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No. 57

Consensus Document on the Biology of Cassava (*Manihot esculenta* Crantz)

Environment Directorate

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The Environment, Health and Safety Division publishes free-of-charge documents in eleven different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides; Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents;** and **Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/chemicalsafety/).

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FOREWORD

Consensus Documents contain information for use during the regulatory assessment of a particular product. In the area of plant biosafety, these are being published on information on the biology of certain plant species, selected traits that may be introduced into plant species, and biosafety issues arising from certain general types of modifications made to plants.

This document addresses the biology of the Cassava (*Manihot esculenta* Crantz).

Brazil served as the lead country in the preparation of this document, in collaboration with two observer organisations: the African Biosafety Network of Expertise of the New Partnership for Africa's Development (NEPAD-ABNE) and the ILSI Research Foundation, Center for Environmental Risk Assessment (CERA). The draft has been revised on a number of occasions based on the input from other member countries and stakeholders.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

Note: The OECD also deals with the safety of foods and feeds derived from modern biotechnology ("novel" foods and feeds). The Consensus Document on Compositional Considerations for New Varieties of Cassava was published in 2009 and is available at http://www.oecd.org/env/ehs/biotrack/.

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PREAMBLE

The environmental safety/risks of transgenic organisms are normally based on the information on the characteristics of the host organism, the introduced traits, the environment into which the organism is introduced, the interaction between these, and the intended application. The OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on identifying parts of this information, which could be commonly used in countries for environmental safety/risk assessment to encourage information sharing and prevent duplication of effort among countries. Biosafety Consensus Documents are one of the major outputs of its work.

Biosafety Consensus Documents are intended to be a "snapshot" of current information on a specific host organism or trait, for use during regulatory assessments. They are not intended to be a comprehensive source of information on everything that is known about a specific host or trait; but they do address the key or core set of issues that member countries believe are relevant to risk/safety assessment. This information is said to be mutually acceptable among member countries. To date, 48 Biosafety Consensus Documents have been published. They include documents which address the biology of crops, trees and micro-organisms as well as those which address specific traits which are used in transgenic crops.

In reading the Consensus Documents, it is useful to consult two additional texts. The first, entitled *An Introduction to the Biosafety Consensus Document of OECD's Working Group for Harmonisation in Biotechnology* explains the purpose of the Consensus Documents and how they are relevant to risk/safety assessment. It also describes the process by which the documents are drafted using a "lead country" approach. The second text is *Points to Consider for Consensus Documents on the Biology of Cultivated Plants.* This is a structured checklist of "points to consider" for authors when drafting or for those evaluating a Consensus Document. Amongst other things, this text describes how each point is relevant to risk/safety assessment.

The Consensus Documents are of value to applicants for commercial uses of transgenic organisms, regulators in national authorities as well as the wider scientific community. As each of the documents may be updated in the future as new knowledge becomes available, users of Consensus Documents are encouraged to provide any information or opinions regarding the contents of this document or indeed, OECD's other harmonisation activities. Comments and suggestions can be sent to:

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SECTION I - SPECIES OR TAXONOMIC GROUP

1.1 Classification and nomenclature

1. The scientific name of cassava is *Manihot esculenta* Crantz (ITIS, 2012), synonym *Manihot utilissima* Pohl (Nassar and Ortiz, 2006). Cassava is a member of the spurge family, and its taxonomic hierarchy is:

Order Malpighiales
Family Euphorbiaceae
Genus *Manihot*
Species *Manihot esculenta* Crantz
Subspecies *M. esculenta* Crantz ssp. *esculenta*
M. esculenta Crantz ssp. *flabellifolia* (Pohl) Cifferi
M. esculenta Crantz ssp. *peruviana* (Müeller) Allem (Allem, 2002)

2. Three subspecies of cassava have been recognized: *Manihot esculenta* ssp. *esculenta* is the cultivated strain, and *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana* are wild forms (Allem, 1999, 2002). In this document, "cassava" will be used to refer to the cultivated strain, *M. esculenta* ssp. *esculenta*. Common synonyms in other languages are manioc (French), mandioca, macaxeira, and aipim (Portuguese), yuca (Spanish), and manioca (Italian).

3. Approximately 98 species were originally identified in the *Manihot* genus, using morphological and botanical characteristics, and there is one species in a closely related genus, *Manihotoides pauciflora* (Rogers and Appan, 1973; Byrne, 1984). As modern molecular genetics tools are used in the analysis of the genus, the number of true *Manihot* species is expected to decrease (Duputié et al., 2007). In addition, due to the conversion of native habitat to agriculture, and the resultant destruction of wild species, some of the previously identified species may now be extinct in the wild (Nassar, 2000).

4. The wild subspecies *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana*, as well as *M. pruinosa* have been identified as close relatives of cultivated cassava and are interfertile with cassava (Roa et al., 1997; Allem, 1999; Olsen and Schaal, 1999; Andersson and de Vicente, 2010). Several additional species are included in cassava's secondary gene pool (Table 1), and experimental crosses are possible with all these species, although F1 hybrids tend to be sterile (Andersson and De Vicente, 2010).

Table 1. **Species within the secondary gene pool of cassava**

Species	Origin and distribution
M. carthagenensis ssp. *carthagenensis* (Jacq.) Müll. Arg.	Venezuela, Bolivia, Colombia, Argentina, Paraguay, Trinidad and Tobago, Antilles
M. carthagenensis ssp. *glaziovii* (Müll. Arg.) Allem (*M. glaziovii* Müll. Arg.)	Native to Brazil, cultivated and naturalised elsewhere (Africa, Asia, Pacific Islands)
M. carthagenensis ssp. *hahnii* Allem	Brazil
M. aesculifolia (Kunth) Pohl	Mexico, Costa Rica, Belize, Panama, El Salvador, Guatemala
M. anomala Pohl	Brazil, Bolivia, Peru, Argentina, Paraguay
M. brachyloba Müll. Arg.	Throughout Central and South America (from Nicaragua to Brazil)
M. chlorosticta Standl. & Goldman	Mexico
M. dichotoma Ule	Brazil
M. epruinosa Pax & K. Hoffm.	Brazil
M. gracilis Pohl	Brazil
M. leptophylla Pax & K. Hoffm.	Brazil, Ecuador, Peru
M. pilosa Pohl	Brazil
M. pohlii Wawra	Brazil
M. tripartita (Spreng.) Müll. Arg.	Brazil, Bolivia, Paraguay
M. triphylla Pohl	Brazil

Source: Andersson and De Vicente (2010)

1.2 Description

5. Cassava typically grows as a perennial shrub, one to five metres in height (Figure 1), with palmate leaves bearing 3 to 9 lobes and covered with a shiny, waxy epidermis. The mature plant generally takes one of two forms: either spreading stems or erect stems with various amounts of terminal branching (Byrne, 1984; Alves, 2002). Species in the genus *Manihot* are generally well adapted to tropical regions, where they take the form of subshrubs to small trees, forming large, woody roots.

6. Due to the high level of morphological variability among cassava varieties, it is difficult to reliably distinguish individual varieties using only morphological characteristics (Alves, 2002). To an increasing extent, DNA molecular markers are being used to characterise varieties and measure genetic diversity within the species (Fregene, 2002). Germplasm preservation programs in numerous countries worldwide have a combined collection of over 20 000 accessions of cassava (Lebot, 2009).

7. Cassava is grown primarily for its enlarged storage roots, which are used for human consumption, following a variety of traditional processing methods including boiling, roasting, processing into flour, and fermentation (Salick et al., 1997; Hillocks, 2002). Although cassava has the lowest protein-to-carbohydrate ratio among major crops (Sayre et al., 2011), it plays an important dietary role in the diets of almost 1 billion people, worldwide (Prochnik et al., 2012). In some regions, particularly in Africa and Brazil, the foliage may also be harvested for human consumption and animal feed, providing supplemental dietary protein (Hillocks, 2002). Cassava is also grown for industrial purposes, such as the production of starch and for fermentation into ethanol (El-Sharkawy, 2004; Adelekan, 2010).

