion of the population and offer an opportunity for improving nutritional status of many families (Olaposi and Adunni, 2010). The importance of indigenous knowledge and traditional crops in the survival strategies of rural people have only recently been recognized by research (Vorster et al., 2007). According to Smith and Eyzaguire (2007), this indigenous knowledge of the health promoting and protecting attributes of TLVs is clearly linked to their nutritional and non-nutrient bioactive properties.

Recent studies conducted in Benin led to the recognition of 187 species of traditional leafy vegetables (TLVs) among which *Launaea taraxacifolia*. *Bidens pilosa* and *Alternanthera sessilis* are highly consumed and are of great importance for local communities in the centre and south of Benin (Dansi et al., 2008). According to Grubben and Denton's (2004) report, *L. taraxacifolia* is well recognized as a TLV with a great need of promotion by research and popularization services while *B. pilosa* and *A. sessilis* remain with low importance as leafy vegetables but more so for medicinal applications. However, these three species are simply neglected and underutilized by research and extension work in Benin and more recently according to Dansi et al. (2012), *L. araxacifolia* and *B. pilosa* were reported among the 19 species considered as priority neglected and underutilized crops for research in Benin. To improve food security and poverty alleviation in line with the Third Millennium Development Goals, by promotion and better utilization of these three genetic resources with great importance in south and central Benin, there is a need to:

- Document indigenous knowledge and farmers' know-how related to *A. sessilis*, *B. pilosa* and *L. taraxacifolia* leafy vegetables across villages and ethnic groups of central and south Benin;
- Map the distribution and extent of the three species across the study area;
- Explore the diversity, importance and domestication level of these three species.

Materials and methods

Study area

The present study was conducted in the central and southern parts of the Benin Republic. The Republic of Benin is situated in West Africa between the latitudes 6°100 N and 12°250 N and longitudes 0°450 E and 3°550 E. The south and the centre are relatively humid agro-ecological zones with bimodal rainy seasons and mean annual rainfall varying from 1.1 to 1.4 mm/year (Adam and Boko, 1993). Mean annual temperatures range from 26° to 28°C. Vegetation types are semi-deciduous forest (south), woodland and savannah (centre-east), dry semi-deciduous forest (centre-west and south).

Site selection and survey

Nineteen villages (7 in the centre and 12 in the south) belonging to diverse ethnical groups and humid agro-ecological zones were selected and surveyed. Surveyed villages and geographical location are listed in Table 1.

Table 1. List of administrative locations and ethnic groups of the villages surveyed

N°	villages	Districts	Regions	Ethnic groups
1	Sehouè	Toffo	South West	Aïzo/Fon
2	Vèdji	Dassa-zoumè	Centre	Idaasha/Mahi
3	Illèman	Dassa-zoumè	Centre	Idaasha/Mahi
4	ouèdèmé	Glazoué	Centre	Idaasha/Mahi
5	Kpakpaza	Glazoué	Centre	Idaasha/Mahi
6	Naogon-aga	Covè	Centre	Mahi/Fon
7	Bognongon	Zogbodomey	South	Fon
8	Ayétédjou	Kétou	South East	Holy
9	Towé	Pobè	South East	Holy
10	Ita-djèbou	Sakété	South East	Yoruba
11	Ikpédjilé	Sakété	South East	Yoruba
12	Gbezoumè	Houéyogbé	South West	Sawhè
13	Assèdji	Athiémè	South West	Cotafon
14	Dahoue	Dogbo	South West	Adja
15	Lalo-centre	Lalo	South West	Adja
16	Gangnigon	Kétou	South East	Holy
17	Houègbo	Toffo	South West	Fon
18	Késsounou	Dangbo	South East	Goun
19	Kpodédjilé	Adjohoun	South East	Goun

Data were collected during field work in different sites through the application of participatory research appraisal tools and techniques such as direct observation, focus group discussions (20 to 25 persons) and field visits using a questionnaire (Dansi et al., 2010; Adjatiin et al., 2012). Through discussions, the following key information on the three leafy vegetables species, *L. taraxacifolia*, *B. pilosa* and *A. sessilis*, was recorded: the vernacular name of the species and its meaning; status – wild or cultivated; habitat; season of availability; procurement practice of each species; period of consumption; frequency of consumption – measured on an ordinal scale as follows: more than two times a week (very frequently), 1-2 times a week (frequently), 1-2 times a month (moderately), 1-2 times per six months (rarely), once a year (very rarely); modes of consumption; storage practices; intraspecific morphological diversity; cultural importance and medicinal properties related to traditional knowledge. The level of domestication attained by the species in each village was determined following the seven steps described by Vodouhè et al. (2011).

Data collected were analysed through descriptive statistics (frequencies, percentages, means. etc.) in order to generate summaries and tables at different levels (villages, ethnic groups, households). The Kruskal Walis test was done to compare different means obtained.

Results

Geographical distribution

Across the study area and among the three species studied, *L. taraxaicifolia* was found alone in 89.5 % (17/19) of villages surveyed while *A. sessilis* and *B. pilosa we*re encountered in 52.6% (10/19) and47.4% (9/19) of surveyed villages respectively (Figure 1).

Following the Benin analytic flora (Akoegninou et al., 2006) and report of Achigan-Dako et al. (2009) on traditionally leafy vegetables in Benin and folk nomenclature, the site where the presence of the three leafy vegetables studied was recorded and thus their geographical distribution maps established for the whole of Benin (Figure 2).

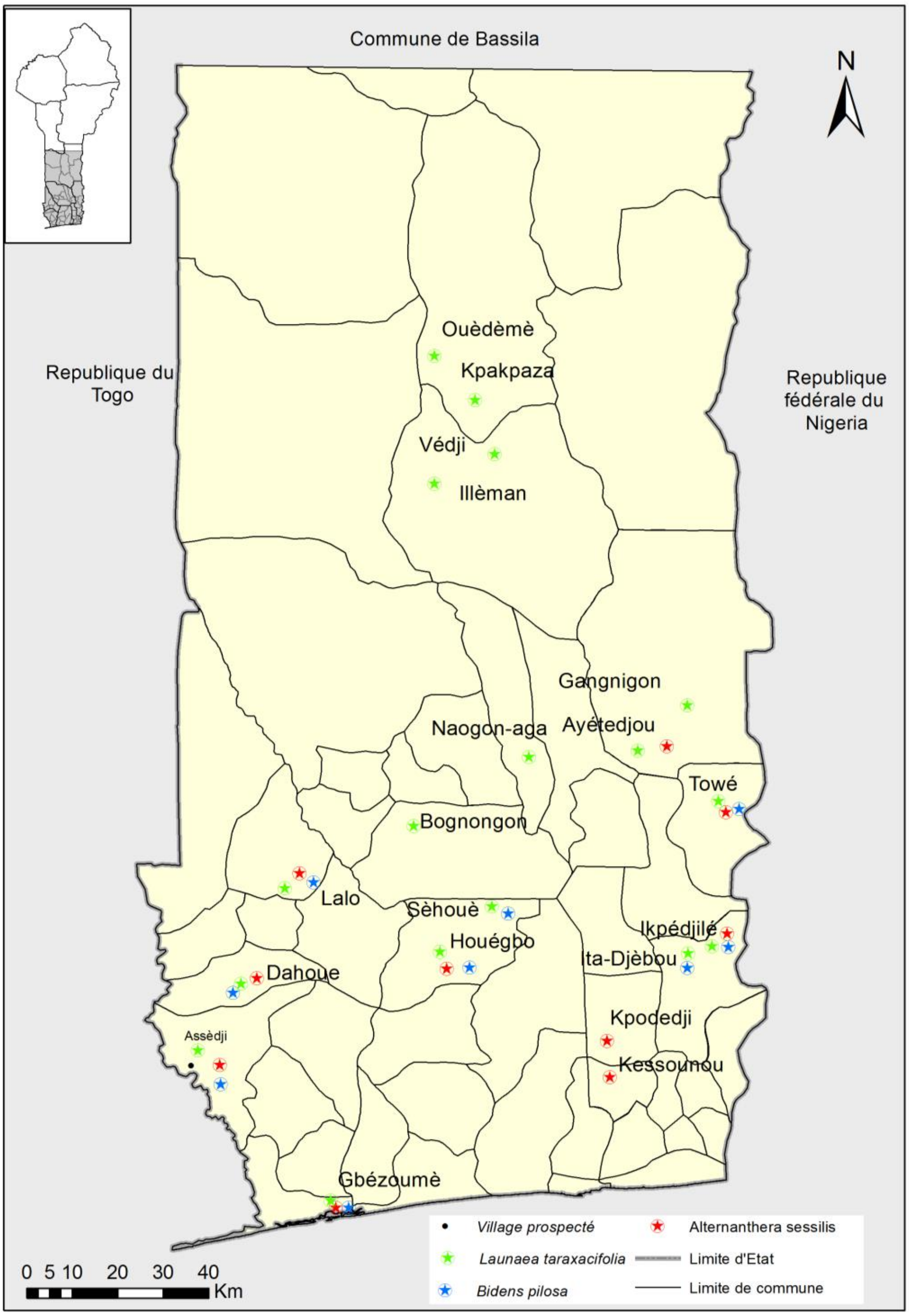

Figure 1. Distribution of *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis* in surveyed villages across central and southern Benin.

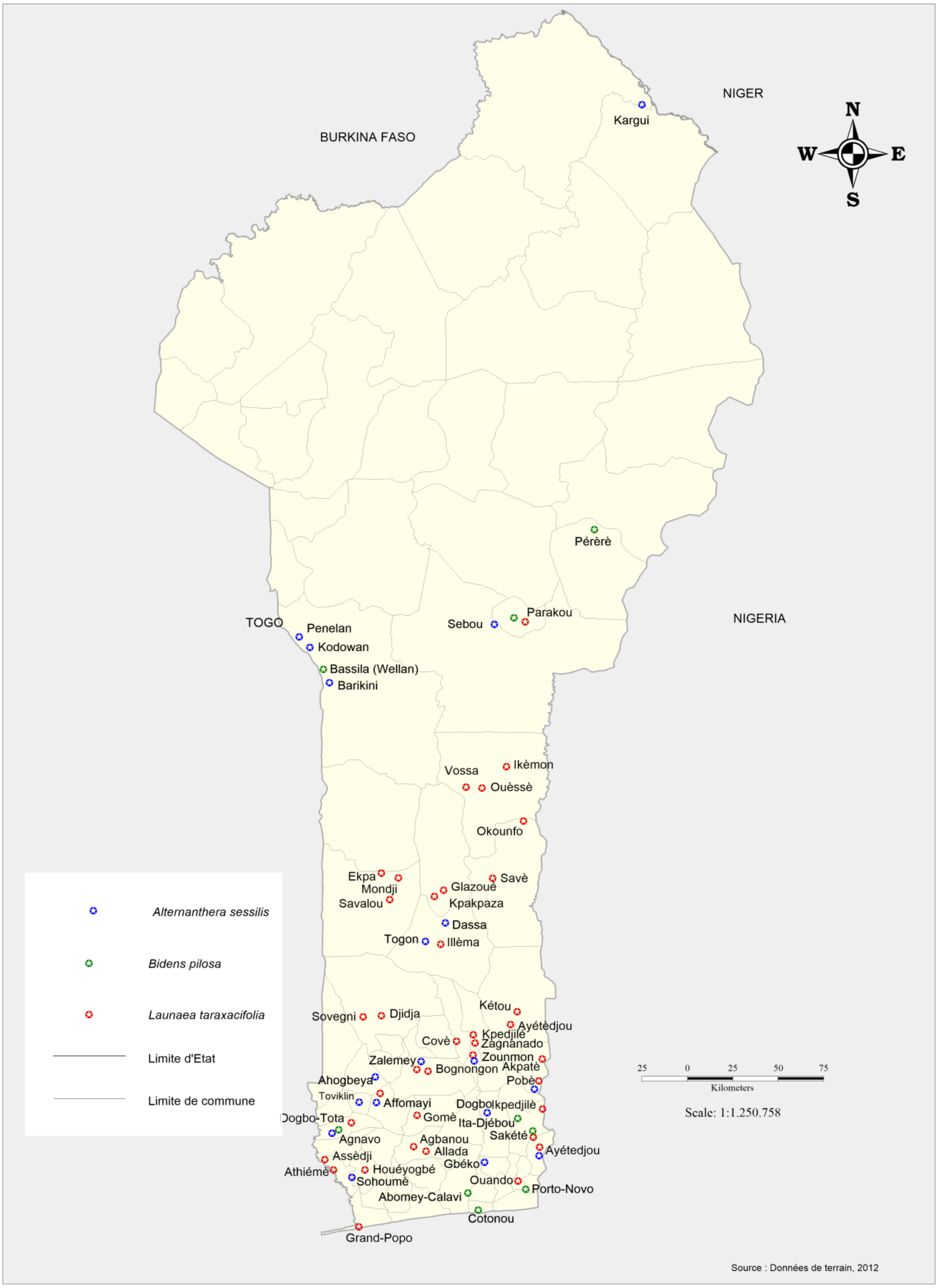

Figure 2. Geographical distribution map of *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis* in Benin.

Folk nomenclature and diversity of species

A total of 27 vernacular names (10 for *L. taraxacifolia*, 9 for *A. sessilis* and 8 for *B. pilosa*) were utilized by respondents for the three leafy vegetables. The vernacular names of species and the analysis of the meanings of vernacular names is compiled in Table 2. The main criteria for the meaning of the names for *L. taraxacifolia* are organoleptic and technological, while morphology traits of the plants were the most used criteria for *B. pilosa,* and habitat for *A. sessilis*.

Table 2. Vernacular names of *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis* and its meanings in the surveyed areas

Species	Vernacular name	Ethnic groups	Meaning of names	Criteria of denomination
Launaea taraxacifolia	Yantotoé or yantoto or latotoé	Fon	Soft like meat after cooking	Organoleptic quality
	Lantoto or yantotoé	Mahi	Leaves consumed instead of meal in ancient times	Organoleptic and technologic qualities
	Odôdô or Odôdôlodôdô	Idaacha	The genuine leafy vegetable	Organoleptic and technologic qualities
	Efô gnanri	Holy/yorouba	The genuine leafy vegetable	Organoleptics quality. technological trait
	Wontou	Adja	Plant with rich biomass	Technological traits
Bidens pilosa	Gningbé/ gninman or Gnintonou	Fon/mahi/Oueme	Herbs with thorn	Morphology of plant
	Abèrè oloko	Yoruba/Nago	The sting of farmers	Morphology of plant
	Djanhounkpi	Sahouè	The plant with sting on mature flowers	Morphology of plant
Alternanthera sessilis	Houngbé	Fon	Blood provider leaves	Medicinal uses
	Idé	Holy	None. Heritage from long ago	
	Gomi	Adja	Plant with high multiplication capacity due to its important number of nodes	Natural habitat, morphology
	Agouègbé, Agouèman	Cotafon, Sahouè	Plant originating from Agoué river, which likes humid zone	Natural habitat. origin
	Agwè-houngbè	Goun	Leafy vegetable prohibited for «Agossou» (abnormally born baby) parents	Cultural uses

	Ossoun odô. Agômayan Goudé	Yoruba	None. Heritage from long ago	-

Within each of these three species and with regard to their morphological traits related to the shape, stem, colour and odour of leaves, different morphotypes were recorded. In the study area, the results showed that there are three different morphotypes and two respectively for *L. taraxacifolia. B. pilosa and A. sessilis* (Figure 3).These results are in accordance with the observation of Dansi et al. (2008) and those of Adjatin et al. (2012), who reported that with traditional leafy vegetables, intraspecific diversity is frequent.

The distinguish traits used by respondents to identify the different morphotypes of each species as well as ethnic groups of respondents are shown in Table 3.

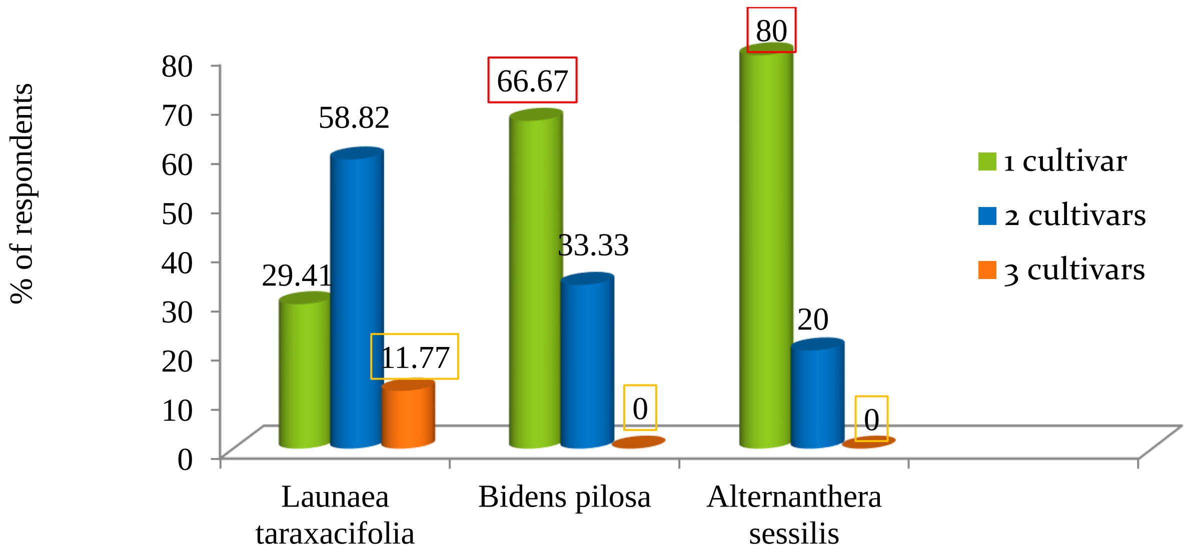

Figure 3. Diversity of *L. taraxacifolia*. *B. pilosa* and *A. sessilis* in the surveyed zone

Table 3. Morphotypes of species studied per ethnic group and traits used to distinguish species

Species	Number of cultivars	Local names	Distinguishing traits
Bidens pilosa	2	Djanhounkoui Adjatô	Odour of leaves
		Djanhounkoui Yovotô	Odour, colour, height of leaves; important ramifications on stem
Launaea taraxacifolia	3	Yantotoé wéwé	Colour of leaves (light green), forms of leaves (lobes), emptiness of stem
		Yantotoé Vôvô	Colour of leaves (green, green-redish), forms of leaves (lobes), stem
		Yantotoé wouiwoui	Colour of leaves (Darkness green)
Alternanthera sessilis	2	Gomi Agwè-aguéton	Height and colour of leaves Development of leaves and colour

Habitat of species and their evolution in domestication process

The perceived natural habitat of the TLVs by farmers are surveyed and reported (Figure 4), though *L. taraxacifolia* was found in the majority of villages surveyed and seem to be adaptable to all types of soil. *B. pilosa* and *A. sessilis* seem to prefer respectively clayey land (45.45% of responses) and dregs (42.85% of responses). These observations may explain the large distribution of *L. taraxacifolia* across the national territory in contrast to the other TLVs.

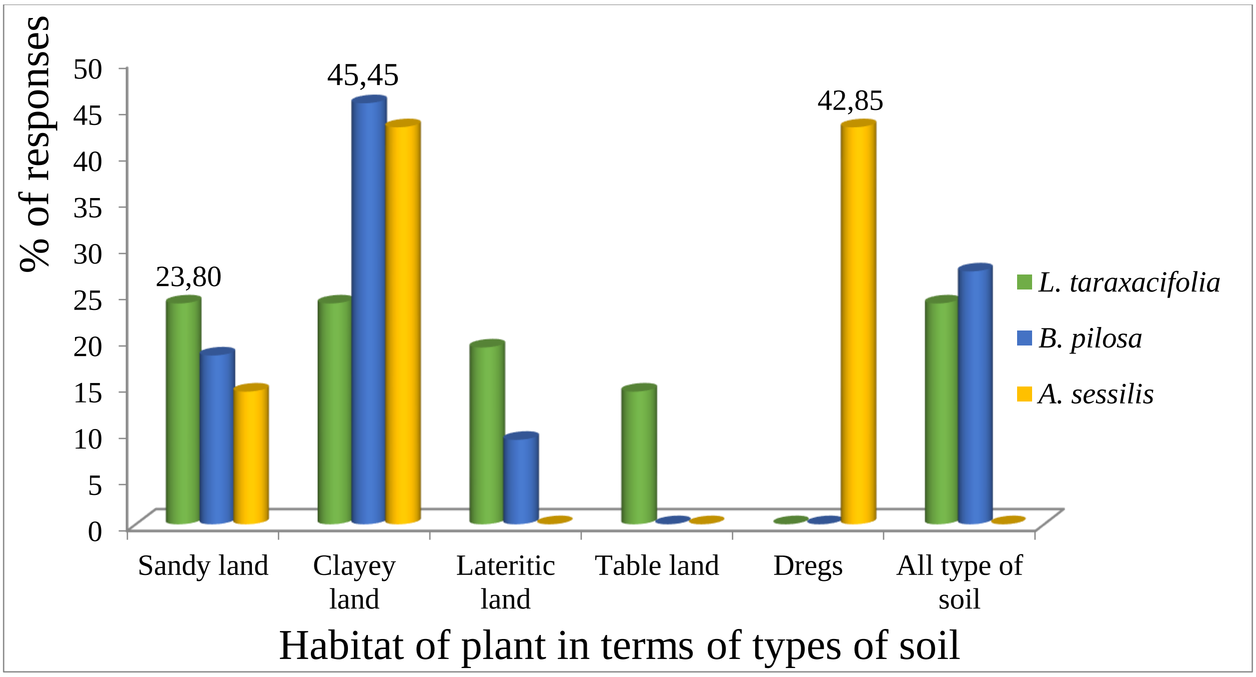

Figure 4. Habitat of *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis* as perceived by farmers

For the three species, domestication stage at the level of community management practices varied among respondents (Table 4). For instance, respectively 11.11%; 55.56% and 90% of the respondents reported that *L. taraxacifolia, B. pilosa and A. sessilis* are still harvested from the wild (level 0 of domestication) while 22.22% and 16.67% of respondents (belonging to Mahi/Holly/Nago and Adja ethnic groups respectively) reported that *L. taraxacifolia* and *B. pilosa* are being cultivated (level 4 of domestication).

The reasons for domestication reported by respondents were: consumption as a vegetable during the dry season where other TLVs are scarce, scarcity of the species in the fields around the villages, high perceived organoleptic quality and medicinal value, contribution to household income through commercialization (in the case of *L. taraxacifolia* and *A. sessilis*).

Table 4. Variation of the domestication levels of *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis*.

Species	Number of villages	Level of domestication (% of villages)				
		N0 (harvested from the wild)	N1	N2	N3	N4 (cultivated)
Launaea taraxacifolia	17	11.11a	61.11c	5.56	5.56	16.67a
Bidens pilosa	9	55.56b	22.22b	-	-	22.22b
Alternanthera sessilis	10	90.00c	10.00a	-	-	-

Harvesting methods and procurement practices

The harvesting methods of the three species were investigated and reported in Table 5. Of the respondents, mostly women were involved in harvesting and the most common harvest method was by uprooting the plant and cutting plant stems. To harvest *L. taraxacifolia*, uprooting (35.3% respondents) is the most practised while plant stem cutting (25% and 40% of respondents) is the most used respectively for *B. pilosa* and for *A. sessilis*. Due to their status of domestication, two major procurement methods were identified for the three TLVs studied: picking from the natural habitat and purchasing from the seller. The results showed that within local communities, certain market values are linked to *L. taraxacifolia* and *A. sessilis* TLVs.

Table 5. Different methods used to harvest *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis* TLVs in prospected areas

Species	Harvesting methods (%)					
	Plant Stem cutting	uprooting	Defoliation	Plant stem cutting and uprooting	Defoliation uprooting	Defoliation and plant stem cutting
L. taraxacifolia	11.76	35.29	5.88	29.41	11.76	5.88
B. pilosa	33.33	11.11	11.11	22.22	0.00	22.22
A. sessilis	40.00	30.00	10.00	10.00	10.00	0.00

Consumption methods and post-harvest storage

The frequency and method of consumption of these three leafy vegetables varied according to the ethnic group. Regarding methods of cooking, sauce made from fresh triturate leaves (17.07% of responses) as well as consumption of raw leaves as salad were reported only for *L. taraxacifolia* while the step of pre-cooking of leaves seems otherwise necessary for the rest of the leafy vegetables studied (Table 6).

According to the ethnic group, the frequency of consumption of each of three leafy vegetable studied vary significantly (Figure 4). The people belonging to Idasha and Mahi (23.52%) were the most frequent consumers of *L. taraxacilia,* while the Adja, Cotafon and Sahouè people were the the most frequent consumers of *B. pilosa*. For the majority of respondents

(82.35%, 88.88% and 80%) for *L. taraxacifolia*, *B. pilosa* and *A. sessilis*, there were no properly defined post-harvest storage practices for conservation of the three TLVs studied.

Table 6. Consumption practices of *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis* in central and southern Benin

Consumption methods (%)	Pre-cooked and added to sauces or non-cooked ingredients	Triturate and added to sauce or non-cooked ingredients	Raw as salad
Launaea taraxacifolia	68.28	17.07	14.63
Bidens pilosa	100.00	0.00	0.00
Alternanthera sessilis	100.00	0.00	0.00

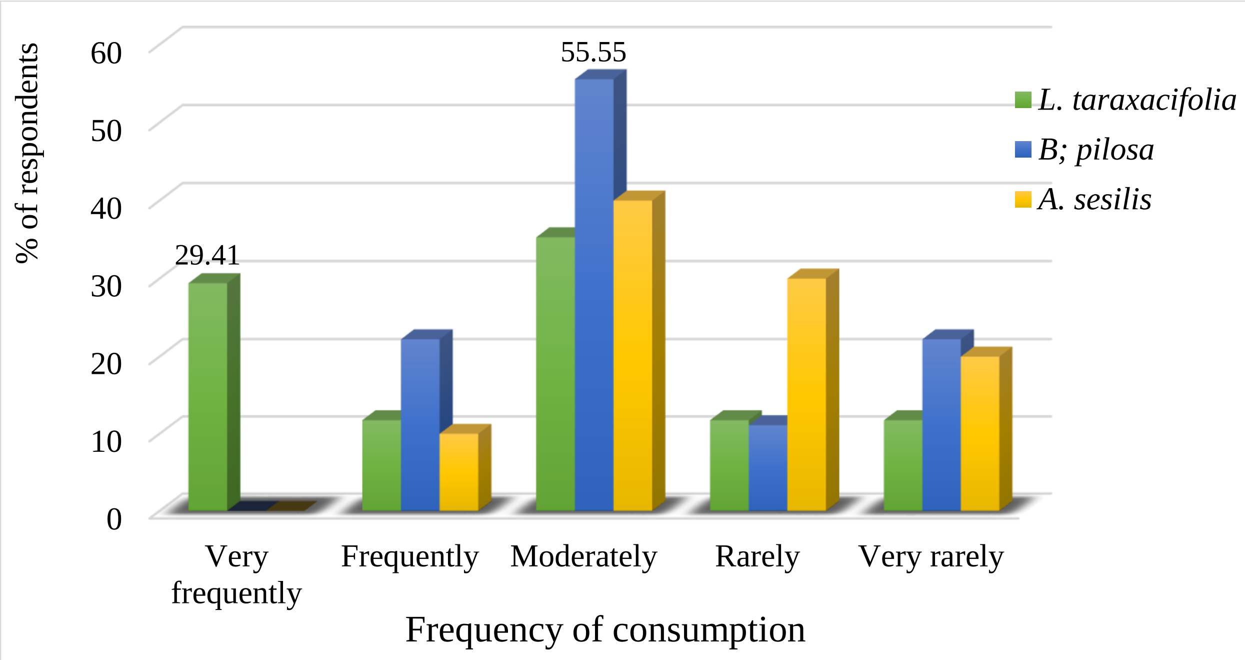

Figure 5. Frequency of consumption of *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis* in the surveyed area

Cultural importance and medicinal values of the species

For all respondents, no particular rituals before consuming the three species studied were necessary and they can be consumed freely at all times. However, *L. taraxacifolia* and *A. sessilis* are prohibited for Hêviosso, Tron and Djaguidi divinity disciples and for «Agossou» (abnormally born baby) parents respectively.

The respondents also reported that these leafy vegetables possessed in addition to their culinary value several medicinal virtues, *L. taraxacifolia* being the most valued medicinally, and is used for the prevention or healing of 21 diseases with 16 possible pharmacological functions among which (Table 7) anti-venomous properties are the most reported (21.05% of responses). Among its medicinal virtues, antalgic, febrifuge, fungicide, blood pressure regulation as well anti-diabetic functions are also well recognized. For the three species, antibiotics and laxative properties are common while, in addition, aphrodisiac properties were reported only for *B. pilosa* and anti-colic for *A. sessilis*.

Table 7. Medicinal properties and possibly cured diseases associated with leafy vegetables of *Launaea taraxacifolia*, *Bidens pilosa* and *Alternanthera sessilis*

Medicinal properties (% of responses)	Possible cured Diseases	*Launaea taraxacifolia*	*Bidens pilosa*	*Alternanthera sessilis*
Antibiotic	Wound, sore throat, abscess	5.26	33.33	10
Anti-venomous	Scorpion/snake bite	21.05	-	-
Anti-poisonous	poison	5.26	-	-
Anti-anaemic	Anaemia	2.63	-	10
Anti-inflammatory	Cramp, navel cicatrizing (baby)	2.63	-	10
Antalgic	Head, eyes and earaches	10.53	-	-
Fungicide	Tetter, tinea, Mycosis	13.16	-	10
Febrifuge	Fever	7.90	-	-
Sedative	Convulsive attack	5.26	-	-
Parasiticide	Guinea worm	2.63	-	-
Blood pressure regulator	Blood pressure	7.90	-	-
Anti-diabetic	Diabetes	2.63	-	-
Anti-coughing	Cough	2.63	-	-
Anti-colic	Stomach disorders	-	-	10
Anti-dizziness	Dizziness	5.26	-	-
Galactogen	Breast milk production fault	2.63	-	-
Laxative	Indigestion, constipation	2.64	33.33	50
Aphrodisiac	Aphrodisiac	-	33.33	-

Discussion

By observation of distribution maps and the geographical localization of species, southern Benin would be the recommended area for carrying out *in situ* conservation of these plant genetic resources. These observations seem different to the findings of Adjatin et al. (2012) and those of Adéoti et al. (2009) who all proposed central Benin for carrying out *in situ* conservation of respectively two species (*Crassocephalum rubens* and *Crassocephalum crepidiodes*) and four species (*Acmella uliginosa*. *Ceratotheca sesamoides*. *Justicia tenella* and *Sesamum radiatum*) of important traditional leafy vegetables in Benin. However, this situation may be explained by the fact that south and central Benin are relatively humid agro-ecological zones (Adam et Boko, 1993), while in the same agro-ecological zone each species requires particular edapho-climatic conditions (soil and climate) to grow well.

Across both villages and ethnic groups surveyed in the study area, variations were recorded among vernacular names while some ethnic groups use a common vernacular for the same species. However, such observed variations of vernacular names are common and were already reported in many crops including cassava (Dansi et al., 2010), sorghum (Mekbib, 2007), cowpea (Gbaguidi et al., 2013), pepper (Orobiyi et al., 2013) and traditional leafy vegetables (Adjatin et al., 2012).

The status (cultivated or not) of domestication of these three TLV species investigated varies among the surveyed villages as reported by Avohou et al. (2012) for *J. tenella* and *S. radiatum*.

The observed progression of domestication level of species may be linked to the economic importance of the species.

The most common harvesting methods of TLVs reported by respondents were uprooting and plant stem cutting. These practices could contribute significantly to loss of species especially by uprooting. It is important to train respondents in different harvesting methods which correspond to the best way to avoid genetic erosion.

According to Grubben and Denton (2004), 50% of sub-Saharan Africa leafy vegetables are harvested from the wild. This is a similar to the situation revealed in this study whereby for each of the three species, more than 50% of respondents reported that leaves are procured by picking from nature. However, if specific measures are not taken to maintain wild diversity, the picking methods may contribute to the genetic erosion of species. Harvesting and procurement methods are thus important factors in conservation and management of genetic resources.

Eating raw leafy vegetables was only reported for *L. taraxacifolia* and may be linked to probable non-toxicity of the species for human consumption as reported for *Crassocephalum* spp. TLVs were eaten as raw green salads in some areas in Nigeria (Grubben and Denton, 2004) and in Benin (Adjatin et al., 2012). Undoubtedly, the frequency of consumption of each TLV is specific for ethnic groups.

The medicinal values reported for *L. taraxacifolia* agree with observations of Arawande et al. (2013) who revealed that leaf extracts mixed with mother's breast milk is administered medically to cure partial blindness resulting from snake bites. According to Yang et al. (2006), bioactive compounds isolated from *B. pilosa* reportedly possess antibiotic and antimalarial properties, and inhibit prostaglandin synthesis. The plant is known in folk medicines, and is used in herbal tea for inflammation, antisepsis, liver protection, blood pressure lowering and hypoglycemia (Deba et al., 2007). There is a close similarity between local knowledge and findings of research reports on the medicinal properties of these three vegetables, and so we can conclude that the respondents have a reliable knowledge on the various ways of their use and so such knowledge can be used in their promotion.

Conclusion

The three species studied here are still mainly wild in Benin and their production is still traditional and organic. Southern Benin may be the best location to carry out *in situ* conservation of these species. Among each of TLVs plants studied, the existence of morphotypes was reported and could be used for breeding purpose. The domestication process as found across surveyed areas is still ongoing and should be encouraged for intensive and optimal production. The people surveyed have a good knowledge of medicinal values of the species and this knowledge can be exploited in their promotion.

Further research is required on the biochemical and phytochemical characterization of the genetic diversity of these species as well as the effects of processing methods on their nutritional value. There is also a need to create awareness among local communities of the importance to apply good harvesting practices for promotion and conservation of the existing diversity.

References

Achigan-Dako E.G., Pasquini M.W., Assogba-Komlan F., N'danikou S., Yédomonhan H., Dansi A., Ambrose-Oji B. 2010. (Editors). Traditional Vegetables in Benin. Institut National des recherches Agricoles du Bénin. 286p. ISBN 978-99919-334-4-3

Adam S., Boko M. 1993. Le Bénin. Les éditions Flamboyant / EDICEF 6p.

Adéoti K., Dansi A., Ahoton L., Kpèki B., Ahohuendo B.C., Ahanchédé A., Vodouhè, R., Hounhouigan J.D., Sanni A. 2009. Selection of sites for the *in situ* conservation of four traditional leafy vegetables consumed in Bénin. International Journal of Biological and Chemical Sciences 3(6):1357-1374.

Adjatin A., Dansi A., Eze C.S., Assogba P., Dossou-Aminon I., Akpagana K., Akoègninou A., Sanni A. 2012. Ethnobotanical investigation and diversity of Gbolo [*Crassocephalum rubens* (Juss. ex Jacq.) S. Moore and *C. crepidioides* (Benth.) S. Moore], a traditional leafy vegetable under domestication in Benin. Genetic Resources and Crop Evolution DOI 10.1007/s10722-012- 9901-z.

Adjatin A., Dansi A., Badoussi E., Loko Y.L., Dansi M., Azokpota P., Gbaguidi F., Ahissou H., Akoègninou A., Akpagan K., Sanni A. 2013a. Phytochemical screening and toxicity studies of *Crassocephalum rubens* (Juss. ex Jacq.) S. Moore and *Crassocephalum crepidioides* (Benth.) S. Moore consumed as vegetable in Benin. International Journal of Current Microbiology and Applied Sciences 2(8): 1-13

Adjatin A., Dansi A., Sanoussi A.F., Badoussi E., Gbaguidi F., Azokpota P., Vodouhe R., Akoègninou A., Akpagana K., Sanni A. 2013b. Proximate, mineral and vitamin C composition of vegetable Gbolo [*Crassocephalum rubens* (Juss. Ex Jacq.) S. Moore and *C. crepidioides* (Benth.) S. Moore] in Benin. Journal of Biology and Life Sciences 7 (1):319-331

Ahmad S.S., Javed S. 2007. Exploring the economic value of underutilized plant species in Ayubia national park. Pak. J. Bot., 39(5): 1435-1442, 2007.

Andzouana M., Mombouli J.B. 2012. Assessment of the Chemical and Phytochemical Constituents of the Leaves of a Wild Vegetable-*Ochthocharis dicellandroides* (Gilg). Pakistan Journal of Nutrition 11(1): 94-99.

Afari-Sefa V., Tenkouano A., Ojiewo C.O., Keatinge J.D.H., Hughes J. d'A. 2012. Vegetable breeding in Africa: constraints, complexity and contributions toward achieving food and nutritional security. Food Security 4:115-127

Arawande J. O., Amoo I.D., Lajide L. 2013. Chemical and phytochemical composition of wild lettuce (*launaea taraxacifolia*). *Journal* of Applied Phytotechnology in Environmental Sanitation 2 (1): 25-30.

