BOTANICAL AND AGRONOMIC EVALUATION OF A COLLECTION OF *SESBANIA SESBAN* AND RELATED PERENNIAL SPECIES

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Proefschrift

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in de landbouw- en milieuwetenschappen,
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Stellingen

1. De door Brewbaker geopperde veronderstelling dat zelf-incompatibiliteit de norm zou zijn in de houtige, diploïde (2n = 12) soorten die behoren tot de familie *Sesbania* is niet bewaarheid.

Dit proefschrift.

Brewbaker, J.L., 1990. Breeding systems and genetic improvement of perennial Sesbanias. In: Macklin, B. and Evans, D.O. (Eds.) *Perennial Sesbania Species in Agroforestry Systems.* Proceedings of a workshop held in Nairobi, Kenya, March 27-31, 1989. pp39-45. Nitrogen Fixing Tree Association, Hawaii.

2. De aanwezige variatie in morfologische, agronomische en nutritionele kenmerken in *S. sesban* kan gebruikt worden voor de selectie van hoog produktieve accessies met een hoge voederwaarde.

Dit proefschrift.

3. Uit de karyotypen analyse en hybridisatie experimenten blijkt dat er een nauwe phylogenetische relatie bestaat tussen de soorten *S. sesban, S. goetzei* en *S. keniensis.* De classificering volgens erfelijke eigenschappen (cladogram) zoals beschreven door Ndungu en Boland zou dus aangepast moeten worden.

Dit proefschrift.

Ndungu, J.N. and Boland, D.J., 1994. *Sesbania* seed collection in Southern Africa. Developing a model for collaboration between a CGIAR Centre and NARS. *Agroforestry Systems* 27:129-143.

4. Morfologische verschillen tussen de *Sesbania* variëteiten *nubica* en *sesban* worden vooral gevonden in de blaadjesgrootte en -aantal, zaadgrootte en -kleur en in de groeivorm.

Dit proefschrift.

5. Er bestaat een goede correlatie tussen de totale droge stof opbrengst in *S. sesban* en diameter van de stam op 30 cm hoogte en boomhoogte. Deze snel groeiende boom volgt daarmee de traditionele vergelijkingen, zoals die in de bosbouw ontwikkeld zijn.

Dit proefschrift.

6. Bij de evaluatie en selectie van multipurpose boomsoorten, hoort een multidisciplinaire onderzoeksbenadering.

Dit proefschrift.

7. De intensivering van de gewas-vee interacties in sub-Sahara Afrika zal de introductie en het gebruik als veevoer van bomen en struiken met meerdere gebruiksdoelen versnellen.

8. De grootste uitdaging voor fundamenteel onderzoek op het gebied van de voeding van meermagigen met ruwvoer van bomen en struiken is het ontwikkelen van simpele *in vitro* screening methodes op anti-nutritionele factoren, die een hoge correlatie hebben met de *in vivo* voederwaarde.

9. In hoeverre kan valsheid in geschriften strafbaar worden gesteld voor regeringsleiders die vredesakkoorden of overeenkomsten tot een staakt het vuren ondertekenen en vervolgens negeren?

10. De uitspraak 'voetbal is oorlog' is van toepassing op wat er zowel op het voetbalveld als er omheen gebeurt.

11. In landenorganisaties kunnen de afzonderlijke lidstaten niets doen, maar tezamen kunnen ze het besluit nemen dat er niets gedaan kan worden.

12. De informatie van een kabelkrant op televisie zal meer tot zijn recht komen als de individuele gebruiker zelf kan bepalen welke onderwerpen hij wil lezen.

Stellingen van Hero Heering, behorend bij het proefschrift: 'Botanical and agronomic evaluation of *Sesbania sesban* and related perennial species'.
Wageningen, 21 april 1995.

Abstract

Heering, J.H., 1995. Botanical and agronomic evaluation of a collection of *Sesbania sesban* and related perennial species. Doctoral thesis, Wageningen Agricultural University, Wageningen, The Netherlands, x+127 pp, English and Dutch summaries.

The species *Sesbania sesban* has many attributes which make it attractive as a multi purpose tree for different agricultural production systems. This thesis focuses on the evaluation and classification of a *S. sesban* collection on morphological, agronomic and nutritional characteristics. It provides information on the chromosome numbers, breeding system and interspecific relations of *S. sesban* and the related perennial species *S. goetzei* and *S. keniensis*. It also demonstrates the multi disciplinary research approach which is required for the development of multi purpose tree germplasm.

The three species have a chromosome number of $2n=12$ and are able to produce viable seeds after interspecific hybridization. They are both self and cross compatible, although outbreeding is thought to be the common method of reproduction under natural conditions. The classification shows that the *S. sesban* accessions in the collection contain large variation in morphological, agronomic and nutritional characteristics. Through numeric analysis it is possible to identify distinct groups of accessions within the collection. This group structure can be used for the selection of accessions low in polyphenolics and with a high agronomic productivity.

The species is at the moment relatively underutilized and there seems to be scope for a greater use of it, particularly in alley farming or for improved fallow. *S. sesban* has definite potential for the highlands and could further be introduced to the higher rainfall areas of the semi-arid zone and in some subhumid areas. It could be very useful to reclaim some of the areas with saline and alkaline soils and could also be grown in places prone to seasonal waterlogging and flooding.

The areas which require future research attention are among others, the collection of germplasm, the identification of anti-nutritional factors and the further adjustment of the management techniques to the systems in which the species is being used.

Key words: *Sesbania sesban, Sesbania goetzei, Sesbania keniensis,* agroforestry, karyotypes, hybridization, reproductive biology, classification, morphology, agronomy, fodder, seed production, HPLC fingerprints, polyphenolics.

Acknowledgements

The topic of this thesis originated in 1989 when I started my work as a bilateral associate expert funded by the Directorate-General International Co-operation (DGIS) of the Dutch Ministry of Foreign Affairs at the Forage Genetic Resources Section of the International Livestock Centre for Africa (ILCA) in Addis Ababa, Ethiopia. I am very grateful to Dr. Jean Hanson, Head of the Forage Genetic Resources Section for her guidance, support and encouragement in carrying out this research. She also introduced me to the finesses of plant genetic resources and genebank management.

Professor L. 't Mannetje of the Department of Agronomy of the Agricultural University Wageningen accepted the subject as a possible topic for a thesis and for his long distance supervision as well as for his suggestions and encouragements I am deeply grateful. I also thank the Department of Agronomy for accepting me as a guest worker and for providing financial support for publishing this thesis. Mr. Koop Wind assisted with the final lay out of the document.

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Last but not least, I am grateful to my wife Marinda, whose understanding and confidence in my abilities were real encouragements; without these I would not have succeeded.

Hero Heering.

Related publications

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General introduction

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General introduction

The use of tree legumes for livestock

Livestock and especially ruminants are an essential component of most of the agricultural production systems in sub-Saharan Africa. The productivity and sustainability of the livestock industry is influenced by technical and socio-economic factors. One of the major constraints to increasing livestock productivity in this region is the feed supply, followed by animal health and management practices (Winrock, 1992). In the drier areas, the quantity of natural forages is often insufficient, whereas in the wetter areas the feed supplies are usually ample but their protein and energy concentrations are low and they are therefore of poor quality. In both areas feed shortages and nutrient deficiencies occur mainly in the dry season.

The increased utilization of leguminous and other tree and shrub species, fed as a high quality supplement to the low quality natural pastures and crop residues, has been recognized as one of the means of improving the forage supplies to ruminants in pastoral and crop-livestock systems. Although a wide range of shrubs and trees has been identified as suitable for feeding ruminant animals in the tropics (Le Houérou, 1980; Topark-Ngarm, 1990) special attention has been given to leguminous species (Brewbaker, 1986; 1989). There are many reasons for the interest in the use of tree legumes in animal agroforestry. The possession of a long tap root enables the trees and shrubs to provide high quality green foliage even during most part of the dry season and once established, recycle plant nutrients from depths inaccessible to crops and pastures. Leguminous tree species can improve soil fertility through nitrogen accumulation and help prevent soil erosion. As well as providing forage they can also be an important source of fuelwood, a commodity which is often in short supply especially in the areas with high population density.

Most research on leguminous trees has been confined to the two species *Leucaena leucocephala* and *Gliricidia sepium*, which are used to a large extent in alley cropping systems in the humid zone of sub-Saharan Africa (Atta-Krah and Kolawole, 1987; Atta-Krah and Sumberg, 1988). *Leucaena* is however less adapted to acid soils and to the arid and highland zones (Jones, 1979), whereas the introduction and use of *Gliricidia* is limited by palatability problems (Simons and Stewart, 1994). Several other species are therefore being evaluated for use under these agro-ecological conditions. The infestation of *L. leucocephala* with a sap sucking psyllid *Heteropsylla cubana* which caused considerable reductions in yield and in some cases even severe mortality of the trees, has stimulated a further effort to obtain species diversification (MacDicken, 1990).