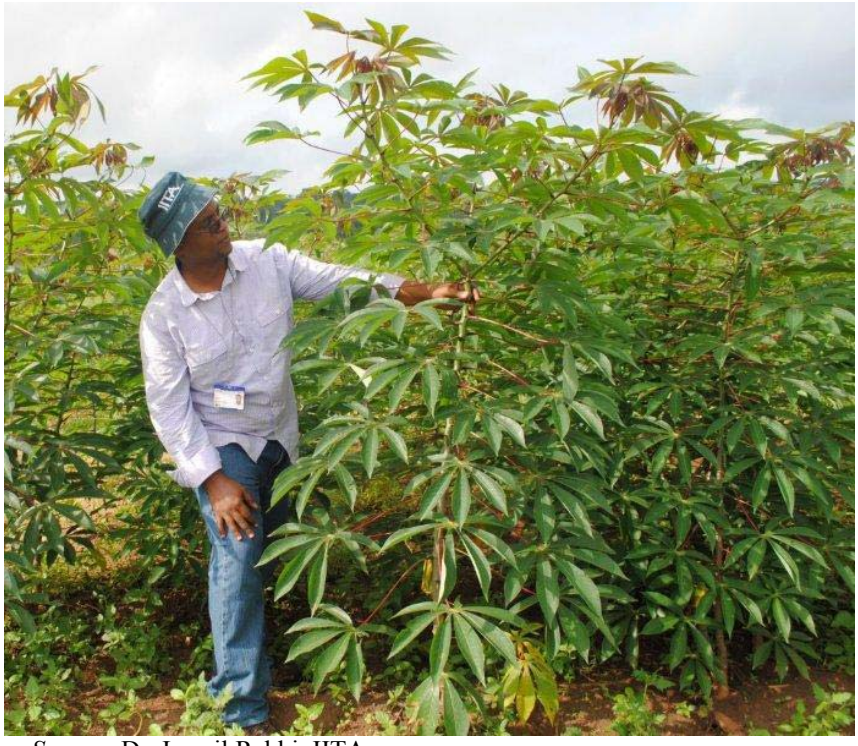
8. Analyses of the susceptibility of crops to the impacts of climate change indicate that cassava may be better suited to survive climatic variations than most major tropical staple crops, which would make it a key food security crop for the future. However, while calculations indicate that cassava has the potential to produce and store more carbohydrate than any other major grain or root crop, it typically

fails to reach that potential due to poor-quality planting material, sub-optimum agronomic practices, and disease and insect pests (El-Sharkawy, 2004; Fermont et al., 2009; Jarvis et al., 2012).

9. The roots and leaves of cassava and other *Manihot* species are known to release hydrogen cyanide (HCN), which can be toxic to humans and animals when consumed, although incidence of cyanide poisoning is rare (OECD, 2009). Cassava varieties are classified as "bitter" (glucoside content > 100 mg/kg fresh wt) or "sweet" (glucoside content < 100 mg/kg fresh wt) according to their level of HCN production (Alves, 2002; Peroni, Kageyama and Begossi, 2007). Cassava breeding programs actively select for varieties which produce lower levels of HCN (Byrne, 1984), but some farmers favour cassava with a high cyanide content due to the belief that such varieties are more insect and stress resistant and less prone to theft by humans and predation by mammals (Byrne, 1984; Fregene, 2002; Lebot, 2009). Most traditional processing methods of cassava enable the safe dissipation of any HCN produced by the plant, and industrial processing methods also remove HCN; however, when large amounts of cassava are processed, toxic effluents can be generated (Taylor et al., 2004). The food and feed processing and use of cassava are described in the OECD *Consensus Document on Compositional Considerations for New Varieties of Cassava* (OECD, 2009).

10. HCN is released through the hydrolysis of two cyanogenic glycosides, primarily linamarin, with lower levels of lotaustralin, and hydrolysis is initiated by physical disruption of plant tissues. Linamarin is hydrolysed by linamarase to release HCN. Linamarin is contained in the vacuoles of intact plant cells, while linamarase is located in the cell walls. Tissue disruption allows the two compounds to react (Alves, 2002).

Figure 1. **Cassava growing in Nigeria**



Source: Dr. Ismail Rabbi, IITA

SECTION II - GEOGRAPHIC DISTRIBUTION, ECOSYSTEMS AND HABITATS, CULTIVATION AND MANAGEMENT PRACTICES, CENTRES OF ORIGIN AND DIVERSITY

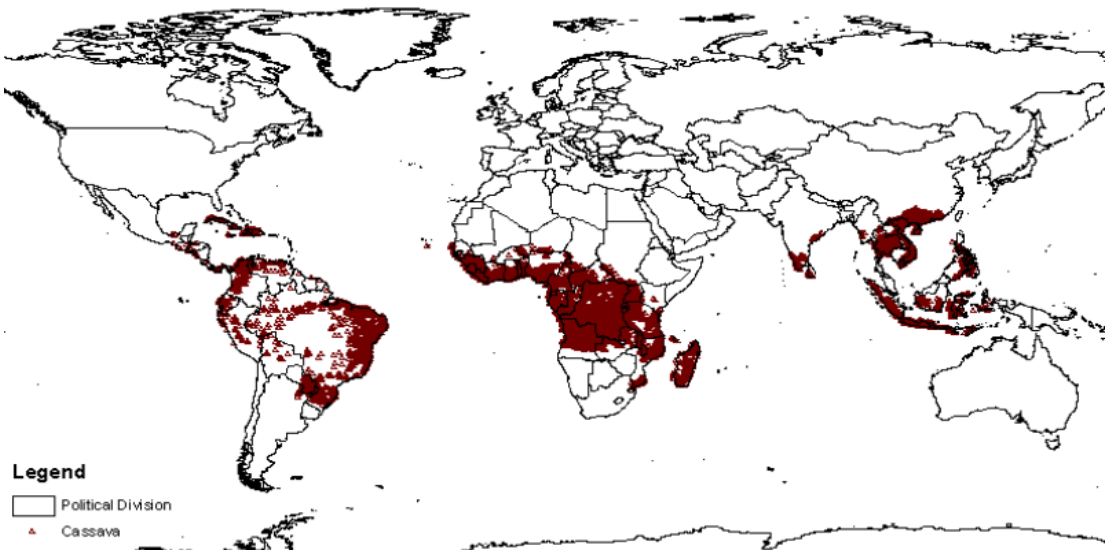
2.1 Geographic distribution

11. Nineteen countries (9 in Africa, 3 in Latin America, and 7 in Asia) are considered to be major global cassava growers, each producing 1 million tonnes or greater annually. The top five cassava producing countries are Nigeria, Thailand, Brazil, Indonesia, and the Democratic Republic of the Congo (FAO, 2009). The species in the genus *Manihot* are native to the New World, falling into two distinct groups, one in Central America and the other in South America. Mexico and Brazil have the greatest number of *Manihot* species, and there are several recognized centres of diversity: central Brazil, north-eastern Brazil, western Mato Grosso, Brazil, south-western Mexico, and Bolivia (Nassar, 2000).

12. Cultivation of cassava is largely limited to the tropics, where the annual mean temperature is greater than 18°C (Figure 2) (Kawano, 1980). Only a few *Manihot* species (e.g. *M. neusana* and *M. grahamii*) can survive in areas where frost occurs (Nassar and Ortiz, 2006). Cassava can tolerate drought but performs well at annual rainfall of 600 to 1500 mm and temperatures of 25 to 29°C (Nassar and Ortiz, 2006). It is grown throughout all tropical regions of the world between latitudes 30°N and 30°S and at up to 2000 m altitude, where day length is between 10 to 12 hours (Alves, 2002). After centuries of cultivation and landrace selection, there are many varieties developed for specific landscapes, elevations, temperatures, and soil types (Salick et al., 1997; El-Sharkawy, 2004).

13. *M. glaziovii* (*M. carthagenensis* ssp. *glaziovii*) was brought to Africa as a source of rubber. It is the only species within *Manihot* that is known to have naturalised in Africa.

Figure 2. **Cassava distribution map showing the widespread cultivation of cassava**



The dark dots represent cassava cultivation points with over 1000 ha.

Source: CIAT (2002)

2.2 Ecosystems and habitats where cassava natively occurs and has been naturalised

14. Cassava itself does not grow wild, nor does it volunteer well in cultivation, and it does not compete well with other plants in abandoned fields or feral environments, seldom persisting more than a few growing seasons (Pujol et al., 2002; Andersson and de Vicente, 2010). Low seed production and seed dormancy limit the ability of cassava to spread to unmanaged ecosystems and persist there (Chavarriaga-Aguirre and Halsey, 2005). Cassava appears to possess only one of Baker's characteristics of weeds, namely, discontinuous germination and long-lived seeds, and cassava is not considered to be a weedy species, either in an agricultural setting or in the wild (Halsey et al., 2008). Originally, *M esculenta* ssp. *flabellifolia* had been proposed as an escapee from cultivated cassava (Nassar, 2002), but various taxonomic and biosystematic studies seem to agree that *flabellifolia* is most likely the wild progenitor of cassava (Roa et al., 1997; Olsen and Schaal, 1999; Allem, 2002; Andersson and de Vicente, 2010).

15. Some species (e.g. *M. pohlii*, *M. zehntneri*, and *M. grahamii*) can be invasive in newly disturbed areas (Nassar and Ortiz, 2006; Andersson and de Vicente, 2010), while others are known to survive drought and fire (Byrne, 1984).