Avohou H.T., Vodouhe R.S., Dansi A., Bellon M., Kpeki B. 2012. Ethnobotanical factors influencing the use and management of wild edible plants in agricultural environments in Benin. Ethnobot Res Appl 10:571–592.

Dansi A., Vodouhè R., Azokpota P., Yedomonhan H., Assogba P., Adjatin A., Loko Y.L., Dossou-Aminon I., Akpagana K., 2012. Diversity of the Neglected and Underutilized Crop species of importance in Benin. ID 932947, DOI 10.1100/2012/932947, Sci. World J., p. 19.

Dansi A., Adoukonou-Sagbadja H., Vodouhè R. 2010. Diversity, conservation and related wild species of fonio millet (*Digitaria* spp) in the northwest of Benin. Genetic Resources and Crop Evolution 57: 827-839.

Dansi A., Adjatin A., Adoukonou-Sagbadja H., Faladé V., Yedomonhan H., Odou D., Dossou B. 2008. Traditional leafy vegetables and their use in the Benin Republic. Genetic Resources and Crop Evolution 55: 1239-1256.

Deba F., Xuan T. D. Yasuda M., Tawata S. 2007. Herbicidal and fungicidal activities and identification of potential phytotoxins from *Bidens pilosa* L. var. *radiata* Scherff. Weed Biology and Management 7: 77–83

Gbaguidi A.A., Dansi A., Loko L.Y., Dansi M., Sanni A. 2013. Diversity and agronomic performances of the cowpea (*Vigna unguiculata* Walp.) landraces in Southern Benin. International Research Journal of Agricultural Science and Soil Science 3(4): 121-133

Grubben G.J.H., Denton O. A. 2004. Plant Resources of Tropical Africa vegetables. Ponennad Looijenhv, Wageningen, pp. 667

Mekbib F. 2007. Infra-specific folk taxonomy in sorghum (Sorghum bicolor (L.) Moench) in Ethiopia: folk nomenclature, classification, and criteria. Journal of Ethnobiology and Ethnomedicine 3:645–663.

Olaposi A.R., Adunni A.O. 2010. Chemical composition of three traditional vegetables in Nigeria. Pakistan Journal of Nutrition 9(9): 858-860.

Orobiyi A., Dansi A., Assogba P., Loko L.Y., Dansi M., Vodouhè R., Akouègninou A., Sanni A. 2013. Chili (*Capsicum annuum* L.) in southern Benin: production constraints, varietal diversity, preference criteria and participatory evaluation International. Research Journal of Agricultural Science and Soil Science 3(4): 107-120.

Smith I.F., Eyzaguirre P. 2007. African leafy vegetables: their roles in the World Health Organization"s global fruits and vegetables initiative. African Journal of Food Agriculture Nutrition and Development, Vol. 7 (3) 1-17.

Ukpong IG, Idiong IC. 2013. Maximum likelihood estimates and determinants of technical efficiency of leafy vegetable producers in Akwa Ibom State, Nigeria. Journal of Agricultural Science; doi:10.5539/jas.v5n3p139 (online first)

Vodouhè R., Dansi A., Avohou H.T., Kpèti B., Azihou F. 2011. Plant domestication and its contributions to *in situ* conservation of genetic resources in Benin. International Journal of Biodiversity and Conservation 3(1): 40-56.

Vorster I.H.J, Van Rensburg W.J., Van Zijl J., Venter S.L. 2007. The importance of traditional leafy vegetables in South Africa. AJFND 2007, 7(4):1–13.

Yang H., Chen S., Chang N., Chang J., Lee M., Tsai P., F, H., Kao W., Chiang H., Wang H., Hseu Y. 2006. Protection from oxidative damage using *Bidens pilosa*. Food and Chemical Toxicology 44: 1513–1521.

Yang R-Y., Keding G.B. 2009. Nutritional contributions of important African indigenous vegetables. In: Shackleton C.M., Pasquini M.W., Drescher A.W. 2009. African indigenous vegetables in urban agriculture. Earthscan, London

Progress in agronomic, nutritional and engineering development research on the tree crop, *Treculia africana*

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Abstract

African breadfruit (*Treculia africana* subsp. *africana* Decne) is an underutilized tree crop in the family Moraceae. An evergreen forest fruit tree in tropical Africa, it produces large round compound fruits which are covered with rough pointed outgrowths. The seeds are buried in the spongy pulp of the fruits. It is an important food item in parts of tropical West Africa, and is variously cooked as pottage, or roasted and sold with palm kernel (*Elaeis guineensis* Jacq.) or coconut as a roadside snack. The flour has high potential usage for production of pastries. The seeds are very nutritious and constitute a vital source of vitamins, minerals, proteins, carbohydrates and fats. This paper reports recent efforts to upgrade the value chains of some varieties of *Treculia africana* found in parts of Ghana and Nigeria. Particular attention is paid to progress in agronomic, nutritional and engineering development research, as well as consumer preferences after alternative processing operations. The agronomic studies showed that seed sterilization resulted in a lower proportion of deformed seedlings. About 63% of seedlings arising from seeds previously treated with 10% dilution of NaOCl had true leaves and each seedling thereof had more leaves. The nutritional studies determined the best methods of seed extraction and demucilagination for use in high-quality flour production. The production of pasta, breakfast meal and good quality oil are also demonstrated. Design, construction and testing of a continuous flow machine for depulping the partially fermented fruits resulted in a potentially significant reduction in the drudgery associated with manual processing. The best conditions for dehulling the seeds after parboiling were determined. Consumer preferences for several derived products were very high. For more effective widespread introduction of the tree crop into the food chain, efforts to extend the mature technologies and full mechanization of depulping and other post-harvest operations should be encouraged.

Introduction

Importance

African breadfruit (*Treculia africana* subsp. *africana* Decne) belongs to the family Moraceae. It is an evergreen forest fruit tree in tropical Africa. As shown in Figure 1, the plant produces a large compound fruit, usually round, and covered with rough pointed outgrowths. The seeds are buried in the spongy pulp of the fruits.

Some varieties of *T. africana* and *Artocarpus altilis* (also called breadfruit) are produced and used in Ghana, as shown in Figure 2. The seed is an important food item, popularly known as "Ukwa" by the Igbo tribal group of southeastern Nigeria. Three varieties of the seeds reported by Akubuo (2006) are shown in Figure 3.

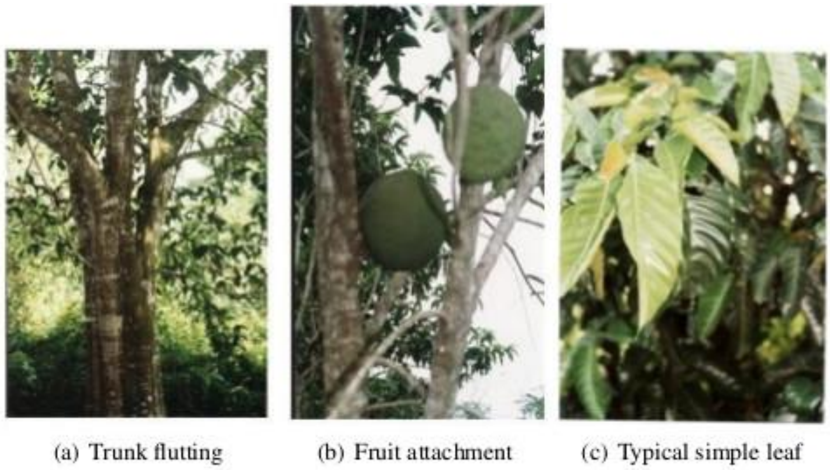


Figure 1. *Treculia africana* tree in Nigeria showing fruit attachment and leaf structure (Source: Mbah, 2005)

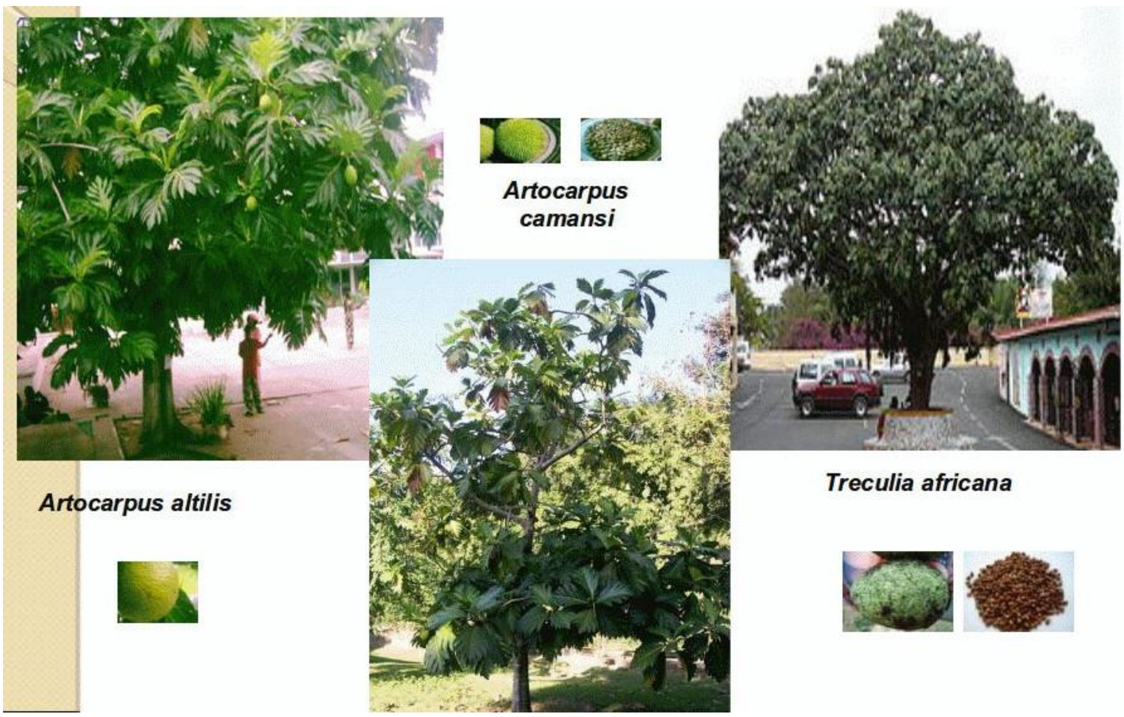


Figure 2. Breadfruit varieties in Ghana. (Source: Oduro et al., 2007)

(a) Large sized (*Var africana*) (b) Medium sized (*Var inverse*) (c) Small sized (*Var molis*)

Figure 3. Different varieties of the breadfruit seeds. (Source: Akubuo, 2006)

The seed is variously cooked as pottage, or roasted and sold with palm kernel (*Elaeis guineensis* Jacq.) as a roadside snack, as shown in Figure 4(a). The flour has high potential usage for pastries. Figure 4(b) shows samples of processed *T. africana* on sale, while Figure 4(b) shows cooked breadfruit on sale as pottage in a restaurant.

(a) Roasted (b) Parboiled (c) Cooked

Figure 4. Processed breadfruit on sale in Nigeria. (Source: Enibe, 2013)

The seeds are highly nutritious and constitute a vital source of vitamins, minerals, proteins, carbohydrates and fats (Okafor and Okolo, 1974). African breadfruit is an important natural resource in parts of tropical West Africa, contributing significantly to the income and dietary intake of the people.

TARP (The *Treculia africana* Research Project)

The *Treculia africana* Research Project (TARP) was conceived to:

1. Improve the widespread use and acceptability of the crop.
2. Develop early maturing varieties of the crop to facilitate the development of commercial plantations and orchards.
3. Develop mechanical equipment for its processing (especially depulping & dehulling).
4. Develop modern foods and beverages from the crop.
5. Upgrade the value chain of the crop.

The Project team comprises scientists and engineers drawn from Universities in Nigeria and Ghana. The present report summarizes the most recent results obtained by members of the team facilitated by an initial funding from the African Forestry Research Network (AFORNET) of the African Academy of Sciences (AAS).

Seedling quality

Baiyeri and Mbah (2006a) evaluated the effects of factorial combinations of four storage durations (in days after seed extraction) and surface sterilization with three dilution levels of sodium hypochlorite on seedling emergence and quality. The specific objective was to identify the after-ripening treatment that could boost seedling emergence percent and the quality of seedlings obtained thereof.

The experiment was conducted in a controlled environment between July and September 2003. Seeds were extracted from a single ripe fruit of *T. africana* subsp. *africana*. Seeds were washed and only viable seeds, determined by floatation method, were used. The seeds were air-dried for two hours and only fully filled seeds were sorted out for use. Six hundred well-filled seeds were finally selected for the experiment.

The experiment was a factorial laid out in a completely randomized design. The factors were number of days in storage and sterilization with sodium hypochlorite (NaOCl, 3.5% active ingredient). Storage durations were 0, 3, 6, and 9 days, while levels of sterilization were 100% water (control), 90% water plus 10% NaOCl (10% dilution) and 95% water plus 5% NaOCl (5% dilution). There were therefore, 12 treatment combinations, each replicated 5 times, and each replicate was sown with 10 seeds.

Parameters measured included number of days to seedling emergence, percent cumulative emergence, total number of true leaves produced by emerged seedlings per treatment combination, percent emerged seedlings that had produced true leaves and percent deformed seedlings.

Seeds stored for three or six days before planting emerged earlier than those planted immediately after extraction from the fruit pulp or those stored for nine days. Cumulative percent seedling emergence was statistically similar if seed planting was not delayed beyond six days of extraction.

Seed sterilization resulted in a lower proportion of deformed seedlings. About 63% of seedlings arising from seeds previously treated with 10% dilution of NaOCl had true leaves and each seedling had more leaves. The combined effects of storage and sterilization on days to seedling emergence and percent cumulative seedling emergence are shown in Figure 5. A higher proportion of seedlings arising from seeds sterilized with 10% dilution of NaOCl and planted within six days of extraction, produced true leaves (Figure 6(A).

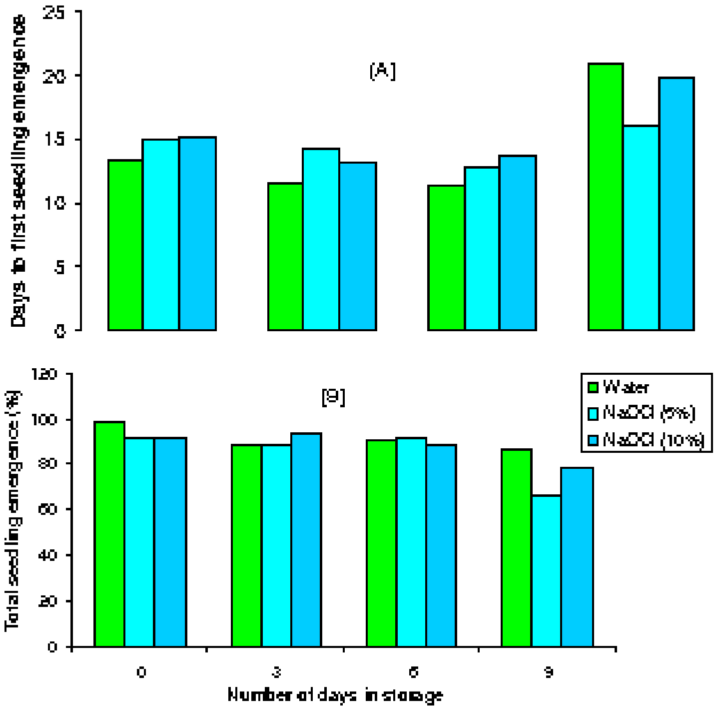


Figure 5. The effects of number of days of seed storage and surface sterilization with sodium hypochlorite at different dilutions on (A) days to first seedling emergence and (B) percent total seedling emergence.

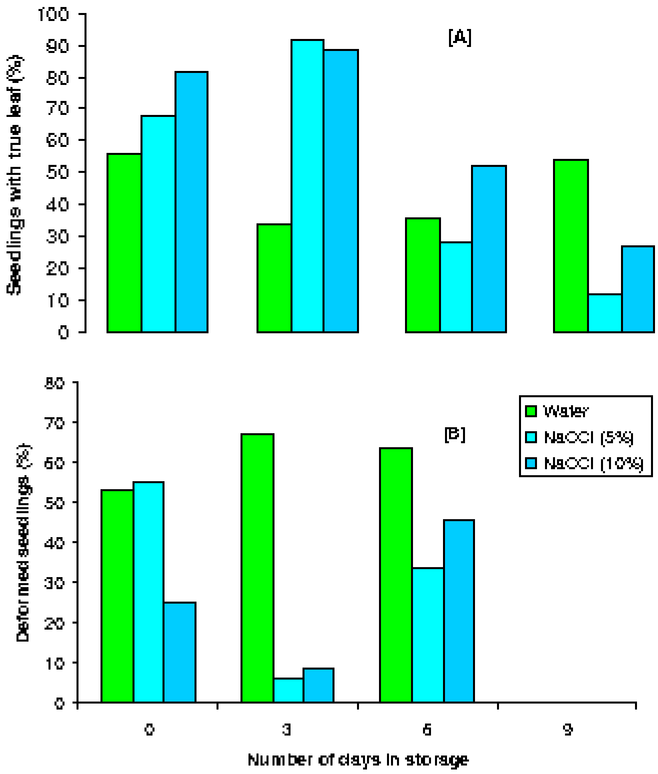


Figure 6. The effects of number of days of seed storage and surface sterilization with sodium hypochlorite at different dilutions on (A) percent seedlings with true leaves and (B) percent deformed seedlings.

Seeds planted three days after extraction showed distinctively the effect of sterilization on foliage development. Deformity is of course unwanted; it results in seedlings unfit for field establishment. Figure 6(b) showed that there were no deformed seedlings arising from seeds stored for nine days.

Nursery media

The effects of soilless and soil-based nursery media on seedling emergence, growth and response to water stress of African breadfruit is reported in Baiyeri and Mbah (2006b).

Seedling emergence: the percent emergence was generally low and occurred during a span of eight weeks (Table 3).

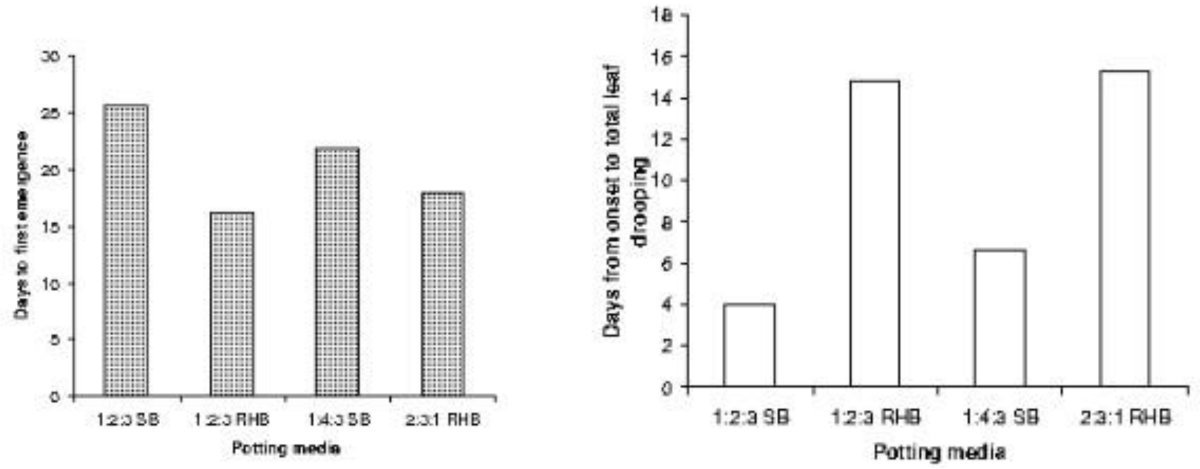
Table 3. Effect of potting media and weeks after planting on African breadfruit (*Treculia africana*) seedling emergence

Weeks after planting							
Media*	2	3	4	5	6	7	8
1:2:3 SB	0.0	8.0[¥]	13.0	14.0	17.0	18.0	18.0
1:2:3 RHB	3.0	37.0	45.0	53.0	56.0	57.0	57.0
1:4:3 RHB	0.0	11.0	30.0	34.0	38.0	39.0	41.0
2:3:1 RHB	1.0	26.0	39.0	42.0	46.0	51.0	52.0
LSD(0.05)	ns	15.5	13.6	13.0	13.8	13.3	13.4

¥Seedling emergence (%).

Percent emergence: percent seedling emergence was consistently highest in medium 1:2:3 RHB and lowest in medium 1:2:3 SB. There was no appreciable increase in percent emergence after the sixth week of planting.

Onset of seedling emergence: Figure 7 shows apparent variability in days to onset of seedling emergence as influenced by potting media. The soil-based medium which had the poorest total emergence similarly had the longest days to first seedling emergence. The earliest days to seedling emergence was obtained in medium 1:2:3 RHB. The duration between the onset of loss of turgidity and when all leaves have drooped as influenced by potting media is shown in Figure 7(b). All leaves on seedlings raised in the soil-based medium lost turgidity within four days, whereas, it took about 15 days between onset and complete loss of turgidity by all leaves for seedlings raised in media 1:2:3 RHB and 2:3:1 RHB. Leaf yellowing followed a similar trend of loss of turgidity.



(a) Onset of seedling emergence (b) Loss of turgidity

Figure 7. Variations in number of days to onset of seedling emergence and loss of turgidity as influenced by potting media.

Seedlings raised in medium 1:4:3 RHB had more leaves, longer stem and thicker stem girth at 24 weeks after planting. However, seedlings that grew in the soil-based medium had longer roots. Total dry matter was higher in soil-based medium followed by those grown in 1:4:3 RHB.

However, seedlings that grew in medium 1:4:3 RHB had higher values for leaf mineral elements except percent nitrogen. On the other hand, seedlings used for the water stress experiment were statistically similar in height and they produced similar number of leaves during the stress period.

Evidence from the seedling emergence, seedling growth, and seedling dry matter content and distribution, and seedling responses to water stress suggested that media 1:2:3 RHB and 2:3:1 RHB were adjudged the best soilless media. Seedling grown in these media had delayed water stress symptom expression suggesting a better water economy.

Depulping

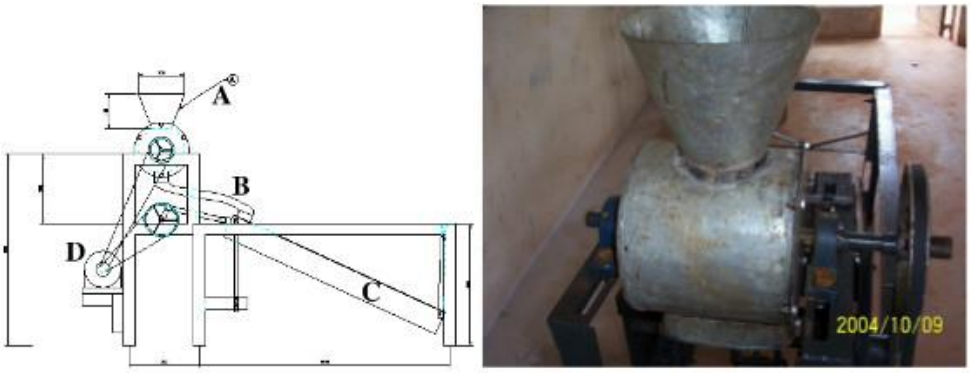
Depulping is the most labour-intensive and least mechanized post-harvest processing operation for *T. africana*. This necessitated the development of a batch depulping machine. Improvements on this initial version resulted in the continuous-flow version (Enibe et al., 2011).

The continous-flow machine consists of four main units, namely the hopper/depulping chamber, the connector-pipe, the separation chamber and power system. It was designed to achieve the following objectives:

1. *Low cost:* the machine was conceived to be cheap to fabricate, operate and maintain, and this was achieved by the use of readily available materials in constructing the machine.
2. *Ease of fabrication, operation, assembly and de-assembly for maintenance*: this was achieved by the extensive use of screw fasteners to hold different components together.

3. *Durability:* the various components were designed to be durable in order to eliminate frequent breakdown of the machine.
4. *Minimal water consumption*.
5. *Minimal manual handling*: this was in order eliminate the messy nature of the traditional processing method, and this was achieved by the continuous flow process of operation.

The general appearance is shown in Figure 8.



(a) Schematic diagram (b) Hopper/depulping chamber

Figure 8. General view of the continuous flow depulping machine. **A:** hopper/depulping chamber; **B:** connector; **C:** separator; **D:** power transmission system.

A computer program for the sequence, "SIXBAR" reported in Norton (1999) was used to analyse the system and obtain values of linear and angular positions, velocities, accelerations and forces in the links in the system. Kinematic and dynamic analyses were also carried out. An inside view of the separation chamber is shown in Figure 9(a), while top and bottom views of the water tank constituting the cover are shown in Figure 9(b) and (c). The baffles may be clearly seen inclined at an angle to the tray axis.

(a) Separator tray (b) Top View (c) Bottom View

Figure 9. Separator tray and water tank doubling as separation chamber cover.

As a preliminary evaluation of the machine performance, the effect of the level of fermentation and the method of feeding were investigated for the de-pulping chamber only and for a shaft speed of 316.7 rpm.

In order to determine the effectiveness of the de-pulping chamber, 2042 cm^3 (2 litres) of well fermented fruit were introduced into the hopper, the machine was started and water supplied. The machine was kept running until all the fruit introduced had passed through the de-pulping chamber and collected at the chamber outlet. The machine was turned off. The quantity of water consumed as well as the time taken to complete the operation was determined.
The seeds in the slurry collected at the outlet were sorted to obtain the number of seed completely de-pulped, N_1, the number of seeds partially de-pulped, N_2 and the number of broken seeds, N_3. Fractions of completely de-pulped seeds, N_1 / N_0, partially de-pulped seeds, N_2 / N_0 and broken seeds, N_3 / N_0 were computed and expressed as percentages. The effectiveness was given by the first expression, N_1 / N_0 and the ineffectiveness by [100 - effectiveness]. The total number of seeds collected, $N_0 = N_1{+}N_2{+}N_3$.

The effect of the level of fermentation and method of feeding on the machine performance is shown in Figure 10. It may be seen that the effectiveness of the machine improved with intermittent feeding of the fruit into the machine as opposed to choke/batch feeding. Also, the effectiveness improved when processing partially fermented fruit compared to well fermented fruit.

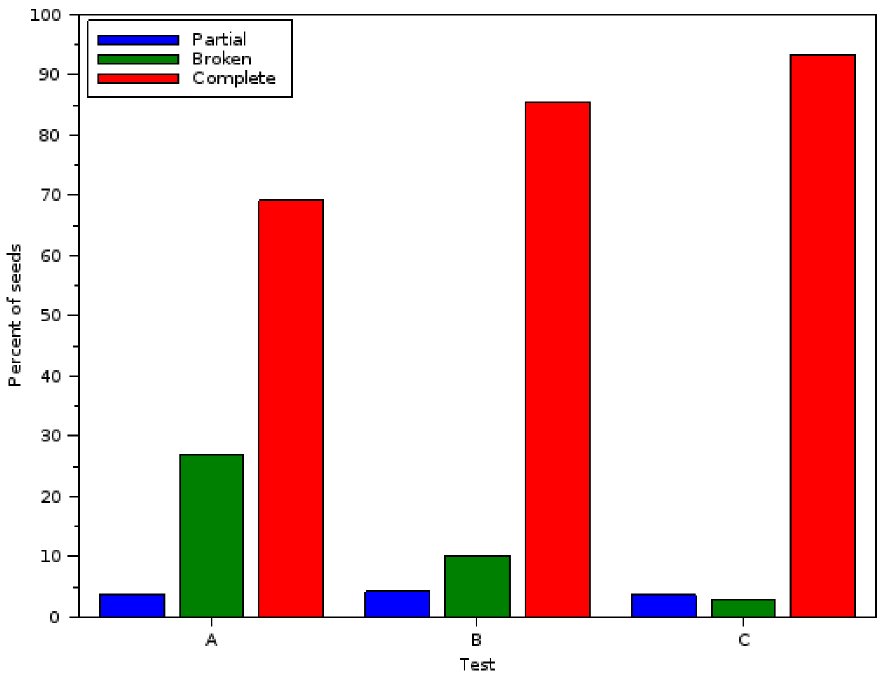


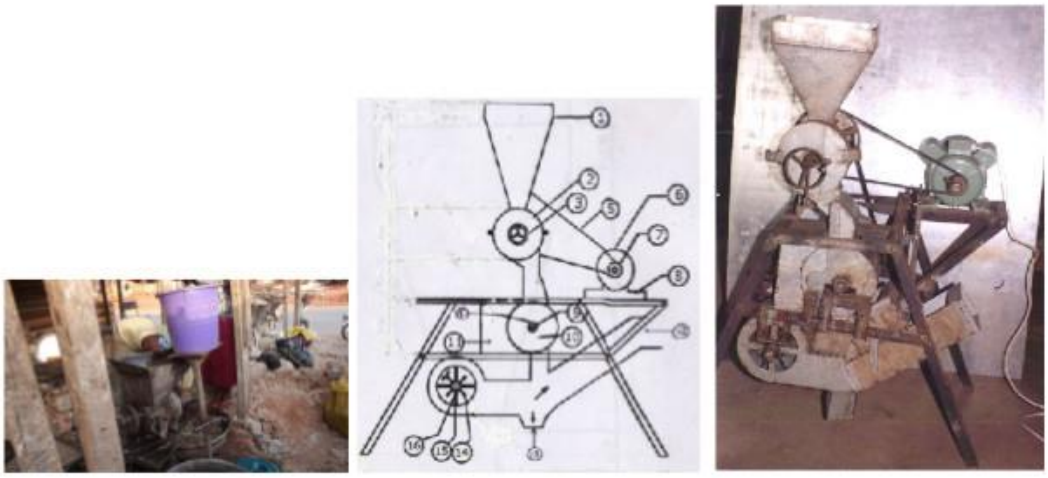
Figure 10. De-pulper performance vs feed method and fermentation level. A = batch feeding of completely fermented fruit; B = intermittent feeding of completely fermented fruit; C = intermittent feeding of partially fermented fruit.

It was observed during the tests that the wire mesh fixed to the outlet of the de-pulping chamber for controlling the flow of the de-pulped fruit out of the chamber tended to prevent the flow of the slurry altogether, and thus no conclusive deductions can be made of the times indicated for the tests. The same also applies for the measured volume of water used up for the processes. Further work is underway to develop an improved version of the depulping machine which is suitable for commercialization.

Dehulling

Dehulling of parboiled seeds is now routinely achieved using domestic grinding machines, but engineering studies of the mechanical processes involved received attention only recently. A detailed study on the development of a customized dehulling machine for *T. africana* seeds has been reported by Akubuo (2006).
Three varieties of the African breadfruit seeds as identified were collected separately: these were the var. *africana* – large sized seeds; var. *inverse* – medium sized seeds and the var. *molis* – small sized seeds (see Figure 3).

The physical properties for which data were collected are: moisture content, seed characteristic dimensions, gravimetric composition, density characteristics and shape factor. The samples used were parboiled for five, seven and ten minutes respectively. The terminal velocities for the kernel and hull (chaff) were determined using the experimental set-up by Emah (2006). Selected physical and mechanical properties of the seeds were determined and utilized in the development of the improved version. Part (a) of Figure 11 shows the domestic grinding machine used as a dehuller, while parts (b) and (c) show the schematic diagram and photograph of the improved breadfruit dehuller.



(a) Domestic grinder as dehuller (b) Schematic diagram (c) Photograph

Figure 11. Schematic diagram and photograph of the breadfruit dehuller.

Key: 1 feed hopper; 2 dehulling chamber casing; 3 handle for disk adjustment; 4 roller shaft; 5 motor disk belt drive; 6 electric motor; 7 electric motor pulley; 8 electric motor stand; 9 roller; 10 roller shaft bearing; 11 concave plate; 12 hull outlet chute; 13 seed outlet chute; 14 fan blade; 15 fan casing; 16 fan shaft bearing. Sources: (a) Enibe (2013); (b & c) Akubuo (2006).

The seed is introduced through the feed hopper and dehulling is achieved in stages as the seed passes through the three major units of the machine. At the time of completion of the construction of the breadfruit seed dehuller, only large-sized breadfruit seeds could be obtained locally. The preliminary tests were conducted with the large-sized seeds.

About 850 g of the seeds were parboiled separately for 5, 7 and 10 minutes, and used for the tests. Each test run lasted for about 45 s. The samples were spread out for about 3 minutes and allowed to dry in order to remove the surface water before using them for the tests. Their moisture contents were determined using the oven-dry method. The dehuller was run at the speed of 200 rpm for each test. Calculations were made to determine the percentages of completely dehulled, partially dehulled, undehulled, breakages and dehulling efficiency which are measures of the effectiveness of the dehuller. Results are shown in Table 4. The results at present indicate that dehulling is best after parboiling the seeds for seven minutes followed by the ten minutes parboiling result.

Table 4. Preliminary performance result of the breadfruit seed dehuller using large sized breadfruit seeds (*Treculia africana* var.*africana*)

	Parboiling time (min)		
	5	7	10
Moisture content % wb	28.4	33.3	37.5
Completely dehulled, %	67.73	74.92	70.71
Partially dehulled, %	10.27	3.70	5.02
Undehulled, %	28.0	18.38	24.42
Breakages, %	21.6	12.48	15.65
Dehulling efficiency, %	53.0	75.46	65.46
Throughput, kg/h	66.23	69.70	74.20

Pasta production

Table 5. Flour composition of blends (composite flour)

Blend	711	721	731	741	751	761
Wheat flour %	100	90	80	70	60	50
Breadfruit flour %	0	10	20	30	40	50

The composite flours, produced as shown in Table 5 were evaluated for physico-chemical properties and proximate composition.

The proximate composition of the wheat flour and breadfruit flour are shown in Table 6. Moisture content of the breadfruit flour was low (5.04%) relative to the wheat flour (10.08%). Swelling power, water binding capacity and solubility increased from 10-50% breadfruit substitutions.

Sensory evaluation of the pasta product showed that sample 731 had the most preferred appearance, colour and firmness by hand, whilst sample 721 was the most preferred in terms of aftertaste and firmness by teeth. However, sample 741 was the most preferred overall. Sensory evaluation results of the pasta product showed that the overall evaluation of pasta from 100% wheat was the most preferred relative to the other products.

Table 6. Nutritional composition of the various blends of wheat-breadfruit flour

Sample	Moisture (%)	Crude fat (%)	Crude fiber (%)	Crude protein (%)	Ash (%)	Carbohydrate (%)
721	9.48 (0.04)	1.65 (0.03)	2.28 (0.01)	13.84 (0.05)	0.78 (0.03)	71.97
731	9.13 (0.07)	1.76 (0.10)	2.23 (0.02)	12.65 (0.03)	0.84 (0.30)	73.39
741	8.58 (0.03)	1.80 (0.30)	2.56 (0.03)	11.80 (0.02)	0.88 (0.10)	74.38
751	8.05 (0.05)	2.10 (0.03)	2.86 (0.01)	10.57 (0.02)	1.07 (0.07)	75.35
761	7.53 (0.01)	2.16 (0.20)	3.08 (0.01)	9.40 (0.04)	1.18 (0.03)	76.65
Wheat flour 711	10.08 (0.01)	1.50 (0.70)	2.00 (0.03)	14.50 (0.03)	0.58 (0.10)	71.34
Breadfruit flour	5.04 (0.05)	2.82 (0.04)	4.06 (0.05)	3.23 (0.02)	1.96 (0.03)	82.84

Standard deviations are given in parenthesis and values are means of duplicate determinations.

Results showed that there was a significance difference among composite samples $P < 0.05$ for all the variables. There was increase in crude fat, crude fibre, ash and carbohydrate content with increase in percentage substitution of non-wheat flour. The same trend was observed in swelling power, solubility and water binding capacity for flour composites.
A decrease in moisture content, protein content, and bulk density was observed for flour samples as level of substitution of non-wheat flour increased from 10% to 50%. The least gelation concentration LGC for each composite flour was constant (15%). Pasta made from 70% wheat and 30% breadfruit (741) was the most preferred after the control, 100% wheat (711). Therefore breadfruit can be used in composite flour as an alternative source for pasta production.

Breakfast meal

The use of blends of breadfruit and soybean composite for breakfast meal production is reported in Oduro et al. (2007a). The study investigated the quality and acceptability of breakfast meals produced from various breadfruit-soybean composite flours.
Blends were formulated with a soybean substitution of 10%, 30%, 50%, 70%, and 90% as shown in Table 7.