Multipurpose tree selection and evaluation

There are several desirable agronomic characteristics of shrubs and tree species that are relevant to their potential as livestock forage in agroforestry systems. The trees should produce high yields of edible material, which is of good quality in terms of protein and mineral concentration, palatability and digestibility and remain productive under repeated cutting or grazing. They should be relatively easy to establish, exhibit a rapid early growth and show good competitive ability against weeds. They should also have a deep rooting system with efficient nutrient uptake abilities and be resistant to local pests and diseases. Finally, they should have an adequate seed production or be easily vegetatively propagated.

At present the selection of suitable multipurpose tree germplasm for a particular use in agroforestry systems has mainly been at species level. Experience with *Leucaena leucocephala* however, showed the large genetic diversity that exists within a species and the high gain that is potentially available when evaluation trials with different accessions or provenances are carried out (Bray, 1994). The basis of a forage development program therefore should be a wide range of germplasm to be used for the selection and evaluation of promising material. The plant genetic resources centres collect or acquire, characterize, evaluate, maintain and disseminate plant germplasm which is adapted to the various environments and ecosystems that are encountered and can provide the basic material for selection and or breeding (MacIvor and Bray, 1983)

Genetic resources information and documentation

The usefulness of such germplasm collections for researchers and breeders is largely determined by adequate and relevant documentation and information about the material it contains (IPGRI, 1993) and on a proper taxonomic identification. The need for proper documentation on germplasm collections has been outlined in several publications (Engels, 1986; Stalker and Chapman, 1989). IPGRI now uses the following definitions in genetic resources documentation for collection, characterization and evaluation:

1. Passport data (identifiers and information recorded by collectors);
2. Characterization (consists of recording those characters which are highly heritable, can be easily seen by the eye and are expressed in all environments);
3. Preliminary evaluation (consists of recording a limited number of additional traits thought desirable by a consensus of users of a particular crop);
4. Further evaluation (consists of recording a number of additional descriptors thought to be useful in crop improvement).

The data obtained from the characterization and preliminary evaluation from large groups of accessions are frequently subjected to numerical analysis to understand the variation within that particular collection. In the maintenance and use of large genetic resources collections some group structure is required so that decisions can be made regarding the selection of material for evaluation, seed multiplication and breeding. Although general descriptors of growth form are available for most of the multi purpose tree species, there is often inadequate information at the provenance level (Huxley, 1984).

In order to develop proper seed multiplication techniques to maintain the genetic integrity in germplasm collections information on the cytogenetics, on interspecific relations, and on the breeding system is essential. For many species however, this information is lacking and this is a major constraint to the provision of forage and multipurpose tree germplasm. Finally, information on the seed production capacity of a species and a sufficient supply of seeds is essential for its development and future utilization (Hopkinson and Eagles, 1980).

General objectives and outline of the study

The overall objective of the present study was to provide some of the above mentioned information which is required for the development of the multipurpose tree species *Sesbania sesban*. The evaluation of the potential of multipurpose tree genetic resources requires a broad based multi disciplinary approach. This study ranging from the cytology and reproductive biology, the morphological and agronomic classification and to the *in vitro* characterization of chemical properties of *S. sesban* is aimed to exemplify such an approach.

A description of the multipurpose tree species *S. sesban* its utilization and properties is given in Chapter 2. Chapter 3 delineates the cytology and interspecific relations of *S. sesban* with two other perennial species which might have some potential as a forage resource, *S. goetzei* and *S. keniensis*. In Chapter 4 the flower biology of these three species is presented. Chapter 5 deals with the evaluation of six selected accessions with regard to seed, wood and forage production under two different cutting heights and frequencies. Chapters 6, 7 and 8 report on the characterization of a large *S. sesban* germplasm collection in terms of morphological attributes and their relation with taxonomy, agronomic characteristics, polyphenolic concentration and HPLC fingerprints. Chapter 9 gives a general discussion.

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Chapter 2

Sesbania sesban

Chapter 2

Sesbania sesban

Introduction

Some of the perennial *Sesbania* species have considerable potential for use in agroforestry as they show a rapid early growth, grow under various ecological conditions and do not require difficult management procedures. In Africa, most of the collection and evaluation studies that have been carried out so far were focused on *S. sesban*. Five varieties of *S. sesban* are recognized botanically, but it is not clear whether there is a relationship between botanical differences and their agricultural value. *S. sesban* var. *nubica* Chiov. is the most widely distributed variety in Africa (Figure 2.1). The varieties *sesban* and *bicolor* Wight & Arnot are very similar except for their flower colour. These three varieties have been noted for their rapid growth and high yields. The other lesser known

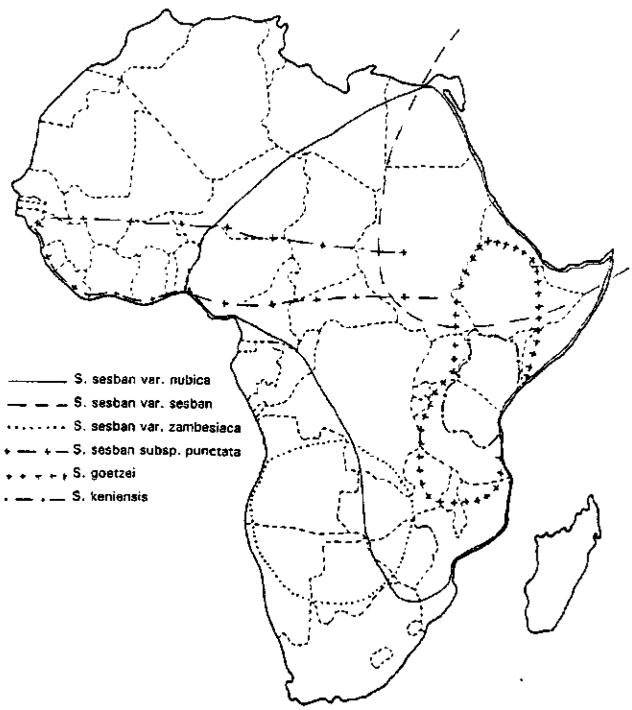

Fig. 2.1. Distribution of three perennial *Sesbania* species in Africa (after Gillett, 1963; 1971; Evans, 1990).

varieties are var. *zambesiaca* Gillett, which in the most recent review of the genus was considered to be synonymous with var. *nubica* (Lewis, 1988) and subspp. *punctata* (DC.) Gillett. Very little is known about the properties of the related species *S. goetzei* and *S. keniensis* which are also native to Africa and could have potential for use in agroforestry. This section will therefore mainly concentrate on the species *S. sesban* with particular emphasis on its environmental adaptation and utilization.

Ecology

S. sesban can be found in areas with a semi-arid to subhumid climate, with a rainfall between 500-2000 mm per year. In the regions with low precipitation however, they occur primarily on poorly drained soils which are subjected to periodic waterlogging or flooding. Because of its good tolerance to low temperatures (but no frost!) it can be grown up to an altitude of 2300 m.
S. sesban is adapted to a wide variety of soil types, ranging from loose sandy soils to heavy clays. It has an excellent tolerance to waterlogging and flooding (Shelton, 1994). The only experiments that have been carried out with regard to its nutrient requirements, studied the effects of phosphorus application which in most cases had a positive effect on growth and nodulation (Dutt and Pathania, 1984; Siaw *et al.*, 1993).

It is particular tolerant to both salinity and alkalinity. Ghai *et al.* (1985) reported that seeds of *S. aegyptica* (Syn. *S. sesban*) showed no reduction in germination up to an electrical conductivity level of 11 mmhos/cm (0.7% salt concentration) beyond which there was a drastic reduction to 67% and 25% germination at EC 15 and 18 mmhos/cm respectively. Hussain (1990) reported that *S. sesban* tolerated salt concentrations from 0.4-1.0% at seedling stage and from 0.9-1.4% as it reached maturity. In a trial by Hansen and Munns (1985) it was demonstrated that the plants are less sensitive to salinity after germination and seedlings could tolerate NaCl concentrations of 100mM with only a slight reduction in growth. The germination of seeds was positively effected under alkaline conditions up to pH 9.0, with only a small reduction in germination at pH 10.0.