2.3 Agronomic, silvicultural and other intensively-managed ecosystems where the species is grown or occurs, and management practices

2.3.1 Cultivation and management practices

16. Generally, cassava requires high levels of sunlight and high temperatures, adequate soil fertility, and rainfall during crop establishment to produce acceptable agronomic yields (Fermont et al., 2009). Typically, the crop is grown with little or no supplemental irrigation, pesticides, or fertilizers (Howeler, 1991, 2002; Leihner, 2002), but inputs such as fertilizer and water, the use of improved varieties, and weed management can significantly improve yields (Fermont et al., 2009). The use of cropping methods such as mulching, intercropping, conservation tillage, and contour planting may improve production under certain circumstances, but the use of these methods varies by locality, and little research has been done to optimize such practices (Byrne, 1984; Howeler, 2002).

17. It is common for cassava to be intercropped with other annual crops such as maize, rice, sorghum, or pulses, or with perennial groundcovers, to minimize the soil erosion that can occur when cassava is grown alone (Leihner, 2002). However, because cassava establishes more slowly than many of the crops it is grown with, the timing of the plantings must be managed so that the developing cassava plants are not subject to excessive shading, causing the plant to divert photosynthesis into the production of shoots and leaves rather than storing it as starch in the roots (Alves, 2002; Lebot, 2009). The cultivar and its associated growth habit also affect the success of intercropping, because taller varieties and those with an erect growth habit may be less affected by the companion crop (Leihner, 2002).

18. Generally, a field intended for cassava production is prepared by slashing and burning or by disking and ploughing. Depending on the size of the farm and the farmer's resources, land preparation may be done by hand or with animal-drawn or mechanized equipment. Smallholder farmers may do little land preparation prior to planting, and some growers may plant the next season's crop while harvesting. However, on large-scale farms under permanent cropping, ploughing to loosen the soil and improve drainage is more common, since cassava does not tolerate waterlogged soils (Lebot, 2009). Ploughing also increases the ease with which the crop can be harvested and therefore may be worth the extra effort for smallholder farmers, who generally harvest by hand (Lebot, 2009).

19. Cassava is grown on a variety of soils, and it tolerates marginal, low-fertility, acid soils better than many other staple crops (Byrne, 1984; El-Sharkawy, 2004). However, cassava is known to be

sensitive to soils with high pH (greater than 7.8) and elevated conductivity and/or sodium (Byrne, 1984; Howeler, 2002). Cassava removes less nitrogen and phosphorous per tonne of dry matter (DM) produced than other common crops, and its efficient use of soil nutrients, especially phosphorous, is attributable to its association with soil mycorrhizae (Howeler, 1991, 2002). Cassava responds favourably to added fertilizer, especially potassium, but over-fertilization, especially with nitrogen, can increase leaf growth at the expense of root formation and increase root cyanide content (Howeler, 1991, 2002; Nassar and Ortiz, 2006).

20. Competition from weeds is recognized as a major limitation on cassava yields (Fermont et al., 2009). Herbicide use, although effective for increasing yields, is more common on larger farms, whereas, on smaller farms, weeds are typically managed by manual weeding, mulching, or other less expensive but more labour-intensive methods (IITA, 2000; Leihner, 2002; Taylor et al., 2004). Disease control in cassava is generally accomplished through the use of resistant varieties, selecting planting materials from plants without disease symptoms, early removal of diseased plants, and crop rotation (Leihner, 2002).

2.3.2 Vegetative and seed propagation

21. Cassava storage roots cannot be used for propagation, since the plant will not regenerate from root tissue; instead, mature, woody stems are harvested and cut into short "stakes" (15 to 30 cm) to be used for planting the next crop (Alves, 2002). A mature cassava plant may provide from ten to twenty stakes (Lebot, 2009). The stakes must be handled with care, as their quality can rapidly deteriorate due to desiccation, bruising, and peeling. Whole stems that have been harvested can be stored for several months in cool, moist conditions and with chemical protection from insects and fungi, without significant loss of viability (Leihner, 2002).

22. Planting is done by placing stakes into the soil vertically, inclined, or buried horizontally, on flat or ridged soil beds, usually at the beginning of a rainy season (Keating et al., 1988) (Figure 3). Depending on soil type, the planting orientation can influence the ease with which the roots may be harvested (IITA, 1990). In addition, vertical or inclined planting of the cuttings encourages plants with a single stem, while horizontal planting often results in multiple-stemmed plants (Lebot, 2009). Germination and early growth of the plants from stakes depends on endogenous nutrients stored in the stems, rather than on soil nutrients, so the success of the planting is determined by the quality of the cuttings (El-Sharkawy, 2004).

Figure 3. **Cassava shoots sprouting from stakes**

Source: Courtesy of ILSI Research Foundation-CERA

23. The cuttings sprout in one to two weeks, and the first leaves begin to expand within 30 days. The canopy closes in three to four months, depending on the variety and the local environmental conditions. For the first 1-2 month(s), the developing plants produce only fine-textured roots, but eventually a number of these roots, depending on the variety, begin secondary growth and starch accumulation. The onset of starch accumulation coincides with a decrease in ability of storage roots to absorb water and nutrients (Alves, 2002; El-Sharkawy, 2004). Development of storage roots begins with secondary growth of fibrous roots and starch deposition which starts about 25 to 40 days after planting (DAP) in many cultivars (Cock, 1984). Storage root thickening begins when the supply of photoassimilates exceeds the requirements for shoot growth (Cock et al., 1979; Lian and Cock, 1979). Onset of storage root bulking is noticeable two to four months after planting when the new storage roots are at least 5 mm thick. Most of the translocation of carbohydrates to the storage roots occurs between 180 and 300 DAP (Lebot, 2009).

24. Planting density can range from 5 000 to 40 000 cuttings per hectare, depending on the cultivar, the soil quality, and the intended use of the crop. Lower planting densities (< 12 500 plants/ha) favour storage root production, while higher planting densities (> 12 500 plants/ha) are used to maximize stake production (Keating et al., 1988; Leihner, 2002; Nassar and Ortiz, 2006; Odedina et al., 2009).

25. Stem cuttings are not necessarily taken from every plant in the field. In fact, only a small minority of the plants may serve as the source for the farmer's next season's crop (Elias, Panaud and Robert, 2000). In addition, it is not unusual for growers to exchange stem cuttings with their neighbours and with neighbouring communities, resulting in fields that contain mixtures of the local landraces (Andersson and de Vicente, 2010). Although farmers typically prefer high yielding varieties, they may maintain lower yielding varieties in parallel with more productive varieties, due to cultural preferences such as taste or cooking quality. This practice of keeping several different varieties in production at the same time, often in the same field, is one way farmers manage the risk of a catastrophic crop loss (Elias, Panaud and Robert, 2000).

26. Botanical seed is not typically used for commercial propagation. Genetically, any particular cassava genotype is extremely heterogeneous (Kawano et al., 1978), and propagation from sexual seed results in a wide and unpredictable diversity of phenotypes. This diversity is of interest to breeders but presents difficulties for farmers (Ceballos et al., 2004). During the growing season, it is not unusual for seedling cassava plants to grow up among the vegetatively propagated plants. These seedlings may have germinated from seeds released by the crop itself or from seeds in the soil seed bank, and it is likely that these new seedlings are genetically different from their parental stock. In addition, because many of the most problematic cassava diseases are passed from one crop to the next via vegetative propagation, such seedlings may be relatively disease-free (Elias, Panaud and Robert, 2000). Farmers may harvest stem cuttings from the seedling-derived plants displaying favourable agronomic characteristics and replant these cuttings with the next season's crop (Olsen and Schaal, 1999). In this way, farmers incorporate genetic variability from sexual reproduction into existing landraces. In regions where wild *Manihot* plants are prevalent, this practice may function to facilitate gene flow between cultivated plants and nearby wild plants (Silva, Bandel and Martins, 2003; Duputié et al., 2007; Olsen and Schaal, 2007; Sardos et al., 2008). Alternatively, some farmers separate stem cuttings from these seedlings to be multiplied as a new variety (Elias and McKey, 2000; Elias et al., 2001).

2.3.3 Harvesting and post-harvest handling

27. Typically, a cassava crop produced in humid, lowland tropical regions can be harvested many months earlier than a crop grown in drier, cool highland areas (Alves, 2002). Depending on the cassava genotype, environment, soil type, and intended use, the storage roots (Figure 4) may be harvested 6 to 36 months after planting. Farmers may leave a percentage of the plants standing,

treating them as a perennial crop, and thereby storing food underground (Byrne, 1984; Nassar and Ortiz, 2006; Sardos et al., 2008). Some farmers may harvest only a few roots from a plant, covering the remaining roots with soil for future harvesting. However, with increasing age and unfavourable conditions, such as moisture stress, storage roots become lignified and less desirable for consumption, and the plants become more susceptible to lodging and rot (Lebot, 2009).