Table 7. Flour composition of blends

Blend	Control	101	102	103	104	105	106
Breadfruit flour,%	0	100	90	70	50	30	10
Soybean flour, %	100	0	10	30	50	70	90

The proximate composition of the blends and the acceptability of the formulated products were determined. The results showed the blends to have a crude protein content between 6.85-36.59%, crude fat content of 4.44-18.12%, carbohydrate content of 33.15-77.84%, ash content of 2.325.06%, and energy value of 378.72-442.04 Kcal/100g.

The sensory analysis showed that the formulated products were acceptable with preference more tilted towards blends with higher soybean content. The response for the overall acceptability (Figure 12) showed no significant difference P > 0.05 and had this order of preference 104 and 105, 102, 103, and 101. This implies that for overall acceptance, with the exception of blend 102, preference was higher for those blends with higher levels of soybean flour relative to those with higher breadfruit flour.

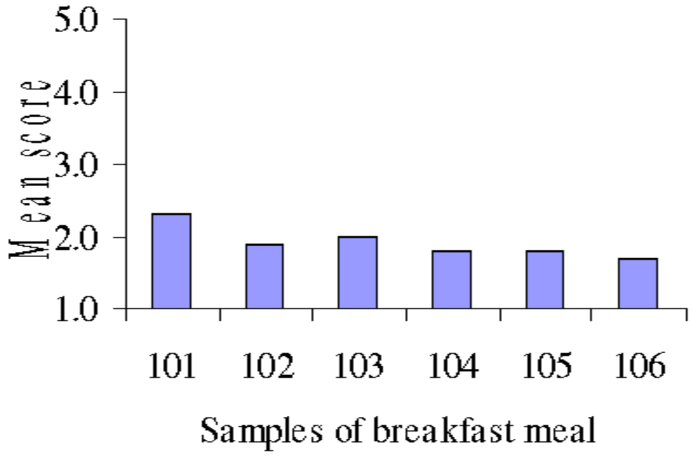


Figure 12. Mean Score for the overall acceptability of breakfast meal

Oil quality

Ellis et al. (2006) carried out studies to assess the quality of oil from *T. africana* seeds. Fresh dried seeds were dehusked and milled, and oil was extracted using the Soxhlet extraction procedure and the yield and quality characteristics of the oil evaluated. Parameters assessed included specific gravity, acid value, saponification value, peroxide value, iodine value, refractive index and free fatty acid content among others. The solubility of the oil in selected organic solvents and the presence of lipid soluble phytochemicals was determined. The nutritional quality of the seeds was also evaluated. The results showed the seeds were high in carbohydrate and proteins with appreciable levels of ash and oil and low fibre content. Potassium and phosphorus levels were relatively high with low calcium and iron levels (see Table 8).

Table 8. Proximate composition and physicochemical properties

(a) Proximate composition of seeds

Parameter	
Husks of seeds (%)	23.40 (0.40)
Moisture (%)	9.72 (0.05)
Crude Protein (%)	13.35 (0.02)
Crude Fat (%)	10.12 (0.02)
Ash (%)	1.96 (0.05)
Crude Fibre (%)	2.83 (0.005)
Carbohydrate (%)	62.01
Energy (kcal/100mg)	392.49
Fe (mg/100mg)	8.70
Ca (mg/100mg)	93.90
K (mg/100mg)	464.60
P (mg/100mg)	1300.00

(b) Physicochemical properties of seed oil

Parameter	Value
Yield (%)	11.82 (1.82)
Refractive index	1.46 (0.14)
Free fatty acid (%)	3.27 (0.02)
Specific gravity	0.89 (0.02)
Peroxide value	2.67 (0.27)
Iodine value	36.55 (0.36)
Acid value	7.29
Saponification value	128.33 (0.33)
Colour	Yellow
Taste	Harsh
Odour	Pleasant

The oil yield was low (11.82%) below the level for commercial sources of oil. The oil had a high specific gravity (0.89), good refractive index (1.47) and an iodine value of less than 100 (35.66). The peroxide value was also low (2.67) but within the range for fats and oils with a relatively high free fatty acids (FFA) (7.26%). The saponification value relative to other oils was low, 128.33 Table 8(b).

Identified lipid-soluble phytochemicals were carotenoids, terpenes, and saponins. Tannins were absent. Even though the yield was low, the quality of the oil from *T. africana* seeds was good and can be used as a supplement with other oils in the food sector.

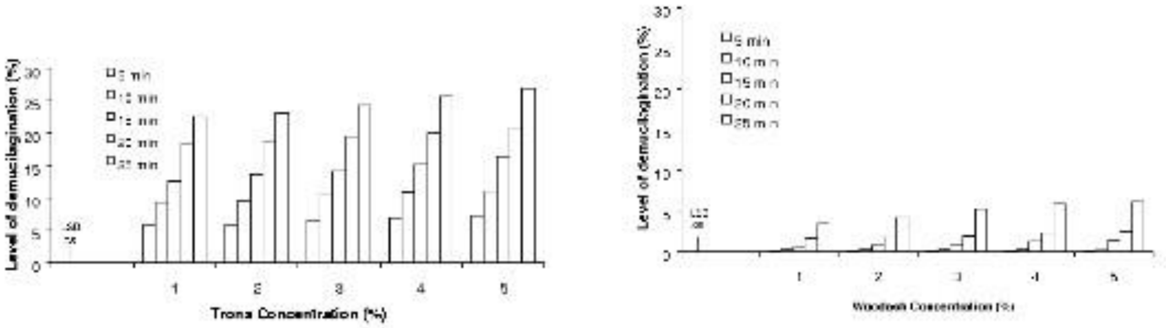
Demucilagination

Onweluzo and Odume (2007) reported studies on the effects of method of extraction and demucilagination of *T. africana* on its composition. Mature fresh fruit heads of *T. africana* were procured and divided into four treatment groups using a randomized complete block design. The first group was allowed to ferment naturally for eight days before the seeds were extracted and washed (fermented control). The second group was quartered, after which the seeds were extracted fresh from the pulp, demucilaginated by brushing with fine sea sand and subsequently rinsed with water (unfermented control). Groups 3 and 4 were also quartered and the seeds were extracted fresh as in group 2 but the seeds were divided into 10 portions and treated with graded concentrations (1% - 5%) of trona and wood ash for times varying from 5 to 25 min.

Following the alkaline treatments the seeds were washed with water and the effectiveness of the treatments in removing the seed mucilage was determined by weight differences. The demucilaginated seeds were dried in a hot air oven at 850^{0}C for 48 h, dehulled and milled into flour to pass through a 40 mm mesh (British Standard Sieves) in an attrition mill. The flours were packed in polyethylene bags, heatsealed and stored at between 0 and 4^{0}C until used for analysis.

Proximate analyses for percentage moisture, crude fat, protein (N x 6.25), crude fibre and ash were done according to the standard method of the AOAC (1990). The nitrogen free extract (total carbohydrate) was calculated by difference. The ether extract was analysed for peroxide value and free fatty acid content using the standard method of Pearson (1991). Trace elements were estimated after wet oxidation of samples (2 g) using concentrated nitric acid and perchloric acid as described by Osborne and Voogt (1978). The concentration of the minerals, calcium, magnesium, copper and zinc in the digested sample were determined with the PyeUnicam Atomic Absorption Spectrophotometer. Potassium and sodium were determined with a Flame Photometer.

Figures 13(a) and 13(b) show the effectiveness of the different concentrations of trona (1% - 5%) and wood ash (1% - 5%) in demucilaginating freshly extracted *T. africana* seeds. Mucilage constitutes about 30 + 2% of whole *T. africana* seeds used in the study on a wet basis.



(a) Trona (b) Woodash

Figure 13. Effect of concentration of Trona/wood ash and treatment time on the demucilagination of *Treculia africana* endocarp.

The effect of the demucilaginating treatments on the colour, bulk density and water absorption capacity of the dehulled *T. africana* seed flour was also determined. Samples from all the treatments showed marginal variations in proximate composition.

Figure 14 shows the effect of treatment on the peroxide value and free fatty acid content of the *T. africana* seed ether extract.

Consumer acceptability

Consumer acceptability studies for *T. africana* conducted in Anambra State of Nigeria were reported by Enibe (2007). Four rural communities were randomly selected for the study. In each community, 30 households were randomly selected and interviewed, yielding a total of 120 households. About 60% of the respondents gave first preference to *T. africana* meals in place of other foods made from cassava, rice or yam. Further work on consumer acceptability and marketing of *T. africana* in Nigeria is ongoing, (Enibe, 2013).

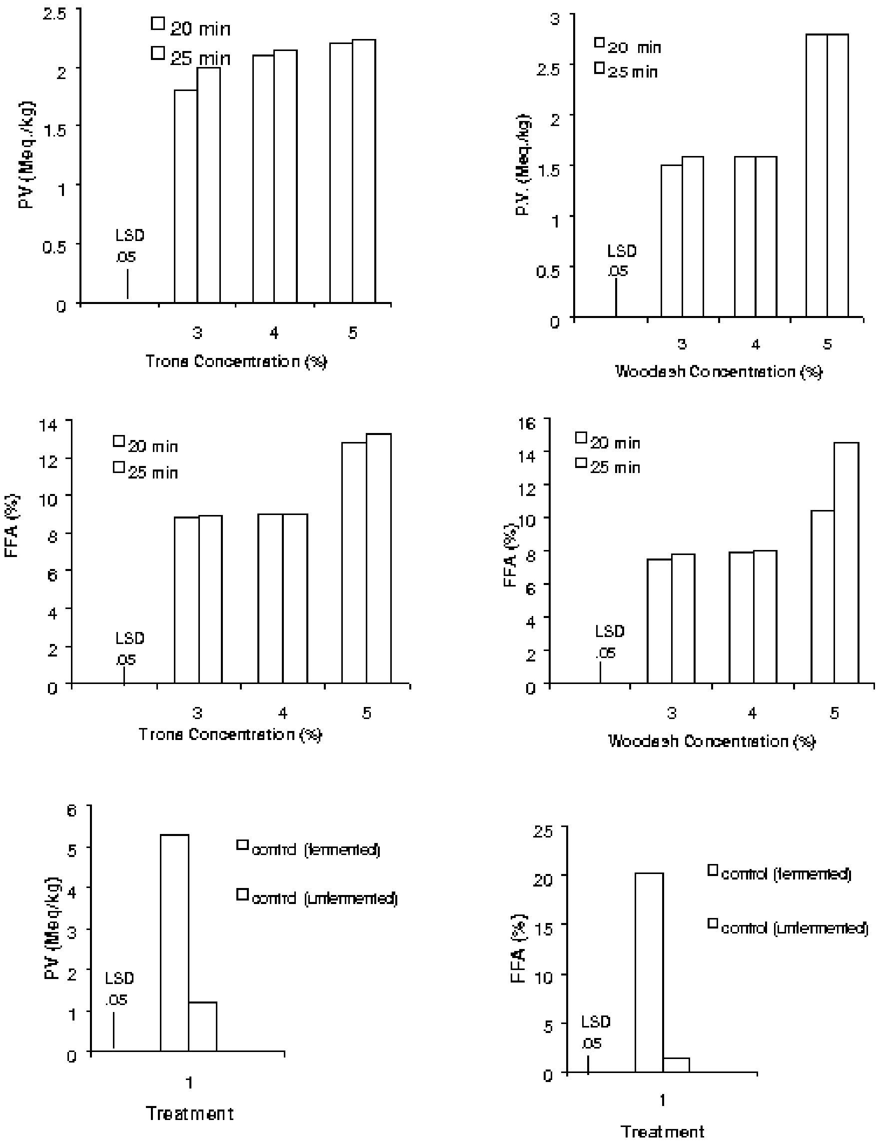


Figure 14. Effect of demucilaginating treatments on the peroxide value (PV) and Free Fatty Acid (FFA) content of *Treculia africana* seed ether extract.

Recommendations

Sustained funding of research and development efforts are required to:

1. Improve the widespread use and acceptability of the crop.
2. Develop early maturing varieties of the crop to facilitate the development of commercial plantations and orchards.
3. Commercialize mechanical equipment for its processing (especially depulping and dehulling.
4. Develop modern food and beverages from the crop.
5. Upgrade the value chain of the crop.

Acknowledgements

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References

Akubuo C.O. 2006 African breadfruit (*Treculia africana*) seed dehulling studies. Technical Report No. TARP/DEHULLING/3, Department of Agricultural & Bioresources Engineering, University of Nigeria, Nsukka, Nigeria.

AOAC. 1990. Official Methods of Analysis 15th edn. Association of Official Analytical Chemists Washington D.C.

Baiyeri K.P., Mbah B.N. 2006a Effects of soilless and soil-based nursery media on seedling emergence, growth and response to water stress of African breadfruit (Treculia africana Decne). African Journal of Biotechnology 5(15): 1405–1410.

Baiyeri K.P., Mbah B.N. 2006b. Surface sterilization and duration of seed storage influenced emergence and seedling quality of African breadfruit (Treculia africana Decne). African Journal of Biotechnology 5(15): 1393–1396.

Ellis, W.O., Oduro, I., Appiah, F. and Antwi, Y. (2007). Quality of oil from *Treculia africana* – an underutilized forest plant. Discovery and Innovation. *19:267-270.*

Emah I. 2006. Design, construction and testing of a vertical wind tunnel for determining the terminal velocity of breadfruit seed. B.Eng. Project report, Department of Agricultual and Bioresources Engineering. University of Nigeria, Nsukka. Nigeria.

Enibe D.O. 2007 Consumer acceptability test in South Eastern Nigeria. Discovery and Innovation; Vol. 19 (AFORNET Special Edition No. 3), pp. 271–273.

Enibe D.O. 2013. Report on phase one fieldwork. Technical Report, School of Agriculture, University of Reading, Reading, England.

Enibe S.O., Asiegbu C.P., Njoku H.O. 2011. Continuous flow depulping machine for *Treculia africana.* Global Journal of Engineering Research, Vol. 10, Nos. 1&2, pp. 77–103. Available online at www.ajol.info/index.php/gjer/article/view/79009

Oduro I., Sulemana A., Ellis W.O., Oti-Boateng, P. 2007a. Breakfast meal from breadfruit and soybean composite. Discovery and Innovation 19:238-242.

Oduro I., Ellis W.O., Narh S.T. 2007b. Expanding breadfruit utilization, its potential for pasta production. Discovery and innovation 19:243-247.

Okafor J.C., Okolo H.C. 1974. Potentials of some indigenous fruits trees of Nigeria. Paper presented at the 5th annual conference of the Forestry Association of Nigeria, December, 1974, Jos, Nigeria.

Onweluzo J.C., Odume L. 2007. Method of extraction and demucilagination of *Treculia africana*: effect on composition. Nigerian Food Journal 25 (1): 90-99.

Osborne D.R., Voogt P. 1978. The analysis of nutrients in foods. Academic Press London. pp. 166-182.

Pearson D. 1991. The chemical analysis of foods. Churchill, Livingstone, London pp. 9-31.

Effect of postharvest losses of amaranth on market participation by smallholders in Tanzania

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Abstract

Smallholder production of traditional leafy vegetables is an important activity even though these crops are perishable and prone to high postharvest losses. We examined postharvest losses in traditional leafy vegetables to estimate the impact of such losses on market participation. The study analysed 160 farm households from the Arusha and Tanga regions in Tanzania that cultivated amaranth (*Amaranthus* spp.) and/or other crops. Systematic random sampling was used to draw samples to assess the postharvest quality of the crop. The study results show that 39% of amaranth growers experienced spoilage in their crops. Mechanical damage ranged from 1.2% to 3.5% depending on locality and type of packaging. Economic losses were reported to be 131 TSh for amaranth per transaction. The field observations showed that market participation in terms of number of amaranth growers varies from region to region. Mitigation measures by smallholders also vary by region. To improve the livelihoods of smallholders, it is important to increase their access to better technologies and infrastructure for amaranth production and value addition, which may result in lower postharvest losses and increased market participation.

Key Words: Postharvest losses, traditional African leafy vegetables, market participation, amaranth

Introduction

Previous surveys conducted by AVRDC – The World Vegetable Centre in Tanzania have highlighted the importance of traditional leafy vegetables both for home consumption and for sale (Weinberger and Msuya, 2004; Barry et al., 2009). Previously, most types of traditional vegetables were collected from the wild, but now many are deliberately cultivated for home consumption, and production for sale is also becoming an important activity. Traditional vegetables contribute up to 27% of the household diet (Weinberger and Msuya, 2004).

It was observed that more than 60% of households surveyed in the Singida, Kongwa, Arumeru and Muheza areas of Tanzania produce amaranth (Weinberger and Msuya, 2004) and it is highly ranked across the regions compared with other traditional vegetables. This study concentrated on leafy amaranth and collected information from producers and traders of this crop.

Leafy vegetables are very perishable and prone to high postharvest losses if not properly handled. Smallholder farmers are usually resource-poor and do not have access to the optimum equipment needed for proper handling, such as cold rooms. Assessment of the extent of postharvest losses is not easy and estimates vary widely (Kader, 2005; Kitinoja, 2010). Results from other surveys conducted on vegetable crops show the importance of looking at price and market information as well as postharvest losses (Genova et al., 2006), however these studies were conducted in Asia and other regions. Very few studies have measured postharvest losses in Tanzania and this study will be an addition to the literature.

Smallholders may have a disadvantage during marketing due to the small volumes each individual produces, so it is important to investigate socio-economic questions such as marketing arrangements (Kader, 2005). Our survey aimed to examine the impact of economic and physical postharvest losses on harvesting, handling and selling of amaranth in the Tanga and Arusha regions of Tanzania.

Materials and methods

The survey was implemented in the Arusha and Tanga regions of Tanzania. One hundred and sixty amaranth growers were identified based on systematic random sampling, while 70 traders were identified through purposive sampling and interviewed. Three questionnaires were administered from March to May 2013 to investigate handling practices and assess losses. The first questionnaire was directed to farmers, the second to traders and the third was for the collection and assessment of amaranth quality in the market and at the farm gate.

Postharvest handling practices of tomato and amaranth producers

A farm household questionnaire was administered to farmers to obtain general information on farm characteristics and sources of information for postharvest handling. General handling practices as well as information on postharvest losses were obtained from growers, who also indicated what they regarded as the most important factors causing losses.

Postharvest handling and marketing practices of traders and wholesalers

The traders' questionnaire was targeted at traders (commission agents, wholesalers and retailers) involved in the marketing of leafy amaranth and included information on trading and postharvest handling practices. This group of respondents was also asked for information on the postharvest losses that they experience and to identify the factors causing the losses.

Assessment of postharvest and qualitative losses

Specific information on qualitative and quantitative losses was obtained by collecting samples of amaranth (20 bundles per sample) at various points in the handling and marketing chain: at the farm gate, from the collector or middleman, and from the marketplace at the point of sale to the consumer. Each sample was assessed visually for physical damage, microbial or insect damage, and stage of maturity. Produce was placed into four categories according to severity of damage: minor (below 10% of surface area), moderate (10-50% of surface area), severe (above 50% of surface area), and no problems observed. Ambient temperature was recorded and the temperature of the leaf surface was measured.

Sampling procedure

Interviews with key informants in each district were used to help select farmers and major production areas. The areas were selected based on key criteria identified by farmers who supplied the main markets.

Results

The survey was conducted from March to May 2013 and the results analysed using STATA software. Interviewees provided information on the postharvest handling problems that they encountered as well as the marketing practices that they followed. In terms of postharvest losses, 39% of the respondents reported that they experienced spoilage of their amaranth crop between harvesting and selling (Table 1).

Table 1. Extent of postharvest spoilage of leafy amaranth in Arusha and Tanga regions of Tanzania

Indicator	Percent
Postharvest spoilage	39
Reasons for Spoilage	
Hot weather	8
Diseases	22
Damage during harvesting	18
Damage during transport	4
Transportation delay	2
No market	6
Poor quality of variety	2
Other	2

Table 2 provides results from the survey of postharvest and qualitative losses on damage to leafy amaranth. The crop from Tanga had a higher percentage of no damage and the percentage of leaves with minor damage was also lower; this indicates less damage in general to leafy amaranth from Tanga compared with leafy amaranth from Arusha.

Table 2. Incidence of damaged amaranth leaves observed in the Arusha and Tanga regions of Tanzania

	Arusha Region	Tanga Region
Percent leaves with no damage	28.46^{b}	47.83^{a}
Percent leaves with minor damage	64.11^{a}	34.57^{a}
Percent leaves with moderate damage	11.43^{b}	14.13^{b}
Percent leaves with severe damage	1.19^{c}	3.48^{b}

Means followed by the same letter are not significantly different (P<0.05)

Respondents were asked to provide information on marketing practices and the differences between the two regions on the location of sales is evident (Table 3). Amaranth from Arusha is almost entirely sold from the farm gate, whereas Tanga has more selling points.

Table 3. Sales locations for amaranth in the Arusha and Tanga regions of Tanzania

Location	Arusha (% of respondents)	Tanga (% of respondents)
Farm gate	95	41
Wholesale	0	11
Local retail	4	48
City retail	1	0

Information from traders on their economic transactions for marketing amaranth was collected (Table 4). This information included the quantity lost during marketing, which was slightly higher during the wet season. It is interesting to note that the price reduction during the wet season is also higher.

Table 4. Impact of postharvest losses on economic transactions of amaranth in the Arusha and Tanga regions of Tanzania (unit: per transaction)

	Dry Season	Wet Season
Quantity wasted (kg)	20	22
Quantity lost from total sales (%)	10	15
Cost (TSh)	5611	6504
Price reduction (TSh)	226	754

Discussion

Most of the postharvest problems reported were for disease incidence and damage during harvesting. Storage of leafy amaranth prior to marketing was not indicated as a common practice and this suggests that the diseases are most likely being carried over from the field and growers are harvesting already diseased material. It was interesting that damage during transportation was not highlighted as a major problem, and it could be that growers and traders are ferrying smaller quantities that are easier to pack and move.

Farmers in the Tanga region experienced a lower level of damage to their amaranth crops, especially for minor cuts and bruises. These growers handle smaller quantities compared to Arusha growers, who harvest and pack larger quantities, which increases the likelihood of more damage to the leaves.

Amaranth from Arusha is mainly sold at the farm gate, and it is possible that there may be more actors involved in handling and marketing. More handling stages allow for more opportunities for damage. Only a few respondents reported damage during transportation as a problem. This could be because there is a delay in damage symptoms becoming visible. The use of improved boxes or packaging that reduces damage during transportation should be investigated.

Amaranth growers reported damage problems during harvesting. This implies the use of poor harvesting practices, or could indicate poor storage facilities in the field prior to shipment to the market. There is an opportunity to introduce good field harvesting and handling practices in both regions.

Price reduction during marketing was higher in the wet season when greater postharvest losses were reported, implying that there is an impact of losses on economic transactions.

References

Barry I.N., Jaenicke H., Pichop G.N., Virchow D. 2009. Production and marketing of African indigenous vegetables in the Arumeru District of Tanzania: assessing postharvest loss and processing otential. Acta Horticulturae, International Society for Horticultural Science 806: 481-488.

Genova C., Weinberger K., Chanthasombath T., Inthalungdsee B., Sanatem K., Somsak K. 2006. Postharvest loss in the supply chain for vegetables – The case of tomato, yard-long bean, cucumber and chili in Lao PDR. AVRDC – The World Vegetable Center, Shanhua, Taiwan. AVRDC Publication No. 06-684, Working Paper No. 17, 49 pp.

Kader A.A. 2005. Increasing Food Availability by Reducing Postharvest Losses of Fresh Produce. Acta Horticulturae, International Society for Horticultural Science 682: 2169-2175 Proc. 5th Int. Postharvest Symp. Eds. F. Mencarelli and P. Tonutti.

Kitinoja L. 2010. Identification of Appropriate Postharvest Technologies for Improving Market Access and Incomes for Small Horticultural Farmers in Sub-Saharan Africa and South Asia. WFLO Grant Final Report (http://ucce.ucdavis.edu/files/datastore/234-1848.pdf).

Weinberger K., J. Msuya. 2004. Indigenous Vegetables in Tanzania—Significance and Prospects. Shanhua, Taiwan: AVRDC – The World Vegetable Centre, Technical Bulletin No. 31, AVRDC Publication 04-600. 70 pp.

Nutritional composition and stability of *Saba senegalensis* fruit extract

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Abstract

Saba senegalensis is an indigenous tree of Africa and is commonly found in the three northern regions of Ghana. Saba fruits are eaten as an appetizer while the leaves and bark of trees are used as medicine. The beverage industry is the largest user of fruit juice and absorbs over 80% of production. The main objective of the study was to assess the stability of fruit juice extract from Saba. Fruit samples of Saba were collected from the Nadowli District of the Upper West Region for juice extraction, proximate analysis, consumer acceptance and storage of the juice. Proximate analyses were carried out following the recommended methods of the Association of Official Analytical Chemists and all analysed parameters were in triplicate. Consumer acceptance assessment was with a panel sample size of 30. Mean values of the proximate composition in percentage were: crude fat (6.2 ± 0.00), crude protein (0.8 ± 0.02), ash (2.0 ± 0.03), carbohydrate (50.0 ± 0.01), moisture content (29.0 ± 0.01) and fibre (12.0 ± 0.10). Consumers showed a level of acceptance for the fruit extract. The fruit juice was pasteurized at a temperature of 65^{o}C for ten minutes. The cooled fruit juice was successfully stored at 4^{o}C for 22 days without fermentation. From the results, fruit extracts could be processed for commercial use based on the nutrient composition and consumer acceptance which would influence the domestication and further utilization of the plant.

Keywords: *Saba senegalensis*, nutrient composition, consumer acceptance, fruit extract

Introduction

Subsistence farmers in the humid lowlands of West and Central Africa achieve food and nutritional security through maintaining multi-strata agroforestry systems. These systems are characterized by the cultivation of high-value indigenous tree species and different farming systems to improve smallholder household nutritional well being and diversify incomes, and by so doing, allow farmers to take care of the forest (Leakey and Tchnundjeu, 2001).

With the recurrent problem of food insecurity and emerging globalization of world trade, more communities around the world are now taking care of their local environment by adding and making exploitation of the environment more sustainable. The processing of indigenous fruits in West and Central Africa holds potential to prolong the shelf life of tree products, making rich macro- and micro-nutrients available and increasing market value for the benefit of smallholder farmer households.

While continuing to assist smallholder farmers to select and use high-value planting materials, it is also vital in the domestication process to initiate product development and management efforts, so that domestication and commercialization effectively affect farmer households' strategies (FAO 1996). This approach would also accelerate the adoption of tree cultivation through continuous capturing of genetic variation in desirable traits and generate considerable environmental benefits (Leakey et al., 2002).

Fortunately, about 48% of African indigenous fruit tree populations serve as a food safety net during the season when people can go hungry, or when there is crop failure since a wide range of edible products such as food beverages can be obtained. They also represent numerous sources of medicinal products against many diseases and infections as well as combating malnutrition disorders in both adults and infants since most edible forest products constitute important and cheap sources of vitamins, minerals, proteins, carbohydrates and fats. Notwithstanding the numerous benefits and potential however, indigenous fruit trees face threats of deforestation and genetic erosion.

The beverage industry is the largest user of fruit juice and absorbs over 80% of production (Manay and Shadaksharaswamy, 2008). This implies that knowing the nutrient composition of the saba fruits which is one of the indigenous wild fruits in Northern Ghana and which has a potential of producing enough juice, may encourage farmers especially in the rural areas to take advantage of going into commercial production of the fruit juice in order to earn extra income and also improve their livelihoods by harvesting fruits from the wild. However, this depends on the fact that the fruit juice, in terms of nutrient composition is rich in minerals, vitamins, carbohydrates, proteins and crude fibre.

Ghana and other developing countries in sub-Saharan Africa could also earn foreign exchange and increase gross domestic product through the sale of juice. Indigenous tree crops can be more lucrative than the traditional cash crops. This research therefore seeks to produce fruit juice from the pulp of the fruit.

Materials and methods

Sample collection

Saba fruit samples were collected in the Nadowli District of the Upper West Region of Ghana. Matured saba fruits were collected randomly from the wild, grouped and were taken to the laboratory of the University for Development Studies (Tamale) for analysis. The pulp with seeds was carefully removed from the pods and soaked in distilled water for 12 h. Pulp with the seeds was mashed and the liquid drained as the extract.

The recommended methods of analysis of the Association of Official Analytical Chemists (AOAC, 1997) were used for the determination of proximate composition. The parameters analysed were vitamin C, ash, crude protein, fruit juice content, moisture content carbohydrate, crude fat and crude fibre. All parameters were analysed in triplicate. Other parameters analysed were pH, titrable acidity and brix concentration.

Consumer acceptance and sensory analysis

Consumer acceptance and sensory analysis were conducted using a taste panel of 30 persons. The parameters considered for the test were colour, aroma, taste and viscosity. The taste panel consisted of staff and students of the University for Development Studies.

Storage of *juice extract*
Fruit juice extract after pasteurization was stored at room temperature (between 33°C-39°C) and refrigeration at 4°C and below 0°C for the determination of stability and quality during storage.

Results and discussion

Proximate composition
Mean values of fruit juice extract after pasteurization indicate the presence of nutrients in varied proportions and are shown in Table 1.

Table 1: Mean proximate composition of fruit extract

Parameters	Mean Values*
Protein	0.8 % ± 0.02
Fat	6.2 % ± 0.00
Carbohydrate	50.0 % ± 0.01
Ash	2.0 % ± 0.03
Fibre	12.0 % ± 0.10
Moisture content	29.0 % ± 0.01
Vitamin C	18 mg
pH	2.3
Titratable Acidity	30.3 g/l
Brix	13.9 °Bx

*± Standard deviation

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. According to Pomeranz and Meloan (1994), the ash content for fresh fruits ranges from 0.2% to 0.8% and is generally inversely related to the moisture content and some dry fruits may contain as much as 3.5% ash. The value from the results could serve as a good source of nutrient for consumers.

Crude protein
The protein content can be compared with other commercial fruits such as apple, mango and papaya which have protein contents of 0.20%, 0.60% and 0.60% respectively (Sundriyal and Sundriyal, 2004).

Crude fat
Fat and oils are a concentrated source of energy with each gram giving about nine calories (Sumati and Rajagopal, 2012). Fats make certain vitamins available for use in the body, they physically protect vital organs, help to maintain body temperature, and make up part of all body cells. Most fruits have fat content <0.5 g/100g edible portion (Watt and Merrill, 1963).

Crude fibre
Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida et al., 2000). The Recommended Dietary Allowance (RDA) of fibre for children, adults, pregnant and lactating mothers are 19-25 g, 21-38 g, 28 g and 29 g respectively (Jimoh et al., 2011).

Carbohydrate

Carbohydrates are the primary source of energy for maintenance, growth, reproduction and help to maintain the body temperature (Daramola et al., 2002). The fruit extract contained 50.0% of carbohydrate, which could serve as a good source of energy.

Moisture content

Moisture determination is one of the most important and most widely used measurements in processing and testing of food. Since the amount of dry matter is inversely related to the amount of moisture it contains, moisture content is hence of direct economic importance to both the processor and the consumer. Fruits that contain too much water are subject to rapid deterioration from mould growth and insect damage. Moisture content determination is important in determining the nutritive value of a food sample, in expressing results of analytical determinations on a uniform basis, and in meeting standards as to how much moisture foods, especially processed foods, should contain.

Vitamin C

Vitamin C is an important vitamin for human nutrition that is supplied by fruits and vegetables. The saba fruit contains about 18 mg of vitamin C which makes it a good source of the vitamin. Vitamin C has also been implicated in the hydroxylation of proline to form hydroxylproline required in the formation of collagen which helps in the healing of wounds, fractures, bruises and bleeding gums and reduces the susceptibility to infections (Manay and Shadaksharaswamy, 2008).

pH

The pH indicates the juice is acidic and could inhibit the multiplication of microorganisms resulting in an enhanced shelf-life. However, the temperature needs to be regulated. High temperatures in storage resulted in fermentation of the juice within 24 hrs.

Titrable acidity

The titrable acidity indicates the finished extract is a weak acid and this could contribute to the flavour and stability of the fruit extract.

Brix

Brix levels gives fair indications of fruit quality as high levels refer to nutrition with high mineral and vitamin content. The level of brix for the fruit extract makes it comparable to other fruits high in natural sugar content.

Sensory analysis of fruit juice

To the consumer, the most important attributes of food include its sensory characteristics such as colour, taste, aroma and viscosity. These sensory characteristics determine an individual preference for a specific product as small differences between brands of similar products can a have substantial influence on acceptability. Consumers showed a high level of preference for all the parameters as no parameter was rated below 'good' affirming an acceptance for the fruit juice extract (Table 2).

Table 2: Sensory and consumer analyses of fruit extract

Parameters	Mean Scores*
Colour	3.9 ± 0.01
Taste	3.0 ± 0.01
Aroma	2.9 ± 0.02
Viscosity	3.2 ± 0.01

* Ranked from 0-4 (0 = dislike, 1 = fair, 2 = good, 3 = very good, 4 = excellent)
± Standard deviation

Stability of fruit extract

Fruit extract without heat treatment and refrigeration fermented within 24 hr. Heat-treated fruit extract was stored at $4^{o}C$ for 22 days without fermentation and the production of undesirable flavours. Frozen fruit extract was kept stable for 90 days. Stability of the extract was enhanced with the use of cold storage.

Conclusion and recommendations for development

Fruit extract from saba can supplement the nutrient needs of consumers. The sensory and acceptance results indicate a high level of acceptance for the fruit extract. The extract was stable during storage and had an enhanced shelf life due to heat treatment and refrigeration. Utilization and use of fruit extract could lead to the processing of *Saba senegalensis* extract for commercial use as fruit juice. In this scenario, domestication of the tree could be considered.

References

AOAC. 1997. Official method of analysis, 15th edition. Association of analytical chemists Washington, DC, USA.

Daramola A. M., Igbokwe E. M., Mosuro G. A., Abdullahi J. A. 2002. Agricultural science for WASSCE and SSCE second edition, Ibadan, Nigeria, pp. 125.

FAO 1996. Product development and management. In; Leakey, R. R. B., Temu, A. B, Melnyk, M. and Vantomne, P. (Eds).(1996). Domestication and Commercialization of Non-Timber Forest Products for Agroforestry, Non-Wood Forest Products No 9, Food and Agriculture Organization of the United Nations, Rome, Italy

Gopalan C., Sastri B. V. R., Balasubramanian S. C. 2004. Nutritive value of Indian Foods. National Institute of Nutrition, Indian Council of Medical research, Hyderabad 500007, Indian pp. 2-58

Ishida H., Suzuno H., Sugiyama N., Innami S., Tadokoro T., Maekawa A. 2000. Nutritional Evaluation of Chemical Component of Leaves Stalks and Stems of Sweet Potatoes (Impomoea batatas poir). Food Chemistry 68: 359-367.

Jimoh F.O., Adedapo A.A., Afolayan A.J. 2011. Comparison of the Nutritive Value, Antioxidant and Antibacterial activities of *Sonchus asper* and *Sonchus oleraceus*. Records of Natural Products 5: 29-42.

Leakey R.R.B., Tchoundjeun Z. 2001. Diversification of tree crops; domestication of companion crops for poverty reduction and environmental services. Experimental Agriculture 37(3): 279-296.

Leakey R.R.B., Atangana A.R., Kengni E., Waruhiu A.N., Usoro C., Anegbeh P.O., Tchoundjeu Z. 2002. Domestication of *Dacryodes edulis* in West and Central Africa: Characterization of genetic variation. Forest, Trees and Livelihoods 12: 57-71.

Manay N.S., Shadaksaraswamy M. 2008. Foods facts and principles, third edition. New age International, Ansari Road, Daryagaj. pp. 68-175.

Pomeranz Y. Meloan E.C. 2000. Food Analysis: Theory and Practice. Springer Science & Business Media. pp. 778.

Sumati R.M., Rajagopal M.V. 2012. Fundamental of Foods, Nutrition and Diet Therapy. 6th edition. New Age International Limited, Publishers, Ansari Road, Daryagayj, New Delhi.

Sundriyal,M., Sundriyal, R. C. 2004. Wild edible plants of the Sikkim Himalaya: nutritive values of selected species. Economic Botany 58: 286-299.

Watt B.K., Merill, A.L. 1963. Composition of Foods: Raw, Processed, Prepared. Agriculture Hand Book No. 8, Consumers and Food Economics Research Division. Agriculture Research Service, USDA, Washington, DC.

Traditional baobab foods from Benin: processing, preservation and gender analysis

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Abstract

The baobab tree, *Adansonia digitata*, is used by local populations in Africa on a daily basis for food and medicinal purposes. The leaves, the fruit containing its pulp, seeds and kernels are all used for food and significantly contribute to food security.

A survey performed in the rural areas of Benin on indigenous knowledge relating to processing and preservation of baobab provided data on the importance of baobab food products and their preservation according to socio-cultural groups and gender. Interviews were held with 253 participants with no or limited education, belonging to 15 ethnic groups in the age range of 15-69 years for men and 15-82 years for women.