Beyond this pH level the germination was decreased as well as delayed (Ghai *et al.*, 1985). Kumar (1983) revealed that *S. sesban* could even grow at pH 9.9, although the yields at 104 days after germination were reduced by 82% when compared to the control trees which were grown at pH 7.6. At pH levels 9.4 and 9.6 the reductions in forage yield were 19% and 60% as compared to the control. Little is known about the adaptation of *S. sesban* to acid soils, though a screening trial by Mugwira and Haque (1993) showed that some accessions yielded reasonably well when grown in a clay loam soil with pH 4.1.

Nutritive value

The leaves of *S. sesban* are high in protein, ranging in general between 20-25% of the dry matter (Table 2.1).

Table 2.1. Chemical composition (g/kg DM) of foliage from *S. sesban*.

crude protein	fat	ash	NDF	crude protein	ADF	lignin	reference+
264		68		387			1
213		88		369			1
306	53	103	272		169	30	2
265	9	100		122			3
260	26	76		144			3
156-287*			113-286			8-53	4
152	16	86		353			5
213		80	219		153	36	6
201-275**		84-142	160-295		136-188	35-54	7
244-300***			263-401		174-249	40-84	8
194		75		329			9

* range of 6 accessions
** range of 12 accessions
*** range of 11 accessions

+ References: 1. Akkasaeng *et al.* (1989); 2. Brown *et al.* (1987); 3. Gohl (1981); 4. ILCA (1989); 5. Lamprey *et al.* (1980); 6. Norton (1994) 7. Nsahlai *et al.* (1994); 8. Siaw *et al.* (1993); 9. Singh *et al.* (1980).

The *in vitro* dry matter digestibility often exceeds 65% because of a relatively low fibre content. The phosphorus and calcium concentrations in the edible portion are in general adequate for animal nutrition (Table 2.2). All the above mentioned properties indicate the potential of this species as a high quality fodder source. Anti-nutritional factors such as polyphenols and saponins might have an adverse effect on its nutritional value, but their role is not clearly understood.

Table 2.2. Mineral concentration (g/kg DM) in the foliage of *S. sesban.*

N	S	P	Ca	K	Na	reference+
42.2	2.7	2.4				1
48.9		3.3	21.5			2
42.4		4.3	27.8			3
41.6		2.7	11.1			3
16.0		2.8	22.1	18.4	0.7	4
39-48*		2.4-4.0	8.0-12.7	21.9-25.5		5
31.0		0.9	14.2			6

* range of 11 accessions.

+ References: 1. Ahn *et al.* (1989); 2. Brown *et al.* (1987); 3. Gohl (1981); 4. Lamprey *et al.* (1980); 5. Siaw *et al.* (1993); 6. Singh *et al.* (1980).

No acute toxicity has been reported when the foliage was either fed as a supplement to sheep (Reed *et al.*, 1990), as a sole feed to goats (Singh *et al.*, 1980) or grazed by heifer cattle (Gutteridge and Shelton, 1991). When fed to non ruminant animals (especially chicks) however, there were reports of high mortality (Topark-Ngarm and Gutteridge, 1990). In conclusion it can be said that the most efficient and safest means of using *S. sesban* foliage is as a supplement to low quality roughages for ruminants.

Soil improvement

S. sesban has also been used to some extent as a green manure in alley cropping systems. The incorporation of its foliage to the soil has contributed to an increase in productivity of several crops. Weerakoon (1990) obtained a rice grain yield of 3.9 t/ha after the incorporation of 4.4 t DM/ha of green manure, compared to 1.9 and 4.4 t/ha of grain for the control and 96 kg N fertilizer applied per ha respectively. It was further stated that its decomposition is fast with 71% of the leaf material decomposed after 14 days.

Onim *et al.* (1990) found that the incorporation of up to 13 t DM/ha of *S. sesban* mulch (adding 448 kg N/ha/year) improved the soils nutrient status considerably and thus gave a significant increase in the yield of maize and beans with residual effects lasting up to 3 years.

Establishment

S. sesban is normally propagated from seeds although research suggested that it can be established from stem cuttings (Evans and Macklin, 1990) and by tissue culture (Hanson, 1993; Zhao *et al.*, 1993). Scarification of the seeds is usually recommended to ensure a uniform germination. The seedlings will normally form root nodules with native rhizobia within 3-4 weeks after planting. Where the effective rhizobia are absent and effective nodulation does not take place inoculation with an appropriate *Bradyrhizobium* strain is necessary. Work by Masafu, cited by Dart (1994) has demonstrated that *S. sesban* accessions respond differently to particular rhizobium strains in terms of the effectiveness of the symbiosis.

S. sesban grows rapidly and normally flowers and produces ripe pods within the first year after planting. Flowering was found to be independent of day length over the range of 11-13 hr, but was inhibited by warm temperatures of 33 °C by day and 28 °C by night (Sedi and Humphreys, 1992).

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Chapter 3

Karyotype analysis and interspecific hybridization in three perennial *Sesbania* species (*Leguminosae*)

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Chapter 3

Karyotype analysis and interspecific hybridization in three perennial *Sesbania* species (*Leguminosae*)

Abstract

The somatic chromosome number in *Sesbania sesban* var *nubica*, *S. goetzei* and *S. keniensis* (*Leguminosae; Papilionoideae*) was found to be 2n=12. These findings were in agreement with earlier reports on *S. sesban* and *S. keniensis*. The chromosome number 2n=12 is a new record for *S. goetzei*. Similarities in karyotypes were found in the three species. All species had one pair of long metacentric chromosomes; the second pair was submedian, followed by four smaller pairs of metacentric chromosomes. Nucleolar organiser regions in the form of satellites were found on the short arm of the fourth chromosome pair in *S. sesban* and *S. keniensis*. Interspecific crosses in all possible combinations were carried out, resulting in pod and viable seed formation for the crosses *S. sesban* x *S. goetzei, S. sesban* x *S. keniensis, S. goetzei* x *S. sesban* and *S. goetzei* x *S. keniensis*. The two crosses with *S. keniensis* as a female parent were unsuccessful. The hybrid plants established normally and produced viable seeds.

Introduction

The genus *Sesbania* Scop. (*Leguminosae*) encompasses about 50 species, the majority of them being annuals, distributed in the tropics and sub tropics. They provide several products such as ruminant fodder, fuelwood, construction material, green manure and human food. Although the annual species have received some attention, recent research has focused on the perennial species.

Of the three species under study, *S. sesban* var *nubica* is the most widely distributed occurring in most of Africa (excluding the countries west of Nigeria) over a broad range of altitudes (100 - 2200 m) and annual rainfall (500 - 1500 mm). It is found near streams, on borders of fresh water lakes and in areas with a high and frequent rainfall. *S. goetzei* grows in Kenya, Tanzania, Ethiopia and North East Zambia in areas at altitudes ranging from 900 - 1900 m and with an annual rainfall between 500 - 1000 mm. It grows on land liable to flooding and close to alkaline lakes. *S. keniensis* occurs at higher altitudes (1200 - 2400 m) in areas with annual rainfall between 700 - 1100 mm. It has the same habitat as *S. sesban* (Gillett, 1963, 1971; Russell-Smith, 1987).

Cytological studies in perennial *Sesbania* species have concentrated on *S. sesban* (L.) Merr. (= *S. aegyptiaca* Poir.) with 2n = 12 reported by many workers. Several authors however, found a variation in the haploid chromosome number with n = 6, 7, 8, 12 and 14 reported for the different varieties of *S. sesban* (Table 3.1). For *S. keniensis* Gillett, a somatic chromosome number of 2n = 12 was reported by Gillett (1963). No reports were found for *S. goetzei* Harms. The chromosome morphology of *S. sesban* (L.) Merr. var *nubica* Chiov. and *S. keniensis* Gillett has not been reported and the chromosome number in *S. goetzei* Harms has not been determined. Hence the karyotypes and phylogenetic relationships of these three species were studied.

Agronomic evaluation at the International Livestock Centre for Africa (ILCA) has shown considerable variation within and between species in vigour, growth form, productivity, coppicing ability and resistance to nematodes and beetles (*Mesoplatys ochroptera*). Differences were also observed in nutritive value, protein and polyphenolic content (Siaw *et al.*, 1993). The presence of polyphenolic compounds at a high level can reduce the intake and digestibility of forages. Selection and evaluation has therefore been focused towards accessions with high agronomic productivity and low anti-nutritional compounds. The possibilities for plant breeding in *Sesbania* aimed at exploiting genetic differences by combining useful traits and broadening the genetic variation have hardly been examined. Few unsuccessful attempts at artificial hybridization have been made in annual species of *Sesbania* (Datta and Sen, 1960; Datta and Bagchi, 1971). These attempts of interspecific hybridization were mainly carried out with species which differed in chromosome numbers. In the present study interspecific crosses in all possible combinations were attempted.

Table 3.1. Previous investigations on the chromosome numbers in *S. sesban* and *S. keniensis*.