Figure 4. **Harvested cassava plant, showing roots**

Source: Courtesy of ILSI Research Foundation-CERA

28. Once harvested, cassava roots of many varieties undergo what is referred to as post-harvest physiological deterioration or PPD (Lebot, 2009). Within 24 to 72 hours of harvest, polyphenol oxidase catalyses the formation of various polyphenolic compounds: pigments, quinones, and tannins. These substances as well as various secondary metabolites, such as coumarin and scopoletin, which are also synthesised at this time, together render the root tissue inedible (Alves, 2002; Reilly et al., 2004). Heat treatments, anaerobic storage, and treatments with polyphenol oxidase inhibitors, such as ascorbic acid, glutathione, and potassium cyanide can reduce the severity of PPD (Alves, 2002). The incidence of PPD can be reduced by pruning the plants to a height of 200 to 300 cm, up to three weeks before roots are harvested (Marriott, Been and Perkins, 1979). This practice seems to increase the sugar/starch ratio in the roots and reduces losses from PPD (El-Sharkawy, 2004); however, pre-harvest pruning can negatively impact taste and cooking quality of the cassava roots (van Oirschot et al., 2000).

2.4 Centres of origin and diversity

29. Pinpointing cassava's origin has been complicated by inconclusive anthropological data and the difficulty in obtaining intact archaeobotanical samples from the humid lowland regions in Central and South America where cassava has been historically grown. Tissue samples are more easily obtained in arid regions, but it is thought that these areas are not the origins of domestication (Byrne, 1984; Olsen and Schaal, 1999; ITIS, 2012).

30. Concerning the origin of cassava, three questions need to be addressed: the botanical origin (i.e. the wild species from which cassava descended); the geographical origin (i.e. the area where the progenitor evolved in the geological past); and agricultural origin (i.e. the area of initial cultivation/domestication of the wild ancestor by Amerindians).

2.4.1 Botanical origin

31. Originally, the entire genus was thought to have arisen via allopolyploidisation, possibly resulting from a cross between two related species (Byrne, 1984; Nassar and Ortiz, 2006). For many years, the accepted hypothesis was that cassava resulted from one or more hybridisation events of wild *Manihot* or other species (Rogers and Appan, 1973). It was proposed that the modern cultivated cassava, *M. esculenta* ssp. *esculenta*, originated directly from the extant wild subspecies *M. esculenta* ssp. *flabellifolia* (Allem, 1994), and this close relationship has since been supported by additional studies (Roa et al., 1997, 2000). The use of molecular tools such as amplified fragment length polymorphism (AFLP) to estimate genetic relationships of *M. esculenta* indicates that the cultivated species has a single ancestor, *M. esculenta* ssp. *flabellifolia* (Olsen and Schaal, 1999; Duputié et al., 2007).

2.4.2 Agricultural origin

32. The origin of domestication of cassava had been disputed for many years. However, recent evidence now points to an origin in the Amazon region of South America (Allem, 2002; Hillocks, 2002). It is currently assumed that there is only one domestication site for cassava, possibly along the southern border of the Amazon basin, where *M. esculenta* ssp. *flabellifolia* plants were originally collected from the wild, domesticated, and multiplied by vegetative propagation (Olsen and Schaal, 1999; Elias, Panaud and Robert, 2000; Allem, 2002). Archaeological findings and other data indicate that the domestication of cassava started approximately 5000 to 7000 years BCE (Lathrap, 1970; Gibbons, 1990). A detailed molecular analysis based on the single-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase (Olsen and Schaal, 1999) indicated that cassava was domesticated specifically from populations of *M. esculenta* ssp. *flabellifolia* occurring along the southern rim of the Amazon basin in the Brazilian states of Acre, Rondônia and Mato Grosso, and likely extending south into Bolivia. Later studies have confirmed a southern Amazonian domestication site (Olsen and Schaal, 2001; Léotard and McKey, 2004).

2.4.3 Geographical origin

33. Central Brazil, with its large number of wild *Manihot* species, is the likely primary centre of diversity of cassava (Nassar, 2000). There is evidence in the literature that cassava has been in cultivation in northern Amazonia for as long as a thousand years and that migration of native peoples from this region to Central America and central Brazil, where wild *Manihot* species were already present, resulted in the creation of new centres of diversity (Nassar, 2000). These domesticated varieties were subsequently moved during migrations of native peoples, allowing hybridisation to occur between the cultivars and local wild relatives (Byrne, 1984; Nassar, 2000, 2002).

34. In the 16th century, the Portuguese brought domesticated varieties of cassava from Brazil to West Africa, from which it was spread across the sub-Saharan region (Hillocks, 2002; Okogbenin et al., 2007). The Spanish brought cassava from Central America in the 16th century to the Philippines, from which it spread to South East Asia, Indonesia, and the Pacific Island countries (Byrne, 1984). Cassava was introduced to East Africa in the 17th century through Madagascar, Zanzibar and other Indian Ocean islands (Jennings, 1976). By the 18th century, movement via ocean routes brought cassava to mainland eastern Africa, and soon after to India, Java and South East Asia (Purseglove, 1968; Byrne, 1984).

SECTION III - REPRODUCTIVE BIOLOGY

3.1 Generation time under natural circumstances and where grown or managed

35. Although some cultivars of cassava can be managed as an annual crop, harvested in six months only after the stem cuttings are planted, it is actually a perennial shrub (Alves, 2002). Cassava undergoes annual cycles of vegetative growth, accompanied by carbohydrate storage in the roots, followed by a period of dormancy during cool, dry conditions (Lebot, 2009). Some growers may leave mature plants in the soil for up to 36 months, storing the roots for harvest later (Byrne, 1984; Nassar and Ortiz, 2006; Sardos et al., 2008; Lebot, 2009).

3.2 Reproduction (production of flowers or cones, fruits, seeds, and vegetative propagules)

36. Flowering time for cassava varies widely with the cultivar. Some varieties flower as early as two months after planting, while others may flower as long as 24 months after planting. Flowering between 6 and 18 months after planting is typical for the species (Byrne, 1984). Once flowering is initiated, an individual plant may produce flowers for over two months (Alves, 2002).

37. Generally, grower selection of cuttings for vegetative propagation has resulted in plants with reduced branching. Since inflorescences form at branch points in the stem, long-term vegetative propagation selects against flower formation and the ability of individual plants to reproduce sexually (Duputié et al., 2007; Olsen and Schaal, 2007; Halsey et al., 2008). In branching varieties, branching begins as early as two months after planting, and flower formation occurs approximately one week later, at the branching points (Halsey et al., 2008). However, early inflorescences are known to abort, so that functional flowers are generally seen emerging from secondary branch points (Lebot, 2009).

3.2.1 Floral biology

38. Cassava is monoecious, bearing separate female and male flowers on the same plant (Figure 5). The flowers are borne together in the inflorescences, with the pistillate flowers beneath the staminate flowers. A flower bud typically forms where the plant branches, so that more-highly-branched genotypes flower more prolifically than those with sparsely-branched habit. The onset of branching, and therefore flowering, is prompted by long days (up to 16-hour day length) in some cultivars (Alves, 2002). The number of flowers produced by a plant varies among varieties, and some genotypes have never been observed flowering (Kawano, 1980; Alves, 2002). Flowering may also be influenced by environmental factors, so that a particular clone may not flower at all in one environment, produce aborted flowers under other conditions, or produce numerous flowers and set seed in another environment (Halsey et al., 2008). It appears that moderate temperature (approximately 24°C) is most suitable for flowering (Alves, 2002).

39. Female flowers have 5 tepals, which can be red, yellow, or purple, and a sticky stigma which secretes nectar on the day the flower opens, attracting insect pollinators (Lebot, 2009). The pistillate flowers are approximately 13 mm by 8 mm in size (Byrne, 1984). The male flowers are half the size of the female flowers, approximately 5 mm, but are more numerous and each flower has 10 stamens, borne in two rings (Byrne, 1984; Alves, 2002).

Figure 5. **Cassava female and male flowers**

a) Female flower

b) Male flower

Source: Tom Rulkens

3.2.2 Pollination, pollen dispersal, pollen viability

40. The female flowers open for approximately one day, and the stigma is receptive throughout that time. Fertilization occurs 8 to 19 hours after pollination (Andersson and De Vicente, 2010).

41. Individual cassava inflorescences display protogyny, with female flowers opening one to two weeks before the staminate flowers on the same inflorescence. However because a single plant usually has more than one inflorescence, male and female flowers on the same plant may open at the same time (Alves, 2002). Therefore, while cassava is generally thought to be an outcrossing species, natural self-pollination may also occur, depending on the cultivar (Byrne, 1984).