Apart from fruit breaking which is performed by both men and women, most of the difficult processing operations namely grinding and sieving operations and the management of by-products (seed decortication, potash preparation) for product recovery are performed exclusively by women. Women take care of all activities related to storage and preservation of baobab fruit and its derived products including the fruit pulp. Because the pulp changes colour during storage, a storage experiment was performed to understand the phenomenon. It showed that colour change during storage is accompanied by vitamin C degradation and that loss of quality was accelerated by increasing temperature, water activity and storage time. Storage techniques that are compatible with local and women's conditions become a priority.

Keywords: Baobab food uses, processing and preservation, socio-cultural groups, storage

Introduction

The baobab, *Adansonia digitata*, belongs to the family of Bombacaceae. It is also known as the monkey bread tree or the upside down tree. It is a multi-purpose tree with products having numerous food uses and medicinal properties, and a fibrous bark that is used for various applications (Codjia, et al., 2001; Sidibe and Williams, 2002; Wickens, 1982). The pulp of the fruit, the seeds and the leaves are all utilized and are essentially wild-gathered foods. They are consumed daily by rural populations in Africa and are also commercialized.

According to Wickens (Wickens, 2008), the baobab is an important source of human nutrition today in Africa. Chemical analysis of baobab parts showed the presence of proteins, amino acids, iron, vitamins C, A and E (in abundant amounts compared to daily needs) in leaves, seeds and fruit pulp (Codjia et al., 2001; Sidibe and Williams 2002).
Baobab pulp and leaves have a high antioxidant activity when compared to other fruits (Besco et al., 2007; Vertuani, et al., 2002) and can consequently be considered as so-called

functional foods, which may have a positive impact on health in addition to their role as a food. In 2008, baobab-dried fruit pulp has been acknowledged as a novel food by the European Union (The Commission of the European Communities, 2008) and approved in 2009 as a food ingredient in the US (Addy, 2009), which may boost the trade of this product from African countries, and thus provide a valuable source of foreign exchange. Despite its international importance, there is no sustained research capitalizing endogenous processing and food knowledge and technological problems. Such research, however, is a prerequisite for any promotion of the products (Sidibe and Williams, 2002), and to better orientate and prioritize further research.
It is consumed by rural communities in Africa as a powder, drink, gruel and as a paste. A survey performed in rural Benin allowed identification of several baobab food products, but also of the processing and storage problems (Chadare et al., 2008). Pulp storage turned out to be one of the most important of these problems. In Beninese rural areas, baobab pulp is stored in its fruit until the time it is used or sold or sometimes processed in advance prior to the sale period. During storage, it happens that the pulp changes colour, making the pulp unsuitable for human consumption. Quantitative information on this phenomenon is lacking and if and how colour change is related to vitamin C in the pulp is not known. The goal of the present work was to assess pulp quality deterioration in terms of pulp colour change and vitamin C content at different water activities and temperatures.

Materials and methods

Survey: sampling of informants

First, a random check was performed on 198 processors offering their foods for sale in local markets to determine the proportion of processors of baobab food products. This proportion was used to compute the sample size N_i of baobab processors to be interviewed, using the following formula: $N_i = \frac{4pi(1-p_i)}{d^2}$, (Dagnelli, 1998) where N_i is the total number of processors to be surveyed for the study, p_i is the proportion of baobab processors among the 198 randomly checked persons and d is the expected error margin in the conclusion, which is fixed at 0.05 (Dagnelli, 1998). Next, the number of processors to be interviewed in each municipality was calculated on the basis of its population size. If T is the proportion of the population of a community among the total number of people living in the study area, N, according to $T_j = \frac{n_j}{N}$ where n_j is the number of people in community j, then N_{ij} is the number of processors to be surveyed in community j, according to $N_{ij} = N_i \times T_j$.

Field data collection

Field data were collected to establish ethno-food knowledge of processing and preservation of baobab food in general and more particularly on its fruit pulp and derived products. Questions were addressed according to gender to informants from different communities in Benin where baobab foods are commonly used. Questionnaires were used which were tested with local inhabitants prior to the formal survey, and adjusted if needed. Discussions were conducted in the villages of the selected localities, based on the adjusted questionnaires. Interviews were conducted in the language/dialect that was best understood by the informants with translation when necessary.

Quality deterioration of baobab fruit pulp during storage

Sample preparation: Baobab pulp was collected and subjected to different treatments. Normal pulp (as collected freshly from the tree (N), humidified pulp (H) and freeze-dried pulp (D) were used for the experiment. Humidified pulp was obtained by putting normal pulp on a thin layer together with water in a desiccator for 72 h to increase its water activity. Normal pulp was freeze-dried to obtain pulp with a low water activity. Water activity was measured using AquaLab Series 3 water activity meter (Decagon, Pullman, WA, USA). At 20 ºC, normal pulp had a water activity (a_w) of 0.45, humidified pulp of 0.74 and freeze-dried pulp, 0.08. The water activities reported below were measured at 20°C, and do not reflect the water activities at the storage temperatures. Each type of pulp, i.e., normal, humidified and freeze-dried was subjected to four different storage temperatures, that is to say 41°C, 55°C, 61°C and 70°C. Samples were stored for 56, 73, 192 and 240 h.

Aliquots of about 4 g of pulp were put into airtight glass tubes, to exclude water exchange. The samples were initially warmed up in a water bath set at a required temperature and as soon as this temperature was reached, they were immediately transferred into an incubator at the required temperature for the remaining duration of the experiment. For each type of pulp, seven tubes were stored at each temperature, resulting in a total of 28 tubes. Two i-button temperature sensors chips (http://www.maxim-ic.com/products/ibutton/ibuttons/) were put in the last tube containing normal pulp to be removed and recorded at different time intervals according to the temperature during the whole experiment. The temperature changes during storage are presented in Figure 1. Tubes were removed from the incubator at different times, quickly cooled down in ice, and kept in the freezer until when they were analyzed for colour and vitamin C content.

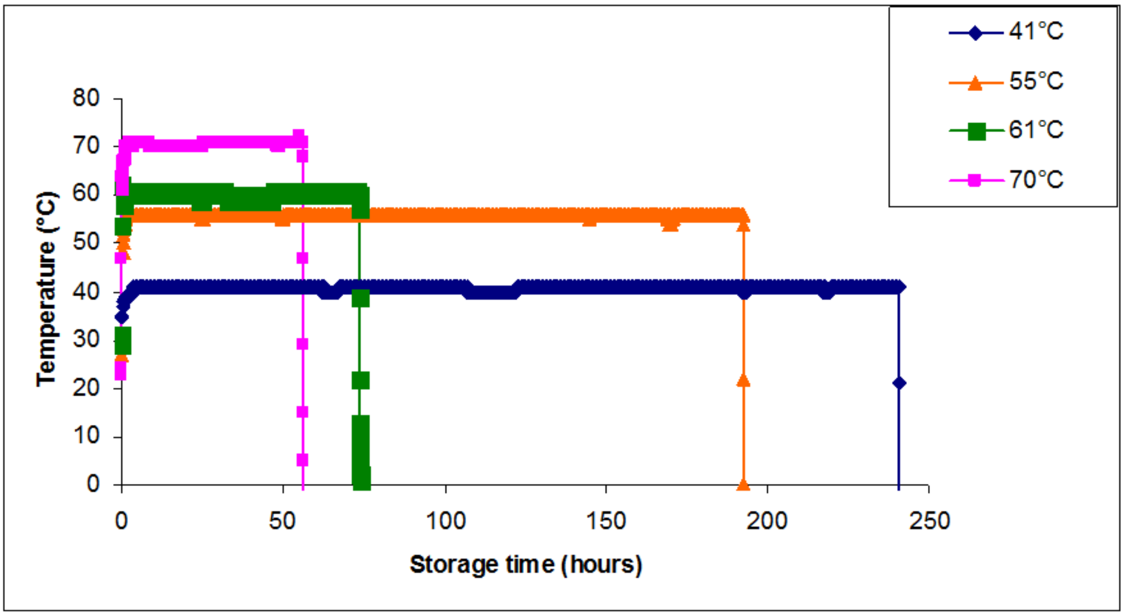

Figure 1. Temperatures recorded during storage experiments.

Colour measurement

Colour was measured using the colour Flex hunterlab (ELSCOLAB, Nederland B.V) and L*, a* and b* values were measured as an expression of colour parameters as follows: L* (+ Light, - dark), a*(+ red, - green) and b*(+ Yellow, - Blue). For each sample, three measurements were made and the average value was reported.

Determination of vitamin C content

Vitamin C (ascorbic acid) was measured by HPLC (Thermo Fisher Scientific) (Hernandez, et al., 2006). Briefly, about 0.12 g of sample was weighed. 2.5 ml of a solution of 3% metaphosphoric acid (MPA) (Merck, Darmstadt, Germany), 8% acetic acid (Riedel de Haën, Morristown, New Jersey, USA) was added. Next, the mixture was homogenized in the Ultra Turrax at the highest speed for 1 min (in ice and darkness) and centrifuged at 4°C for 20 min at 10 500 rpm. This procedure was repeated twice and the two supernatants were pooled. The extracts were filtered in a vial through a 0.45 filter prior to injection in the HPLC column. For calibration, a stock standard solution of 10 mg of ascorbic acid per ml of Millipore-filtered water was prepared. Subsequently, solutions of various concentrations were prepared by diluting the stock solution in 3% MPA - 8% acetic acid. The solutions were filtered and transferred to HPLC vials. Orthophosphoric acid (Merck, Darmstadt, Germany) 0.2% in milli Q water was used as elution buffer. A 20 µl of sample was injected and run at a flow of 1 ml/min and analysed at a wavelength of 245 nm. Samples were analysed in duplicate.

Data processing

Statistical analyses were performed using SAS.v8 software (SAS Institute Inc., 1999)**.** Descriptive analyses and response surface regression, ANOVA, was used to process data and assess the effect of water activity, temperature and storage time on vitamin C degradation during the storage experiment.

Results and discussion

Gender analysis of traditional processing and preservation of baobab fruit pulp in Benin

Results obtained from the survey showed that women are guardians of the knowledge of traditional processing of baobab products especially the fruit pulp. Up to 35 traditional baobab foods were recorded in Benin. The most important foods derived from baobab fruit pulp were: (i) Mutchayan, a cereal dough enriched with baobab fruit pulp. Such a dough is consumed with a sauce or soaked in water and used because it is believed to have a bracing effect. It is known in 11 ethnic groups and 91% of the informants who have mentioned it were women aged from 15 to 25 years; (ii) various gruels (Gruel, Nanganfirou, Yewowi, Norendoorou, Tcho) are traditionally prepared from a mixture of baobab fruit pulp and cereal flour (Chadare et al., 2008). They were mentioned in 15 ethnic groups and 89% of the informants are women aged from 15 to 70 years. These fruit pulp-derived foods are processed only by women and consumed by the whole family at various times of the day. Baobab fruit pulp is a main ingredient in the preparation and processing of all these products. In most villages, pulp retrieval is still manual. The different processing units allowing pulp retrieval are shared according to gender (Table 1).

Table 1. Gender analysis of baobab fruit pulp processing

Difficult process operations	Difficulty	Who does it?
Sieving of pulp	Wind takes product away	Exclusively women
Fruit breaking	Requires strength	Men and women
Management of by-products (seed processing, potash preparation, etc.)	Very difficult and meticulous	Exclusively women

Preservation of baobab fruit pulp products at a local level is also managed by women. Recorded storage time of fruit pulp in local conditions varies from one ethnic group to another depending on the storage circumstances (e.g. packaging, humidity, drying frequency). It was noticed and mentioned that the pulp changes colour during storage and gets dark. Laboratory investigations allowed a better understanding of this phenomenon (see below). Baobab pulp is one of the most important parts of the tree for food production. Its processing and preservation, which are mainly done by women, need to be improved with respect to quality and ease of operations. Moreover, women should be informed on the relation of food preparation and health as they take care of the nutrition of the household.

There is fortunately a recent increasing interest in the mechanisation of traditional processing of some traditional and indigenous food. This will lead to effective promotion through improvement of traditional techniques and products, and production of added value products (i.e. with functional properties) for a larger market and will increase income of rural, poor populations.

Quality deterioration of baobab fruit pulp during storage

Colour change

The water activity of the product, the temperature and the storage duration appeared to affect pulp quality in terms of colour change. As storage time increased, pulp colour lost its lightness with decreasing L* values and became more brownish with increasing a* value (reddish) and b* value (yellowish). The higher the storage temperature, the faster the colour changed. Whatever the temperature, freeze dried pulp with water activity 0.08 showed a slower browning than normal pulp with water activity of 0.45. Browning was more pronounced in humidified pulp with water activity 0.74. Colour change was faster at higher temperatures and at higher water activities (Figures 2 and 3). In fact, initial L*, a*, b* values for untreated baobab pulp were 84.84, 1.78, 16.26 for normal pulp ($a_w = 0.45$); 84.95, 1.78, 16.03 for freeze-dried pulp ($a_w = 0.08$) and 83.69, 2.63, 17.52 for humidified pulp ($a_w = 0.74$), respectively. After 56 h of storage at 70°C, the L* value decreased to 55.34 for humidified pulp, 65.86 for normal pulp and 72.94 for freeze-dried pulp while a* and b* values increased to 4.18 and 29.28 for freeze-dried pulp, 4.89 and 27.6 for normal pulp and 7.36 and 26.64 for humidified pulp. These results show that for the same temperature, browning is faster at higher water activity. Considering the same water activity, e.g., normal pulp with $a_w = 0.45$, L* decreased to 65.86 after 56 h of storage at 70°C, 74.89 after 192 h of storage at 55°C, 83.36 after 240 h of storage at 41°C; a* and b* values increased to 4.89 and 27.6 at 70°C, 4.44 and 24.65 at 61°C, 4.12 and 25.34 at 55°C and 2.07 and 19.26 at 41°C. Browning reaction was thus faster at increasing temperatures. Baobab pulp contains reducing sugars (Nour et al., 1980) and some protein (Lockett et al., 2000; Obizoba and Amaechi, 1993; Osman, 2004). The observed colour change is, therefore, likely to be due to the Maillard reaction (chemical reaction between amino acids and reducing sugars) which is in this case not desirable.

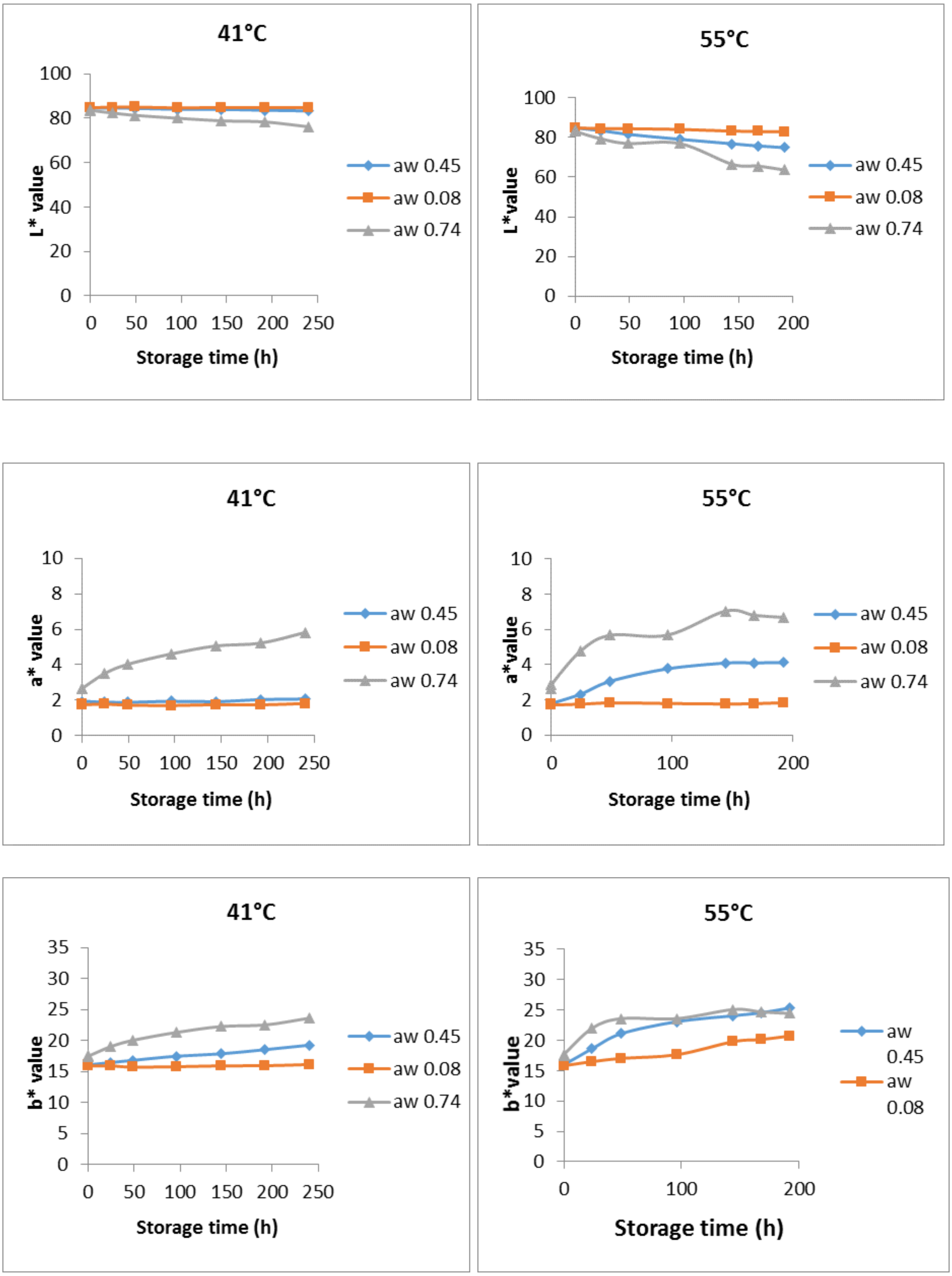

Figure 2. L*, a*, b* values showing colour change of baobab pulp with different water activity (measured at 20°C) during storage at 41°C and 55°C.

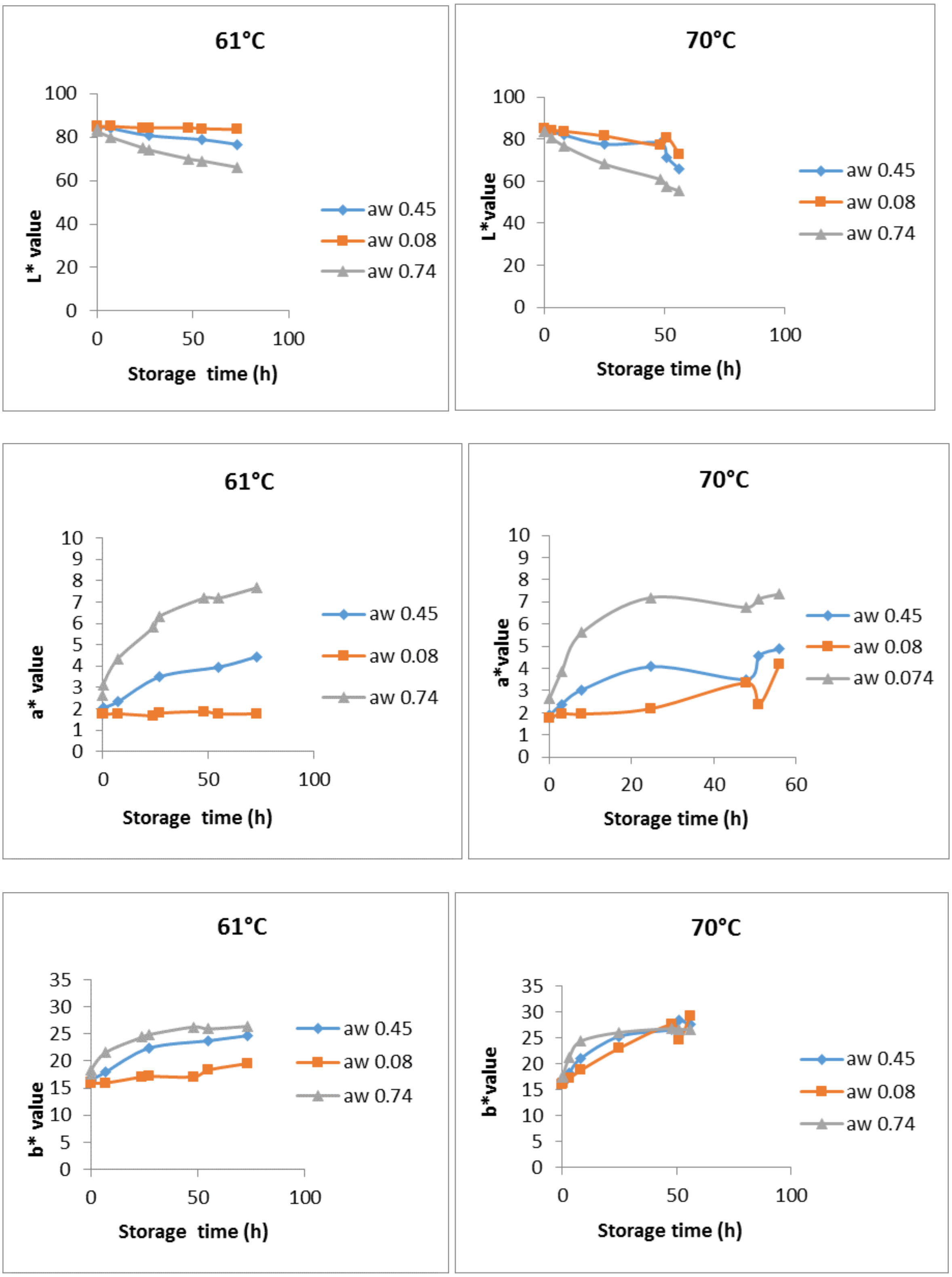

Figure 3. L*, a*, b* values showing colour change of baobab pulp with different water activity (measured at 20°C) during storage at 61°C and 70°C.

Vitamin C degradation

The reported vitamin C content in baobab pulp ranges from 150 to 500 mg/100gdm (Scheuring, et al., 1999). The present data on the unheated samples (397 mg/100gdm for normal pulp, 383 for humidified pulp and 389 for freeze-dried pulp) are within this range.

Results of the analysis of variance on the model expressing vitamin C content as a function of storage temperature, water activity and storage time (Table 2) indicated that both linear and quadratic models as well as interaction effects were significant ($P<0.05$).
In general, whatever the temperature, reaction rate was faster at increasing water activity of baobab pulp. This may be explained by the fact that the available water facilitates several chemical and enzymatic reactions leading to faster quality degradation, or by a faster rate of diffusion (van Boekel, 2009). Moreover, for normal and humidified pulp, the reaction rate increased with increasing temperature. For freeze-dried pulp, reaction rate is rather low.

Table 2. Analysis of variance for vitamin C content

Source	DF	AdjMS	F	P
Regression	9	208247	32.22	0.000
Linear	3	188476	29.16	0.000
Square	3	238927	36.97	0.000
Interaction	3	24330	3.76	0.012
Residual Error	182	6463		

Analysis of each of these effects considering the estimated regression coefficients and significance of linear, quadratic and interaction effects of storage temperature, water activity and storage time on vitamin C content showed that the adjusted value of R-square was equal to 59.5% meaning that water activity, storage temperature and time failed to explain 41.5% of the variations of the vitamin C content. Consequently, the other factors that would contribute to explanation of vitamin C loss need to be further investigated.

Analysis of the response surface plots (Figure 4) indicates that (i) when storage time is fixed, vitamin C degraded faster with increasing water activity and increasing storage temperature; (ii) at a fixed storage temperature, vitamin C degrades faster with increasing water activity and increasing storage time; (iii) finally, when water activity is fixed, vitamin C degrades faster with increasing storage time and increasing storage temperature (Figures 4a, b, c).

In addition to its vitamin C content, baobab pulp is known to have a high antioxidant activity compared to other fruits and it has been suggested that the antioxidant capacity of baobab pulp is mainly due to its vitamin C content (Besco et al., 2007; Vertuani et al., 2002). Consequently, vitamin C degradation could be positively correlated with loss of antioxidant activity by baobab pulp, and hence pulp quality degradation.

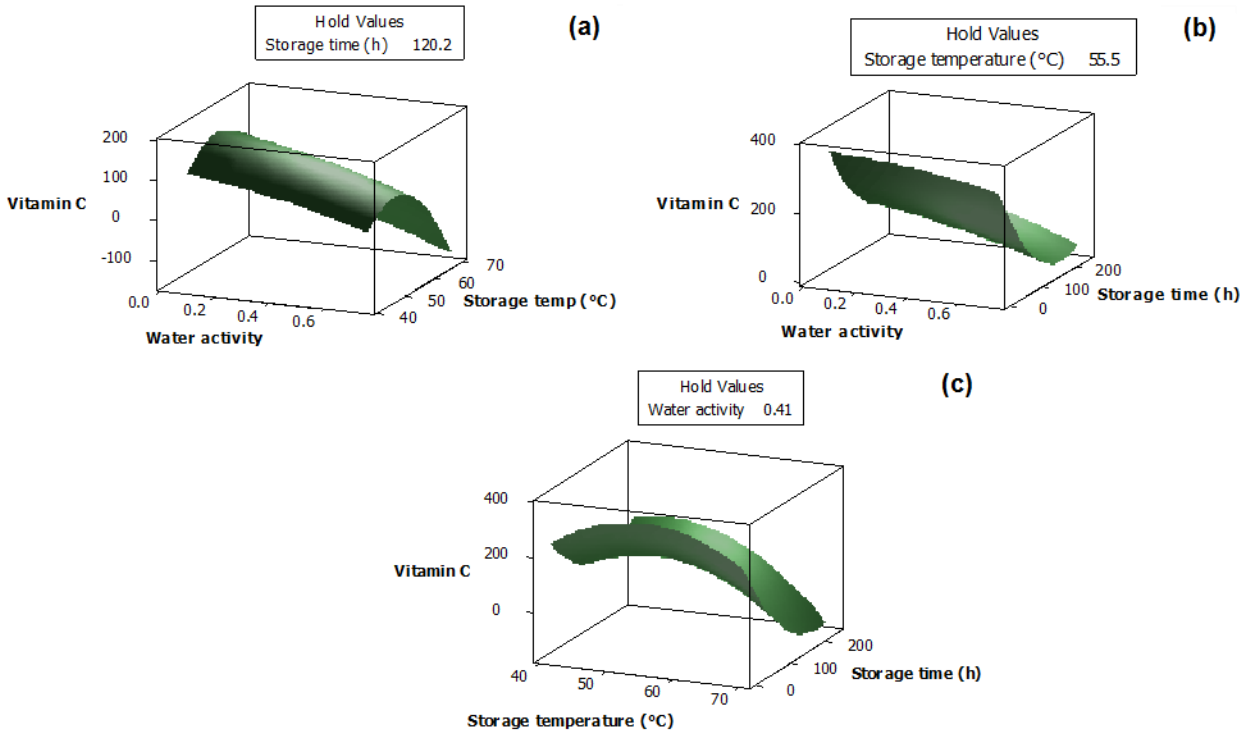

Figure 4. Surface plot of vitamin C content versus (a) Storage temperature and water activity, (b) Storage time and water activity and (c) Storage time and storage temperature.

It was also noticed that in general, the darker the pulp, the lower its vitamin C content. It was found that browning occurs (probably via the Maillard reaction) and that vitamin C degradation takes place. Also knowledge of other factors such as the effect of amino acid content (in particular lysine) and sugar content, in relation to the Maillard reaction and brown pigment formation, would be useful. This would give, in addition, more insight in the nutritional loss of lysine in the same framework of quality degradation. Knowledge of the reactions that lead to quality loss can then also provide the basis for suitable ways of packaging the pulp to preserve its quality.

The present work shows that water activity, storage temperature and time are important factors, and they can be influenced by choosing the right packaging material.

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References

Addy R. 2009. Baobab fruit approved as food ingredient in US In http://www.foodnavigator.com/Publications/Food-Beverage-Nutrition/NutraIngredients-USA.com/Industry/Baobab-fruit-approved-as-food-ingredient-in-US: Food Navigator.com.

Besco E., Braccioli E., Vertuani S., Ziosi P., Brazzo F., Bruni R., Sacchetti G., Manfredini S. 2007. The use of photochemiluminescence for the measurement of the integral antioxidant capacity of baobab products. Food Chemistry 102: 1352-1356.

Chadare F.J., Hounhouigan J.D., Linnemann A.R., Nout M.J.R., van Boekel M.A.J.S. 2008. Indigenous knowledge and processing of *Adansonia digitata* L. food products in Benin Ecology of Food and Nutrition 47: 338-362.

Codjia J.T.C., Fonton-Kiki B., Assogbadjo A.E. , Ekue M.R.M. 2001. Le baobab (Adansonia digitata) Une espèce à usage multiple au Bénin. Cotonou, Bénin: Coco Multimédia.

Dagnelli P. 1998. Statistiques théoriques et appliquées.

Hernandez Y., Lobo M., Gonzalez M. 2006. Determination of vitamin C in tropical fruits: a comparative evaluation of methods. Food Chemistry 96: 654-664.

Lockett C.T., Calvert C.C., Grivetti L.E. 2000. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, Northeastern Nigeria. International Journal of Food Sciences and Nutrition 51: 195-208.

Nour A.A., Magboul B.I., Kheiri N.H. 1980. Chemical Composition of baobab fruit (*Adansonia digitata*). Tropical Science 22: 383-388.

Obizoba I.C., Amaechi N.A. 1993. The effect of processing methods on the chemical composition of baobab (*Adansonia digitata* L.) pulp and seed. Ecology of Food and Nutrition 29: 199-205.

Osman M.A. 2004. Chemical and nutrient analysis of baobab (*Adansonia digitata*) fruit and seed protein solubility. Plant Foods for Human Nutrition 59: 29-33.

SAS Institute Inc. SAS OnlineDoc®, Version 8, Cary, NC: SAS Institute Inc., 1999.

Scheuring J.F., Sidibé M., Frigg M. 1999. Malian agronomic research identifies local baobab tree as source of vitamin A and vitamin C. In Sight of Life Newsletter, pp. 21-24.

Sidibe M., Williams J.T. 2002. Baobab, *Adansonia digitata*. Southampton, UK: International Centre for Underutilised Crops.

The Commission of the European Communities. 2008. COMMISSION DECISION of 27 June 2008 authorising the placing on the market of Baobab dried fruit pulp as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. . Official Journal of the European Union 38-39.

van Boekel M. 2009. Kinetic Modelling of Reactions in Foods. Boca Raton: CRC Press, Taylor & Francis Group.

Vertuani S., Braccioli E., Buzzoni V., Manfredini S. 2002. Antioxidant capacity of *Adansonia digitata* fruit pulp and leaves. Acta Phytotherapeutica 5: 2-7.

Wickens G.E. 1982. The baobab –Africa's upside-down tree. Kew Bulletin 37 173-209.

Wickens G.E. 2008. The Baobabs: Pachycauls of Africa, Madagascar and Australia: Springer Science + Business Media, B.V.

Innovation for diversification: Crops for the Future, Crops for the Future Research Centre and the creation of an underutilized species knowledge network

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Abstract
Crops For the Future (CFF) and its research arm, Crops For the Future Research Centre (CFFRC), deal with the challenges of raising awareness and undertaking research on underutilized species. In doing so, both organizations face challenges not encountered by those engaged in research into major crops where established socio-technical networks exist concerning these crops. By contrast, underutilized species may possess socio-cultural significance in local food networks beyond which there is typically limited knowledge, demand, or socio-economic infrastructure to support their wider production. The nature of underutilized crop networks has influenced the development of both CFF's and CFFRC's programmes. Rather than replicating the development of research into the major crops, the challenge has been to develop a new approach which integrates different forms of knowledge into initiatives aimed at delivering practical benefits employing limited resources. Thought thus needs to be given to the context in which scientific knowledge is generated and the non-scientific validation to which it is subject. Both CFF and CFFRC have been required to develop a broad agenda considering how specific species are integrated into wider food systems. The paper demonstrates how the organizations have attempted to address this challenge through CFFRC's development of the research value chain approach and the advocacy work being undertaken by CFF.

Key words: CFF, CFFRC, research value chain, awareness, advocacy

Introduction
Underutilized species are defined broadly as ones which are currently not used, or are used to only a small extent of their actual potential, for social and economic purposes. Because of their underutilized status these species are often produced and consumed by smallholder households, or traded informally in local supply chains. As a consequence, their importance may go under-reported or unrecognized, yet these species can play an important role in the food security and nutritional wellbeing of farming households, providing a supplementary income to smallholders or acting as a food insurance crop. The fact that these species are often not regarded as cash crops and are part of gendered production systems means that they may play a disproportionately large role in food security and, in particular, in the livelihood strategies of female farmers. In addition many of these species are indigenous to regions which are marginal in relation to the production of major crops and are better suited to adverse environmental and climatic conditions. These facts are relatively well known, and indeed interest in these species is largely justified by their perceived suitability to marginal farming systems. However identifying the potential of these species is only the first hurdle that must be addressed if these crops are to be used more widely. Socio-political obstacles as

well as supply and demand bottlenecks present a considerable challenge to unlocking the potential of these species to support a range of socio-economic goals (Table 1).

Chief among the barriers relating to underutilized species is a lack of knowledge about underutilized crops at all points in a research value chain stretching from molecular biology and genetics through to processing, nutritional value and distribution. The wider use of these crops is dependent on the generation of relevant, credible and salient knowledge about different aspects of the production, consumption and distribution of these crops, evidence of their capacity to meet socio-economic needs and the development of methods to disseminate this information amongst producers, distributors and consumers.

Table 1. Underutilized crop constraints

Political/Policy Constraints	Supply constraints	Demand Constraints
• Inappropriate seed and intellectual properties laws • Unsympathetic food safety and quality standards regimes • Lack of legal frameworks for the protection of farmer products (GIs) • Underutilized species absent from university and school curricula • Inadequate knowledge and investment in underutilized species.	• Productivity • Lack of information on agronomic practices practices • Uncertainty over control of diseases and pests • Lack of economies of scale • Lack of recognized varieties • Poorly organized distribution networks	• Lack of familiarity with preparation and cooking • Demand constraints • High retail prices • Poorly integrated supply chains • Lack of convenience products • No awareness of food value

CFF and its research arm, CFFRC, were established with the complementary goals of undertaking awareness-raising and research activities aimed at promoting the use of underutilized species. The fact that underutilized species occupy a peripheral position in relation to conventional agricultural research networks contributes to, and is itself, a significant obstacle to the wider uptake of these species. Since their inception, both organizations have had to deal with the particular challenges this situation poses to efforts to broaden the use of these underutilized species. This paper argues that doing so requires a re-orientation in approaches to the generation and dissemination of knowledge related to the current and potential role of underutilized species in meeting socio-economic goals, and proposes a research value chain approach in order to achieve this.

Neglected and underutilized species (NUS) and current research paradigms

In its introductory brochure, CFFRC sums up the issues it is seeking to address in the following terms:

> "Agricultural production increasingly relies on a **narrow range of crops** and is becoming more and more uniform with less diversity on the table. This trend involves risks to the production, distribution and availability of food and non-food products….. …..new approaches to agricultural development are urgently needed," (CFFRC 2011).

This echoes wider dissatisfaction with the operation of the global agri-food industry (Sage, 2012). The imperative this industry places on increasing the output of basic commodities, along with the tendency towards increased standardization of production contributes to styles of agriculture which are:

- Over-dependent on a limited range of plant crops.
- Dominated by crop monocultures.
- Disarticulated from the localities in which it operates.
- Dependent on external inputs and outputs.
- Capital intensive.
- Impeding the life chances/opportunities of rural dwellers. (CFFRC 2011).

CFF and CFFRC promote an alternative suggesting that the generation and dissemination of information relating to the cultivation of neglected and underutilized species can result in their wider use. Doing so has the potential to address some of the shortcomings of conventional approaches to agriculture, promoting biodiversity and increasing the range of food and income options available to marginal smallholders. However, significant knowledge gaps in relation to underutilized crops across a wide range of disciplines exist. A critical issue for CFFRC thus involves identifying and addressing these gaps via research in a range of disciplines. Questions concerning the types of knowledge which are required and how this should feed into practical measures aimed at resolving "real world," problems are thus central to the work of both organizations. However Tovey (2008) suggests that agricultural research is increasingly is an expression of what has been defined as Mode 2 knowledge, – *"increasingly 'conceptualized,' shaped and organized around economic and social interests and concerns"* (Tovey 2008). In practice, this means that agricultural research is based on a body of ideas which is incorporated into, and driven by conventional agriculture which determines the questions and issues considered pertinent and thus the type of solution research generates (Alroe and Noe, 2010). These reflect commercial agriculture's interest in such considerations as cost reduction and increased productivity.