Species	Origin	n	2n	Authority
S. aegyptiaca Pers.	India	6	12	Haque, 1946; Rao, 1946
	India		12	Sampath, 1947
	India	7		Sarkar *et al.*, 1978
	Vietnam		12	Tixier, 1965
S. aegyptiaca Poir.	India	6		Baquar *et al.*, 1965
S. sesban	Java		12	Lubis *et al.*, 1981
S. sesban (L)	India		12	Datta & Neogi, 1970
S. sesban Mill.	Java		12	Tjio, 1948
S. sesban (L) Merr.			12	Berger *et al.*, 1958
	India	6	12	Jacob, 1941
(yellow flowered)	India	6	12	Jacob, 1941
(purple flowered)	India	6	12	Jacob, 1941
	Iraq	6		Al Mayah & Al Shehbaz, 1977
	Nigeria	7,8		Gill & Husaini, 1985
var bicolor (Wt and Arn) FW Andr.	India	6,7,12		Bir *et al.*, 1975
	India		12	Sareen & Trehan, 1979; Parihar & Zadoo, 1987a
	India	6		Parihar & Zadoo, 1987b
	W Pakistan	6,7		Baquar & Akhtar, 1968
var concolor (W and A) Baquar	W Pakistan	6,7,8		Baquar & Akhtar, 1968
var nubica Chiov.	Kenya		12	Gillett, 1963;Goldblatt & Davidse, 1977
	N Rhodesia		12	Gillett, 1963
var picta Santapau	India	6		Bir & Sidhu, 1966, 1967; Parihar & Zadoo, 1987b
	India	6,7,8, 14		Bir *et al.*, 1975
	India		12	Sareen & Trehan, 1979
var sesban Linn.	India	7,8		Bir *et al.*, 1975
	India		12	Sareen & Trehan, 1979
	India	6		Parihar & Zadoo, 1987b
(yellow)	W Pakistan	6,7,8		Baquar & Akhtar, 1968
S. keniensis Gillett			12	Gillett, 1963

Materials and Methods

Cytology

Sesbania germplasm was obtained from the ILCA genebank. *S. sesban* accession ILCA 15019, collected in Zaire; *S. goetzei* ILCA 1278 originating from Kenya and *S. keniensis* ILCA 13092 from Tanzania were used in this experiment. Young and healthy root tips were taken from young plants that had been germinated in pots. The root tips were pre-treated for 3-4 hours in a 0.003M 8-hydroxyquinoline solution at room temperature, washed with distilled water and fixed in 3:1 absolute ethyl alcohol : glacial acetic acid overnight. The root tips were hydrolysed for 6 minutes in 1M HCl at 60°C, washed with distilled water and stained in Feulgen's solution for 1-2 hours. Stained tips were squashed in a drop of 1% acetocarmine. Chromosome counts were made of 20 plants per species. Cells with a good spread of chromosomes during mitosis were photographed and prints were enlarged to a magnification of 3000 times. Karyotypes of the species were made by cutting out individual chromosomes and arranging them in homologous pairs in descending order of their length and arm ratio. The chromosomes were classed by the arm ratio (long arm / short arm) as median = 1.00-1.63 and sub median = 1.64-4.26 as suggested by Levan *et al.* (1965) and refined by Adhikary (1974). The relative length of chromosomes was calculated by dividing the length of each chromosome by the mean length of all chromosomes in the complement.

Hybridization

Different accessions of *S. sesban*, *S. goetzei* and *S. keniensis* were grown in separate plots at the ILCA seed multiplication site at Zwai in the Rift Valley of Ethiopia. Flowers were emasculated at the bud stage, labelled and bagged with polypropylene pollination bags. The next morning the emasculated flowers were pollinated with fresh pollen from the desired parent. One week after pollination the bags were opened and the number of pods formed was recorded. Pods were checked regularly until they were harvested at maturity when they were opened and the fate of each ovule was recorded. Three categories were recognised: ovules which showed no sign of development, seeds that had started to develop but failed to complete full development and fully formed seeds. The full seeds when obtained were weighed. The results were compared with the seed formation and seed weights in pods taken randomly after selfing the female parent plants. The full seeds were then germinated on agar, planted in pots and transferred to the field. Ripe pods produced from the hybrids of *S. sesban* x *S. goetzei*, *S. sesban* x *S. keniensis*, *S. goetzei* x *S. sesban* and *S. goetzei* x *S. keniensis* were harvested and the seeds formed were tested for germination. The germination test was carried out with four replicates of 50 seeds on top of filter paper in petri dishes at alternating temperatures of 20 and 30 °C.

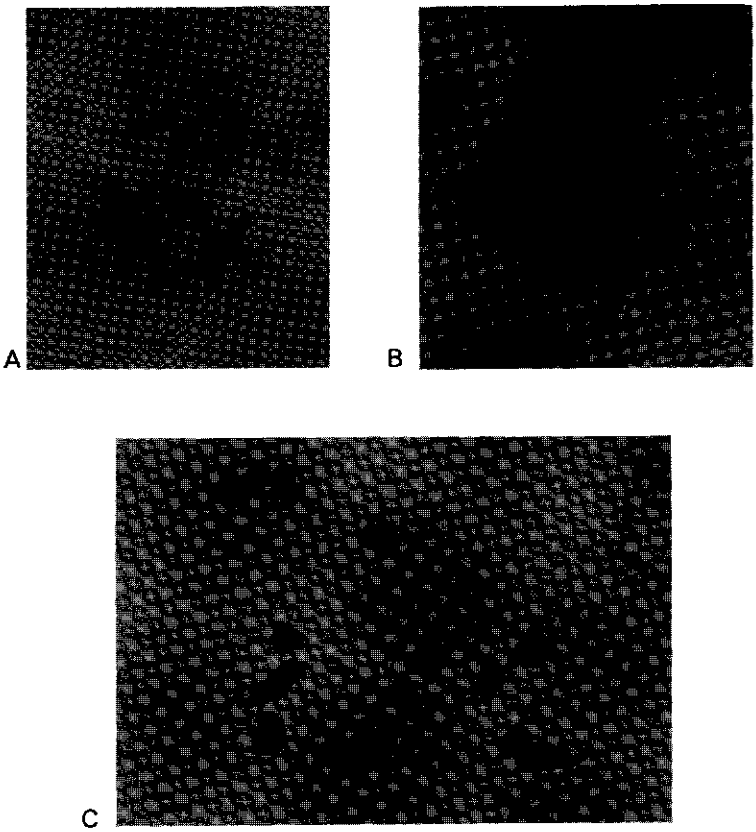

Fig. 3.1. Metaphase complement of *Sesbania sesban* (a), *S. goetzei* (b) and *S. keniensis* (c) with 2n = 12.

Results

Cytology

All three species under study were diploids and their somatic complement was composed of 12 chromosomes (Figure 3.1 and 3.2).

The longest chromosome pair of the complement was about double the size of the shortest pair (2.0, 1.8 and 2.0 times longer for *S. sesban*, *S. goetzei* and *S. keniensis* respectively). The first pair was much longer than the other pairs and was metacentric, the second pair was sub median and the other four pairs were metacentric for all three species. Two of these four pairs of short chromosomes were almost of uniform length, and hence, indistinguishable from one another. Nucleolar organisers in the form of satellites were found on the short arm of the fourth chromosome pair in *S. sesban* and *S. keniensis* (Table 3.2).