42. The pollen grains are large, ranging from 90 to 148 µm in size (Hahn, Bai and Asiedu, 1990; Alves, 2002; Halsey et al., 2008; Vieira et al., 2012a). Typically, pollen viability is lost quickly after shedding; for example Leyton reported 97% seed set with newly-collected pollen, 56% seed set with pollen stored for 24 hours at 25°C, and only 0.9% seed set after 48 hours of storage (Leyton, 1993; Nassar and Ortiz, 2006). As a result, cassava breeders typically use pollen for crosses within one hour after collection (Halsey et al., 2008; Andersson and de Vicente, 2010).

43. Vieira et al. conducted a study on viability, production and morphology of the pollen grains of five varieties of cassava and accessions of six *Manihot* wild species and subspecies (*M. anomala, M. dichotoma, M. esculenta* ssp. *flabellifolia, M. esculenta* ssp. *peruviana, M. tomentosa*, and *M. violacea*). In general, the wild accessions produced more (579 to 3638 grains per flower) and larger (132 to 163 µm) pollen grains compared with the cassava varieties (613 to 1193 grains and 129 to 146 µm). The number of pollen grains for the cultivated cassava varieties was similar to *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana*, but significantly smaller than the wild accessions of *M. dichotoma, M. tomentosa*, and *M. violacea*. The lower pollen production in the cultivated cassava varieties, *M. esculenta* ssp. *flabellifolia,* and *M. esculenta* ssp. *peruviana* could represent one of the consequences from the initial steps in the domestication process. The process favours the vegetative propagation of the species to the detriment of sexual propagation and, consequently, the production of pollen (Vieira et al., 2012a).

44. The pollen grains of cassava are sticky, which limits wind pollination (Halsey et al., 2008). Various species of bees and wasps appear to be the main pollinators of cassava in both Africa and Latin America, including *Apis mellifera*, *Polybia* spp., and *Polistes* spp. (Byrne, 1984; Nassar and Carvalho, 1990; Halsey et al., 2008; Andersson and De Vicente, 2010). Most pollen foraging occurs over

a distance of 1 to 5 metres (Andersson and De Vicente, 2010), and when cassava plants were grown spaced at 2 x 2 m, both insect-mediated outcrossing, as well as a smaller amount of self-pollination, is observed (Silva, Bandel and Martins, 2003).

45. Reproductive isolation of cassava can be effectively accomplished by a number of means, including isolation distance, destruction of plants prior to flowering, removal of flower buds, and bagging of flowers. Kawano et al. conducted detailed research on reproductive isolation distances in cassava using a very large germplasm collection as a pollen source to eliminate biases related to flower opening, potential genetic incompatibilities, and limited pollen pool. The researchers observed measurable gene flow at 1 m, but found no gene flow at 30m and 500 m, suggesting that an isolation distance of 30 m is adequate to ensure genetic isolation of cassava in field experiments (Kawano et al., 1978). Other data indicates that reproductive isolation of wild *Manihot* (the pollen source) and feral cassava could be accomplished using a distance of 60 m (Duputié et al., 2007). Because wild *Manihot* species begin flowering earlier and flower more profusely than cassava, measurements of gene flow from wild *Manihot* to cassava would likely overestimate actual gene flow that may occur in cassava to cassava situations (Fregene, 2010).

3.2.3 Seed production, and natural dispersal of fruits, cones and seeds

46. The fruit of cassava (Figure 6) is a tricarpellary capsule, and each locule contains one ovule; however, it is common for capsules to contain fewer than 3 seeds (Kawano, 1980). The fruit reaches maturity two to three months after pollination, and the fruit dehisces explosively, although seed typically falls to the ground near the mother plant (Alves, 2002).

Figure 6. **Fruit of cassava**

Source: Tom Rulkens

47. Ants are attracted to the seeds, which bear an edible oil body called the caruncle. The ants assist in seed dispersal by bringing the seeds to their nests, resulting in seed movement up to several meters, however ants' contribution to cassava seed dispersal appears to vary by species and distance to nest entrances (Elias and McKey, 2000; Elias et al., 2001). Ant dispersal is associated with fire-adapted species, since the movement of seeds into ants' underground burrows protects the seeds from high temperatures occurring during bush fires (Pujol et al., 2002).

48. Some birds, specifically doves, may also have a role in the dispersion of the seeds (Elias and McKey, 2000; Andersson and De Vicente, 2010).

3.2.4 Seed viability, longevity and dormancy; natural seed banks; germination; seedling viability and establishment

49. Cassava seed is subject to a dormancy period of various lengths, depending on the genotype. Seeds falling to the soil become dormant, forming seed banks from which plants may germinate (Pujol et al., 2002; Andersson and de Vicente, 2010). Seeds can remain viable when stored under ambient conditions for up to one year, although germination percentages may decline substantially after six months (Kawano, 1980; Rajendran, 2000). Seeds will remain viable and dormant for several years under cool (4°C), humid (70 to 80% relative humidity), and dark conditions, which are unfavourable for germination (Byrne, 1984; Pujol et al., 2002; Halsey et al., 2008; Lebot, 2009).

50. Seed scarification has mixed success in breaking dormancy, but several successful thermal treatments, involving exposure to 35°C, have been developed to shorten dormancy and increase germination frequency (Pujol et al., 2002; Nassar and Ortiz, 2006). The fact that germination is stimulated by dry heat suggests that cassava has evolved where fire cycles were common (Pujol et al., 2002).

3.2.5 Asexual propagation (apomixis, vegetative reproduction)

51. Because of the propensity for natural inter-varietal and interspecific hybridisation, cassava varieties are preserved through vegetative propagation. Farmers generally do not establish cassava crops using seed (Byrne, 1984; Halsey et al., 2008). As previously stated, many cassava varieties have become adapted to vegetative reproduction and flower little, if at all (Lebot, 2009).

52. Apomixis occurs frequently in *Manihot* species, including *M. esculenta*, and data indicates that the mechanism is apospory, the development of the gametophyte from the sporophyte without meiosis (Nassar, 2000). Apomixis is thought to have contributed to the rapid speciation of the genus by enabling interspecific *Manihot* hybrids living in naturally occurring micro-environments to develop into new species (Nassar, 2002).

SECTION IV - GENETICS

53. All *Manihot* species have the same chromosome number (2n = 36), and the species generally display normal diploid meiosis (Rogers and Fleming, 1973; de Carvalho and Guerra, 2002). Although *M. esculenta* has also been described as an allotetraploid with basic chromosome number 1n = 9 (Umanah and Hartmann, 1973), studies conducted on the meiotic behaviour of several cassava genotypes observed the formation of 18 bivalent chromosomes typical of a diploid. The amount of hybridisation noted between cassava and its wild relatives suggest that interspecific hybridisation barriers are fairly weak. In fact, no incompatibility systems have been identified in *Manihot* that prevent or inhibit crossing between species, and cassava chromosomes are observed to pair with those of even distant relatives (Byrne, 1984). Natural and artificial hybrids of cassava and *M. glaziovii* have been recorded (Lefèvre and Charrier, 1993; Second et al., 1997), and additional discussion of intraspecific crosses within the genus is presented under Section V.

54. Cassava does occasionally exhibit meiotic irregularities, possibly due to the almost exclusive use of vegetative propagation to produce the crop, which can result in the accumulation of somatic mutations (Hahn, Bai and Asiedu, 1990; Olsen and Schaal, 2007; Sardos et al., 2008). As a result, many cultivars display some sterility, typically due to one of several mechanisms by which the male flowers fail to mature and produce viable pollen (Byrne, 1984; Olsen and Schaal, 2007; Lebot, 2009).

55. The genetics of cassava and its relatedness to wild *Manihot* species has been examined using a variety of molecular tools, including isozyme analysis (Olsen and Schaal, 1999; Cabral et al., 2002); RAPD (Random Amplified Polymorphic DNA) (Nassar, 2000); RFLP (Restriction Fragment Length Polymorphism) (Beeching et al., 1993; Fregene et al., 1997); AFLP (Amplified Fragment Length Polymorphism) (Roa et al., 1997; Elias, Panaud and Robert, 2000); and SSR (Simple Sequence Repeat) and microsatellite markers (Elias et al., 2001; Duputié et al., 2007; Otti et al., 2011). Marker-assisted cassava breeding can assist with the selection of appropriate parentals and ultimately in the production of improved varieties (Lebot, 2009). Approximately 96% of the protein-coding sequences of one variety of cassava have been sequenced, revealing over 30,000 predicted genes (Prochnik et al., 2012). There are currently no studies available that show evidence of organellar inheritance of agronomically important traits in cassava.