In many instances, the research undertaken on underutilized crops is based on approaches informed by this frame of reference. For this reason it may be worth considering whether this research is any more likely to result in outcomes that address the problems inherent in conventional agriculture. Or, is it likely to be the case that such information will simply be subsumed into the general information bank of commercial agriculture (Lockie and Halpin, 2005). There, bigger farmers *"operating at larger economies of scale,* (have) *the potential both to erode price premiums and to displace smaller farms"*(Alroe and Noe, 2010). Research design in relation to underutilized crops thus needs to embody a recognition of the values and desired outcomes of specific beneficiaries in the formulation of research problems and policy interventions based on them. This is particularly so given the current use of these crops in marginal farming systems. As Gruère et al. (2006) express it, in economic terms it may be necessary to find ways *"to avoid pressures toward commoditization and declining prices"* in order to *"preserve minimum rents for the producers once… …products from underutilized plant species become profitable"*. The challenge is thus to develop research in a range of disciplines that links underutilized species to the solution of applied problems while serving, or at least safeguarding, the interests of current producers. This has required CFF and CFFRC to adopt a broader perspective, considering how underutilized crops are integrated into wider supply chains and how its research agenda impacts on the dynamics of these relationships. This involves developing novel research approaches which lie outside of current agricultural research paradigms and incorporate a range of disciplines into programmes that deliver on a range of social and economic objectives.

Efforts to re-orientate agricultural research away from conventional agriculture are not new and have resulted in numerous "alternative agricultures". In the case of organic agriculture, such efforts have been associated with perspectives which establish a *"a binary division between growth, market, globalization and industrial production on the one ('bad') side and values, localness and artisanal production on the other ('good'),"* (Alroe and Noe, 2008). In their most extreme form such perspectives embody a rejection of functionally specialized science (Noe et al., 2007). However as Alroe and Noe (2010) argue "*disciplinary specialisation and sophistication is the basis for the strength of science in technological development and problem solving*". Adopting an approach which rejects the genuine merits of established research practices may lead to a considerable reduction in the efficiency of the research processes.

In other instances, researchers have sought to address the problems in existing agricultural research paradigms through enhanced forms of multi- and inter-disciplinary collaboration. Such multi-disciplinary processes can lead to the dilution of the quality of the specialised work brought to the table as:

- Results and outcomes becoming subject to "micro-political," negotiation and bargaining (McAreavey, 2006).
- Certain specialities achieve an hegemonic status thus undermining the idea of multi-disciplinary work (Noe et al., 2007).
- Certain figures develop specific skills − effectively new specialisms in multi-disciplinary work.
- Quite often the actual role and function of the supposed "multi-disciplinary" component of projects is poorly defined and untheorized leading to a lack of clarity as to the aims, objectives and methods employed (finding its expression in the dreaded inter-departmental meeting).

CFFRC's work involves developing a framework to integrate different forms of evidence-based research concerning underutilized crops and their use in addressing socio-economic needs. In doing so the organization must avoid the pitfalls mentioned above. CFFRC is also faced with another reality in which underutilized crop research does not now, nor is likely to command the same levels of resources as are available for research into major crops. Instead, the organization will have to devise ways to make efficient use of limited resources. In this context the question of how different forms of knowledge are brought together involves the adoption of approaches which involve forms of social innovation: *"changes of attitudes, behaviour or perceptions of a group of people joined in a network of aligned interests that in relation to the group's horizon of experiences lead to new and improved ways of collaborative action within the group and beyond*" (Neumeier, 2011). Elaborating such an approach is in itself an important and discrete research and operational challenge, requiring its own resources and as importantly the commitment and genuine engagement of the knowledge communities involved.

A value chain approach to building knowledge networks

As a research organization, CFFRC's programme involves the delivery of three sets of outputs which are interlinked and potentially mutually reinforcing. These are:

- Scientific outputs – generated through specific scientific research projects.

- Applied outputs – this involves the delivering of practical outputs such as for example identifying and devising cropping systems which can be used in dryland systems.
- Policy outputs − this concerns, in the broadest sense the question of determining the ends to which the scientific and applied knowledge generated in the programme should be used.

To genuinely deliver, CFFRC must achieve a balance between these three sets of outputs (Figure 1).

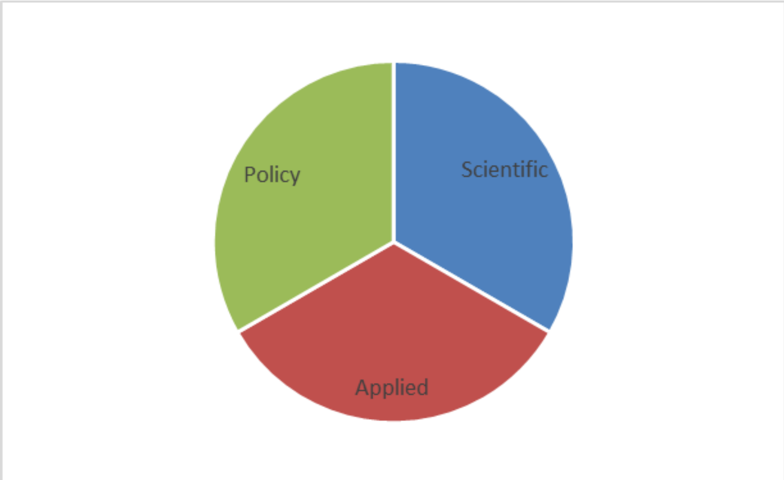

Figure 1. The outcomes balance for CFFRC

The primary mechanism through which CFFRC operates is via high quality scientific research projects (Figure 2 illustrates such a project). It involves a number of steps through which information is extracted from a particular problem field (all the possible research problems concerning a specific research object). The output is the production of scientific knowledge concerning some aspect of the problem field, such as for example the findings of a genetic study. In this sense, the role of the research project is relatively straightforward and involves addressing a particular question in a problem field. The routes through which this research finds its way into industry, society and policy are, however, less clearly defined and more diffuse.

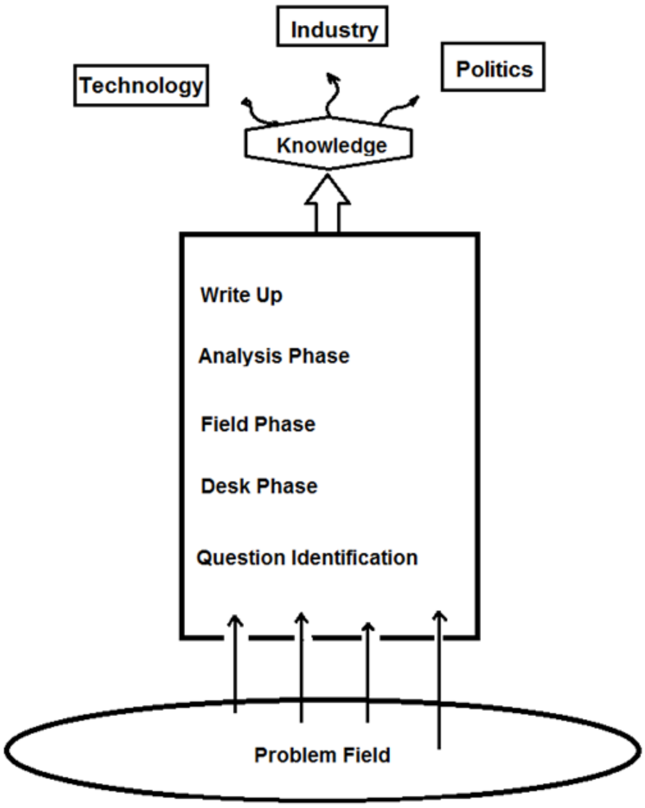

Figure 2. Research project.

Within CFFRC, a significant number of research projects have already been initiated. However, if such research is to contribute to achievement of both applied and normative goals then they need to be combined with others (Figure 3).

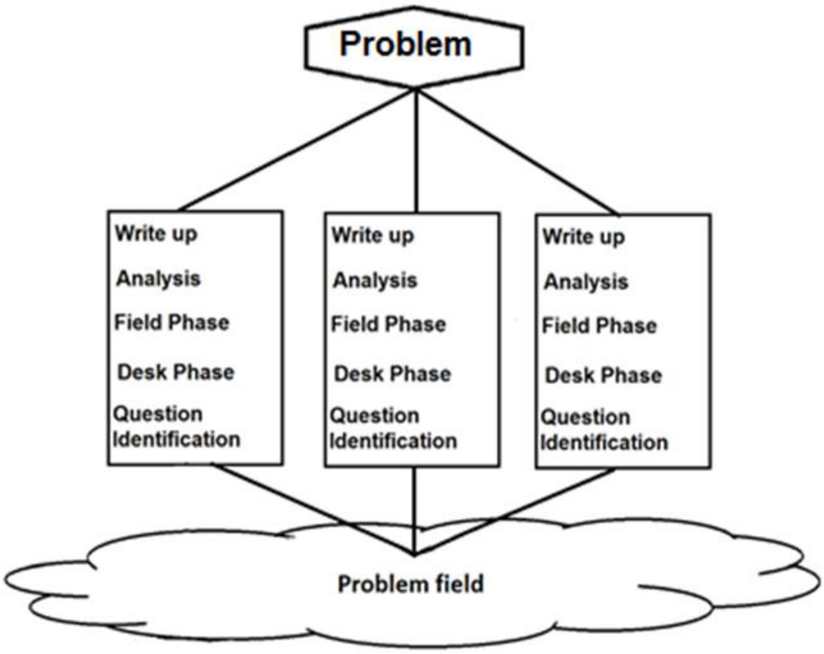

Figure 3. CFFRC project.

Notice too that in the case of CFFRC, the route envisaged between scientific research and the applied problem is more direct than is the case in Figure 1. The challenge of combining projects in this way in order to achieve a series of applied and normative goals will need to be addressed if CFFRC is to be successful.

CFFRC Programmes

CFFRC Programmes have been developed as a means of bringing together knowledge drawn from multiple disciplines. CFFRC programmes involve combining projects in order to deliver applied results linking fundamental research in areas such as genetics to issues such as economics and development studies. In doing so, the experience of the CFFRC programmes is intended to shed light on how complex research programmes in underutilized crops can be managed more efficiently. Table 2 details the differences between projects and programmes.

Table 2. Characteristics of projects and programmes in CFFRC

Research Project	CFFRC Programme
• Focused on answering specific scientific questions • Examines specific objects in the problem field • Primarily employs expert knowledge in research contexts • Tightly planned around the achievement of scientific objectives • Criteria for validity are primarily scientific • Well understood and recognized perspective on what constitutes the issue	• Focused on employing a range of perspectives to tackle broader applied and normative issues • Examines multiple objects in a problem field • Employs different forms of knowledge in a range of socio-cultural contexts • May embark with less clearly defined objects (broad aims) • Multiple criteria for assessing the outputs of the programme • Multiple perspectives on a combination of technical, applied and normative issues identified through the trans-disciplinary process

A critical aspect of the approach being developed by CFFRC is the need to identify and address critical gaps in knowledge. Concerning underutilized crops, this requires a number of research issues to be addressed ranging from questions in bioscience disciplines through to questions in the social sciences. While functionally specialized research tends to result in the creation of disciplinary "silo's" (Azam-Ali, 2010), this makes interdisciplinary co-ordination difficult. CFFRC programmes must combine research in different disciplines in developing solutions to applied problems. This approach is encapsulated in the notion of a research value chain. The concept of "value chain" originates in the business world. At its simplest it refers to the all the stages through which a firm processes raw materials and/or other inputs ultimately producing goods. At each stage, value is added, contributing to the final product. The concept of a research value chain as employed in CFFRC programmes bears some similarities to the traditional notion of a value chain; it envisages research as a series of interlinked processes which collectively contribute to the achievement of an agreed outcome. As with value chain management strategies, there is a focus in CFFRC in minimizing waste and maximizing quality by identifying precisely those research inputs that are needed and eliminating duplication of effort. The research value chain approach also places emphasis on the need for effective communication and co-operation between those involved in different activities in the chain as a means of achieving these goals. By organizing disparate disciplinary research and the inputs of other knowledge communities into programmes aimed at the solution of practical challenges, the research value chain directly links high quality scientific research to the delivery of inter-related scientific, normative and applied outputs.

The idea of a research value chain also differs from the commercial value chain in important respects. Commercial value chains are understood as a linear process with a clearly defined beginning, middle and end. They are organized around well-defined objectives. Firms tend to have a comparatively high level of control over the different elements of the production process. However, a research value chain creates a context within which researchers from different disciplines interact with each other and other knowledge communities. At the outset, ideas concerning the key issues to be addressed and the key desired outputs of a research value chain may vary between the different disciplines and other knowledge communities involved. Moreover these value chains are dynamic. Rather than a discreet beginning, middle and end, they involve a constant re-appraisal of research objectives. As programmes employing a research value chain progress, additional information gaps may be identified as objectives change or new issues emerge. A research value chain approach links the activities of researchers and inputs of other knowledge groups directly to the production of scientific, applied and normative outputs compelling researchers to engage with other knowledge communities providing a more effective mechanism for fostering cooperation between different disciplines.

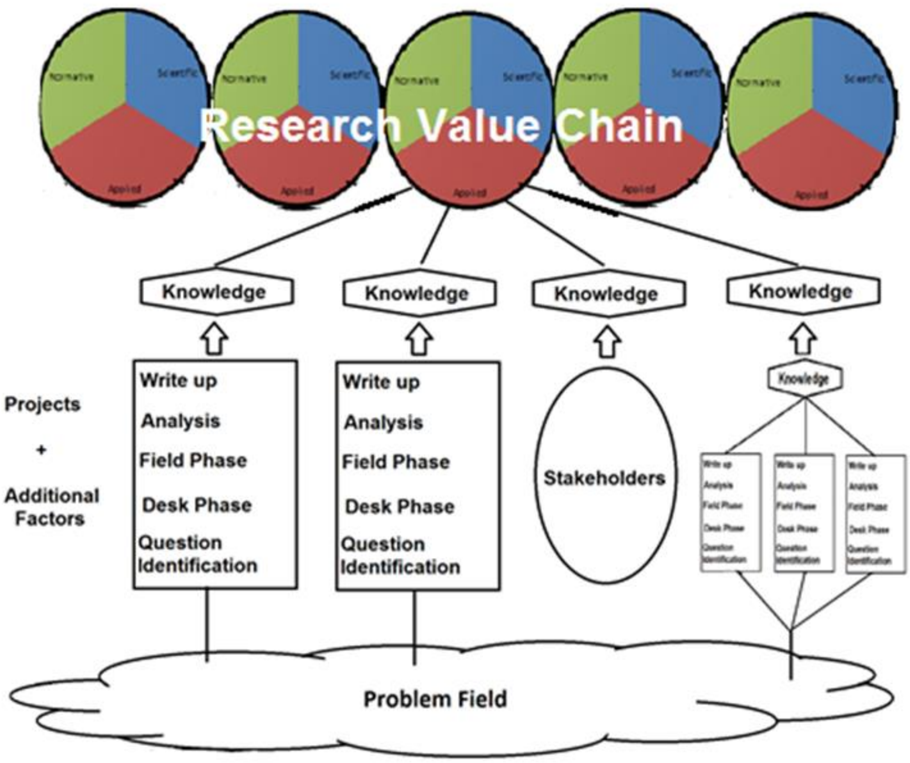

Figure 4. CFFRC Programme.

CFFRC themes identifying generic issues

The CFFRC research value chain differs from commercial value chains in another important way. While the latter are primarily focused on the production of a product, the applied outputs of CFFRC research value chains are based on scientific outputs which are themselves significant outputs of the programme. An important element of CFFRC's programme includes the need to ensure the quality of the scientific outputs generated within the research value chain, and the development of generic research methods based on them, while the programmes provide a means through which research can be combined to produce applied and normative outputs. A mechanism is also needed to ensure the quality of scientific outputs in different programmes and ultimately the production of proven generic methods which can be applied to a range of crops. CFFRC has developed a number of scientific themes, linking research in related scientific disciplines being undertaken in different research value chains. The themes act as the guarantors for the quality of the scientific outputs of the different CFFRC programmes. In addition, the themes provide an overview allowing for the identification links between research in different programmes. The themes also identify key issues relevant to a range of underutilized crops thus facilitating the development of generic methods for addressing them.

CFF and knowledge dissemination

While CFFRC focuses on knowledge generation, CFF fulfils the complementary role of knowledge dissemination. These roles are interdependent and it is only through the generation of hard scientific evidence that a case can be made for the promotion of underutilized species. However, it is equally the case that without effective dissemination the capacity of the evidence generated by CFFRC to contribute to the solution of economic and social challenges is likely to be exceptionally limited. As an advocacy organization, CFF defines its role as being:

- To add value to the work of the neglected and underutilized species (NUS) community in terms of facilitating collective action.
- To become a global champion and voice for NUS R&D.

This involves a community with a range of different target audiences including NUS researchers, producers, consumers and policy makers. This is reflected in the operational objectives of CFF which embody a range of measures tailored to the specific needs of different target audiences which include:

1. Facilitating access to knowledge on NUS, through web portal, monographs, synthesis papers, and innovative uses of the Internet.
2. Providing information services to NUS stakeholders.
3. Engaging in policy research and advocacy to promote the use of NUS (e.g. highlighting the significance of addressing market access barriers).
4. Raising awareness concerning the current and potential and contribution of NUS to livelihoods and well-being.
5. Strengthening capacity amongst NUS researchers.
6. Providing greater public access to information concerning underutilized crops.

Since its inception, CFF has placed a strong emphasis on linking evidence to advocacy in its approach to engaging with policy makers, the NUS research community and the general public. In addition however, it has focused in particular on the significant potential of ICT as dissemination tools. However, given the plethora of agricultural information services, CFF has attempted to avoid replicating existing capacity and has instead focused on maximizing access to and the quality of existing services, through the development of its site as a portal and the use of existing, tried and tested services such as Google books to disseminate information.

Conclusion

There is a widespread view that underutilized species offer an alternative to conventional agriculture which has considerable capacity to support marginal farming communities and address some of the broader environmental challenges that confront the globe. However the capacity of these crops to deliver on this promise is unlikely to be achieved via conventional approaches to agricultural research and development. The proponents of underutilized crops have neither the time nor the resources to devote to doing research in the established way nor are the outcomes of their investigations likely to appeal to conventional agriculture. What is needed are novel approaches to undertaking quality research in a range of disciplines that links underutilized species to the solution of complex applied problems while also protecting the interests of current producers. The research value chain approach which CFFRC has developed is intended to target the delivery of research inputs to address critical knowledge gaps related to underutilized crops. This involves enhanced systems of interdisciplinary communication and collaboration. It also involves new strategies for communicating with user groups, policy makers and other actors involved in agricultural and rural development. By linking research in different disciplines in value chains it is intended to generate a balance of scientific, applied and normative outputs. The research value chain approach compels scientists in different disciplines to engage more meaningfully with each other around the delivery of applied and normative as well as scientific outputs. By delivering such outputs CFFRC research value chains contribute to the development of generic approaches to solving real world problems using underutilized species. Doing so is essential to providing a credible evidence base to support the wider use of these crops.

References

Azam-Ali S.N., 2010. Fitting underutilized crops within research-poor environments: Lessons and approaches. South African Journal of Plant and Soil, 27(4): 293–298.

Alroe H. F., Noe E. 2010. Multiperspectival science and stakeholder involvement: Beyond transdisciplinary integration and consensus. In 9th European IFSA Symposium pp. 527-533.

Alroe H.F., Noe E. 2008. What makes organic agriculture move: protest, meaning or market? A polyocular approach to the dynamics and governance of organic agriculture. International Journal of Agricultural Resources, Governance and Ecology 7(1): 5-22.

CFFRC. 2011. CFFRC strategy document (unpublished)

Gruère G., Giuliani A., Smale M. 2006. Marketing Underutilized Plant Species for the Benefit of the Poor: A Conceptual Framework EPT Discussion Paper 154.

McAreavey R. (2006. Getting Close to the Action: The Micro-Politics of Rural Development Sociologia Ruralis 46: 85-103

Neumeier S. 2011. Why do Social Innovations in Rural Development Matter and Should They be Considered More Seriously in Rural Development Research - Proposal for a Stronger Focus on Social Innovations in Rural Development. Sociologia Ruralis 52: 48-69.

Noe E., Alroe H.F., Langvad A.M.S. 2008. A polyocular framework for research on multifunctional farming and rural development Sociologia Ruralis 48(1): 1–15.

Lockie S., Halpin D. 2005. The 'Conventionalisation' thesis reconsidered: structural and ideological transformation of Australian organic agriculture. Sociologia Ruralis 45 (4): 284-307.

Tovey H. 2008 Introduction: rural sustainable development in the knowledge society era. Sociologia Ruralis 48(3): 185- 199.

Contract farming by African traditional vegetable seed producers in Tanzania: implications for household cropping income

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Abstract

Traditional African leafy vegetables typically have been considered minor crops and have attracted little marketing attention compared to major crops such as maize and cash crops such as tomato and banana. Although demand for traditional African leafy vegetables has increased in recent years, the vegetable supply currently cannot meet demand due to a lack of quality seed of preferred varieties. To address this bottleneck in seed availability, it is important to understand the structure of the marketing system for traditional African leafy vegetable seed, and to develop strategies to improve the system. Evidence suggests that contract farming is an efficient mode of integrating smallholder farmers into the agricultural marketing chain. We examined the determinants of traditional African leafy vegetable seed producers' participation in contract farming systems and sought to identify the impact of contract farming on seed producers' income. A primary survey of 90 farm households producing traditional vegetable crop seeds in the Arusha, Tanga, Morogoro, Dar es Salaam and Dodoma regions of Tanzania were surveyed using a systematic random sampling approach. Among 90 farm households, 16 farm households participated in contract farming systems. Out of the 16 farm households, 9 were headed by women. On average, income from seed sales was 186.6 USD per household, whereas it was 109.8 USD per acre. Contract farmers received higher crop income per season and acre (USD 293.7) than in the Quality Declared Seed (QDS) and non-QDS systems. The results show that small-scale farmers receive higher income from crop sales than large-scale farmers. Our results show that farmers who engaged in contract farming realized more income from crop sales than those that participated in QDS and non-contract farming systems. Policy options to improve marketing systems for seed producers and growers of traditional African leafy vegetable crops are discussed.

Keywords: smallholders, contract farming, QDS, participation, Tanzania

Introduction

In recent years, the demand for African traditional vegetables including amaranth (*Amaranthus* spp.), African eggplant (*Solanum aethiopicum, S. anguivi* and *S. macrocarpon*), African Nightshades (*Solanaceae*) and jute mallow (*Corchorus* spp.) has increased but lack of availability and accessibility of quality seeds of preferred varieties is a key constraint to improving delivery of produce to consumers. While many African traditional vegetables are well adapted to local conditions and highly valued in local markets, the informal markets and networks that smallholders rely on to obtain African traditional vegetable seeds typically fail to provide predictable or good quality varieties. Most species are open-pollinated and their seeds are therefore easily saved by farmers over many seasons, which have discouraged commercial investment. Small companies are starting to invest in production of African traditional vegetable seed as a business but often have inadequate land to bulk commercial seed. Evidence suggests that contract farming system can be an efficient model for seed supply chains that integrate smallholder farmers and small-scale enterprises. The main objective of the paper is to understand the structure of the marketing system for African traditional vegetable seed, and to develop strategies to improve the system and livelihoods of smallholders who grow African traditional vegetable seeds in Tanzania.

Seed policies in Tanzania

Tanzania has introduced several policies and regulation for improving seed varieties, production and marketing. These policies and regulations include the Seed Act of 2003 and its regulations (2007), the Plant Protection Act of 1997, the Protection of New Plant Varieties Act of 2002 (plant breeder varieties) and its regulations (2008). To improve seed quality, the government has introduced the Quality Declared Seed (QDS) programme, which was developed by the Food and Agriculture Organization of the United Nations (GRAIN 2005, Food and Agriculture Organization, 2006). The objective of the QDS program is to improve the availability of quality seeds to farmers in seed deficit areas. It functions where formal seed markets are not active and government resources are too limited. As part of the seed regulatory process, the government has established an independent institute called the Tanzania Official Seed Certification Institute (TOSCI) to regulate seed businesses in accordance with the Seed Act of 2003. The government has further established an independent body called the Agricultural Seed Agency (ASA) with the key mandates of promoting the use of improved seeds as well as promoting private sector participation in seed production processing and marketing.

Despite several challenges, these policies and regulations have changed the face of seed production and marketing in Tanzania, particularly after trade liberalization. However, the country still must contend with low production and productivity due to limited use of inorganic fertilizers, seeds and pesticides, accessibility and availability of quality inputs due to inefficient input distribution systems, poor infrastructure facilities and climate change.

Contract farming system in Africa

According to Minot (2011) contract farming can be defined as “agricultural production carried out according to a prior agreement in which a farmer commits to producing a given product in a given manner and the buyers commits to purchasing it”. Contract farming plays a significant role in integrating smallholders in agri-business chains; it can promote commercially orientated smallholders and brings agriculture to markets (World Bank, 2007). Contracts solve several constraints smallholders face in producing and marketing their output. However, several studies criticize contract farming as it creates income inequality, which in turn creates inequality in the community (Minot, 2011; Freguin-Gresh et al., 2012), or excludes smallholders, as traders prefer to contract with medium and large farmers due to characteristics of the crops that make them particularly unsuited to smallholders (Nankumba and Kalua, 1989; Saenger et al., 2012). Some studies show that contract farming can strengthen value chain relations (Schipmann and Qaim, 2011) and improve smallholders’ access to better technology, improve seed varieties and other inputs, increase income, and reduce transaction costs (Minten et al., 2009; Barrett et al., 2012; Abebe et al., 2013).

Contract farming in sub-Saharan African countries tends to improve farmers' income (Little and Watts, 1994; Warning and Key, 2002; Minten et al., 2009; Meijerink, 2010; Minot, 2011). Institutional development may make smallholders more desirable partners for firms. Poor institutional development restricts smallholders' access to better production information and makes them depend on the firm for the high fixed costs of extension services. However, a few studies show mixed results (Warning and Key, 2005; Simmons et al., 2005; Miyata et al., 2009). Oya (2012) suggests some research questions tend to be absent on contract farming systems under the framework of current dynamics of agrarian change and transitions to capitalism in African countries. Two models of farmer-led seed enterprises that require market-oriented collective action between traders and farmers exist in Tanzania: the community-led Quality Declared Seed production system, and contract farming. Afari-Sefa et al. (2012) conclude that in Tanzania, average community seed producers have a lower input cost and higher returns than contract seed growers. Although the authors identified challenges and constraints faced by these two models, they did not investigate the determinants of smallholders' participation and income. In Tanzania, few studies have analysed linkages between smallholders and contract farming systems. Therefore, this paper aims to understand the structure of the marketing system for African traditional vegetable seed, and to develop strategies to improve the constraints and challenges faced by farmers in Tanzania.

Materials and methods

Survey data

A survey of 90 farm households which cultivate African traditional vegetable seed production in the Arusha, Tanga, Morogoro and Dodoma regions in Tanzania was conducted from January to May 2013. Lists of contract and QDS farmers were received[1] and samples were selected randomly from the lists. Similarly, a list of non-contract farmers was received from village leaders and appropriate sample sizes were drawn purposively from the list. Socio-economic and cropping pattern information as well as input-output data on the two farmer-led seed enterprise models were collected for the 2012-13 cropping year. A semi-structured questionnaire was administered following expert interviews with officials in several departments and academic institutions. Primary data were obtained through face-to-face interviews.

Results

The descriptive statistics (Table 1) show the type of seed distribution channels across farm size category and regions. Marginal, small- and medium-scale farmers constituted 96% of the sample. Marginal farmers were defined as owning cultivated land between 0-1 ha, small farmers 1-2 ha, medium farmers 2-4 ha and large farmers 4 ha above. Out of the total of 90 farm households, 15% and 33% engaged in contract farming and QDS systems, respectively, while 52%, including smallholders, sold their seed through the informal system. The high percentage of smallholders selling seeds through the two formal systems may indicate a preference for the low risk factors of the formal arrangements and timely availability of good

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[1] List of contract farmers was received from private seed companies; the QDS farmers list was received from the district (DAICOS) in collaboration with INADES, a local NGO that works with farmers on QDS seed production and distribution.

quality seeds compared with the informal open market system. On a regional basis, in Arusha it was observed that although almost all types of seed marketing systems exist, the major seed distribution channel was through contract farming since Arusha is considered to have a friendly agro-climatic zone for seed cultivation and commercial access. In Tanga and Morogoro, only the informal seed marketing system was in existence. In Dodoma, all farmers produced seeds under a QDS system. In the Arusha region, agro-climatic conditions and infrastructure accessibility are considered to be better for seed production, which likely give it a comparative advantage and to be more commercially viable over the other regions. Table 2 provides details on landholding size characteristics across farm size and seed marketing distribution channels. Small and medium farm categories accounted for most of the landholding volume, which indicates that small- and medium-scale farmers play an important role in seed production. In the contract farming system, there is little difference between net operated and irrigated area, which indicates farmers under the contract farming system have sufficient access to water and perhaps irrigation facilities.

Table 3 depicts basic characteristics of farm households. Out of 90 farm households, 38% were headed by women. Interestingly, contract farming had the highest level of women's participation. The average age of respondents was 45 years. Contract farmers had lower levels of education compared with QDS and non-QDS farmers.

On average, income from seed sales was 186.6 USD per household, whereas it was 109.8 USD per acre. However, contract farmers received higher crop income per season and acre (USD 293.7) than in QDS and non-QDS systems (Table 4). All categories of farmland holders in contract farming were higher for both output and total marketed surplus for seeds. Those farmers who produced under contract marketing systems tended to sell more produce compared with farmers who used QDS and non-QDS systems. Farmers who sold their crops through contract farming received more crop income than selling their crops through the other systems. This might be an indication that the QDS system might not improve crop income.

Farmers' perceptions about social norms, perceived control, and adoption of new agricultural technologies under different seed marketing systems are shown in Table 5. The values were measured using a 5-point Likert scale. Those farmers participating in contract farming systems had higher attitude scores than farmers who produced seed under the QDS and non-QDS systems. Scores for social norms and perceived control did not vary across seed systems. Farmers with high scores for attitude and perceived control might not be concerned about what other farmers thought (social norms).

Discussion

This paper aims to understand the structure of the marketing system for traditional African vegetable seeds, and to develop strategies to improve the constraints and challenges faced by farmers in Tanzania. In general, more women farmers than men farmers participated in farmer-led enterprises, particularly in the contract farming system. Younger farmers preferred to be part of farmer-led enterprises, and more participated in contract farming than in QDS. Finally, the results show that under a contract farming system, income from crop sales is higher than income generated through QDS and non-contract systems. Strengthening the contract farming system in Tanzania will help to increase incomes for marginal smallholders, who prefer to engage in contract farming**.**

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References

Abebe G.K., Bijman J., Kemp R., Omta O., Tsegaye A. 2013. Contract Farming Configuration: Smallholders' Preferences for Contract Design Attributes. Food Policy 40 (June): 14–24.

Afari-Sefa V., Chagomoka T., Karanja D.K., Njeru E., Samali S., Katunzi A., Mtwaenzi H., Kimenye L. 2013. Private contracting versus community seed production systems: Experiences from farmer-led seed enterprise development of indigenous vegetables in Tanzania. Acta Horticulturae 1007: 671-680.

Barrett C., Bellemare M.F., Bachke M., Michelson H., Narayanan S., Walker T. 2012. Smallholder market participation in evolving agricultural value chains: Comparative evidence from five countries. World Development 40(4):7 15.

Food and Agriculture Organization. 2006. Quality Declared Seed System, FAO Plant Production and Protection, (Paper No. 185). FAO, Rome.

Freguin-Gresh S., d'Haese M., Anseeuw W. 2012. Demythifying contract farming: evidence from rural South Africa. Agrekon 51(3): 24-51.

GRAIN. 2005. Africa's seed laws: red carpet for the corporations, Available online at: http://www.grain.org/article/entries/540-africa-s-seeds-laws-red-carpet-for-corporations [accessed August 1, 2014].

Little P., Watts M. eds. 1994. Living Under Contract: Contract Farming and Agrarian Transformation in Sub-Saharan Africa. Madison: University of Wisconsin Press.

Meijerink G. 2010. Contract Farming: Some Lessons for Ethiopia, Ayelech Tiruwha Melese, VC4PD Brief, 28th June.

Minot N. 2011. Contract Farming in Africa: Opportunities and Challenges. Presented at the AAMP Policy Seminar "Successful Smallholder Commercialization" on 22nd April, 2011, Rwanda.

Minten B., Randrianarison L., Swinnen J.F.M. 2009. Global retail and poor farmers: evidence from Madagascar. World Development 37: 1728–1741.

Miyata S., Minot N., Hu D. 2009. Impact of contract farming on income: linking small farmers, packers, and supermarkets in China. World Development 37:1781-1790.

Nankumba J.S., Kalua B. 1989. Contract farming in Malawi: smallholder sugar and tea authorities, Eastern Africa. Economic Review. (Aug). 42-58.

Oya C. 2012. Contract farming in sub-Saharan Africa: a survey of approaches, debates and issues. Journal of Agrarian Change 12(1): 1-33.

Saenger C., Qaim M., Torero M., Viceisza A. 2012. Contract farming and smallholder incentives to produce high quality: experimental evidence from the Vietnamese dairy sector. Global Food Discussion Paper No. 10, Transformation of Global Agri-Food Systems: Trends, Driving Forces, and Implications for Developing Countries Georg-August-University of Göttingen.

Schipmann C., Qaim M. 2011. Supply chain differentiation, contract agriculture, and farmers' marketing preferences: The case of sweet pepper in Thailand. Food Policy 36, 666–676.

Simmons P., Winters P., Patrick I. 2005. An analysis of contract farming in East Java, Bali, andLombok, Indonesia. Agricultural Economics 33: 513-525.

Warning M., Key N. 2002. The social performance and distributional consequences of contract farming: an equilibrium analysis of the arachide de bouche programme in Senegal. World Development 30(2): 255-263.

World Bank, 2007. World Development Report 2008: Agriculture for Development. World Bank, Washington DC.

Table 1. Type of seed distribution channels by farm size category and regions
(QDS - Quality Declared Seed)

By Farm Size Category	Households (No.)	Contract Farming	QDS	Non-QDS/ Contract	Overall
Marginal Farm (0> to 1 ha)	24	31	27	26	27
Small Farm (1> to 2 ha)	32	38	27	40	36
Medium Farm (2> to 4 ha)	30	23	40	32	33
Large Farm (4 ha above)	4	8	7	2	4
Total	90	100	100	100	100
By Regions					
Arusha	18	100	10	6	20
Tanga	19	-	-	40	21
Morogoro	25	-	-	53	28
Dodoma	28	-	90	0	31
Total	*90*	100	100	100	100

Table 2. Land size details by farm size category

By Farm Size Category	Households (No.)	Contract Farming	QDS	Non-QDS/ Contract	Average
Land Size (ha)					
Own Area	90	1.5 (1.5)	2.0 (1.3)	1.6 (0.9)	1.7 (1.6)
Net Operated Area (NOA)	90	2.0 (1.9)	1.9 (1.2)	1.6 (0.9)	1.7 (1.2)
Net Operated Irrigated Area (NOIA)	90	2.0 (1.9)	0.4 (0.3)	0.8 (1.0)	0.8 (1.0)
Area under Seed Cultivation	90	1.7 (1.1)	0.4 (0.3)	0.6 (0.5)	0.7 (0.7)
NOA by Farm Size Category		Land Size (Ha)			
Marginal Farm (0-1 ha)	24	0.7 (0.2)	0.6 (0.2)	0.5 (0.3)	0.6 (0.3)
Small Farm (1-2 ha)	32	1.5 (0.2)	1.4 (0.3)	1.3 (0.2)	1.4 (0.3)
Medium Farm (2-4 ha)	30	2.8 (0.7)	2.6 (0.5)	2.5 (0.5)	2.6 (0.5)
Large Farm (4 ha above)	4	7.7 (0.0)	4.9 (1.1)	4.5 (0.0)	5.5 (1.6)
Overall	90	2.0 (1.9)	1.9 (1.2)	1.6 (0.9)	1.7 (1.2)
Pie-Value: Land Size by Farm size category		(Share %)			
Marginal Farm (0-1 ha)	24	11	8	8	9
Small Farm (1-2 ha)	32	29	20	33	29
Medium Farm (2-4 ha)	30	32	55	50	51
Large Farm (4 ha above)	4	30	17	6	14
Overall	90	100	100	100	100

Note: SD in brackets

Table 3. Basic characteristics of farm households (HH)
(QDS - Quality Declared Seed)

By Farm Size Category	HH	Contract Farming	QDS	Non-QDS/Contract	Average
By Gender					
Female	35	54	47	29	38
Male	56	46	53	71	62
		100	100	100	100
Age Group of Respondent					
0-35 years	23	15	27	27	25
35-50 years	39	38	57	35	43
50 above	29	46	17	38	32
		100	100	100	100
Level of Education					
Number of Years	90	5.5	7.1	7.3	7
Family Size					
Number of People	90	4.8	5.9	5.6	5.6

Table 4. Production and crop income per crop season

Indicators	QDS	Contract Farming	Non-QDS/contract	Average
Production & Crop Income per Household				
Production (Quantity per Household - Kg)	103.2	98.9	15.1	57.5
Sales (Quantity per Household - Kg)	41.2	97.2	8.2	33.4
Net Crop Income per Household (USD)	215.3	587	59	186.6
Production & Crop Income per Acre				
Production (Quantity - Kg)	54.3	49.5	9.4	33.8
Sales (Quantity - Kg)	21.7	48.6	5.1	19.6
Net Crop Income (USD)	113.3	293.7	37.0	109.8

Table 5. Surveyed farmers' psychological constructs on attitude, social Norms and perceived control (Likert Scale; 1=highly disagree, 5=strongly agree).