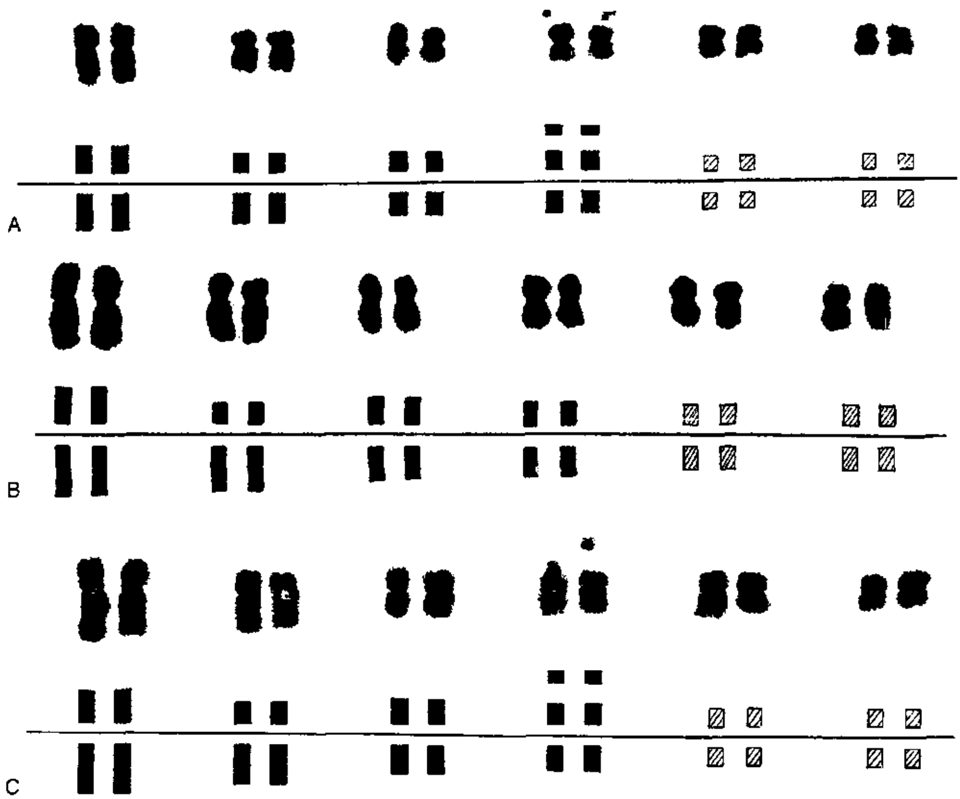

Fig. 3.2. Karyotype of *Sesbania sesban* (a), *S. goetzei* (b) and *S. keniensis* (c).

Table 3.2. Chromosome length in μm of the three investigated species (m = number of metaphases measured).

Species	Chromosome pair									
	1		2		3		4		5 and 6	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
S.sesban (m=8)										
long arm	2.53	0.17	1.94	0.13	1.50	0.09	1.35	0.09	1.22	0.07
short arm	1.73	0.11	1.12	0.08	1.25	0.07	1.14	0.07	1.00	0.06
satellite							0.53	0.03		
total	4.26	0.28	3.06	0.21	2.75	0.16	3.02	0.19	2.22	0.13
arm ratio	1.46		1.73		1.20		1.18		1.22	
rel length	1.46		1.05		0.94		1.03		0.76	
S.goetzei (m=8)										
long arm	3.20	0.17	2.84	0.11	1.94	0.07	1.85	0.06	1.66	0.05
short arm	2.31	0.11	1.43	0.05	1.73	0.04	1.57	0.05	1.44	0.05
satellite										
total	5.51	0.28	4.27	0.16	3.67	0.11	3.42	0.11	3.10	0.10
arm ratio	1.39		1.99		1.12		1.18		1.15	
rel length	1.43		1.11		0.95		0.89		0.81	
S.keniensis (m=10)										
long arm	2.80	0.08	2.20	0.07	1.53	0.03	1.46	0.04	1.34	0.05
short arm	1.87	0.07	1.27	0.03	1.40	0.03	1.25	0.04	1.16	0.04
satellite							0.62	0.08		
total	4.67	0.15	3.47	0.10	2.93	0.06	3.33	0.16	2.50	0.08
arm ratio	1.50		1.73		1.09		1.17		1.16	
rel length	1.45		1.07		0.90		1.03		0.77	

Hybridization

The crossing of *S. sesban x S. goetzei* and *S. sesban x S. keniensis* resulted in a 10% and 12% pod production (Table 3.3). Similarly, the *S. goetzei x S. sesban* and *S. goetzei x S. keniensis* cross yielded a 8% and 9% pod production. The *S. keniensis x S. sesban* cross was carried out with 86 flowers and the *S. keniensis x S. goetzei* cross with 83 flowers, of which no pods were produced. The final number of pods produced is significantly lower ($P<0.05$) in the interspecific crosses and the percentage of pods that dropped is in all cases higher than in the selfed parents, with the exception of the *S. sesban x S. keniensis* cross.

The average number of non developed ovules was higher in all four crosses that produced pods than in the selfed parents (Table 3.3). The same applied for the average number of partly developed ovules which were, except for the *S. sesban x S. keniensis* cross, higher compared with their selfed parents. The average number of fully formed seeds per pod was lower than after selfing in all crosses. The calculated 100 seed weights were lower than those produced after selfing for all the crosses.

Table 3.3. The pollination results, seed formation per pod and hundred seed weight of the selfed parents and their interspecific crosses.

Parents	No. of flowers pollinated	No. of pods set	Final no. of pods produced	No. of NDO[a]/pod		No. of PDO[b]/pod		No. of full seeds/pod		100 seed weight (g)
				mean	SE	mean	SE	mean	SE	
S.sesban x										
S.sesban	75	25	18	10.6	1.2	2.2	0.5	23.4	1.6	0.69
S.goetzei	82	14	8	12.1	3.0	12.7	4.4	11.8	2.4	0.49
S.keniensis	60	8	7	13.4	2.0	1.4	0.8	12.9	2.2	0.40
Chi-squared prob.			$P<0.05$							
S.goetzei x										
S.goetzei	60	28	15	7.4	1.1	3.1	0.6	28.6	1.6	1.07
S.sesban	52	10	4	11.6	3.1	20.4	3.1	0.4	0.3	-
S.keniensis	58	12	5	8.0	1.6	14.0	6.6	19.3	9.6	0.81
Chi-squared prob.			$P<0.05$							
S.keniensis x										
S.keniensis	74	23	15	4.7	1.2	5.3	0.8	25.3	1.5	0.97
S.sesban	86	0	0	-	-	-	-	-	-	-
S.goetzei	83	0	0	-	-	-	-	-	-	-

a) NDO = non developed ovules
b) PDO = partly developed ovules

The germination percentages of the fully developed seeds were 100% for the *S. sesban x S. goetzei* cross; 73% for the *S. sesban x S. keniensis* cross and 84% for the *S. goetzei x S. keniensis* cross. The *S. goetzei x S. sesban* cross yielded four mature pods, but only 2 full developed seeds were found of which one germinated. The young hybrid plantlets were transplanted to pots and after establishment transferred to the field where they developed normally. All hybrids reached maturity and produced pods. The mean germination percentage of seeds from the *S. sesban x S. keniensis* F1 hybrid was 91%; for the *S. sesban x S. goetzei* hybrid seeds 86%; for the *S. goetzei x S. sesban* hybrid seeds 99% and also 99% for the *S. goetzei x S. keniensis* seeds. In each of the above cases, the random variation was within the tolerance limits given by Ellis *et al.* (1985). This indicates that the estimates can be treated as reliable.

Discussion

The somatic chromosome number of 2n = 12 as found in the study for *S. sesban* var *nubica* and *S. keniensis* is in agreement with earlier reports on these species (Gillett, 1963; Goldblatt and Davidse, 1977). The chromosome number of 2n = 12 is a new report for *S. goetzei*. The results in the present study confirm the opinion that the base number of the genus is x = 6.

In the karyotypic study of *S. sesban* var. *nubica* nucleolar organisers were found on the short arm of the fourth homogenous pair. This finding is in line with earlier reports (Datta and Neogi, 1970; Sareen and Trehan, 1979; Parihar and Zadoo, 1987a) as far as the number is concerned, but differs in the position of the nucleolar organiser. The first two publications report a nucleolar organiser in the form of secondary constrictions in the longest pair of chromosomes, whereas the latter authors observed satellites in the fifth pair in homologues. Lubis *et al.* (1981) however, found two pairs of chromosomes with satellites and Jacob (1941) reported as many as three pairs of nucleolar chromosomes in *S. sesban*.

Observing the karyotype of the three species in this study it can be concluded that the general morphology of the complements is comparable. This suggested that hybridization between these species may be possible. Datta and Sen (1960) unsuccessfully crossed *S. aculeata* (4n race) with *S. speciosa* (2n race) and attributed the main cause of seed failure as due to the genomic differences of the two species. Similarly Datta and Ghoshal (in Datta and Bagchi, 1971) crossed *S. sesban* (2n) with *S. speciosa* (2n) resulting in non viable seed formation and *S. sesban* (2n) with *S. aculeata* (4n) which resulted in no pod formation. They stated that the diploid species were phylogenetically wide apart resulting in seed lethality in the former cross and that the quantitative and qualitative imbalances between the genomes led to seed failure in the latter experiment. Datta and Bagchi (1971) gave the same reason for the failure of pod production after crossing *S. benthamiana* (4n) with *S. brachycarpa* (probably 2n). The interspecific hybridization in this study resulted in pod and viable seed formation for the crosses *S. sesban* x *S. goetzei*, *S. sesban* x *S. keniensis*, *S. goetzei* x *S. sesban* and *S. goetzei* x *S. keniensis*. No pods were produced in those crosses where *S. keniensis* was the female parent. In these crosses the flowers would wilt and drop within one or two days after pollination. No explanation can be given for this.