56. Because of the propensity for natural interspecific hybridisation, cassava is highly heterozygous (Byrne, 1984; Alves, 2002). Many traditional varieties, when tested using microsatellite markers, have been found to be polyclonal (Sardos et al., 2008). Outcrossing within and between fields is common, and although cassava is vegetatively propagated using stem cuttings, seeds produced during the growing season or in previous seasons may fall to the ground and germinate. Because of the extent of the cassava seed bank in areas where the crop has been in cultivation for many years, some of these seedlings may represent varieties that are no longer grown (Elias et al., 2001). Thus, even with vegetative propagation, cassava fields may contain significant genetic diversity (Andersson and De Vicente, 2010).

57. Another source of variability comes from the difficulty in distinguishing different cassava varieties, and even different species of *Manihot*, solely by the use of morphological characteristics (Elias et al., 2001). Although some varieties have local names, the names are not indicative of genetic background, as names may be assigned to multiple varieties, or the same variety may bear several different names, depending on the region where it is grown (Elias, Panaud and Robert, 2000; Sardos et al., 2008).

Even the concept of a "variety" may vary from one culture to another, further complicating the understanding of cassava genetics (Peroni , Kageyama and Begossi, 2007).

SECTION V - HYBRIDISATION AND INTROGRESSION

5.1 Natural facility of interspecific crossing (extent, sterility/fertility)

58. The ancestry of cassava and its relatedness to other members of the *Manihot* genus remains a topic of active research, and additional light will be shed on these questions as more sophisticated genetic tools are employed (Allem, 2002). A relatively high rate of hybridisation, combined with the naturally occurring micro-environments in South and Central America, has contributed to rapid speciation (Nassar, 2000). Apparent hybrids between cassava and its wild relatives, such as *M. zehntneri*, have been observed growing at the margins of cultivated cassava fields. Pollen movement from cassava to wild relatives and vice-versa have been proposed as mechanisms by which both cultivated varieties and wild species can obtain new genetic diversity (Nassar and Ortiz, 2006).

59. Introgression may result in genetic enhancement of local landraces via gene flow from wild *Manihot* species, however evidence indicates that the genetic diversity of cassava is contained within the diversity of *Manihot*, so it appears that gene introgression from wild populations into cassava is not the primary driving force for the crop's evolution globally (Olsen and Schaal, 2007). Although field observations indicate that hybrids grow larger and more vigorously than the parents, heterosis may be limited to vegetative characteristics and may not be expressed as increased fertility or reproductive fitness (Duputié et al., 2007). It is possible, however, that such hybrids may exploit new ecological niches better than the parents, eventually resulting in speciation (Nassar and Ortiz, 2006; Duputié et al., 2007).

60. Although manual interspecific crosses have been documented by many researchers, there is little available information regarding natural hybrids between cassava and its wild relatives (Nassar, 2003; Duputié et al., 2007). The absence of synchronous flowering has been proposed as one reason why hybridisation between cassava and its wild relatives is not seen more frequently (Nassar, 2003; Andersson and De Vicente, 2010). Many varieties of cassava have extended flowering periods, which could overlap with those of nearby wild plants, and it is proposed that greater evidence of hybridisation will be found with the increased use of molecular genetics tools (Duputié et al., 2007). Data on the viability of the seeds from presumed hybrids is generally lacking (Andersson and De Vicente, 2010), but fertile hybrids between cassava and its presumed progenitor, *M. esculenta* ssp. *flabellifolia* have been found in nature (Duputié et al., 2007). *M. glaziovii* (*M. carthagenensis* ssp. *glaziovii*) is the only species within *Manihot* that is known to have naturalised in Africa, and natural hybrids between cassava and *M. glaziovii* have been found in Africa, although pollination frequencies are low (Halsey et al., 2008; Lebot, 2009; Andersson and de Vicente, 2010).

5.2 Experimental crosses

61. No genetic barriers to crosses between cassava genotypes have been identified, but manual crosses can be difficult to make due to the need for synchronous flowering (Halsey et al., 2008). In addition, the high heterogeneity of cassava can complicate breeding efforts due to uncertainty about the precise pedigree of the parental lines (Okogbenin et al., 2007). To address this heterozygosity, various molecular techniques, such as the use of microsatellite markers, are employed to verify genotypes of parental plants (Otti et al., 2011).

62. Some data indicate that the use of insect vectors for pollination, rather than pollination by hand, results in a greater number of successful hybridisations (Nassar, 2000). Bridge species, such as *Manihot neusana* which more readily cross both with *M. esculenta* and other wild *Manihot* species, can be used to move genes between species which do not cross well (Nassar, 2003). Another technique that has been observed to increase the success of manual crosses is the use of "mentor" pollen —pollen of the same species as the maternal plant that is devitalised by freezing and mixed with the pollen from the desired male parent (Nassar, 2003).

63. Experimental interspecific crosses, between cassava and its wild relatives, have been documented in literature. Very often, considerable effort such as embryo rescue is needed to ensure success of the interspecific crosses. These crosses result in varying levels of hybrid fertility (Nassar, 2000). Spontaneous tetraploids and triploids have also been observed in the progeny of crosses between cassava and the related species *M. epruinosa* and *M. glaziovii* (Hahn, Bai and Asiedu 1990). Some triploids show desirable qualities, such as increased vigour, higher starch accumulation, longer lasting leaves, and some farmers select such triploids for vegetative propagation (Lebot, 2009).

64. Interspecific crosses have been used in a few cassava improvement programs. For example, genes conferring resistance to cassava mosaic disease (CMD) and cassava bacterial blight (CBB) have been moved from *M. glaziovii* into cassava (Hahn, Bai and Asiedu, 1990), and backcross derivatives from interspecific hybrids between cassava and *M. glaziovii* have been released as successful varieties in Africa, for instance TMS 30572 ("Migyera") (Jennings and Iglesias, 2002). Hybrids between cassava and *M. oligantha* show increased protein levels and reduced cyanide production in the roots (Lebot, 2009). An interspecific hybrid between cassava and *M. walkerae* was identified with delayed onset of post-harvest physiological deterioration, and several other *Manihot* species have been identified with high protein, high DM content, and green mite resistance (Fregene et al., 2006).

65. Three accessions of *M. esculenta* ssp *flabellifolia* were hand-crossed with seven varieties of cassava, and the paternity of the interspecific hybrids was investigated using 24 microsatellite markers (SSRs). The rate of hybridisation success varied from 17 to 92% and the data demonstrated that SSR markers can be routinely used in breeding programs to verify the paternity of interspecific crosses of cassava (Vieira et al., 2012b).

5.3 Information and data on introgression

66. There are limited studies on introgression in cassava. For natural hybridisation to take place between a wild relative and cassava, they must be in close proximity, i.e. less than 30 m (Andersson and De Vicente, 2010) and they must also be flowering simultaneously, with the concurrent presence of effective insect pollinators. When cassava was inter-planted with either *M. anomala* or *M. neusana*, putative hybrid seed was produced but seedling viability was poor, and the few surviving hybrids were identified by morphological characteristics, not by molecular methods (Nassar, 2003). Recent data has shown that through controlled hybridisations, genes for high DM content, high protein content of storage roots, and delayed post-harvest physiological deterioration were introduced from wild relatives to cultivated cassava varieties (Ojulong et al., 2008; Morante et al., 2010; Okogbenin et al., 2012). In such cases, the interspecific hybrids were semi-fertile and recovered through embryo rescue techniques (Akinbo et al., 2010).

SECTION VI - PLANT DEVELOPMENTAL STAGES

67. Cassava is a perennial shrub that can grow indefinitely, alternating periods of vegetative growth, storage of carbohydrates in the roots, and periods of dormancy. During its growth, there are distinct developmental phases. The occurrence, duration and existence of each phase depend on several factors related to varietal differences, environmental conditions and cultural practices. The plant developmental stages under favourable conditions in the field, expressed in days after planting (DAP), are as follows:

i) Sprouting from stem cuttings, 5 to 15 DAP

From 5 to 15 DAP, the first adventitious roots arise from the basal cut surface of the stake and occasionally from the buds under the soil. The first sprouting occurs 10 to 12 DAP, followed by the emergence of small leaves within 15 DAP (Conceiçao, 1979).

ii) Beginning of leaf development and formation of root system, 15 to DAP

The true leaves start to expand around 30 DAP when the photosynthesis process starts to contribute positively to plant growth. Before 30 DAP, shoot and root growth depends on the reserves of the stem cutting. The fibrous roots start to grow, replacing the first adventitious roots. These new roots penetrate the soil, reaching 40 to 50 cm deep, and function in water and nutrient absorption (Conceiçao, 1979). A few fibrous roots (3 to 14) will become storage roots, which can be distinguished from fibrous roots, beginning from 60 to 90 DAP (Cock et al., 1979). At 75 DAP the storage roots represent 10–15% of total DM.

iii) Development of stems and leaves (canopy development), 90 to 180 DAP

Maximum growth rates of leaves and stems are achieved in this period, and the branching habit and plant architecture is defined. From 120 to 150 DAP the leaf canopy closes (Veltkamp, 1985). Maximum canopy size and maximum DM partitioning to leaves and stems are accomplished (Távora et al., 1995). The storage roots continue to bulk. The most vegetative growth occurs during this period (Ramanujam, 1985).

iv) High carbohydrate translocation to roots, 180 to 300 DAP

Photoassimilate partitioning from leaves to roots increases, accelerating the bulking of storage roots. The highest rates of DM accumulation in storage roots occur within this period (Peressin et al., 1998). Leaf senescence increases, hastening rate of leaf fall, and in this stage the stem becomes lignified (Conceiçao, 1979).

v) Dormancy, 300 to 360 DAP

Rate of leaf production is decreased in this stage. Almost all the leaves fall and shoot vegetative growth is finished. At this stage maximum DM partition to the root is attained. This phase occurs primarily in the regions with a distinct cool, dry season (Lebot, 2009).