Psychological Indicators	QDS	Contract	Non-QDS/Contract	Overall
Attitude				
I consider myself as a progressive farmer	4.1	4.2	3.6	3.8
I like to try new agri-tech	4.4	4.7	4.2	4.3
I actively seek information from others	4.4	4.2	4.1	4.2
I like new ideas in general	4.4	4.4	4.2	4.3
	4.3	*4.4*	*4.0*	*4.2*
Social Norms				
Other farmers think I am a progressive farmer	4.0	3.7	3.6	3.7
Other farmers ask my opinion	3.9	4.0	3.8	3.8
Other farmers will not object to my farming activities	3.9	3.8	3.4	3.6
	3.9	*3.8*	*3.6*	*3.7*
Perceived Control				
It is easier for me to collect information about tech.	3.6	3.4	3.1	3.3
I have good contacts with extension officers	4.0	4.3	3.8	3.9
I can adopt new agri-tech if it is profitable	4.4	4.5	4.3	4.4
	4.0	*4.1*	*3.7*	*3.9*

Research capacity for neglected and underutilized species: a situation analysis in ten African countries

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Abstract

Hundreds of traditional minor crops exist in Africa which are highly nutritional and often well adapted to local environments, such as marginal soils and drylands, but their broader use, e.g. for food security and in climate change adaptation is constrained by weak scientific capacity to work on such crops. Between 2010 and 2012 more than 1300 young African scientists applied to seven training courses on neglected and underutilized species (NUS) that targeted ten countries in West and Southern/East Africa. The high demand raised questions about the availability and quality of capacity development in this field, what NUS research is currently taking place, and the main constraints NUS researchers face. To investigate these questions, a survey was administered by email to the scientists who had applied to the training courses. The survey had 383 respondents between 24 and 57 years of age. The majority held a Master's (61%) or PhD (31%) in agriculture or a related field. Most respondents were involved in NUS research at the time of the survey, or wanted to research NUS in the future. A broad range of NUS crops and trees were being studied in their research, including legumes and pulses, cereals, leafy vegetables, fruits, nuts, roots and tubers. The main issue addressed was nutrition, followed by characterizing and conserving genetic diversity, and aspects of NUS production and marketing. Funding was the most frequent constraint to NUS research. Poor quality of facilities and lack of information were other limitations. External support for NUS research, including policy support, was uneven; about half ranked it as poor or non-existent while one tenth found such support very good or excellent. Overall, this survey reveals a strong interest in, and skills base for NUS research among African scientists. Increased opportunities for training, networking, and funding would strengthen this emerging and important field of research. These results could guide decision makers regarding strategies and action plans for developing capacity for NUS research, development and education with the aim of diversifying agriculture sustainably.

Key words: Capacity development, training, mentoring, neglected and underutilized species, research methods and themes, West Africa, Eastern/Southern Africa, expert survey

Introduction

Green Revolution technologies and farming practices have led to dramatic yield increases in many parts of the world. In sub-Saharan Africa, however, yield increases have been more modest; the increase in production has been mainly due to expansion of the area cultivated (Evenson and Gollin, 2003; Tadele and Assefa, 2012; Otsuka and Larson, 2013). The failure of the Green Revolution in Africa has been attributed, among other factors, to variable and harsh environmental conditions, and inconsistent access to inputs, labour and markets. Without the yield rewards of Green Revolution practices, Africa has still suffered many of the consequences of simplified, intensive production systems, including an impoverished dietary diversity. This is linked to a breakdown of traditional food systems and a shift towards Western-type cereal-based, energy-rich diets, which also leads to increases in diet-related non-communicable diseases (Frison et al., 2005).

Hundreds of minor crops have historically been valued in traditional cuisine and cultures across Africa and remain an important food source for the rural poor today. These crops are often well adapted to local environments, such as marginal soils and drylands, and they provide many vital macro- and micro-nutrients that enhance human well being (Padulosi et al., 2013). These neglected and underutilized species (NUS) of crops and trees have great potential to improve nutrition, livelihoods, and the sustainability of farming systems in sub-Saharan Africa but they have not received adequate investment by research and development efforts, which have tended instead to promote displacement of indigenous crops with major staples and commodity crops (Frison et al., 2005; Padulosi et al., 2013).

In many cases, social attitudes have also shifted away from traditional foods as they are considered 'poor-man's crops' or are no longer accessible or convenient for rapidly urbanizing populations. NUS crops are also statistically neglected; national food supply data both generalize (e.g. lumping together several species) and underestimate food crop diversity (focusing mostly on major food crops), and FAO data lack the resolution necessary for tracking trends in geographically restricted food species (Khoury et al., 2014). Awareness is growing in Africa and elsewhere, however, regarding the importance of diversifying agricultural production (FAO, 2011) to address hunger, poverty and climate change adaptation and it is recognized that NUS are key assets in these pursuits (Tadele and Assefa, 2012; Chivenge et al., 2015).

Enhancing the cultivation and use of NUS in Africa requires a strong research and development effort that crosses disciplines and includes all value chain stakeholders from seeds to final use. Farmers are central stakeholders in these efforts due to their roles as producers – and managers of NUS genetic resources – and also often as traders and consumers. Their participation helps identify constraints in the value chains and priorities for upgrading. In turn, improving farmers' links to markets provides economic incentives for on-farm conservation of these marginalized resources (Garcia-Yi, 2014; Padulosi et al., 2012; Smale, 2006).

As women are often key producers and users of traditional crops, effective research and development of NUS also requires a gender-responsive approach (Elias, 2013). It is unclear whether researchers are receiving sufficient training to foster the strong interdisciplinary, collaborative, participatory and gender-responsive research required for NUS development. Current training and research environments primarily serve the major staple crops and commodities, and the concept of agricultural biodiversity is poorly covered in higher agricultural education (Rudebjer et al., 2013), which hinders progress in NUS research and development.

In addressing the need for increased capacity on NUS research and development in sub-Saharan Africa, a series of seven training workshops were organized between 2010 and 2012, on the following topics, all with a focus on NUS plants:

- Scientific proposal writing (two courses)
- Experimental design and data analysis (two courses)
- Food systems: from agronomy to human health
- Value chain research
- Scientific writing and communication

The courses targeted young scientists from ten countries: Benin, Ghana, Mali, Nigeria, Senegal, and Ethiopia, Malawi, Mozambique, Kenya and Uganda. More than 1300 scientists in total applied for the courses, of which approximately 170 were accepted. Thus, for the majority of applicants, due to funding constraints, their training needs could not be met. This high demand for training showed a surprisingly strong interest in NUS research among young scientists. It also raised questions about the availability and quality of NUS training, what NUS research is currently being conducted, and the main constraints researchers face in this field. We investigated these questions through a survey that was administered by email to scientists who had applied to the training courses.

The courses were part of the project '*Building human and institutional capacity for enhancing the conservation and use of Neglected and Underutilized Species (NUS) of crops in West Africa, and Eastern/Southern Africa'*, supported by the African, Caribbean and Pacific (ACP) Science and Technology Programme, funded by the European Union. The project was implemented by Bioversity International, Italy; International Foundation for Science (IFS), Sweden; Regional Universities Forum for Capacity Building in Agriculture (RUFORUM), Uganda; African Network for Agriculture, Agroforestry and Natural Resources Education (ANAFE), Kenya; Institut de Recherche et de Développement sur la Biodiversité des Plantes Cultivées, Aromatiques et Médicinales (IRDCAM), Benin; Plant Genetic Resources Research Institute (PGRRI), Ghana; University of Nairobi, Kenya and the University of Malawi.

Methods

A survey of 44 questions was prepared using 'SurveyMonkey', an online questionnaire software, taking approximately 20 minutes to complete. The survey was sent to 592 scientists in Eastern, Southern and West Africa, who had documented their interest in NUS research by their application to the aforementioned NUS training courses in 2010 and 2012 (many of the 1300 applicants had changed email addresses and could not be reached). The data collection took place in July and August 2013.

All analyses were performed in R version 2.15.2. The analysis involved calculation of summary statistics and contrasting results from East vs. West Africa and students vs. non-students using chi-square tests. There were very few significant differences between these groups of respondents, and so we only describe results for the full group of respondents.

Survey respondents

A total of 383 researchers (a response rate of 64.7%) responded to the survey, of which one third were women (Table 1). The age of respondents ranged from 24 to 57, with an average age of 37. Respondents came from 15 countries in Africa with 38% from eastern and southern African countries and the rest from West Africa. The greatest number of respondents came from Nigeria, followed by Ethiopia and Kenya. Combined, these three countries accounted for 63.7% of the responses. Benin, Ghana and Senegal were also well represented. By contrast, four countries, Côte d'Ivoire, Gabon, Togo and Zimbabwe, were represented by only one scientist each. Almost all (90%) of respondents resided in their home country. Of the 10% that were ex-patriot, half were residing in another African country and half elsewhere, usually studying for a higher degree.

Table 1. Nationality and gender of respondents.

Nationality	Female	Male	Total
Eastern and Southern Africa			
Ethiopia	6	50	56
Kenya	21	30	51
Malawi	5	10	15
Mozambique	0	4	4
Uganda	8	9	17
Zimbabwe	0	1	1
West Africa			
Benin	12	26	38
Cameroon	0	3	3
Côte d' Ivoire	0	1	1
Gabonese	1	0	1
Ghana	6	19	25
Mali	3	6	9
Nigeria	54	83	137
Senegal	9	14	23
Togo	0	1	1
Not provided	0	1	1
Total	**125**	**258**	**383**

The majority of participants in the survey (61%) had achieved a Master's degree and about a third (31%) had obtained a PhD. Only 2% had a Bachelor's degree alone and 6% did not indicate their level of education. Respondents' most common field of study was, by far, agriculture (59%). Many had also studied health and nutrition (10%), ecology/environmental science (10%), or forestry (8%). Fewer respondents had studied economics (4%) or social science (2%). The rest comprised other fields of study such as food science/technology, biotechnology, plant breeding, bioengineering, and pure biology fields like botany and microbiology. Nearly half of respondents (45%) were currently studying for a higher degree. Most respondents were affiliated with a university/college in a developing country (59%) or a national research institute (29%). The private sector was poorly represented in this survey.

A majority, 240 respondents (63%) were currently performing research on NUS. Another 100 (26%) were planing to do so in the future. Only 1% of respondents indicated they had no plans of engaging in NUS research and 11% did not respond to the question. These groups, 43 respondents were excluded from the analysis.

Current NUS research

The results described in this section are based only on the respondents who were currently researching NUS (n=240).

Students dedicated a higher proportion of their time to NUS research than did the non-students: 46% of students and 23% of non-students allocated at least 60% of their working time to NUS. Half of the non-students spent 40% or less of their time on such crops. NUS research is often a part-time job (Figure 1).

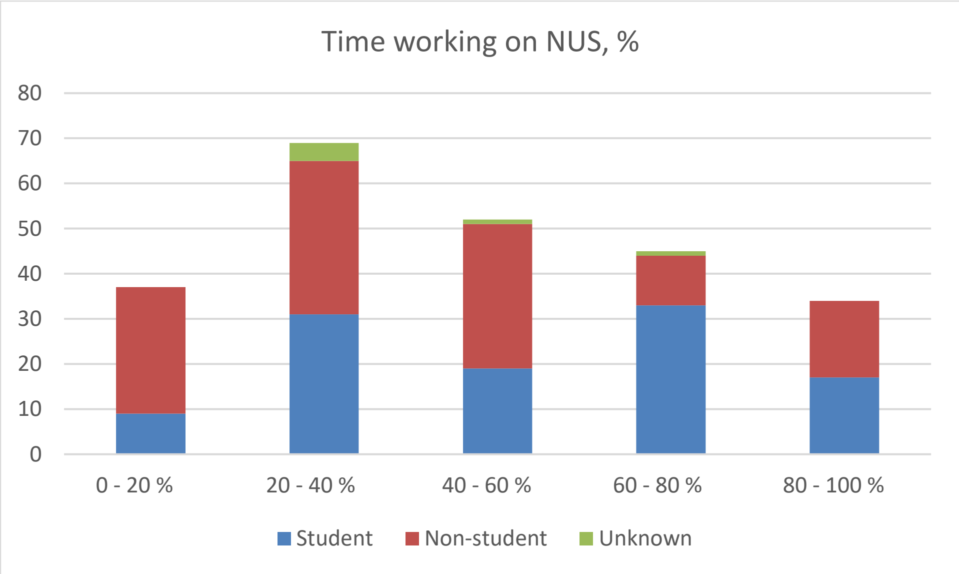

Figure 1. Proportion of working time dedicated to NUS research by students and non-students.

A wide diversity of NUS species was represented in respondents' research. The majority of researchers (61%) focused on just one type of NUS but over a third (39%) were researching a combination of NUS types, for example cereal and legume species. The most popular types of NUS studied were legumes and pulses, leafy vegetables and roots and tubers (Table 2). Among the most studied species were leafy amaranths, Bambara groundnut (*Vigna subterranea*), cowpea (*V. unguiculata*), yams (*Dioscorea spp.*), sorghum (*Sorghum bicolor)* and the multi-purpose *Moringa oleifera* tree, studied for its leaves, seeds, roots and oil. A small group of respondents was studying animals, which in some cases involved looking at the contribution of NUS plants to the nutrition and health of livestock. In total, the 240 researchers conducted research on 184 species in 148 genera and 64 families.

Table 2. Types and species of NUS considered in current research.

NUS Type	Total (N=240)	Most popular species (no. of respondents)	# species mentioned
Legumes	75	*Vigna subterranea* (15) *Sphenostylis stenocarpa* (10) *Vigna unguiculata* (8) *Phaseolus vulgaris* (5)	32
Leafy vegetables	67	*Amaranthus sp.* (22) *Solanum sp.* (10) *Telfairia occidentalis* (10) *Crassocephalum sp.* (6) *Corchorus olitorius* (6)	43
Roots and tubers	62	*Dioscorea sp.* (13) *Manihot esculenta* (10) *Colocasia esculenta* (10) *Ipomoea batatas* (10) *Cyperus esculentus* (6)	21
Cereals and pseuodcereals	52	*Sorghum bicolor* (9) *Pennisetum glaucum* (9) *Eleusine coracana* (7) *Digitaria exilis* (7)	12
Fruits and nuts	37	*Vitex. sp.*(4) *Vittelaria paradoxa* (3)	42
Plants with other uses*	52	*Moringa sp. (7)*** *Cola sp. (5)* *Jatropha sp.(5)*	32
Animals	10	All unique, including *Apis mellifera*, livestock, and farmed fish	8
Fungus	7	*Pleurotus sp.* (5)	9
Algae and seagrass	4	All unique, including *Arthrospira platensis*	3
No reply	5		

*Medicinal, timber, oil seeds, biodiesel, fibre, etc.
** There were actually 11 people studying *Moringa* but only seven people indicated that the purpose was for 'other' uses. The tree was also being studied for its edible leaves (5), seeds (3), and roots (3 respondents).

Most respondents' NUS research was purely biophysical in nature (36%) or combined biophysical and socio-economic research (47%). Only 13% of respondents' research focused only on socio-economic issues.

The most popular topic of NUS research was nutrition (36%; Figure 2). Other common topics were genetic diversity characterization and conservation, and aspects of NUS production such as agronomy, post-harvest processing, and value addition. The least addressed topics were gender and policy aspects, which were considered by only about 5% of the respondents. Most respondents' research (72%) addressed more than one topic.

In accordance with the fact that multiple topics were addressed by respondents' NUS research, the majority, 62%, of researchers also used more than one method to approach their NUS research. Experiments conducted in laboratories and research stations were the most commonly used methods, followed by diversity characterization and socio-economic surveys (Figure 3). Participatory methods were among the least common, used by 23% of respondents, and seemed notably low among those investigating nutrition, conservation and value chains (Figure 4).

Gender was considered in 79% of NUS research projects at various stages of research. However, few considered gender in the formulation of research questions and hypotheses (25%), choice of methodology and approaches (22%), and data analysis stages (22%). Very few respondents (10%) considered gender in all these research stages and even fewer (7%) then also included gender in the dissemination phase of the research.

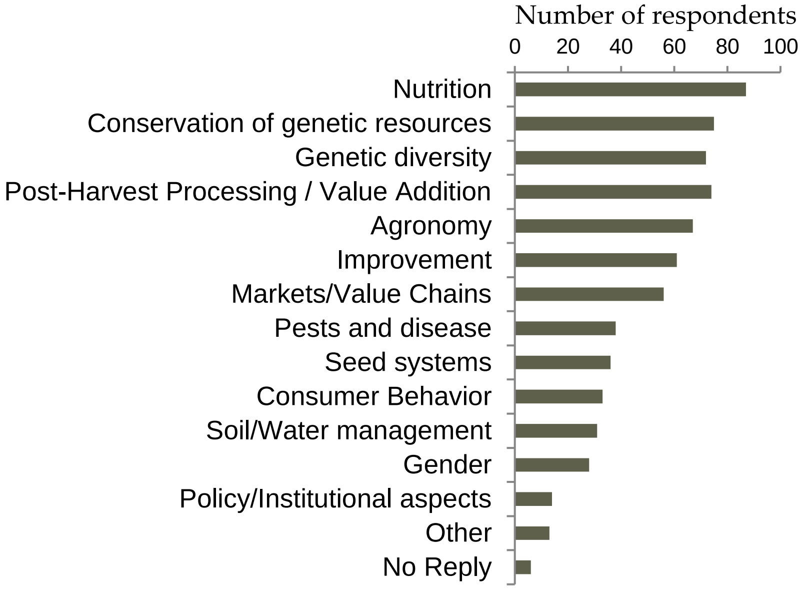

Figure 2. Topics addressed in NUS Research (n=240)

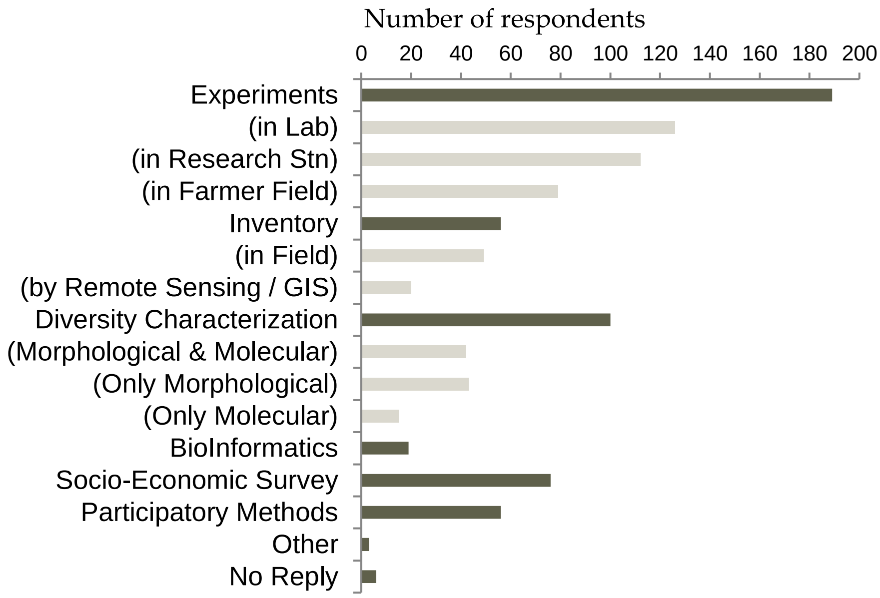

Figure 3. Methods used in NUS Research (n=240)

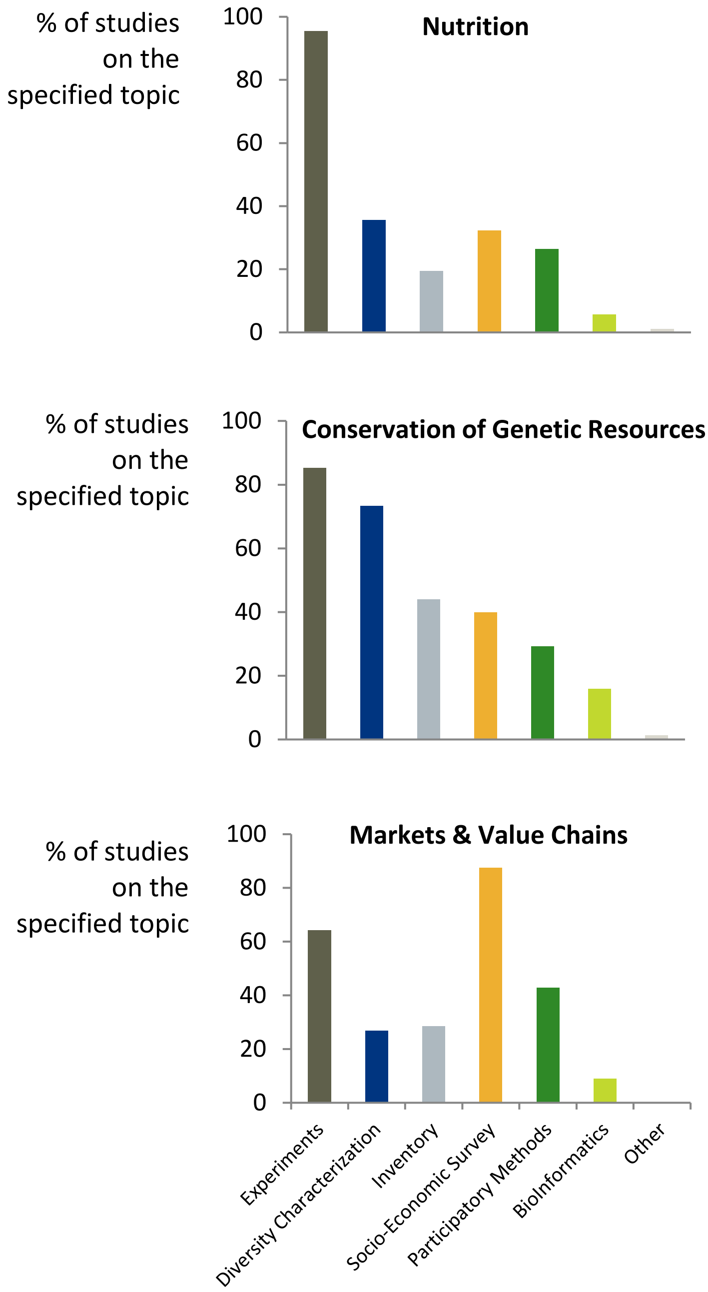

Figure 4. Percent of studies on nutrition (n=87), conservation of genetic resources (n=75) and markets and value chains (n=56) that used different methods of investigation.
Note: studies on a particular topic often addressed topics in addition to those shown.

NUS research capacity

This section and the following are based on responses from both current and potential NUS researchers (n=340).

How well did universities prepare respondents for NUS research? About half of respondents (51%) felt their education had prepared them well or very well to conduct research on crops including NUS. Another 30% reported that their education had given them adequate preparation. A much smaller number (18.8%) felt that their education did not prepare them very well or at all for NUS research. One third (35%) did not encounter NUS in the curricula of their most recent academic degree (Figure 5).

Thesis research and short training courses were important in developing NUS research capacity. Most respondents (81%) said their theses were related to NUS (Figure 5). Many (39%) had also attended short training courses on NUS in the past five years. Typically (22%) these were international training courses but relatively few took these courses nationally (10%) or in their own institution (9%).

In many cases, researchers were investigating topics they were not trained for in their previous degree. For instance, nutrition was the most popular topic of NUS research but, at most, 24% of those studying nutrition had been trained in this field (Figure 6). Be that as it may, it was common for NUS research to be carried out in interdisciplinary teams (65%) and the teams often made up the discrepancy in training to some extent. For instance, 61% of researchers investigating nutrition included someone on their team who was trained in the field. Researchers investigating value chains were, likewise, rarely trained in economics (14%), but 59% of these researchers had an economist on their team.

Only 7% of researchers looking at gender issues were trained in this topic in their previous degree but 71% had a gender-specialist on their research team. Many respondents (28%) had also taken a training course on gender in the past five years. Overall, 89% of researchers investigating gender had either been trained in gender in their previous degree, had a gender specialist on their team or had taken a supplementary training course on gender.

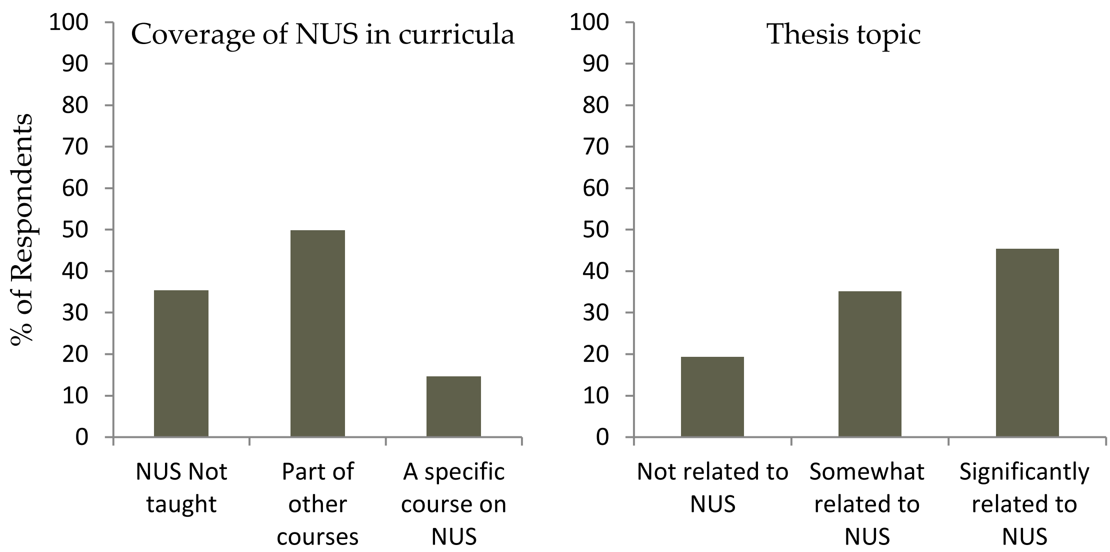

Figure 5. Coverage of NUS conservation or use in the curriculum of respondents' most recent academic degree and the extent to which their thesis related to NUS crops/trees.

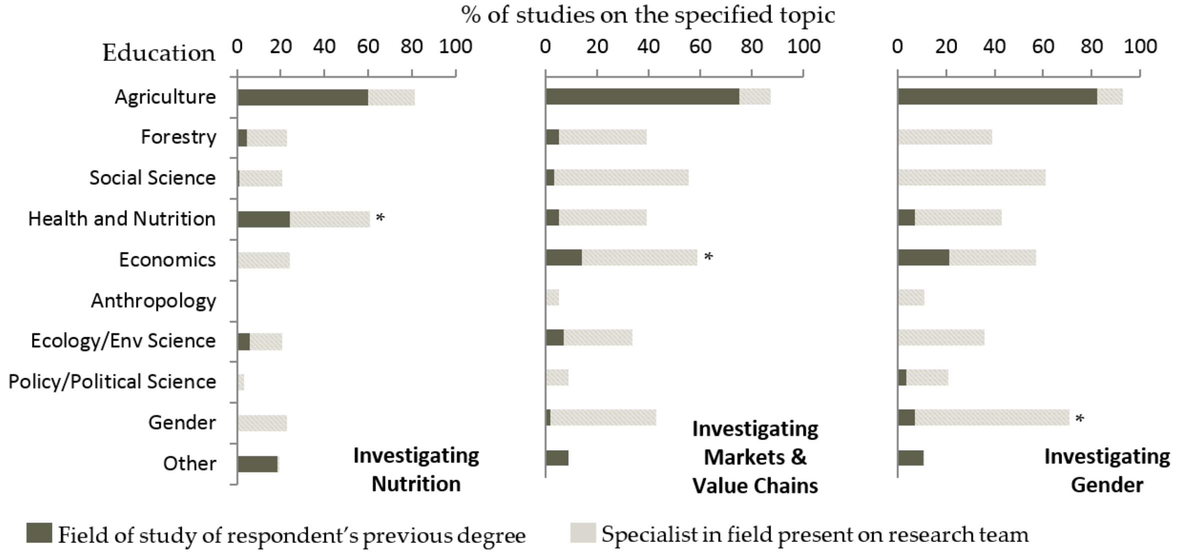

Figure 6. The percentage of researchers investigating nutrition (n=87), markets and value chains (n=56) and gender (n=28) who were educated in different fields of study (dark grey bars), and those who had other people of a specific specialization on their teams (light grey bars).

Organizational capacity and external support

NUS researchers were supported by their own organization more strongly in some aspects than in others (Figure 7). Collaboration within institutions was fairly common (57%). Access to mentorship on NUS was, however, rated as poor to non-existent by almost half of respondents. Less than a quarter of respondents indicated that their access to mentorship on their NUS research was very good or excellent. Access to mentorship on statistics and gender-responsive research were also seen as less than adequate by more than half of respondents.

In terms of facilities, laboratory quality was seen as poor to very poor by the majority of respondents. This was indeed recognized by 29% of respondents as a major constraint in pursuing NUS research. Lack of information on NUS constrained many respondents (19%), including information to help in identifying species and methodologies for cultivating them, which could relate to issues with internet and library access as well as the understudied nature of these species (Figure 7).

Regarding financial resources, 35% had received funding from their organization. About half of respondents currently engaged in NUS research (46%) were at least partially self-funded. We also asked respondents to describe the two or three most critical constraints they face as NUS researchers. By far, financial constraints were the most common, reported by as many as 70% of respondents. Inadequate facilities, equipment and materials were mentioned by 29%, and human resource constraints by 25% of respondents, respectively (Table 3).

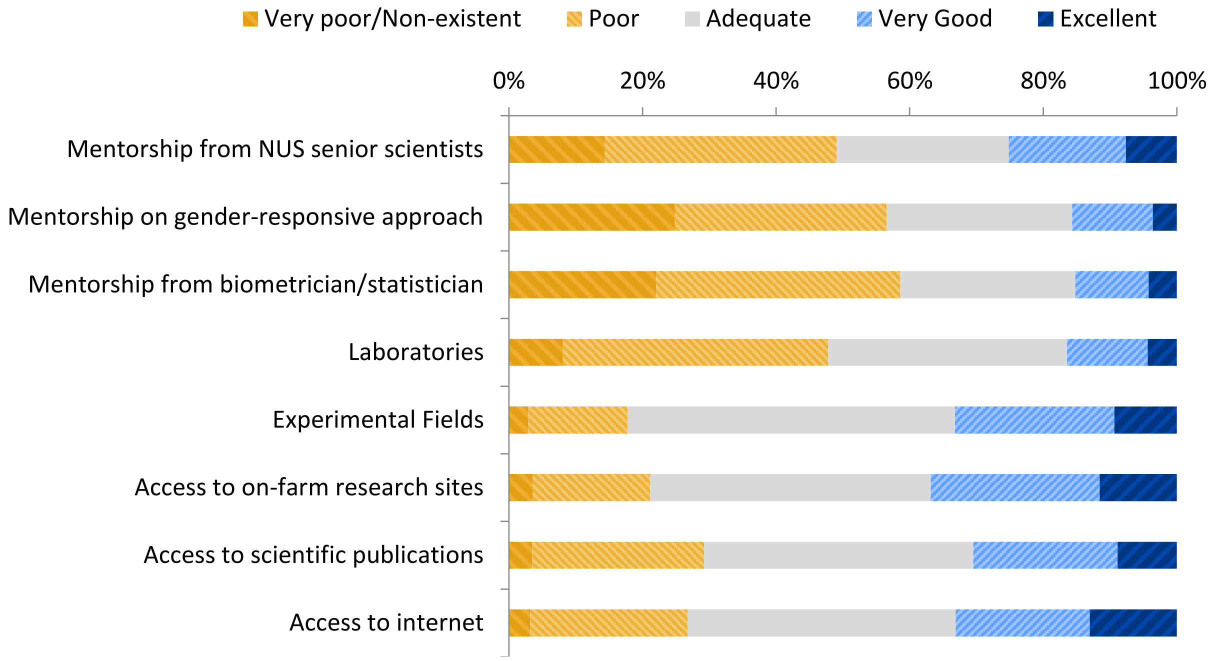

Figure 7. Perceived support within home organization for NUS research in terms of access to mentorship and quality of facilities. Missing values omitted from percentage calculations.

Table 3. Constraints to NUS research.

Constraints to NUS research	**% of respondents**
Financial	**70%**
Facilities/materials	**29%**
Inadequate equipment and lab materials	18%
Inadequate facilities	15%
Human resources	**26%**
Lack of necessary skills in research team	9%
Missing supportive networks and collaboration	6%
Non-availability of training	6%
Few experts and mentors	4%
Insufficient time	3%
Information, methods and data on NUS	**17%**
External support	**16%**
Lack of interest/priority by donors, government stakeholders	14%
Lack of supportive policy	4%
Lack of germplasm or access to germplasm	**8%**
Lack of seed/germplasm	6%
Access to seed/germplasm	2%

External support for NUS research

The extent to which the external environment was supportive of NUS research varied greatly (Figure 8). Sixty-one per cent of respondents found it poor or non-existent, while 13% reported that it was very good or excellent. Half of respondents rated the level of national policy support as poor or non-existent, while one tenth experienced very good or excellent policy support.

A majority reported that their participation in networks or professional associations related to NUS was poor or non-existent both at national and international levels (Figure 8), indicating a certain scientific isolation. Still, many researchers benefitted in various ways from collaboration and partnerships outside of their own organization. For instance, 37% received funding from national or international sources and 60% reported collaboration outside of their own organization in their NUS research, either with national (37%) or international (33%) organizations. One third (33%) of respondents had attended a conference related to NUS in the previous five years.

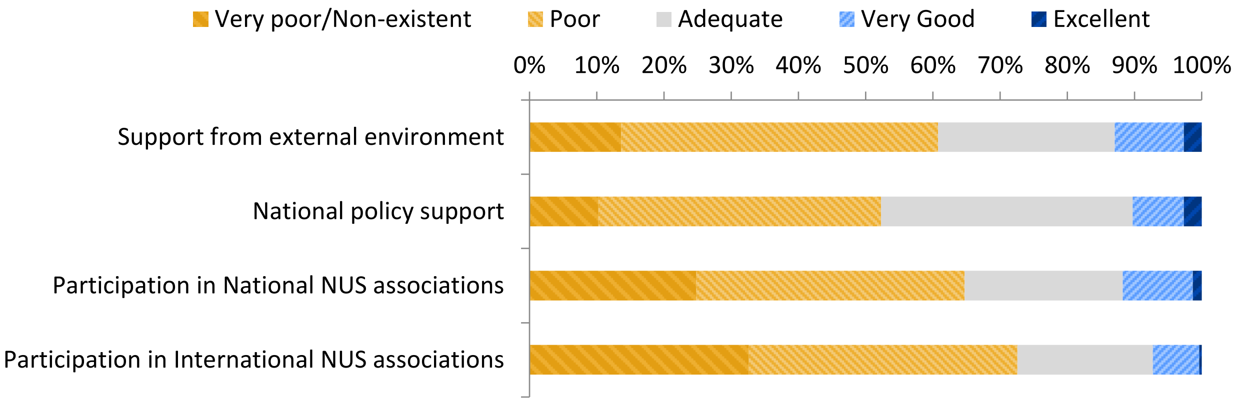

Figure 8. The external environment for NUS research as perceived by African researchers interested in NUS (n=340). Missing values omitted from percentage calculations.

Discussion

This survey demonstrated that African researchers are studying a wide diversity of NUS from a variety of angles including their genetic diversity, nutritional, agronomic, and market potential. The fact that respondents were studying 184 different species indicates as well that new knowledge is being generated for a wealth of crop and tree diversity in sub-Saharan Africa, which could contribute to enhancing the resilience, nutritional value, and sustainability of food systems on this continent. The high number of species under study also hints, however, that research efforts are likely to be fragmented and lacking a critical mass in terms of addressing the numerous constraints to value chains of each specific crop.