The results from both the karyotype analyses and the hybridization experiment lead to the conclusion that the three species under study are phylogenetically closely related. There is an overlap in the distribution of these three species which indicates the possibility that natural hybrids may occur. However, there have been few reports on the existence of natural hybrids. Lewis (1988) in his review of the genus *Sesbania* stated that it seemed possible that interspecific hybrids could be common. Further studies on herbarium specimens and germplasm collected from areas where these species naturally grow together are needed to identify natural hybrids.

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Chapter 4

The reproductive biology of three perennial *Sesbania* species (*Leguminosae*)

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Chapter 4

The reproductive biology of three perennial *Sesbania* species (*Leguminosae*)

Abstract

The reproductive biology of *Sesbania sesban, S. goetzei* and *S. keniensis* (*Leguminosae; Papilionoideae*) was studied. Fifty percent flowering was observed at 102 to 153 days after germination for *S. sesban* accessions; 96 to 146 days for *S. goetzei* and 131 to 176 days for *S. keniensis* accessions. Flowers opened in the afternoon and remained fresh for 2 - 3 days. Bee species including *Xylocopa sp.*, *Apis mellifera*, *Megachile bituberculata* and *Chalicodoma sp.* visited the flowers. Hand pollination experiments showed that all three species were self compatible. *S. sesban* and *S. goetzei* were also cross compatible. The percentage of fully developed seeds was greater in pods formed after cross pollination compared to self pollination. No evidence was found for stigmatic or stylar self incompatibility. Outcrossing is probably the common method of reproduction under natural conditions, although in isolated trees substantial seed set by selfing might occur. Pod production under natural conditions was 34% for *S. sesban*; 49% for *S. goetzei* and 39% for *S. keniensis*. Considerable variation was found in pod production under open pollination between accessions of the same species. Selective abortion was observed within pods, with more mature seeds formed at the distal end of the pod.

Introduction

Knowledge of the pollination biology and breeding system is essential to determine patterns of geneflow and genetic recombination within and between populations. It is important to know whether a species is self or cross pollinated as to determine the appropriate sampling procedure to collect the maximum amount of genetic variation within a limited number of samples during plant collection. This information is also required to ensure that the characteristics of an accession are reproduced as exactly as possible during multiplication and regeneration for genetic resources conservation. The Forage Genetic Resources Section of the International Livestock Centre for Africa (ILCA) maintains a large *Sesbania* collection consisting of 400 accessions of 24 species. However, information on reproductive biology of this multipurpose tree is very limited (Brewbaker, 1990). Perennial *Sesbania* species are used in various agroforestry systems around the world. The value of these species as a multipurpose tree in agricultural systems for use as a livestock fodder, fuelwood and green manure has recently been emphasised (Evans and Rotar, 1987; Macklin and Evans, 1990). This study aimed to provide some basic information on the reproductive biology in three perennial *Sesbania* species, *Sesbania sesban* (L) Merr., *S. goetzei* Harms and *S. keniensis* Gillett.

Materials and Methods

Five accessions of *Sesbania sesban*, three of *S. goetzei* and two of *S. keniensis* were grown in separate plots at the Zwai seed multiplication site of ILCA in the Rift Valley of Ethiopia. Dates to 50% flowering were recorded. Individual flowers were tagged and examined to record development. The time of day when the flowers opened and time of pollen release were determined. Insect activity was observed and insects visiting the flowers were identified. The breeding system was examined by cross pollinating and selfing flowers from which insects had been excluded by polyethylene pollination bags. The flowers used for cross pollination were emasculated at the bud stage and pollinated the next day. Some flowers were bagged and left untouched while control flowers were left untouched but without bags. Self pollination was done by tripping the flowers just before opening.

The number of pods formed was recorded. After ripening the pods were opened and the fate of each ovule, numbered from the proximal to the distal end was determined. Three categories of development were recognised as ovules which showed no sign of development, seed that had started to develop but aborted and fully formed seed.

Fertilization was estimated by both pod set and examining styles under the fluorescence microscope. Styles and stigmas of self and cross pollinated flowers were collected between 12 and 24 hours after pollination and examined for compatibility. The styles and stigmas were fixed in absolute ethyl-alcohol:acetic acid (3:1) solution for 24 hours, softened in 4 M sodium hydroxide overnight and stained in a 0.1% solution of water-soluble aniline blue dye dissolved in 0.1 M tri-basic potassium phosphate for 4 hours. The squashed stigma and styles were examined under ultra violet light (Martin, 1959).

Results

Fifty percent of the trees of the *S. sesban* accessions started flowering after 102 days from germination for the first accession and 153 days for the last (mean = 125). For *S. goetzei* flowering started between 96 and 146 days (mean = 115) and for *S. keniensis* the first accession flowered after 131 days and the second after 176 days. It took on average 7 days for a pin head size bud (±3 mm) of *S. sesban* to develop into an open flower. For *S. goetzei* this took 8 days and for *S. keniensis* 10 days. The flowers were arranged in axillary racemes, which were 3 to 20 flowered in *S. sesban*; 2 to 4 flowered in *S. goetzei* and 1 to 2 flowered in *S. keniensis*. Flowering in these racemes is indeterminate.

Flowers open mainly in the afternoon and remain fresh for 2 to 3 days, after which the petals wither and the flowers either drop or the ovary enlarges and develops into a pod. The development of the ovary into a ripe pod took on average 63 days for *S. sesban*; 90 days for *S. goetzei* and 73 days for *S. keniensis*. Abortion of set pods occurs mainly when they are young. After maturity the pods split along the sutures and the seeds are released by gravity.

Sesbania has nine fused staminal filaments (diadelphous) and one upper stamen which is free. Long and short filaments alternate so that the anthers fit tightly around the stigma in a double ring. The style and stigma are glabrous in *S. keniensis*. However, in some accessions of *S. sesban* and *S. goetzei* the style is pubescent. The anthers dehisce and release their pollen in a lethargic way a day before the flower opens. When examining buds in the late bud stage the highest percentage of dehisced anthers was found in the afternoon. At this stage the stigma is already shiny and appears to be receptive. Examination of these buds under fluorescent microscopy showed in a few cases that pollen had already germinated on the stigma and had penetrated the style before the flower had been open for pollination. Some flower buds were found to be infested with fly larvae (*Chloropidae*) which were damaging the anthers.

Sesbania flowers at Zwai were visited by a variety of bees. The carpenter bees *Xylocopa flavorufa kristenseni* Friese and *Xylocopa somalica* Magretti contribute to pollination because they are large enough to force the keel down and enable tripping. Honey bees (*Apis mellifera*), mason bees (*Megachile bituberculata* Smith) and leaf cutter bees (*Chalicodoma sp.*) were also frequent visitors to the Sesbania flowers. Occasionally bees were observed piercing the calyx in order to rob the nectar which does not lead to tripping.

In *S. keniensis* no pods were formed after bagging without tripping (Table 4.1). Pod formation after emasculation and hand pollination was poor in this species, both after self and cross pollination. The flowers drop early in many cases, even before anthesis is completed. Fluorescent microscopy showed a normal germination of pollen grains and a similar growth of the pollen tubes through stigma and style in both self and cross pollinated flowers of the three species.

Table 4.1. Pod production of flowers of *S. sesban, S. goetzei* and *S. keniensis* after self and cross pollination.

Treatment	*S. sesban*		*S. goetzei*		*S. keniensis*	
	No of flowers	% of pods set	No of flowers	% of pods set	No of flowers	% of pods set
Natural conditions (control)	570	34	253	49	87	39
Flowers tripped and bagged	478	22	196	27	103	16
Flowers bagged, without tripping	493	2	234	1	82	0
Flowers cross pollinated	398	5	234	7	95	0

Marked differences were observed in pod production between accessions of the same species (Table 4.2). The percentage of flowers which produce pods under natural conditions ranges between 23 and 45. The accession with a low percentage pod set is consistently low for all treatments.

Table 4.2. Differences in pod production between 3 accessions of *S. sesban* after self and cross pollination.

Treatment	No of flowers observed			% of flowers producing pods		
	ILCA 2000	ILCA 15368	ILCA 2024	ILCA 2000	ILCA 15368	ILCA 2024
Natural conditions (control)	162	141	122	41	23	45
Flowers tripped and bagged	149	112	102	17	2	55
Flowers bagged, without tripping	134	129	112	3	1	29
Flowers cross pollinated	130	73	85	1	0	19

On average a *S. sesban* flower grown under natural conditions had 32 ovules (Table 4.3). This number varied considerably between accessions, ranging from a mean of 27.9 (for accession 15368) to 38.8 (for accession 2057). In *S. keniensis* the percentage of aborted seeds was about 12% higher than in the other species. Seed damage by larvae of beetles (*Coleoptera; Bruchidae*) was observed in some of the pods of all three species. The number of non developed ovules in pods of *S. sesban* set under natural conditions is significantly lower ($t = 7.33$, $P < 0.001$) when compared with pods formed after tripping, resulting in a significantly greater ($t = 5.70$, $P < 0.001$) number of fully developed seeds per pod (Table 4.3). Also for *S. goetzei* a significantly lower ($t = 2.17$, $P < 0.05$) number of non developed ovules and a higher ($t = 2.60$, $P < 0.01$) number of fully formed seeds were found. *S. keniensis* showed similar results, although differences were not significant.