SECTION VII - GENERAL INTERACTIONS WITH OTHER ORGANISMS

68. Because canopy closure in cassava fields can occur fairly late in the growing season, there is a window of time, as long as 4 months, during which weeds can establish, competing with the developing crop for water and nutrients. Vigorous, fast-growing cassava varieties are less sensitive to competition from weeds, but they tend to produce greater amounts of above ground mass at the expense of storage root mass (Leihner, 2002).

69. Significant root yield losses can be caused by predation by mites, thrips, scales, whiteflies, and mealybugs. Major diseases include cassava mosaic disease, cassava brown streak disease, cassava root rot diseases, cassava bacterial blight, anthracnose and super-elongation. Common pests and pathogens are presented in Annex I to this document. Cassava breeders have identified resistance genes to some of the more significant insect pests and diseases of cassava in several wild *Manihot* species (Byrne, 1984). Cassava breeding and improvements obtained through biotechnological techniques, or contemplated for future developments, is presented in Annex II to this document.

ANNEX I - COMMON PESTS AND PATHOGENS

70. Because cassava is a low-value crop, it is typically grown with minimal inputs, and insecticides and fungicides are seldom used by smallholder farmers (Bellotti, 2002). In addition, yield reductions due to insects and diseases may be overshadowed by those caused by low soil fertility and moisture stress (Hillocks and Wydra, 2002). To date, smallholder farmers have relied on cultural practices and native resistance in cassava to mitigate insect pests and pathogens (Byrne, 1984), but crop losses from pests and diseases are often significant (Bellotti, 2002). Due to the genetic heterogeneity of cassava, resistance to major pests and pathogens varies widely among the hundreds of varieties in common use across the tropics. Unfortunately, even when resistant varieties are identified, farmers may be unwilling to switch varieties, mainly because the new varieties do not have other traits they prefer. The situation is worsened by the almost universal practice of vegetative propagation for cassava, which results in the accumulation of systemic infections in the crop (Bellotti, 2002; Okogbenin et al., 2007). As cassava production shifts to large-scale farms, disease and insect pressure are expected to increase.

1.1 Diseases

71. Cassava mosaic disease (CMD) is the most significant cassava disease in Africa. It is caused by geminiviruses that are vectored by the whitefly, *Bemisia tabaci* (Taylor et al., 2004). Several cassava geminivirus species, distinguishable by serological and molecular tools, and genome sequence information, affect cassava in Africa, India, and Sri Lanka. The prevailing view is that CMD is endemic to Africa and did not co-evolve with cassava in Latin America (Calvert and Thresh, 2002). The viruses causing CMD distort the leaves and restrict growth thereby reducing root yields, but quantifying losses is difficult (Calvert and Thresh, 2002). Overall, storage root yield losses across sub-Saharan Africa were estimated at 15 to 24 % annually, which is equivalent to 12to 23 million tonnes or an annual loss of US$ 1.2 to 2.3 billion (Calvert and Thresh, 2002). There are several resistant varieties available, but some farmers choose to grow traditional varieties instead, in spite of their susceptibility to the disease. Cultural practices such as using virus-free planting material and culling diseased plants, can help manage losses from CMD (Calvert and Thresh, 2002). Central and South American varieties are susceptible to CMD but the vector for the viruses is largely absent from the region, although *B. tabaci* and a new biotype, *B. argentifolia* have been found in the Americas, making the need for CMD-resistant varieties even more crucial (Bellotti, 2002; Okogbenin et al., 2007). The use of resistant varieties is the most effective measure for the control of CMD in many African countries (Mahungu, Dixon and Kumbira, 1994). Two major sources of resistance genes have been used; genes derived from *Manihot glaziovii* and the CMD2 gene from West African landraces (Fregene et al., 1997; Akano et al., 2002), and some success has been achieved in moving these genes into cassava to produce highly resistant varieties (Calvert and Thresh, 2002). However, CMD often results in significant storage root yield losses that can occur even in resistant genotypes that show only mild or no foliar symptoms (Seif, 1982).

72. Cassava bacterial blight disease (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (*X. campestris* pv. *manihotis*), kills both leaves and young shoots via systemic infection (Hillocks and Wydra, 2002). The disease can not only cause the loss of the root crop, but also make the stems unusable for propagation. Although less common in Asia, CBB is present in most areas where cassava is grown. The bacterium is vectored by grasshoppers and can also be spread via contaminated stakes and seed (Hillocks and Wydra, 2002; Lebot, 2009). CBB can be managed via crop sanitation, cultural practices, and crop rotation, and seeds can be effectively disinfected using heat treatments (Hillocks and Wydra,

2002). Resistant varieties have been identified, but resistance has been overcome by increasingly virulent strains of the bacterium (Fregene, 2002; Hillocks and Wydra, 2002).

73. Cassava brown streak disease (CBSD) is a viral disease that causes elongated necrotic lesions in storage roots (Figure 7), and variable symptoms on stems and leaves. CBSD is caused by two virus species: *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV), classified in the genus *Ipomovirus* (Family *Potyviridae*) (Mbanzibwa et al., 2009; Monger et al., 2010; Winter et al., 2010). Yields are reduced by severe infections (Hillocks et al., 2008), but the more important impact is on the quality of storage roots, since the necrotic lesions render roots unusable (Calvert and Thresh, 2002). The disease is spread through the planting of infected stem cuttings (Taylor et al., 2004), and there is evidence of spread via an arthropod vector, possibly whiteflies (Calvert and Thresh, 2002). Although known to have been present in East Africa since the 1930s, CBSD was mostly confined to the coastal regions and around Lake Malawi (Legg et al., 2011) until a new outbreak was identified in Uganda in 2004 (Alicai et al., 2007). Since that time, the disease has developed to epidemic proportions, representing a significant constraint to cassava production throughout Eastern and Central Africa (Lebot, 2009; Campo et al., 2011; Legg et al., 2011; Jarvis et al., 2012).

Figure 7. **Healthy cassava root tissue (left), brown streak disease infected (right)**

Source: ASARECA

74. Three other viral diseases, cassava common mosaic disease, cassava vein mosaic disease and cassava frogskin disease, are of lesser importance in term of crop loss. They occur in South America and are generally controlled by planting virus-free stock and culling infected plants. There is no effective resistance to any of these diseases (Calvert and Thresh, 2002; Lebot, 2009).

75. Angular leaf spot, caused by *X. campestris* pv. *cassavae* is prevalent in Africa and can cause defoliation when severe (Lebot, 2009). However, unlike cassava bacterial blight, angular leaf spot does not result in systemic infection of the plant (Hillocks and Wydra, 2002).

76. Stem and root rots, caused by *Erwinia carotovora* ssp. *carotovora*, occur in South America and Africa and result in yield losses and the destruction of planting stock. The bacterium is vectored by fruit flies (e.g., *Anastrepha* spp.), and the planting of fruit fly resistant varieties and spraying to kill the flies can help control the disease (Hillocks and Wydra, 2002; Lebot, 2009).

77. Various leaf spot and stem diseases of cassava, occurring worldwide, are caused by several species of fungi such as *Cercospora*, *Phoma*, and *Colletotrichum*. Disease severity is generally worse in humid regions, but infestations resulting in significant yield loss are uncommon. Some resistant varieties have been identified, but there are no other effective control measures (Hillocks and Wydra, 2002; Lebot, 2009).

78. Several fungal root rot diseases are caused by *Phytophthora, Pythium, Fomes, Sclerotium*, and *Armillariella*, and when severe, these pathogens can cause significant or complete loss of storage roots. However, these diseases occur sporadically and usually under specific conditions, such as in poorly drained soils, or in recently cleared forest land. Varieties differ in resistance to these diseases (Hillocks and Wydra, 2002).