Researchers used a range of methods to investigate NUS, but these mostly did not involve participatory methods. Most of the studies involved laboratory analyses of nutritional value, or characterization of diversity. These methods alone are insufficient to address the many value-chain bottlenecks that these crops face for their use enhancement. More studies orientated towards value chains and involving participatory methods that engage communities in the research are necessary to ensure that actions are targeted and relevant, and would increase the likelihood of research results being put into use.

There was significant awareness of gender issues among the African scientists surveyed, but gender was not strongly considered in most research projects. NUS research should ideally be gender-responsive as women are often the main producers of traditional crops. Many respondents said that they considered gender at some point in their research but very few did so throughout the research process. Gender aspects were rarely addressed at the design stages of the study. For best results, gender should not be tagged on at the analysis phase; rather it should be embedded in the methodologies from conceptualization of the research to the dissemination of results. Increased emphasis on participatory, gender-responsive research approaches and training in this field, could address this issue.

The survey was not conclusive regarding the coverage of NUS issues in formal education. Half of the respondents felt that their formal education had prepared them well or very well for NUS research. Still, more than one third had not encountered university courses that covered NUS. Integrating NUS issues in higher education could also help build capacity to research native crop species or upgrade their value chains. Teachers' training, provision of good training materials, and curriculum review could support this process.

Conducting thesis research on NUS played an important role in developing capacity for work in this field. Further strengthening of NUS thesis research opportunities would be an effective way of quickly increasing overall research capacity on NUS. Offering short training courses on NUS for working professionals would also be important in developing capacity. These training opportunities should be supported and increased.

Access to mentorship on NUS, gender and biometrics/statistics was an issue. These findings highlight a capacity limitation for NUS research, which could be addressed via on-the-job training, institutional strengthening, improved networks and formation of communities of practice. Networks and collective action also help to build a critical mass for more rapid research progress on selected priority crops. Social networks can play a role in connecting scientists working in isolation, and sharing knowledge and ideas within and between countries.

Funding limitations and inadequate access to, or quality of, facilities often constrain NUS researchers. However, this study did not make a comparison with the situation facing scientists working on major staple crops and commodities. This finding could then reflect a general problem in academic institutions in sub-Saharan African, rather than one specific to NUS. It is however likely that funding opportunities for NUS research might be poorer than for the dominant crops, given that investment in agriculture research already is low in many African countries.

There is much room for improvement of external support, including policy support for NUS research, according to a clear majority of respondents. In particular, there is a deficiency of national and international associations and networks dedicated to NUS.

The fact that hundreds of scientists in this subset of countries in Eastern, Southern and West Africa conduct research on NUS is encouraging. Their combined effort will generate new knowledge on a large number of NUS of importance to food and nutrition security and livelihoods on the continent. Such knowledge on crops that are often locally adapted can contribute to climate change adaptation. The capacity to harness such knowledge and bring it into use for development through proper sharing among researchers and effective two-way communication with non-scientists are also other important aspects that would in fact need to be strengthened.

Acknowledgements

This study was linked to the project 'Building human and institutional capacity for enhancing the conservation and use of Neglected and Underutilized Species (NUS) of crops in West Africa, and Eastern/Southern Africa' under the ACP-EU Science & Technology Programme, implemented by the African, Caribbean and Pacific Group of States (ACP) Secretariat and financed by the European Union.

References

Chivenge P., Mabhaudhi T., Modi A.T., Mafongoya P. 2015. The potential role of neglected and underutilized crop species as future crops under water scarce conditions in sub-Saharan Africa. International Journal of Environmental Research and Public Health. 12: 5685-5711.

Elias M. 2013. Practical tips for conducting gender-responsive data collection. Bioversity International, Rome (Italy).

Evenson R.E., Gollin D. 2003 Assessing the Impact of the Green Revolution, 1960 to 2000. Science, May 2003: 300(5620): 758-762.

FAO. 2011. Second global plan of action for plant genetic resources for food and agriculture. Commission on genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations.

Frison E., Smith I.F., Cherfas J., Eyzaguirre P., Johns T. 2005. Using biodiversity for food, dietary diversity, better nutrition and health. South African Journal of Clinical Nutrition 18: 112-114.

Garcia-Yi J. 2014. Market-based instruments for the conservation of underutilized crops: in-store experimental auction of native chili products in Bolivia. Sustainability 2014, 6(11), 7768-7786; doi:10.3390/su6117768

Khoury C.K., Bjorkman A.D., Dempewolf H., Ramirez-Villegas J., Guarino L., Jarvis A., Rieseberg L.H., Struik P.C. 2014. Increasing homogeneity in global food supplies and the implications for food security. Proceedings of the National Academy of Science USA 111(11): 4001-4006.

Otsuka K., Larson D.F. eds. 2013. An African Green Revolution: Finding ways to boost productivity on small farms. The International Bank for Reconstruction and Development/The World Bank. New York/London.

Padulosi S., Bergamini N., Lawrence T. (eds). 2012. On farm conservation of neglected and underutilized species: trends and novel approaches to cope with climate change. Proceedings of an international conference, Frankfurt, 14-16 June 2011. Bioversity International, Rome.

Padulosi S., Thompson J., Rudebjer. P. 2013. Fighting poverty, hunger and malnutrition with neglected and underutilized species (NUS): needs, challenges and the way forward. Bioversity International, Rome, Italy.

Rudebjer P., Chakeredza S., Dansi A., Ekaya W., Ghezae N., Aboagye L.M., Kwapata M., Njoroge K., Padulosi S. 2013. Beyond commodity crops: strengthening young scientists' capacity for research on underutilized species in sub-Saharan Africa. In: Massawe, F., Mayes, S., Alderson, P. (eds) 2013. Proceedings of the second international symposium on underutilized plant species, 'çrops for the future – beyond food security'. Volume 2. Acta Horticulturae 979. International Society for Horticultural Science. Leuven.

Smale M. (ed) 2006. Valuing crop biodiversity: on-farm genetic resources and economic change. CABI. 336 pp. ISBN-10: 0-85199-057-6

Tadele Z., Assefa K. 2012. Increasing food production in Africa by boosting the productivity of understudied crops. Agronomy 2: 240-283.

Information for small holders in Africa

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Abstract

The Food Plants International (FPI) database of edible plants of the world has increasingly focused on Africa as a region of food insecurity and undernutrition, but it is a continent that is rich in underutilized species and traditional knowledge that is often not widely accessible. The database currently contains information on 26 350 edible species from the whole world. Of these, 7300 species are currently known in Africa. The information has been written in easy-to-understand English and can, for example be searched for food plants suitable for arid zones in Africa (1574 species). Increasingly, information is being added to highlight food plants that can be processed and stored for use during the dry season. Where available, nutritional information is included.

In the presentation delivered at the '3rd International Conference on Neglected and Underutilized Species (NUS) for a Food-Secure Africa', in Ghana, the focus was on how to use the database to deliver appropriate information for any country or region in Africa. Selecting plants on an agro-ecological basis with close attention to nutrition has the potential to ameliorate food shortage, malnutrition and develop capacity to cope with climate change. The database layout allows illustrated booklets on selected plants in specific locations to be produced quickly and easily. (Some models of this are available on Food Plant Solutions, a Rotarian Action Group website, www.foodplantsolutions.org). The global scope of the database offers unique features where information available for another region or a related species can be adapted and applied to African locations.

Although an older version of the database is available for perusal on our website (www.foodplantsinternational.com), using the database from a DVD or on the computer hard-drive is much faster and easier. Copies were made available at the Ghana conference. These are covered with a Creative Commons copyright allowing them to be copied and distributed freely.

Edible species diversity in Africa

The Food Plants International (FPI) web site maintains the world's largest database of edible food plants. The database has increasingly focused on Africa as a region of food insecurity and undernutrition. It is a continent that is rich in underutilized species and traditional knowledge that is often not widely accessible. The database currently contains information on over 26 350 edible species from the whole world. Of these, 7300 species are currently known in Africa (Table 1).

Table 1. Edible species diversity in Africa

Algeria	277	Madagascar	913
Angola	1066	Malawi	1360
Benin	676	Mali	492
Botswana	841	Mauritania	241
Burkina Faso	492	Mauritius	346
Burundi	355	Morocco	295
Cameroon	1202	Mozambique	2066
Cape Verde	120	Namibia	759
Central African Republic	383	Niger	525
Chad	349	Nigeria	1367
Comoros	130	Rwanda	392
Congo	1893	Sao Tome	89
Djibouti	79	Senegal	845
Egypt	644	Seychelles	190
Equatorial Guinea	253	Sierra Leone	1150
Eritrea	189	Somalia	573
Ethiopia	1344	South Africa	2155
Gabon	594	South Sudan	169+
Gambia	384	Sudan	1019
Ghana	1130	Swaziland	1138
Guinea	1001	Tanzania	1781
Guinea-Bissau	775	Togo	515
Ivory Coast	763	Tunisia	212
Kenya	1434	Uganda	1078
Lesotho	263	Zambia	1636
Liberia	464	Zimbabwe	1978
Libya	225		

I am not a historian so am not particularly interested in whether it is indigenous or not (although I have often indicated the plants' origin with an asterisk), but as an agriculturalist I am greatly concerned whether it is easily available locally and well adapted. Local well adapted plants have already adapted to the local conditions so provide a more stable and sustainable production system. Usually, if people can select and save their own seed locally, the plant will develop broadly based and therefore stable genetic resistance to local pests and diseases and become increasingly adapted to existing soil and climatic conditions. In poorer, rural villages, a stable yield is far more important for family survival than a maximum yield. When crises occur with droughts, epidemics and "hungry seasons" it is then that families need to be kept alive and well fed.

Overcoming language barriers

Africa has 2146 languages. To bridge the information gap, scientific names are essential. "The Plant List" (www.theplantlist.org) is becoming a reasonably reliable and comprehensive source for scientific names and synonyms. Increasingly local language names are being included in the FPI database although the specific language has not been documented. Searching on a local language basis sometimes works! Given the 26 000 edible plant species, there are probably another 100 000 older names or synonyms and these can be searched for, to give the currently adopted name. (Under the tab, "How else is it known?")

Checking for similar related species in other places

One advantage of a global database is that often a plant is better known or more researched in one country or region than in another. Also, similar or related food plant species occur in diverse locations and indications for improvement or development can be gained by looking at related species. The popular West African *Gnetum africanum* species has related attractive species in Asia, the Pacific and South America. Of the 41 accepted *Gnetum* species names, 23 are in the FPI database as being used for food. Similarly African edible plants such as *Terminalia (*13 in Africa out of 51 edible globally), *Canarium* (4 out of 43), *Parinari* (9 out of 22), *Syzygium (22 out of 58), Sterculia* (14 out of 59), *Strychnos* (23 out of 28), *Vitex* (31 out of 55) etc., have related species in other countries. An Australian researcher was explaining to an audience in Papua New Guinea the research done on our traditional Australian nut (*Macadamia*), but then concluded that the global market was already saturated so not to start growing it. My comment was to take all this incredible research and adapt it to a local Papua New Guinean related nut species the *Finschia* group. These nuts are suited to a similar environment and often a 'new' nut can gain a market share.

A range of indigenous African *Acacia* spp. as well a range of species originating in Australia that have edible seeds have been introduced to reduce desertification in arid areas of Africa. In Africa, 102 Acacias are known that have edible plant parts out of 231 edible Acacias worldwide.

Aframomum spp. are a significant group of fruit and spice plants, all 24 edible species occurring in Africa. *Allanblackia* trees are receiving attention for their seeds with edible oil. All 11 of those recorded as edible occur in Africa. *Amaranthus* spp. are popular, productive and nutritious leafy greens. Twenty nine species are recorded as being used as food in Africa out of 53 edible species worldwide. *Amorphophallus* spp. are a taro family root crop that often has the advantage of being storable. Nine poorly understood species are recorded and used in Africa, out of 29 species recorded as edible worldwide.

Of the 166 accepted *Annona* spp. names, 56 are recorded as being edible and 13 are recorded as occurring in Africa. Many in this group are acknowledged as having anticancer properties. This could be extended to the 75 species in the Annonaceae family recorded as being used as food in Africa. There is a website devoted to the Annonaceae in Africa (afroannons.myspecies.info).

Children and nutrition

Many minor fruit and other edible species are often mentioned as not being of commercial significance but these are often significant forage for children, knowing they won't be reprimanded if they harvest and eat them. For Africa, at least 317 species are mentioned as being especially eaten by children. Of these 244 are fruit. Zinc, being a component of 72 enzymes in our bodies are crucial for the healthy development of children. Zinc is most often in significant amounts in seeds and nuts. Eleven nuts are mentioned in Africa as specifically eaten by children, as well as possibly 75 seeds. There are 145 recorded nut species used as food within Africa.

Plants for specific locations

For Africa, 106 species are mentioned which grow on termite mounds, 247 species growing in coastal regions, 411 species growing in savannah and 66 edible species recorded growing in mangrove swamps. The 1578 species recorded as growing in arid regions should become a major focus for the future.

Ficus is a very large genus with 830 species. Of the 257 species recorded globally as having edible parts, 80 species are mentioned as being used for food in Africa. About 120 edible plant species are specifically recorded growing in the Sahel.

A similar strategy applies to species such as the African *Abelmoschus callei* (West African Okra), and *Abelmoschus esculentus* (Okra) and the Asian *Abelmoschus manihot* (Aibika). In fact the best source of information on pests and diseases of these plants is probably from books on pests and diseases of cotton (*Gossypium spp.)* as they are related plants with a similar range of insect pests. Similarly, of the 103 accepted *Amaranthus* species, 53 have so far been recorded as edible, 29 of these are being used as food in Africa. They are an attractive, nutritious, and productive group of food plants. I am unfamiliar with the African winged beans (*Psophocarpus spp.)* and the African *Rungia* spp. but enjoy immensely the related species in Papua New Guinea - *Psophocarpus tetragonolobus* and *Rungia klossii*. I imagine there is a synergy from looking at related species in other tropical countries. The FPI database could at least create an awareness of this potential.

Vernonia species are important in Africa. Of the 650 accepted *Vernonia* species names globally, 22 species are recorded as being used for food in Africa, and 28 worldwide. At least two in Africa are commercially cultivated vegetables, (*Vernonia amygdalina* and *Vernonia hymenolepis*) the first being sold in significant amounts and exported.

Good nutrition is most easily established by eating a diversity of food plants. This needs to include a starchy staple as an energy supply, some beans and legumes for protein, leafy vegetables for iron and Vitamin A, seeds and nuts for zinc and other nutrients, and fruit for Vitamin C and others. For Africa, the choice across the continent is wide. Naturally all these are not available at any specific location nor throughout the year so selections from this diversity need to be made. Some are famine foods, eaten in times of emergency.

Naturally, the humid zone will have many more edible plant species than indicated above; the database has yet to be fully updated.

The database is a collation of other people's work. Nothing is original. An attempt has been made to document the sources of information. Even though people may not always have access to all the references, it is well worth searching this field of the database as well.

Food types from African species

Edible part	Number of food species in Africa
Starchy staple - root/tuber (underground storage)	1120
Starchy staple - cereal	262
Beans	320
Leafy greens	2711
Seeds	1491
Nuts	145
Fruit	2468

Food plants mentioned as in specific locations

Conditions mentioned	Number of food species in Africa
Arid	1582
Savannah	442
Humid	347
Termite mounds	106
Desert	108
Mangrove	65
Desert	115
Mediterranean	282

Some practical guidelines for using the database

Using the "find" button at the top of the database, all the fields will go blank. Then having browsed the fields to see the type of words used, any one of these can then be typed into a blank field space and the "find" field button will now have changed to a "perform find" button and pressing this will give the results. Most people want to find information for their own country. So, putting the country name in the "Where does it grow?" "Found in" field and pressing the "Perform find" button will give all the edible plants that I have located for this particular country. Usually the number is fairly large. But by going to the "Print-Friendly View" these will be laid out ready for printing. Usually, instead of printing them all out (hundreds of pages) it is more practical when in this layout to save them as a PDF document under the "File" draw down menu.

It makes more sense to make a more detailed selection. For example if you wanted to know the trees that grow in arid areas in Africa that have edible fruit, you would have to insert the words "tree" in the Description field, "arid" in the Distribution field, "Africa" in the "Found in" field and "fruit" in the "Edible Portion" field. The results are that 355 fruit trees grow in arid places in Africa.

The database is covered by a Creative Commons copyright, which means that you can copy the whole folder off a disk and give it away free to your friends or colleagues. In fact it works much more efficiently and quickly if you put the whole folder onto your computer hard drive, rather than simply working off a disk. And it is much more useful working from a bound copy on disk than from the database on the internet server on www.foodplantsinternational.com.

PowerPoint presentations of these examples and instructions are included on the disk provided and on the website.

Conclusion

The Food Plants International database aims to give field workers access to preliminary information on edible plants of the world to increase their awareness of the food plant resources available locally. Also, it seeks to raise the potential of sharing knowledge of similarly related plants in other locations and how these are being utilized.

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Prospects and challenges for preserving and mainstreaming underutilized traditional African vegetables

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Abstract

Traditional African vegetables are sources of food and nutrition diversity and can generate substantial income to alleviate poverty in sub-Saharan Africa and elsewhere. Traditional green leafy and fruit vegetables have been part of local food systems in the region, and the diversity currently available can be attributed largely to farmers' conservation and selection efforts. The diversity of traditional vegetables and other neglected and underutilized plant species is threatened by many factors such as population pressure and expansion of land under staple crops. As younger generations migrate to cities, fewer people remain in rural areas to take up farming and conserve traditional crops. A considerable number of accessions have been collected from various countries in sub-Saharan Africa, regenerated, characterized, and stored for the short and long term; pertinent information on each accession has been made available online. Collection and characterization efforts with a special focus on landraces and crop wild relatives continue to fill gaps in existing collections and to preserve this valuable diversity. Existing accessions have been used for breeding research, resulting in the development of many breeding lines and the release of improved cultivars in Africa. Quality seed of purified accessions, breeding lines and improved cultivars have been distributed across Africa and other parts of the world for use in breeding and research and development programmes. Prospects and challenges facing conservation, technology generation and utilization of available technologies are described in this paper.

Keywords: conservation; germplasm development; improved cultivars; selection; traditional vegetables

Introduction

Various types of traditional African vegetable have been important in helping to meet people's nutritional needs (Chweya and Eyzaguirre, 1999; Weinberger and Msuya, 2004; Abukutsa-Onyango, 2010).Traditional vegetables provide sustenance, nutrition and income for rural, urban and peri-urban resource-poor dwellers. Onim and Mwaniki (2008) reported that, with the increase of urbanization in East and Central Africa, there is a demand for all sorts of food including traditional vegetables. Their report indicated that traditional African vegetables have helped alleviate urban food crises, and that the demand for these crops has led urban people to grow vegetables even using poor quality irrigation water, in the absence of an appropriate one, in cities like Nakuru and Nairobi in Kenya, and Dar-es-Salaam in Tanzania. Traditional vegetables have high nutritive value (Kamga et al., 2013; Yang et al., 2013), and help bridge seasons of food shortage (Onim and Mwaniki, 2008). This paper highlights research and development in traditional vegetables in sub-Saharan Africa, mainly related to the activities of AVRDC – The World Vegetable Centre.

Cultivation and constraints

Cultivation

The type and number of traditional vegetables grown varies among and within countries in sub-Saharan Africa. Amaranth (*Amaranthus* spp.), African eggplant (*Solanum* spp.), nightshade (*Solanum* spp.), okra (*Abelmoschus esculentus*), Ethiopian mustard (*Brassica carinata*), jute mallow (*Corchorus olitorius*), vegetable cowpea (*Vigna unguiculata*) and spider plant (*Cleome gynandra*) are important in many countries in the region (AVRDC, 2008). Data on area, production and yield are not available except in a few cases in some countries. In Tanzania, only amaranth, African eggplant and okra were listed in the National Agricultural Sample Census (NASC) of 2007/08 (NASC, 2008). Amaranth covers about 4377 ha at the national level (NASC, 2003; AVRDC, 2008). African eggplant covers from 19 ha in Mwanza to 683 ha in Zanzibar, and okra from 150 ha in Arusha to 906 ha in Morogoro (NASC, 2008).

African eggplant, okra and roselle (*Hibiscus sabdariffa*) are the most important traditional vegetables in Mali, while pumpkin (*Cucurbita moschata*), amaranth, African nightshade and African eggplant are consumed in Madagascar. In Cameroon, traditional vegetables rank second in area after tomatoes (AVRDC, 2008). The gap between farmers' yield and results obtained from research sites is very wide. Amaranth gives 9-19 t/ha leaf yield from five times leaf peaking at two-week intervals during a growing period allowing the plant to grow to produce grain under research conditions in Arusha (AVRDC, unpublished data). However, 2-13 t/ha leaf yields were reported under farmers' conditions usually harvested without leaving the plant to produce grain (Keller, 2004; Weinberger and Msuya, 2004; AVRDC, 2008).

Production constraints

Keller (2004) reported that 36-42% of farmers surveyed in Tanzania consider diseases and insect pests as important production constraints for all vegetables including traditional vegetables. Pest infestations increase when a crop is kept in the field for repeat harvest over a long period of time during a cropping season or when the crop is grown for seed production. Lack of improved cultivars and cultural practices are other major constraints. Cultivars released in some countries did not reach farmers due to less focus on traditional crops from National Agricultural Research and Extension Systems (NARES) and the seed sector. In the absence of improved cultivars, most farmers grow local cultivars with minimum inputs; they are usually less productive, but better adapted to local growing conditions.

Conservation

In sub-Saharan Africa, there are many plant species whose green leaves or fruit can be consumed as vegetables. Only a few national and international genebanks have included traditional vegetables in their collection and conservation programmes, mostly with only a few crops and a limited number of accessions. The National Plant Genetic Resources Centre hosted by the Tanzanian Tropical Pest Research Institute conserves some accessions of selected traditional vegetables (Keller, 2004). Farmers, as sole curators of the crops since the dawn of agriculture, have rich knowledge of the cultivation and uses of these plants. However, with growing urbanization, the number of people in agriculture in general and in traditional crops in particular is declining. As a consequence, indigenous knowledge in the collection, conservation, cultivation and processing of traditional vegetables is being lost (Keding et al., 2007; Keller, 2004; Weinberger and Msuya, 2004).

The introduction and expansion of global vegetables halted the domestication, cultivation and consumption of traditional vegetables (Abukutsa-Onyango, 2010). With large areas of agricultural land planted with just staple crops, the area left to neglected and underutilized traditional vegetables is shrinking, resulting in the reduction of agricultural biodiversity at the level of crop types as well as within each crop. Traditional vegetables must compete with staple crops as well as with global vegetables, and are thus threatened with extinction (Chweya and Eyzaguirre, 1999).

Since its establishment in 1971, AVRDC – The World Vegetable Center has focused on the collection and conservation of traditional vegetables as well as the development of improved germplasm for use in breeding programmes and release by NARES and private seed companies. A total of 1758 accessions of the crops have been collected and are conserved at AVRDC's Regional Center for Africa under short-term storage conditions (Table 1). A total of 360 accessions from 11 crops were characterized from 2010-2012 (personal communication, T. Stoilova, Genbank Manager, AVRDC Regional Center for Africa).

Table 1. Summary of AVRDC Regional Center for Africa germplasm collections up to 2012

Crops	Family	Scientific name	No. of Species	No. of accessions/ Lines	No. of countries of origin*
African eggplant	Solanaceae	*Solanum* spp.	5	353	22
African nightshade	Solanaceae	*Solanum* spp.	8	128	3
Amaranth	Amaranthacea	*Amaranthus* spp.	8	141	13
Jute mallow	Tiliaceae	*Corchorus olitorius*	1	38	8
Ethiopian mustard	Cruciferae	*Brassica carinata*	1	60	3
Spiderplant	Capparidaceae	*Cleome gynandra*	1	93	7
Sunn hemp	Fabaceae	*Crotolaria* spp.	8	22	1
Cowpea	Leguminosae	*Vigna unguiculata*	1	126	4
Mungbean	Leguminosae	*Vigna radiata*	1	73	4
Pumpkin/squash	Cucurbitaceae	*Cucurbita* spp.	3	56	6
Moringa	Moringaceae	*Moringa oleifera*	1	10	1
Okra	Malvaceae	*Abelmoschus* spp.	3	315	11
Roselle	Malvaceae	*Hibiscus sabdariffa*	1	298	8
Vegetable soybean	Leguminosae	*Glycine max*	1	4	-
Hyacinth bean	Hyacinth bean	*Lablab purpureus*	1	41	3
		Total		**1758**	

Source: AVRDC-RCA Genebank database; *Total number of countries from where collections obtained was 35.

Germplasm improvement at AVRDC

Germplasm development, evaluation and release

AVRDC initiated a traditional vegetable improvement programme in sub-Saharan Africa with the establishment of its Regional Centre for Africa in Arusha, Tanzania in 1992. The programme was started with line development from germplasm collections and evaluation of promising lines. The centre collaborates with and supports NARES and the private sector in evaluation and testing of germplasm for cultivar release. Regardless of limited support available for traditional vegetables from national and international research and development programmes, a number of cultivars based on AVRDC lines have been released by NARES and private seed companies in Tanzania, Mali and other countries (Table 2). Simlaw and East Africa Seed companies, for example, have released cultivars of traditional vegetables. Some of the recently released cultivars in different countries in sub-Saharan Africa are presented in Table 2. Participatory cultivar selection involving farmers ensures the cultivars developed are acceptable to farmers, consumers and markets, and has been used as a breeding strategy of traditional vegetables at AVRDC.

More research must be done to fully exploit the potential of traditional vegetable crops. For instance, yield components—traits primarily contributing to yield—are not well identified. Product profiles need to be established or better defined. AVRDC's germplasm and cultivar development strategy focuses on breeding research to fill the gaps.

Germplasm distribution

The types of germplasm dispatched from the Regional Centre for Africa included improved cultivars, advanced lines and accessions. Germplasm has been distributed to various NARES, private seed companies, universities and non-governmental organizations in countries in Africa and beyond. Interested individuals participating in training programmes, field days and seed fairs have received a number of improved cultivars. A total of 1098 germplasm seed samples of 26 vegetable crops (467 kg seed) were distributed to 20 countries (16 Africa countries, 3 in Asia and 1 in Europe) in 2009; 226 seed samples (91 kg) to 13 countries (12 African plus 1 Asian) in 2010; 118 kg seeds of 1543 samples of 21 vegetables crops to 9 countries (8 African countries and 1 Asian) in 2011; 453 kg of 651 samples of 18 crops to 9 countries (6 African counties, and to Republic of China/Taiwan, USA and Australia) in 2012 (personal communication, T. Stoilova, Genbank Manager, AVRDC Regional Centre for Africa).

Seed systems

In a study conducted in 2003 in Tanzania, 69% of seed of traditional vegetables is farmer-saved seed and only 20% is purchased either from local seed dealers or seed companies (Keller, 2004; Weinberger and Msuya, 2004). However, the situation has changed somewhat in the last few years as the number of private seed companies involved in vegetable seed production increases. Today, Alpha Seed, East Africa, Kibo, Simlaw, Lagrotech, Victoria and FICA Seed companies in East Africa are marketing some traditional vegetables along with global vegetables. However, not all crops are offered, and not all areas, especially many remote areas, are reached. For example, in the Lake Victoria Islands in Tanzania, farmers neither have seed production experience nor are companies available to supply seed (Dinssa, 2013). Developing capacity in individuals and/or groups of farmers in seed production or linking with a private seed company has been suggested for sustainable vegetable production in such areas.

The public seed sector is usually not active in producing seed of traditional vegetables, except in some cases for the supply of breeder and pre-basic seeds. Local seed companies and farmers themselves seem to be the sole potential sources of commercial seeds – in the case of farmers, quality declared seed (QDS). Strengthening interested private sectors in traditional vegetables is as important as establishing and strengthening seed grower farmers. Emerging multinational vegetable breeding companies like RijkZwaan-Afrisem in Tanzania may add value, but they mainly focus on global crops and hybrid cultivars. NARES and public seed enterprises are the only potential partners, at least at this point, to provide initial seeds.

Table 2. List of traditional vegetables registered in some countries in Africa since 1983

Crop	Name	Release year	Country released
African eggplant	Tengeru White	1999	Tanzania
African eggplant	Mshumaa	2011	Tanzania
African eggplant	Tessa Oval egg	-	Tanzania/Uganda
African eggplant	Cleo	-	Tanzania/Uganda
African eggplant	Soxna	2011	Mali
African eggplant	L10	2011	Mali
Amaranth	Madiira 1	2011	Tanzania
Amaranth	Madiira 2	2011	Tanzania
Amaranth	A2002	2011	Mali
Amaranth	A2004	2011	Mali
Amaranth	White Elma	-	Uganda
Amaranth	Green Gina	-	Uganda
African nightshade	Nduruma	2011	Tanzania
African nightshade	Olevolosi	2011	Tanzania
Africannightshade	Managu	2012	Kenya
African nightshade	Giant Nightshade	2006	Kenya
African nightshade	Medium leaf (Long Lasting)	-	Kenya
Ethiopian mustard	Rungwe	2011	Tanzania
Ethiopian mustard	Arumeru	2011	Tanzania
Okra	Spear/Victoria Spineless	2007	Uganda
Okra	- ARP1	2007	Tanzania
Okra	Sasilon	2011	Mali
Okra	Batoumambe	2011	Mali
Okra	Safi	2011	Mali
Roselle	TSH22	2011	Mali
Roselle	L28	2011	Mali
Roselle	Samandah	2011	Mali
Grain soybean	GC 30229-8 (AGS 19)	1983	Zimbabwe
Grain soybean	Nyalab	1992	Zimbabwe
Vegetable soybean	VSS 1	1999	Mauritius
Vegetable soybean	VSS 2	1999	Mauritius
Vegetable soybean	AGS 292	2002	Sudan
Vegetable soybean	Edamame1	2006	Zimbabwe
Mungbean	Filsan	1987	Somalia
Mungbean	Imara	1983	Tanzania
Cowpea	Tumaini	-	Tanzania
Jute mallow	Edeliana	-	Uganda

Source: AVRDC, cultivar registration database

Technology dissemination

Uptake of improved technology is very important to justify investment in technology generation. AVRDC gives due attention to technology dissemination through mobilization of NARES, private seed companies, NGOs and relief organizations in collaborative projects. Up to 2008, more than 10 000 Healthy Diet Gardening Kits containing 14 vegetables rich in vitamins and minerals were distributed in southern Sudan, Zambia, Tanzania, Uganda, Malawi and Rwanda with financial support from UNICEF, the Federal Ministry for Economic Cooperation and Development (BMZ) through Gesellschaft für Internationale Zusammenarbeit (GIZ, previously called GTZ), the United States Agency for International Development (USAID) and in collaboration with NARES (AVRDC, 2008). The Lake Victoria Islands have been supplied with improved traditional vegetables in collaboration with Helen Keller International-Tanzania (NGO). About 9000 seed kits were distributed to farm households in flood-affected areas in Tanzania in 2011/2012 in collaboration with FAO.

Consumption and marketing

Traditional vegetables serve resource-poor farmers not only as subsistence crops providing both food and nutrition diversity, but also by generating income as high value crops − which substantiates their potential contribution to poverty reduction in sub-Saharan Africa. They have been part of the food systems of the people for generations (Abukutsa-Onyango, 2010). The strategic importance of the crops in providing sustainable balanced diets for a healthy life has been described by Afari-Sefa et al. (2013). In Tanzania, the value of traditional vegetables consumed by resource-poor and resource-rich households reaches 11% and 2% of the value of all food consumed, respectively. Resource-poor families obtain approximately half of their vitamin A and one-third of their iron requirements from traditional vegetables (Weinberger and Msuya, 2004).

The consumption of vegetables differs significantly between seasons, countries, income levels and accessibility to markets (AVRDC, 2008). Ruel et al. (2005) reported that Kenya stands out in vegetable consumption, at 147 kg and 73 kg/person per year in urban and rural areas, respectively, while the amount is only about 25, 50 and 40 kg/person in Ethiopia, Ghana, and in each of Malawi, Tanzania and urban Guinea respectively. Consumption is higher in urban than in rural areas due to fluctuations in availability across the year and low purchasing power of rural populations (Ruel et al., 2005). Urban dwellers obtain vegetables from different sources and are more aware of the nutritional values. The consumption of traditional vegetables increased in Nairobi, Kenya from 31 t in 2003 to 6000 t in 2006 (Mwangi and Kimathi, 2006); similarly, consumers' willingness to pay a premium price for these foods reached 79% over the average base price of Kshs 15 per bunch in Eldoret, Kenya (Chelang'a et al., 2013). In the Arusha region of Tanzania, farmers' willingness to adopt AVRDC seed kits was the highest for amaranth, nightshade and tomato followed by African eggplant, Ethiopian mustard and cowpea leaves (personal communication, Dusabeyezu M. Rose, AVRDC Regional Center for Africa).

Vegetable producers are more integrated into markets than staple crop producers. Farmers involved in crop production in three regions of Tanzania − Arusha, Dodoma and Tanga − marketed 98% of their total vegetables produced, 88% of traditional vegetables, and 49% of cereal crops (Weinberger and Msuya, 2004; Keller, 2004). Weinberger and Msuya (2004) reported that the share of traditional vegetables marketed and consumed at home in terms of total household income on average from three districts in three regions of Tanzania is 13%, ranging from 10 to 20%. They reported that about 82% of African eggplant produced and 63-67% of nightshade, okra, amaranth and Ethiopian mustard are sold.

Opportunities and challenges for the improvement and development of traditional vegetables

Opportunities

There is a general understanding about the role of traditional vegetables in food and nutritional security, and in income generation. The recent focus of the world not only on food security but also on nutrition is an opportunity for traditional vegetables to gain the attention of policy makers and donors. Traditional vegetables are relatively cheap sources of micronutrients, especially for resource-poor populations for whom animal products are only rarely available. Traditional leafy vegetables reach first harvest within one month after their planting due to their early maturity and hardiness; most sprout rapidly after the first seasonal rainfall. They escape disease and insect pest outbreaks as well as drought or low moisture conditions that occur at late stages of a crop cycle. We believe that traditional vegetables have a comparative advantage under the current unreliable rainfall conditions and the associated pest outbreaks due to climate change.

There is a growing interest on the part of scientists on various topics related to traditional vegetables. Currently, several projects are being conducted on nutritional characterization, genetics, crop morphology/physiology, crop management, seed policy and postharvest supported by USAID, Australia and Germany in collaboration with AVRDC. More companies are starting to market seeds of traditional vegetables and are also developing their own cultivars based on AVRDC lines. There is a growing interest in the area of postharvest handling, an opportunity to make use of vegetables over longer periods of time during surplus production or a period of market glut.

Challenges

Carbohydrate crops still draw the attention of policy-makers and the donor community, who have yet to absorb the value of traditional vegetables. The benefits of traditional vegetables, which can be grown on a small area of land, and the speed at which they can reach needy populations during poor-harvest seasons, needs to be well communicated to consumers, policymakers and donors.

Support from the scientific community, NARES and the seed sector is insufficient. More awareness creation is needed for the seed sector, vegetable producers and consumers on the benefits of traditional vegetables through the dissemination of technology, knowledge and information. Private seed companies only become involved with traditional vegetables after a cultivar becomes popular and peaks at the market; most are not interested in popularization and dissemination activities for open-pollinated cultivars due to the lack of cultivar protection and a royalty system. Cultivars from public institutions are public goods; anybody can market them once a market has been established, and this provides little benefit for those who have invested in the initial dissemination activity. Moreover, the seed sector is interested in popular cultivars that can be sold over wide geographical areas and regions for market control and to simplify seed production and handling. While traditional vegetables are thought to be specific in their adaptation, there is a need for more thorough investigation of genotype x environment interaction for individual genotypes and crops. Greater support from the public sector and donors is needed to train seed growers and farmer groups to produce traditional vegetable seed as their main commodity, with the hope that some may grow into small-scale local seed companies.

References

Abukutsa-Onyango M.O. 2010. African indigenous vegetables in Kenya: Strategic repositioning in the horticultural sector. Second inaugural lecture, 30th April 2010. 63 pp. Nairobi: JKUAT.

Afari-Sefa V., Tenkouano A., Ojiewo C.O., Keatinge J.D.H., Hughes, J. d'A. 2012. Vegetable breeding in Africa: constraints, complexity and contributions toward achieving food and nutritional security. Food Security 4:115–127.

AVRDC. 2008. Vegetable breeding and seed systems for poverty reduction in Africa. Baseline study on vegetable production and marketing. Synthesis report. 60 pp. Arusha: AVRDC.

Chelang'a P.K., Obare G.A., Kimenju S.C. 2013. Analysis of urban consumers' willingness to pay a premium for African Leafy Vegetables (ALVs) in Kenya: a case of Eldoret Town. Food Security 5: 591–595.