Table 4.3. Seed development in pods of *S. sesban, S. goetzei* and *S. keniensis* under natural conditions (=control) and after self pollination (n = sample size).

	S. sesban				*S. goetzei*				*S. keniensis*			
	Control n=236		Selfed n=99		Control n=267		Selfed n=48		Control n=77		Selfed n=15	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Non Developed Ovules	3.6	0.3	8.8	0.7	6.8	0.3	8.7	0.9	4.8	0.5	6.7	1.5
Partly Developed Ovules	2.3	0.2	2.3	0.3	2.5	0.2	3.5	0.9	6.6	0.6	6.3	1.0
Full Developed Ovules	26.4	0.6	20.8	0.7	32.0	0.5	28.8	1.0	22.4	0.8	19.9	2.1
Total Ovule Positions	32.3	0.5	31.9	0.5	41.3	0.3	41.0	0.7	33.8	0.5	32.9	1.5

The number of mature seeds per pod varied considerably in *S. sesban* (ranging from 1 to 48, n = 236) with an average of 26 seeds. For *S. goetzei* this number ranged between 9 and 50 (n = 267) and for *S. keniensis* between 7 and 35 (n = 77). Within pods there were clear positional effects on the development of ovules with a greater proportion of the ovules developing into mature seeds in the distal two thirds of the pod (Figure 4.1). The number of non developed ovules was particularly high in the first two proximal positions. The number of aborted ovules was similar in all positions within the pods. However, as fewer ovules begin to develop at the proximal positions of the pod this means that their rate of abortion is higher. Only the result of the analysis for *S. goetzei* with 40 ovules per pod is illustrated, but similar patterns were found for the different ovule numbers in the other species.

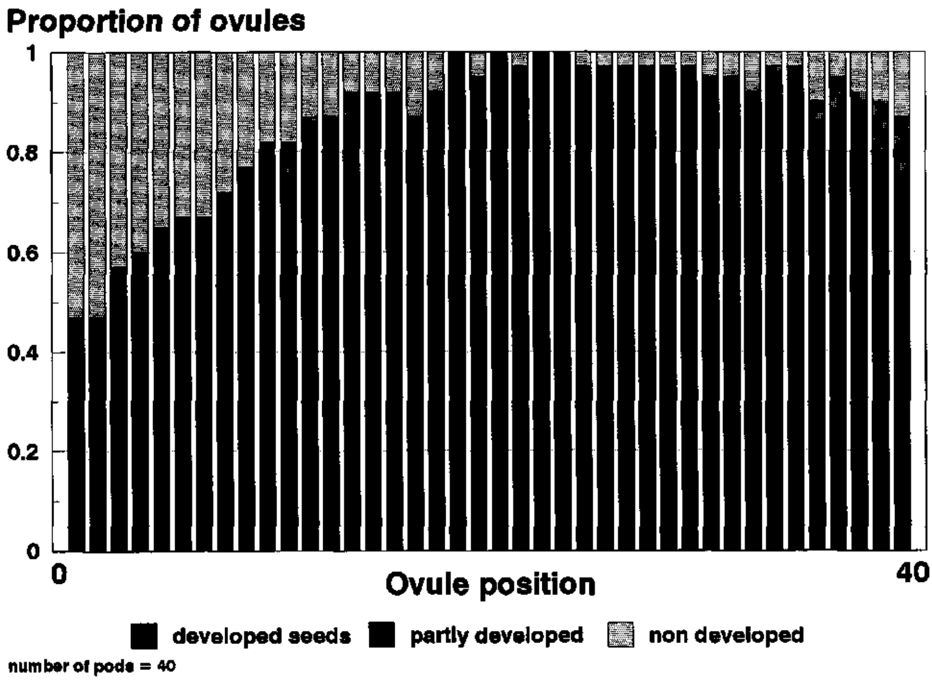

Fig. 4.1. Seed formation and abortion within pods of *S. goetzei* (n = 40).

Discussion

The flowers of these *Sesbania* species have some characteristics which suggest that they can set seed through selfing. The anthers shed their pollen before the flower opens. At this stage the stigma is shiny and seems to be receptive. The flowers of *S. sesban* and *S. goetzei* do not require tripping before they become receptive. This ability for selfing is in agreement with a FAO report (1961) which states that all *Sesbania* species are self compatible and require no isolation for pure seed production. On the other hand however, Burbidge (1965) observed that the floral structure with pollen and nectar concealed in the papilionoid flower seems to be suited to cross pollination by bees. Indeed, the

Sesbania flowers were visited by a wide range of bee species, especially by the heavier bees of the genus *Xylocopa*, a common pollinator in leguminous flowers (Arroyo, 1981) and reported earlier as a pollinator in *Sesbania* (Evans and Rotar, 1987; Brewbaker, 1990). Also the fact that honey bees visit *Sesbania* flowers has been mentioned by these authors. However, foraging by the mason bee *Megachile bituberculata* and a leaf cutter bee *Chalicodoma sp* has not been reported before. It was found that some of the smaller bees were not strong enough to trip fresh mature flowers and relied on visits through the side of the flower.

Brewbaker (1990) assumed that the perennial *Sesbania* species are largely outcrossing and that self incompatibility would be the norm. In the *Leguminosae* the one S locus multi allelic incompatibility system is frequently found. Since most incompatible species belonging to the same family have a common incompatibility system (Frankel and Galun, 1977; Nettancourt, 1977) it would be appropriate to assume that the genetically controlled self incompatibility mechanisms which are expressed in the stigma and style prevail in *Sesbania*. In the experiment however no evidence was found for stigmatic or stylar incompatibility. In all three species self pollen germinated, pollen tubes penetrated the stigma and grew through the full length of the style up to the ovules. It is possible that in some accessions a pollen-ovule incompatibility reaction exists, but more work is needed to determine this. The observed reduction in self seed set could result from a late acting self incompatibility system that causes self pollen to be less effective in fertilization or leads to early embryonic failure. The latter would also explain the higher rates of non developed and aborted seeds after self pollination compared to crossing. Such a reduction in selfed seed formation after self and cross pollination was found in other experiments (Barnes *et al.*, 1972; Stephenson, 1981).

It is also possible that the reduction in self fertility at the ovule level is due to post zygotic inbreeding depression (Seavey and Bawa, 1986) as this also effects later stages of the life cycle. Observations on selfed seedlings showed that they grew slower and were less vigorous than crossed seedlings (J.H. Heering, unpublished data). Research to study the effect of the pollination system on seedling vigour is continuing. Almost all plants examined showed some degree of self fertility. The proportion of self fertile plants appears to be too high to be attributed only to incomplete incompatibility due to mutations at the S locus or chance combinations of alleles at the locus. It is unclear what degree of outcrossing occurs in natural conditions and morphological or biochemical genetic markers should be used to determine the exact level of outbreeding. Outcrossing is probably the preferred method of reproduction under natural conditions, but selfing is clearly possible and it is assumed that isolated trees produce selfed seeds. Pod production under natural conditions is relatively high, especially when compared with studies on other legumes in which only 10% and sometimes even less than 1% of the flowers produce a pod (Stephenson, 1981; Bawa and Webb, 1984). Flower and pod abortion is common in shrubs and trees with hermaphrodite flowers and need not be attributed to the lack of compatible pollen or a limitation of resources. Access of compatible pollen clearly does not limit pod production in these species because self pollination and fertilization can occur.

The existence of selective abortion of ovules in all three species is apparent by the small number of pods containing 1-10 seeds which are retained on the tree till maturity. Selective abortion is also indicated by the pattern of seed production within pods. There was a trend to greater seed production at the distal end of the pod. This was earlier reported in legumes by Bawa and Webb (1984). Linear non random seed set patterns with hardly any seed formation at the first basal ovule position were also found in pods of bee pollinated *Lupinus nanus* and *Medicago sativa* (Horowitz *et al.*, 1976). It is possible that ovules at the proximal end seldom start to develop because they do not receive pollen tubes and are not fertilised. It is also likely that the distal ovules are the first to be fertilized therefore they can develop early and successfully compete for resources within the pod.