1.2 Arthropods

79. Many arthropod pests have co-evolved with cassava, and these species are much more prevalent in South America than in either Asia or Africa. However cassava is also subject to predation by generalist feeders wherever it is grown. Generally, insect damage is more severe in drier climates and during dry seasons in humid climates, and plants may be able to recover from predation with adequate rainfall or irrigation (Bellotti, 2002).

80. Managing insect damage in cassava is extremely challenging, especially for smallholder farmers. Pesticide use is usually precluded by the high cost; moreover, pesticides may disrupt the activity of existing natural enemies in the environment. For specific pests, cultural practices such as intercropping may mitigate crop losses, but these practices are not universally effective, and large-scale production may preclude the use of some of these practices (Bellotti, 2002). Resistant cultivars are not available for most arthropod pests, and while some resistance has been found in wild *Manihot* species, moving these traits into desirable cassava varieties is a long process (Bellotti, 2002).

81. The cassava green mite is the common name for approximately 40 different mite species, for example *Mononychellus tanajoaI* present in South America and Africa, and *Tetranychus urticae* present in South America and Asia. Mites, which harm the growing points and young leaves, can cause stunting when infestations are severe (Bellotti, 2002). They can be controlled by predatory mites (*Typhlodromalus aripo* and *T. manihoti*). Control by a fungus (*Neozygites tanajoae*) is the subject of ongoing research (Bellotti, 2002; Nassar and Ortiz, 2006; Lebot, 2009), and a few varieties have been identified with low to moderate mite resistance (Bellotti, 2002).

82. *Phenacoccus manihoti* and *P. herreni* are the predominant species of mealybug in South America and Africa, causing leaf damage, shoot malformation, and even yield losses when infestations are severe. There is little native resistance to mealy bugs (Bellotti, 2002), but various parasitic wasps (e.g. *Apoanagyrus lopezi*) have been effective in their control (Lebot, 2009).

83. Many species of whiteflies, of which *Aleurotrachelus socialis* is predominant, cause significant cassava crop losses due to photosynthate loss from phloem feeding. Research is underway to identify varieties with useful resistance as well as appropriate whitefly parasitoids (Bellotti, 2002). The whitefly, *B. tabaci*, is a major pest of cassava, particularly in Eastern Africa, where it is responsible both for the transmission of viruses that cause CMD and CBSD, and increasingly for direct damage due to feeding by high populations (Omongo et al., 2012).

84. In South America, the cassava stem borer (*Chilomina clarkei*) causes damage by feeding internally on stems, resulting in stem breakage. Although borer damage does not usually result in significant yield loss, they do reduce the amount and quality of planting material available for

the next year's crop (Bellotti, 2002). Traditional pesticide sprays are ineffective against the borer because the insect causes much of its damage while inside the stem, protected from externally applied sprays (Taylor et al., 2004; Lebot, 2009). Research is ongoing to identify effective natural enemies and resistant varieties (Bellotti, 2002).

85. The hornworm (*Erinnyis ello*) is a serious cassava pest in South America, which feeds on young leaves and stems and can completely defoliate the plant. Although the plants typically recover, the weakening of the plant can result in large yield losses. Effective and inexpensive control of hornworm has been achieved using sprayed suspensions of a Baculovirus (Bellotti, 2002; Lebot, 2009). Control by natural predators has limited effectiveness, due to the migratory behaviour of the hornworm adults, resulting in the deposition of large numbers of eggs that hatch while predator populations are too low to provide control. Better monitoring of hornworm migrations and synchronizing predator release with egg laying may result in more effective control (Bellotti, 2002).

86. *Cyrtomenus bergi* is polyphagous, feeding on storage organs of many crops. In cassava growing areas, it is known as the cassava burrower bug. Cassava is not its preferred host, and the bug tends to avoid cassava varieties that produce higher levels of cyanogenic glucosides. Root feeding allows infection by any of several soil-borne fungal pathogens, causing lesions in the root tissue and reducing starch content. Severe infestations by the burrowing bug can cause significant crop losses (Bellotti, 2002).

1.3 Other

87. Yield losses due to nematodes such as *Meloidogyne* and *Pratylenchus* are difficult to measure, and nematodes are not generally regarded as serious pests on cassava. However, crop damage can increase over many seasons, as nematode populations build up, and when this occurs, the planting of resistant varieties is advisable (Hillocks and Wydra, 2002).

ANNEX II - BIOTECHNOLOGICAL DEVELOPMENTS

88. Given the importance of cassava as a source of dietary calories in the tropics, there is a great deal of interest in using biotechnology to improve the crop to increase nutritional quality, reduce pre- and post-harvest losses, decrease cyanogenic potential of the edible parts of the plant, and to develop disease-resistant varieties (Taylor et al., 2004, 2012; Lebot, 2009).

89. Although cassava was at first recalcitrant to plant tissue culture methods, using plant transformation to obtain transgenic cassava plants became possible in the mid-1990s. Typically, researchers use embryogenic tissue from a variety of explants, and cell transformation is accomplished using biolistic methods or *Agrobacterium tumefaciens* (Fregene, 2002; Taylor et al., 2004). Challenges to the use of biotechnology to produce improved cassava varieties include the requirement that gene expression remain at effective levels after many generations of vegetative reproduction, and the difficulty in achieving homozygosity in a largely heterozygous crop. The development of double haploid cassava lines is under investigation to assist with this limitation (Taylor et al., 2004; Aerni, 2006). Also, transgenic traits must be made available in a wide range of varieties that farmers want to use. Ideally, important landraces would be transformed with traits of agronomic significance, but there can be considerable variability in the culturability of individual landraces, even when the landraces are related (Taylor et al., 2004).

2.1 Nutritional improvements

90. There is ongoing research into the enhancement of micronutrient and vitamin content (such as zinc, iron, and vitamin A/ß-carotene) of cassava through genetic engineering (Fregene, 2002; Taylor et al., 2004; Sayre et al., 2011). Modifying starch quality and enhancing the production of sugars in the storage roots is also under investigation (Fregene, 2002).

91. To increase protein content of cassava storage roots, tissue-specific production of an artificial storage protein is being attempted (Fregene, 2002; Taylor et al., 2004; Sayre et al., 2011). Efforts are underway to increase starch synthesis and accumulation, for both food and industrial purposes, and to reduce starch grain size, largely for industrial uses.

92. In addition to directly improving the storage root quality, there are efforts underway to improve foliage quality, specifically the longevity of the leaves. Leaves that remain photosynthetic longer contribute to higher root yields, and in regions where the leaves are also consumed, long-lived leaves add to the overall value of the crop (Fregene, 2002).

93. Efforts to reduce the release of cyanide from cassava tissues focus on either reducing the production of the cyanogenic glycosides, or increasing the rate of breakdown of the glycosides. In the first instance, the approach is to use anti-sense constructs to reduce the synthesis of a cytochrome P450 that catalyses the first step in the synthesis of linamarin and lotaustralin. In the second case, the approach is to increase the synthesis of hydroxynitrile lyase, which catalyses the breakdown of acetone cyanohydrin into acetone and hydrogen cyanide (Fregene, 2002; Taylor et al., 2004).

2.2 Pre- and post-harvest losses

94. Lepidopteran insects, particularly stem borer (*Chilomina clarkei*) and hornworm (*Erinnyis ello*), cause major cassava crop losses in Latin American. Lepidopteran insect control using a transgene from *Bacillus thuringiensis* producing one of the Bt proteins, is under investigation, and experimental plants display resistance to both species (Fregene, 2002; Taylor et al., 2004).

95. CMD and CBSD are the greatest constraints to cassava production, and resistance to both diseases is being addressed from a variety of gene-silencing approaches (Taylor et al., 2004, 2012; Ogwok et al., 2012).

96. Efforts are underway to use genetic engineering to reduce post-harvest deterioration of the storage roots, beginning with elucidating the physiological steps involved in the process (Taylor et al., 2004). The reduction and control of reactive oxygen species is a main focus of these efforts (Sayre et al., 2011).

97. Efforts to create herbicide-tolerant cassava varieties through genetic engineering are ongoing (Fermont et al., 2009). Herbicide-tolerance is a trait perceived to be of particular value for industrial-scale cassava production (Taylor et al., 2004); however, herbicide-tolerant cassava might also reduce the high labour costs of manual weeding for smallholder farmers.

2.3 Other traits

98. There is increasing interest in the development of cassava as an industrial crop, specifically in the use of cassava in the production of biodegradable plastics. Research is underway to produce plastic precursors, such as polyhydroxyalkanoates in cassava (Fregene, 2002; Taylor et al., 2004; Lebot, 2009).

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