Chweya J.A., Eyzaguirre P.B. (eds.).1999. The biodiversity of traditional leafy vegetables. International Plant Genetic Resources Institute (IPGRI), Rome.

Dinssa F.F. 2013. Technical backstopping trip report (29 January - 04 February 2013). Enhanced homestead food production project in Lake Victoria, Hellen Keller International, Tanzania and AVRDC collaborative project.

Kamga R.T., Kouame C., Atangana A. R., Chagomoka T., Ndango R. 2013. Nutritional evaluation of five African indigenous vegetables. Journal of Horticultural Research 21: 99-106.

Keding G., Weinberger K., Swai I., Mndiga H. 2007. Diversity traits and use of traditional vegetables in Tanzania. Technical Bulletin No. 40. Shanhua, Taiwan: AVRDC – The World Vegetable Center. 53 pp.

Keller B.G. 2004. African nightshade, eggplant, spider-flower and other indigenous vegetables production and consumption aspects of traditional vegetables in Tanzania from the farmers' point of view. Gottingen: Georg-August Universitatet (MSc Thesis). 251 pp.

Mwangi S., Kimathi M. 2006. African leafy vegetables evolve from underutilized species to commercial crops. In research workshop on collective action and market access for smallholders, 2–5thOctober 2006, Cali, Colombia. http://www.capri.cgiar.org/pdf/ca-market_mwangi-s.pdf (Accessed 1 August 2013).

NASC. 2008. National agricultural sample census of Tanzania, 2007/2008.

NASC. 2003. National agricultural sample census of Tanzania, 2002/2003.

Onim M., Mwaniki P. 2008. Cataloguing and evaluation of available community/farmers-based seed enterprises on African Indigenous Vegetables (AIVs) in four East and Central Africa countries. (http://www.asareca.org/resources/reports/AIVSTUDYREPT.pdf (Accessed 1 August 2013).

Ruel M.T., Minot N., Smith, L. 2005. Patterns and determinants of fruit and vegetables consumption in Sub-Saharan Africa: a multicountry comparison. Background paper for joint FAO/WHO Workshop on fruits and vegetables for health, 1-3 September 2014, Kobe, Japan. http://www.who.int/dietphysicalactivity/ publications/26v africa economics.pdf (Accessed 29 July 2013).

Weinberger K., Msuya J. 2004. Indigenous vegetables in Tanzania – significance and prospects. Shanhua, Taiwan: AVRDC–The World Vegetable Center, Technical Bulletin No. 31, AVRDC Publication 04-600. 70 pp.

Yang R.Y., Fischer S., Hanson P.M., Keatinge J.D.H. 2013. Increasing micronutrient availability from food in sub-Saharan Africa with indigenous vegetables. In: Juliani H.R., Simon J.E., Ho C.-T. 2013. African Natural Plant Products Volume II: Discoveries and Challenges in Chemistry, Health and Nutrition. ACS Symposium Series 1127: 231-254. American Chemical Society.

Thousands of crops and very little money– how can marker technology be applied to underutilized species?

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Abstract

Many underutilized species have had limited conventional breeding work and many still exist as landraces. Given the substantial progress made in enhancing yields in major crops (e.g. four-fold in 100 years in UK wheat and Malaysian oil palm), it seems likely that current low yields in underutilized crops are not an intrinsic limitation to improvement in the future. Use of molecular markers has the potential to increase the rates of genetic improvement in underutilized species, but their development and application has a cost, both in terms of the consumables used and the physical and human infrastructure required. In addition, are the targets for marker assisted selection (MAS) always the same for major and for underutilized crops? Some markers may be, such as disease resistance/tolerance. However, there is evidence that a landrace structure involving multiple genotypes (which have been adapted to a particularly environment through continuous exposure) could be more resilient to environmental instability. Also, how much capacity and what tools need to be developed within the crop species and how many can be adapted from related species? This raises the related question of how much of that capacity needs to be 'in-house' and how much can be outsourced effectively to a service provider? This paper explores some of these questions with specific examples drawn from on-going work on Bambara groundnut and other legumes

Keywords; molecular markers, marker-assisted selection, genetic improvement, breeding

Introduction

The potential for currently underutilized and neglected crops – NUS (= neglected and underutilized species) to play a greater role in food and nutritional security has been long recognized. Any attempt to develop a new potential crop from a currently underutilized crop requires an integrated and multidisciplinary approach which addresses simultaneously the potential gaps in knowledge relating to the crop across the entire research value chain (RVC). In an analogous way to value chains used to optimize processes in industry, the RVC aims to identify the critical gaps in knowledge as a prelude to focused agricultural research and analysis to help to resolve these. The RVC must, of necessity, stretch from fundamental biotechnology and crop genetics, through breeding and agronomy, ecophysiology, agrometerology, processing and bio-products to social sciences, economics and policy research. Only by addressing all of these stages is it possible to identify underutilized crops with true potential and to begin to address the gaps in the current research records.

The recent explosion in the field of biotechnology, driven primarily by second (and now third) generation sequencing (www.lifetechnologies.com; www.illumina.com;

www.pacificbiosciences.com; www.nanoporetech.com) is making investigation of underutilized crop species more feasible than was the case a decade ago. There are two major ways in which this new technology is contributing to work in NUS – through studies with species to generate new data and through the creation of computer based (*in silico*) or experimental linkages between the underutilized crop species and extensively researched model plants and major crop species.

In addition to the question of the balance between translation of tools and information from major crop/model species and *de novo* generation within species, there is also a question of how much of this information should be generated 'in-house'/close to the research programme and how much can be generated by external service providers/data generating companies without a loss of the associated benefits of in-house development (control of data, biological understanding and expertise, development of research capacity, etc).

The final approach adopted must crucially take into account cost as it is unlikely that the only constraints on enhanced uptake of an underutilized crop will be due to biotechnology and crop genetics, and research in other parts of the research value chain (RVC) needs also to be funded.

Crops for the Future Research Centre (CFFRC) is a joint venture between the Government of Malaysia and the University of Nottingham in Malaysia. CFFRC is the world's first centre dedicated to research on underutilized crops for food and non-food uses. Its research focuses on the uses of agricultural biodiversity to diversify crop and agricultural systems, address changing climates, improve food security and economic well-being, and improve nutrition.

BamYIELD is a multidisciplinary research programme for underutilized legumes using Bambara groundnut (*Vigna subterranea* L Verdc.) as an exemplar species following on from extensive previous work (Linneman and Azam-ali, 1993; Massawe et al., 2005; Basu et al., 2007; Mayes et al., 2009; Mayes et al., 2013);. The programme spans the entire RVC, from production research (genetics, physiology, agronomy, climate) to utilization research (product development, supply chain, and policy). Together with data translation from model and major crops, focussed breeding activities and field trials, BamYIELD will optimize the contribution of this legume to food security and poverty alleviation while developing generic approaches to improve other underutilized legumes based on this detailed example.

Without an active breeding programme, it is difficult to translate research advances into new genotypes available for farmers to use, so at the core of the BamYIELD programme is the development of a trials and breeding programme. The aim is to establish comparable and coordinated field trials in a minimum of seven countries linked by common germplasm. There are currently three partner countries involved in South East Asia (Thailand, Malaysia, Indonesia) and four in Africa (Ghana, Nigeria, Botswana, South Africa) and we expect the first steps of the trials programme to become fully active in 2015, after the award of an International Treaty on Plant Genetic Resources for Agriculture Benefit Sharing Fund award.

BamYIELD will initiate research in areas of the RVC where gaps in current knowledge or bottlenecks to enhanced uptake exist and to provide solutions to alleviate these constraints (Figure 1).
This paper evaluates the main lessons of the work undertaken using Bambara groundnut as an exemplar species with potential to contribute further to food and nutritional security. The

aim is to identify a minimal set of information needed to allow further development of an underutilized crop, here focused on the area of biotechnology and crop genetics. Our current conclusions and approach are presented.

The application of biotechnology to crop species (and particularly the application of molecular genetics and marker assisted breeding) has often been slow to establish applications in practice. This is for a number of reasons, including a certain naivety on the part of molecular researchers as to the ease with which results can be translated to functional selection systems in crop breeding. However, even simple applications of markers can make a difference to the efficiency and accuracy of breeding and research efforts and these simple applications are often dismissed as 'unexciting'. This paper presents a range of both 'unexciting' and more 'exciting' applications of molecular markers in Bambara groundnut.

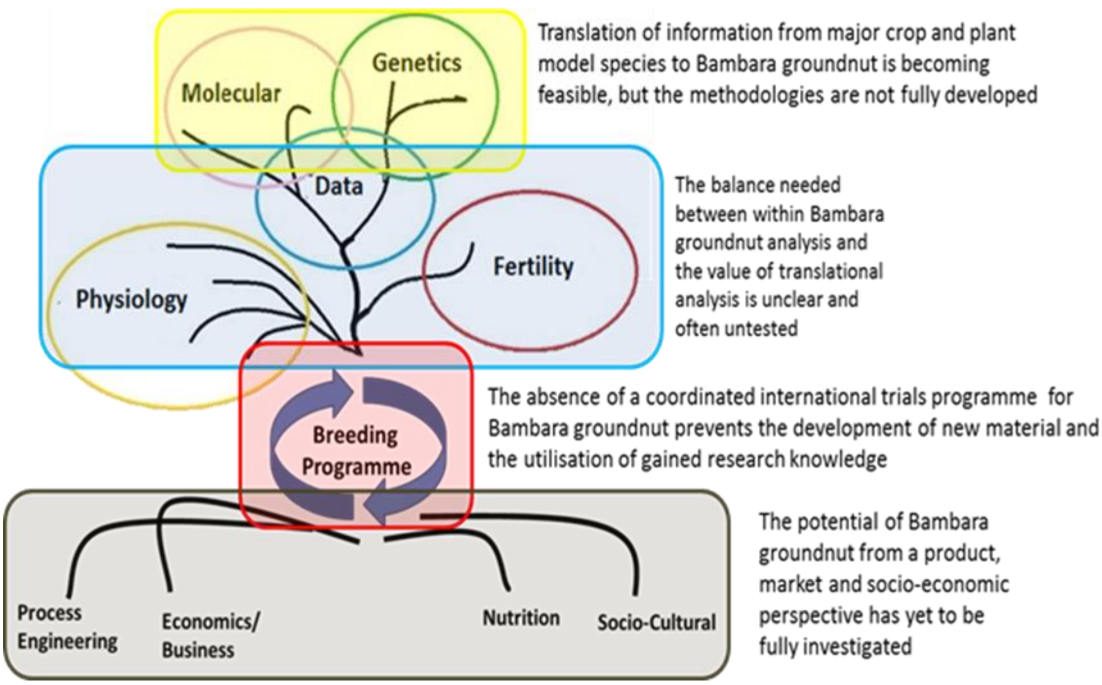


Figure 1: structure of the Bambara groundnut 'BamYIELD' programme and an initial overview 'gap' analysis.

Materials and methods

The development of the results presented are described in the theses by Stadler (2009), Ahmad (2012), Molosiwa (2012) and further background information on materials and analyses can also be found in Mayes et al. (2013), Molosiwa et al. (2013), Chai et al. (2013), Ahmad et al. (2013), Redjeki et al. (2013), Aliyu et al. (2014) and Molosiwa et al. (2015).

Results

Genetic fingerprinting and quality control in research and breeding

The simplest application of molecular markers is to confirm the identity of the material being evaluated. The ability to confirm the genetic identity of material before significant breeding or evaluation work is carried out is one of the most powerful applications of markers, particularly in the context of underutilized crops where large agrochemical corporations are unlikely to commit to running sophisticated breeding programmes.

Figure 2 presents an illustration of the application of SSR markers (pre-screened to identify those which can run on simple agarose gel systems) to identify hybrids in the Bambara groundnut crossing programme. Such approaches can be applied to endosperm samples, allowing hybrids to be confirmed before planting. True hybrids can be given particular attention, allowing large population numbers to be developed from important crosses (recent F_1 hybrids have consistently produced around 300 F_2 seed, due to increased planting space and altering management practices, compared to an average seed yield per plant of <100) in the same controlled-environment glasshouses.

To establish a breeding programme, it is essential to know whether the species is inbreeding or outbreeding and the level of heterozygosity observed in available germplasm. Co-dominant molecular markers, such as SSRs, can be applied to parents and offspring to evaluate the patterns of transmission of parental alleles (potentially identifying any self-incompatibility or other distorters of transmission). Co-dominant markers can also evaluate the residual levels of heterozygosity through an analysis of individual genotypes and the levels of heterogeneity by comparing multiple genotypes drawn from the same landrace accession (Figure 3); for Bambara groundnut, SSRs reveal a residual 2% heterozygosity in single genotype accessions, implying a very strongly inbreeding species, consistent with it being cleistogamous (Molosiwa et al., 2015).

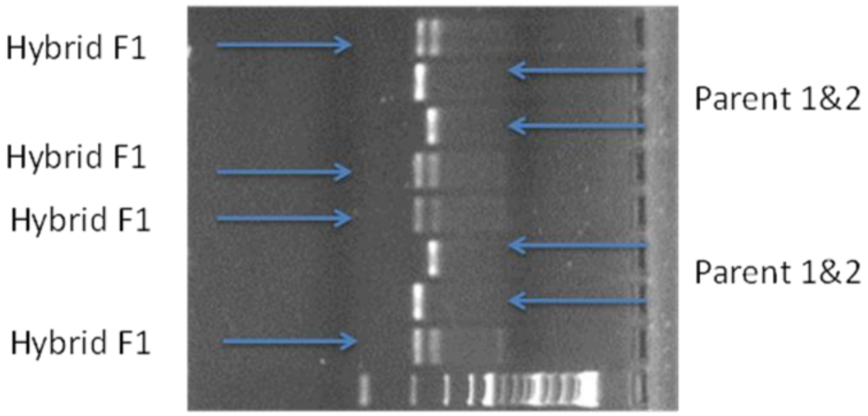


Figure 2: Simple applications of molecular markers in a breeding programme – confirmation of F_1 hybrids.

Understanding the genetic diversity available in the germplasm

Ultimately, breeding progress depends on the availability of trait diversity and the selection of appropriate parents to combine this in the offspring. For many underutilized crops, the available germplasm may exist as landraces (mixtures of genotypes which represent a breeding population).

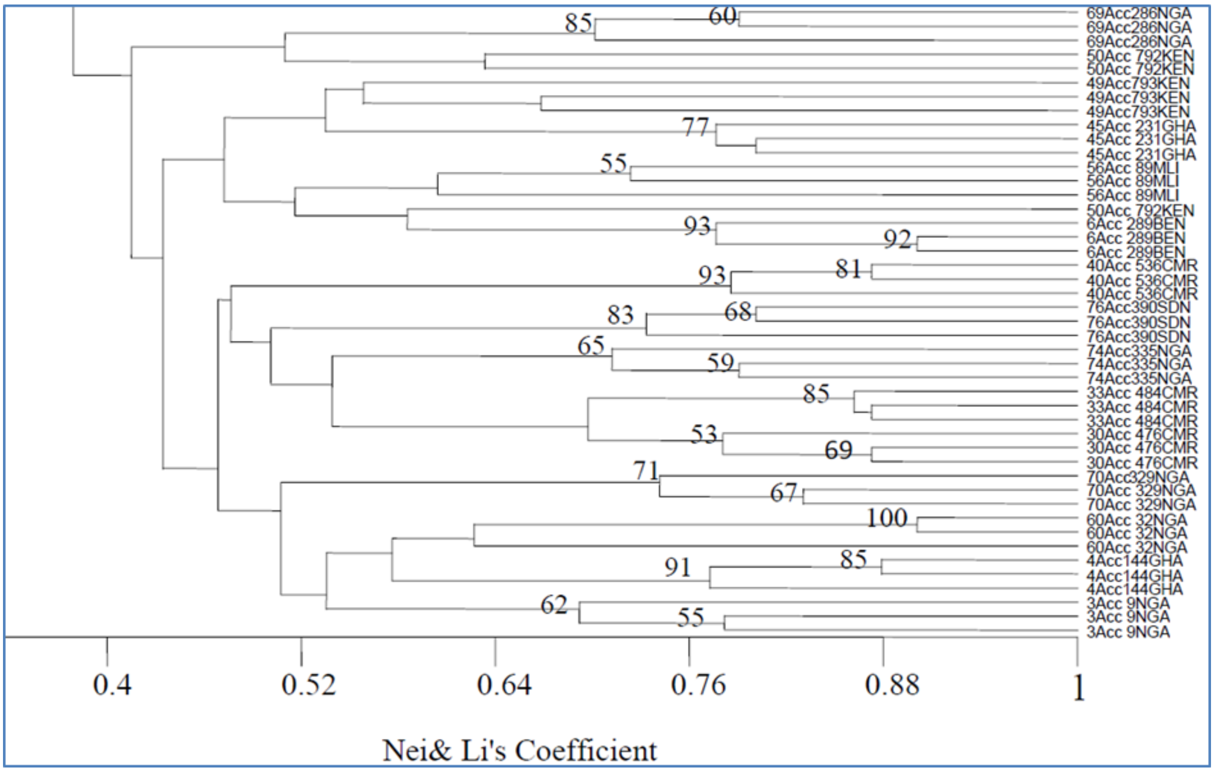
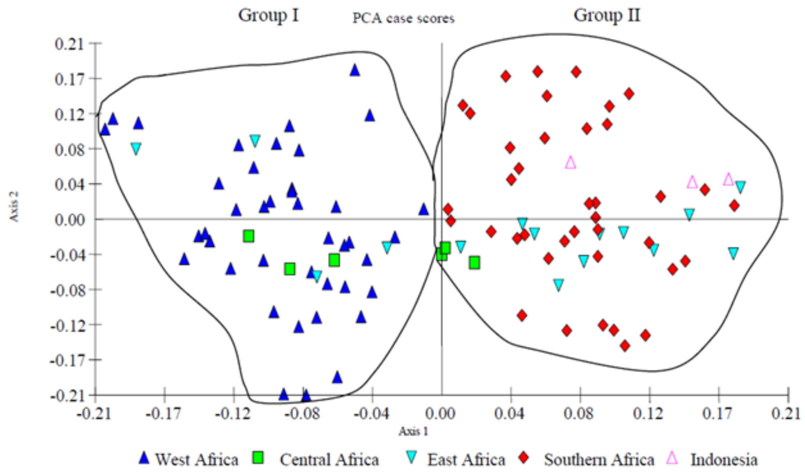


Figure 3: Genetic fingerprinting of three individual seed genotypes derived from 15 landrace accessions for Bambara groundnut (Molosiwa, 2012)

The landrace structure in many underutilized crops makes genetic improvement more complex and evaluation of genetic potential difficult. Figure 3 presents an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis tree based on three genotypes (seed) derived from 15 landraces. Residual heterozygosity as assessed by 20 SSR markers of 2% essentially means that seed derived from a single plant is an unselected variety. On the basis of this we have begun the development of a core genotype-defined germplasm collection using material grown at the University of Nottingham, from the International Institute for Tropical Agriculture (IITA) germplasm bank and material supplied by in-country partners. This will develop approximately 200-300 genotype lines representing specific landraces. Promising traits characterized in the genotype-specific lines can be followed up by evaluating the full landrace accession for further trait variability. The development of a series of lines (or 'pseudo landrace') which may be grown as a population is an option. Such an approach could be evaluated for increased resilience to biotic and abiotic stresses in marginal and low-input agriculture (Zeven, 1998).

Such material will provide the basis for field selection of suitable varieties in-country, for linkage between the different in-country experiments for a combined analysis and for later genome wide association genetics studies.

4A)



4B)

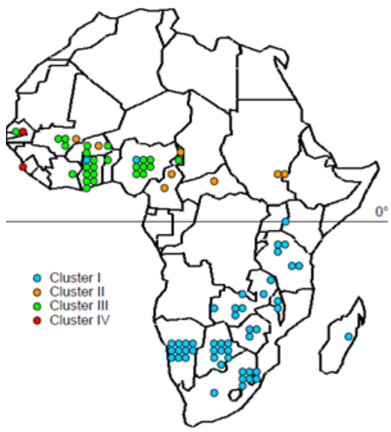


Figure 4A: Principle component analysis of diverse Bambara groundnut germplasm based on 20 microsatellite markers assessed on 123 individual genotypes using a capillary sequencer (Beckmann CEQ 8000; Molosiwa, 2012).

Figure 4B: Overlay of the different UPGMA clusters onto a map of Africa where the accessions were originally reported collected according to IITA data. The UPGMA was based on 201 DArT Array markers (Stadler, 2009)

An understanding of the genetic diversity within a breeding programme is a key requirement for assessing potential sources of genetic variation for breeding (Massawe et al., 2002; Ntundu et al., 2003; Karikari, 2004); Figures 4A and 4B). One concern in many modern breeding programmes is that continual selection within the same material leads to a narrowing of the genetic base, thus limiting future breeding progress. Introduction of new material from unrelated sources while increasing the genetic base of the breeding population may also lead to a loss of breeding progress, as elite complexes of genes are broken up by the introduction of dissimilar genetic material. Crosses between members of different genetic clusters drawn from similar agro-ecological environments could introduce significant genetic variation into programmes without breaking up the adaptive complexes that have been selected by farmers over millenia.

Elucidating the genetic control of traits in underutilized species

An understanding of the genetic basis for trait variation can potentially be used in a number of ways, from modification of conventional selection approaches, to the direct application of marker-assisted selection in the breeding programme (Collard et al., 2005). The latter can often mitigate the need to identify the specific gene responsible. Although developing 'perfect' markers based on the genetic variation giving rise to the trait difference will always be preferable, it is hard to justify the additional expense and time investment in obtaining this in underutilized crops.

Genetic mapping and developing an alignment between the underutilized crop linkage map and the genetic or physical maps of model or major crop species are two linked approaches which could allow translation of positional information (and identification of candidates genes within the model or major crops species, particularly where a full genome is available).

However, the development of extensive genetic resources and microarrays derived from the target species or Single Nucleotide Polymorphism (SNP) chips is harder to justify in underutilized species. DArT Seq is a genotype-by-sequence approach which produces a genomic representation based on the methylation-sensitive restriction endonuclease *Pst*I and is derived from an earlier method using hybridization to detect differences in the genomic representations (e.g. Jaccoud et al., 2001; Wenzl et al., 2004; Alexander et al., 2005; Olukolu et al., 2011) with representations now assessed by direct sequencing rather than hybridization.

In Figure 5, the within species DNA marker map is based upon DArTseq for Bambara groundnut which generates classical DArT (+/-) markers and also SNPs (Ahmad, 2012). In all cases, markers have an associated 64bp within-species sequence tag.

The cross species 'soybean' map is based on hybridization to the soybean Affymetrix GeneChip™ of labelled leaf Bambara groundnut RNA for a population of 60 offspring of the DipC x Tiga Necaru controlled cross (data not shown). Individual oligonucleotides from the soybean chip showing segregation of hybridization signal strength were converted to Mendelian markers and used to construct a genetic map.

For the Gene Expression Marker (GEMs; Hammond et al., 2005; Hammond et al., 2011) map, the Affymetrix probe design sequence could be used to identify a location on the soybean genome (www.affymetrix.com/), allowing a comparison of gene order and position between the Bambara groundnut linkage groups and the soybean physical map.

For the DArT Seq markers, the 64bp within-species sequence tag can be used to screen the available Bambara groundnut leaf transcriptome gene models. Initial analysis suggests that 15% of the 64bp tags have a unique hit to a Bambara groundnut gene model. The gene models can then be used to identify locations on the soybean genome sequence. Both methods may allow alignment of the chromosomes of the underutilized species with major crop or model species. Such alignments will allow the corresponding location of QTL and genetic effects identified in Bambara groundnut to be identified in the major crop or model species. This will allow existing literature to be searched for genes which have similar effects at this location or even the development of a cross-species gene list underlying a QTL for trait effects in the underutilized species.

This approach generates a genetic framework in which candidates genes for a genetic understanding of the same trait in the major crop or model species can be evaluated for an effect in the underutilized species. An important consideration in translation of such information is that the agricultural variation observed in the underutilized species may involve the same basic pathway as exists in the major crop or model species, but might be caused by a different gene in that network or may be due to subtle changes in the underutilized crop gene action. Translation of information between species needs to be a nuanced affair, whether sequence, trait or product development. There is always a danger with large data sets that a superficial comparison is made using formulaic approaches, rather than biologically informed ones, leading to a correct hypothesis being abandoned.

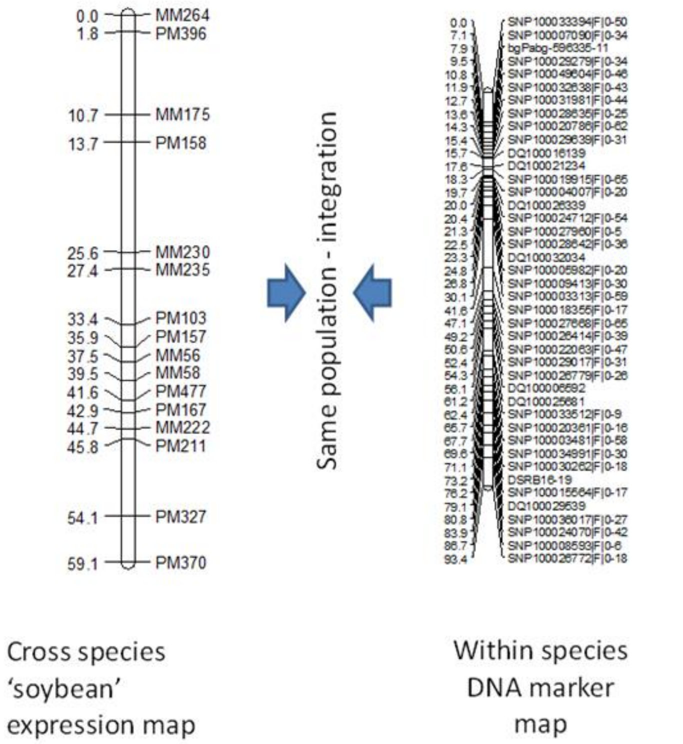


Figure 5. A linkage group from the cross-species GEMs map and the within species DArT Seq map.

Discussion

The major constraint for the application of biotechnology and specifically molecular markers in underutilized crops is likely to be cost (although weak infrastructure and institutional capacity could compound this). Bambara groundnut has been used as an exemplar as we have applied a number of approaches to evaluate their value and tried to identify some initial generic lessons for how to work in other underutilized species.

The need for the development of (limited) numbers of within-species markers which can be used routinely in QC and low level analysis is clear and our current approach is to develop co-dominant SSR markers from a leaf transcriptome. However, SSRs are expensive and technically demanding to apply in large numbers, so the DArT Seq approach has been adopted for genetic diversity, mapping and genome wide analysis. The potential to link the genetic maps developed in the underutilized species through screening the 64bp in-species sequence tag associated with the DArT Seq markers against the gene models in the transcriptome has been tested and appears feasible. This allows the use of the marker associated with the gene model to link to sequenced and published genomes, effectively providing a 'pseudo physical' map for the underutilized crop and integrating the linkage map into the genetic map network for related species. Generating these networks of species allows the location testing and translation of information and candidate gene effects from the major and model species to the underutilized species.

Overall, our current approach will cost around $20K per species, although the costs of sequencing are decreasing. As this continues and Third Generation Sequencing becomes generally available, alternative options (particularly whole genome sequencing) become possible, with long fragment reads facilitating bioinformatics assembly. Besides cost, the latter is probably the main constraint to generating useful genome sequences.

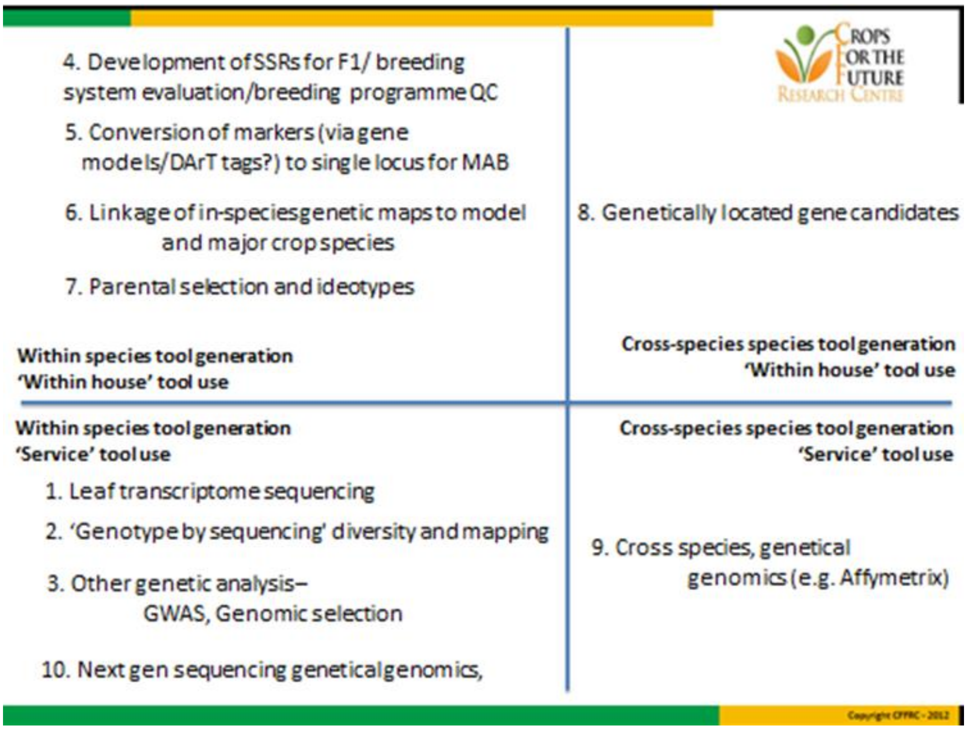


Figure 6: An initial placement of potential approaches for developing molecular tools within species versus translation from major crop and model species and also 'within house' versus from a service provider.

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References

Ahmad N.S. 2012. Thesis submitted in partial fulfilment of the requirements for the award of a Doctor of Philosophy. University of Nottingham, UK.

Ahmad N.S., Basu S., Redjeki E.S., Murchie E., Massawe F., Azam-Ali S.N., Kilian A., Mayes S. 2013. Developing Genetic Mapping and Marker-Assisted Breeding Techniques in Bambara Groundnut (*Vigna subterranea* L)

Alexander H.J., Wittenberg Van Der Lee, T., Cayla, C., Kilian, A., Visser, R.G. and

Schouten H.J. 2005. Validation of the high-throughput marker technology DarT using the model plant Arabidopsis thaliana. Molecular Genetics and Genomics

Journal 274(1):30-39, DOI: 10.1007/s00438-005-1145-6.

Aliyu S., Massawe F.J., Mayes S. (2014) Beyond landraces: Developing improved germplasm resources for underutilized species - a case for Bambara groundnut. Biotechnology and Genetic Engineering Reviews 30: 127-41

Basu S.M. 2005. Genetic mapping and trait analysis in Bambara groundnut *Vigna subterranean* (L.) Verdic. Thesis submitted to the University of Nottingham for the 442 degree of Doctor of Philosophy.

Basu S., Mayes S., Davey M., Roberts J.A., Azam-ali S.N., Mithen R., Pasquet R.S. 2007. Inheritance of 'domestication' traits in Bambara groundnut (*Vigna subterranea* (L.) Verdc). Euphytica 157: 59-68.

Chai, H.H., Lai, H – M., Guo, H., Basu, S., Massawe, F., Azam-Ali, S.N., Graham, N., Broadley, M., May, S. and Mayes, S. (2013). Developing XSpecies Approaches for Genomics and Transcriptomics - Using Resources Developed in Major Species for Research in Bambara Groundnut. In F. Massawe, S. Mayes, and P. Alderson (Eds.), Proceedings of 2nd International Symposium on Underutilized Plant Species "Crops for the Future – Beyond Food Security" Acta Hort. 979 2013 (pp 773 – 778). Belgium: ISHS

Collard B.C.Y., Jahufer M.Z.Z., Brouwer J.B., Pang E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142: 169-196.

Hammond J.P., Broadley M.R., Craigon D.J., Higgins J., Emmerson Z.F., Townsend H.J., White P.J., May, S.T. 2005. Using genomic DNA-based probe-selection to improve the sensitivity of high-density oligonucleotide arrays when applied to heterologous species. Plant Methods 1: 10-18.

Hammond J.P., Mayes S., Bowen H.C., Graham N.S., Hayden R.M., Love C.G., Spracklen W.P., Wang J., Welham S.J., White P.J., King G.J., Broadley M.R. 2011 Regulatory hotspots are associated with plant gene expression under varying soil phosphorus supply in *Brassica rapa*. Plant Physiology 156: 1230-1241.

Jaccoud D., Peng K., Feinstein D. Kilian A. 2001. Diversity arrays: a solid state technology for sequencing information independent genotyping. Nucleic Acids Research 29: 1-7.

Karikari S.K. 2004. Variability between local and exotic Bambara groundnut landraces in Botswana. African Crop Science Journal 8: 145-152.

Linneman A.R., Azam-ali, S.N. 1993. Bambara groundnut (*Vigna subterranea* (L.) Verdc) In: Underutilized Crop Series I. Vegetables and Pulses. Chapman and Hall, London, UK.

Massawe F.J., Dickson M., Roberts J.A., Azam-Ali S.N. 2002. Genetic diversity in Bambara groundnut (*Vigna subterranea* (L.) Verdc.) landraces revealed by AFLP markers. Genome 45: 1175-1180.

Massawe F.J., Mwale S.S., Azam-Ali S.N., Roberts J.A. 2005. Breeding in Bambara groundnut (*Vigna subterranea* (L.) Verdc.): strategic considerations. African Journal of Biotechnology 4(6): 463-471.

Mayes S., Stadler S., Basu S., Murchie E., Massawe F.J., Kilian A., Roberts J.A., Mohler V., Wenzel G., Beena R., Sheshshayee M.S., Azam-Ali S.N. 2009. BAMLINK- a cross disciplinary programme to enhance the role of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) for food security in Africa and India. Acta Horticulturae 806: 137-150.

Mayes S., Basu S.M., Molosiwa O., Redjeki E.S., Ahmad N.S., Khan F., Zehra S., Noah S., Mayes K., Roberts J., Stadler F., Massawe F., Kilian A. and Azam-Ali S.N. 2013. Molecular Analysis of Bambara Groundnut, an Underutilised African Legume Crop as Part of the BAMLINK Project - What Lessons Can We Learn? Acta Horticulturae 979:451 – 458). Belgium: ISHS

Molosiwa O.O. 2012. Genetic diversity and population structure analysis of bambara groundnuts (*Vigna subterranea* (L.) Verdc.) landraces using morphoagronomic characters and SSR markers. PhD thesis, University of Nottingham, UK.

Molosiwa O., Basu S., Stadler F., Azam-Ali S.N., Mayes S. 2013. Assessment of Genetic Variability of Bambara Groundnut (*Vigna subterranea* (L.) Verdc) Accessions Using Morphological Traits and Molecular Markers. Acta Horticulturae 979: 779-790. Belgium: ISHS

Molosiwa O., Aliyu S., Stadler F., Mayes K. Massawe F., Kilian A., Mayes,S. 2015. SSR marker development, genetic diversity and population structure analysis of Bambara groundnut [*Vigna subterranea* (L.) Verdc.] landraces. Genetic Resources and Crop Evolution DOI 10.1007/s10722-015-0226-6

Ntundu H.W., Bach C.I., Christianstein J.L., Anderson B.S. 2003. Analysis of 785 genetic diversity in Bambara groundnut (Vigna subterranea (L.) Verdc.) landraces using amplified fragment length polymorphism (AFLP) markers. African Journal of Biotechnology 3: 220-225.

Olukolu B.A., Mayes S., Stadler F., Ng Q.N., Fawole I., Dominique D., Azam-Ali S.N., Abbott, G.A., Kole, C. 2011. Genetic diversity in Bambara groundnut (*Vigna subterranea* (L.) Verdc.) as revealed by phenotypic descriptors and DArT marker analysis. Genetic Resources and Crop Evolution 59: 347-358.

Redjeki E.S., Mayes S., Azam-Ali S.N 2013. Evaluating the stability and adaptability of bambara groundnut (*Vigna subterranea* (L.) Verd.) landraces in different agro-ecologies. Acta Horticulturae 979: 389 – 400. Belgium: ISHS

Stadler F. 2009. Analysis of differential gene expression under water-deficit stress and genetic diversity in Bambara groundnut [Vigna subterranea (L.) Verdc.] using novel high-throughput technologies. PhD Thesis, Technische Universität München, Germany.

Wenzl P., Carling J., Kudrna D., Jaccoud D., Huttner E., Kleinhofs A., Kilian A. 2004. Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc. Natl. Acad. Sci. USA 101: 9915-9920.

Zeven AC. 1998. Landraces: a review of definitions and classifications. Euphytica 104: 127-139.

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