Differences between accession within species regarding pod production and seed yield exist, as shown in *S. sesban*. Evaluation at ILCA has shown considerable variation within species in agronomic and nutritional characters. This means that there is a great potential for selection. With characters such as early flowering, good seed yield and the ability to produce seeds after selfing these species would also be suitable for plant breeding.

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Chapter 5

The effect of cutting height and frequency on the forage, wood and seed production of six *Sesbania sesban* accessions

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Chapter 5

The effect of cutting height and frequency on the forage, wood and seed production of six *Sesbania sesban* accessions

Abstract

The forage, wood and seed production of six *Sesbania sesban* accessions was assessed under irrigated conditions for two cutting frequencies and cutting heights. Control trees were left uncut to measure their seed production potential. The trial was conducted over an 18 months period. The fastest growing accession produced almost 10 t/ha total dry matter (DM) after six months growth; 40% of it being leaves. Total DM yield was higher at the six months cutting interval compared to the three months interval with yields between 25-42 t/ha/year. Some accessions could not sustain their high level of production but showed drastic drops in yield after repeated cutting. Within cutting heights variation was found between accessions in their response to frequency with some accessions producing most leaf DM at three months cutting interval, whilst others showed the highest production when cut every 6 months. In general leaf DM production increased with increased cutting height. When cut at 150 cm the DM leaf yield at the three months cutting interval ranged from 9.7-18.2 t/ha. More plants survived at the three months cutting frequency. Seed yields varied considerably between accessions (0.02-1.56 t/ha at the six months interval). After 18 months undisturbed growth the trees yielded 36.5-83.7 t/ha total DM comprising of 21% leaves. Fresh wood biomass ranged between 56.4-138.0 t/ha and seed yields were 2.7-6.6 t/ha. Accession 15019 performed best, combining a very high leaf DM yield under repeated cutting with high seed production potential when left uncut.

Introduction

The International Livestock Centre for Africa (ILCA) recognised the potential of the multipurpose tree *Sesbania sesban* as a feed resource for ruminants in 1985.

S. sesban is a fast growing tree with high crude protein content of the leaves (25-30% of dry matter). The foliage is palatable and contributes to an increase in animal productivity when fed to livestock (ILCA, 1985; Tothill *et al.*, 1990). In addition to providing a high protein forage, the species is frequently used to improve soil fertility, to supply fuelwood and construction material and to reduce soil erosion (Macklin and Evans, 1990).

In 1983 ILCA received its first accession of *Sesbania*. Subsequent collections of *Sesbania*, mainly *S. sesban*, were made in Ethiopia and in a wider area of Africa on joint multi species forage collection missions. Additional material was acquired from the University of Hawaii. Evaluation of this material yielded several accessions which out-performed the locally introduced variety (Siaw *et al.*, 1993; Tothill *et al.*, 1990). In 1987 a total of 161 accessions, among which 65 *S. sesban* var *nubica* were collected in Tanzania (Solomon Mengistu, 1990). These accessions were subsequently evaluated at ILCA and in different semi arid and subhumid sites in East and Southern Africa through the African Feed Resources Network (AFRNET) (Kategile and Adoutan, 1990).

Although previous evaluations assessed the forage and wood production under repeated cutting, only few studied the effect of cutting height and frequency on forage and wood yield. Those that were carried out had contradicting results (Galang *et al.*, 1990; Mune Gowda and Krishnamurthy, 1984). Seed production characteristics are important in the evaluation and development of a new species. Even though previous experience showed that under Zwai conditions seeds could be produced under repeated cutting, yields had not been quantified. Further, little information was available on the seed yields of *S. sesban* under undisturbed growth. The study reported here therefore evaluated the effect of two cutting frequencies and heights on forage and seed production of the six best performing accessions from the ILCA evaluation trials. Control trees, which were allowed to develop into full grown trees, were included in the trial in order to assess the seed production potential of these accessions.

Materials and methods

The experiment was carried out at ILCA's seed multiplication site at Zwai in the Rift Valley of Ethiopia (8°00'N; 38°45'E) at an altitude of 1650 m above sea level. The soil at the site was loamy sand and classified as a vitric andosol (King and Birchall, 1975) having pH (H_2O) 8.05, organic matter 1.96%, total N 0.01%, (Bray II) extractable P 4.57 mg/kg, exchangeable Ca 34.02 mg/kg, Mg 2.20 mg/kg, K 4.03 mg/kg, Na 0.27 mg/kg at 0-35 cm depth (Kamara and Haque, 1988). The mean annual maximum and minimum temperatures of the area are approximately 27°C and 18°C respectively. Flood irrigation was carried out once in every two weeks in the dry season.

The trial was laid out in 5 blocks of a three factor split plot design with cutting frequency and height applied to the main plots. Each main plot had 6 rows with a different accession in each 5 m long row. Scarified and inoculated seeds were directly sown at a rate of three seeds per hole 50 cm apart within rows with rows 2 m apart. After one month the seedlings were thinned to one tree per hole. No fertiliser was applied during the trial period.

At 6 months after sowing the trees were cut back to either 75 cm or 150 cm above ground level, whereas the control trees remained uncut. After the primary harvest the regrowth was cut back to their respective heights every three or six months over a period of 12 months. At each harvest the whole 5 m row was cut and the harvested material weighed fresh in the field. A representative sub sample was taken from each row, sorted into leaf (leaf plus fine stem up to 5 mm), wood (any branch with a diameter greater than 5 mm was considered wood) and unripe pods. Each sorted sample was weighed and then oven dried at 70°C for 48 hrs to determine the dry matter yields. The number of surviving plants was counted every 3 months and percentage survival calculated. Ripe pods were harvested from each row on a monthly basis. The seeds were bulked, threshed and weighed after every 3 months. The control trees were also cut to 75 cm at the end of the experiment and forage, stem and unripe pod production determined.

Results

Standardisation cut

The dry matter production for the standardisation cut is presented in Table 5.1. The accession 10865 was about five times more productive than the lowest producer. On average 35% of the total dry matter (DM) consisted of leaf material. The leaf DM produced was lower at six months after planting than the three months coppice regrowth for all accessions. Also the unripe pods contributed considerably to the DM production (on average 13% of the total DM), although large variation existed between accessions.

Table 5.1. Dry matter yields (t/ha) six months after sowing when cut back to 75 and 150 cm height.

Accession Number	DM yields (t/ha)							
	Total		Leaf		Wood		Unripe Pods	
	75cm	150cm	75cm	150cm	75cm	150cm	75cm	150cm
1238	2.91	3.10	1.09	1.34	1.58	1.53	0.24	0.23
10865	9.83	5.69	4.12	2.77	5.59	2.87	0.12	0.06
15019	5.77	4.77	2.01	1.54	2.93	2.23	0.83	1.00
15021	3.44	1.07	1.02	0.36	1.78	0.44	0.64	0.27
15022	4.69	2.44	1.20	0.73	2.46	1.15	1.02	0.57
15036	2.21	2.88	0.56	0.81	1.04	1.45	0.62	0.63
Mean	4.81	3.33	1.67	1.26	2.56	1.61	0.58	0.46
SED	0.80		0.29		0.47		0.16	
F prob.								
accession no.	<0.001		<0.001		<0.001		<0.001	
height	0.001		0.011		<0.001		NS	
acc x height	<0.001		0.001		<0.001		NS	

Regrowth cuts

Mean total DM yield varied significantly ($P < 0.001$) between accessions and cutting frequency, with a higher yield at the six months cutting interval (Table 5.2).

Table 5.2. Total DM yields (t/ha) for the regrowth cuts at three and six months interval.

Frequency (months)	Accession Number						
	1238	10865	15019	15021	15022	15036	Mean
3	16.06	23.69	25.98	17.87	18.11	15.92	19.60
6	24.79	34.91	42.06	31.77	26.41	27.28	31.20
Mean	20.42	29.30	34.02	24.82	22.26	21.60	25.40

SED cutting frequency 2.61 F prob. <0.001
SED accession no. 2.70 F prob. <0.001

No significant difference in DM yield was found between cutting heights. Accession 15019 had the highest total production followed by 10865, both at three and six months.

Most accession reached their maximum production already before or at the second regrowth cut and DM yields decreased rapidly thereafter (Figure 5.1). At the first regrowth cut the total DM yield for the accessions cut every three months ranged from 3.8-8.7 t/ha (11.7-28.5 t/ha fresh yield). In contrast, average DM yield at the last regrowth cut dropped by 3 to 55% (average 38%) to a range of 2.7-4.9 t/ha (9.4-17.2 t/ha fresh). Accession 10865 had the highest yield at 75 cm whereas 15019 produced best at 150 cm. At the end of the trial the total DM production from trees cut at 150 cm was higher than those cut at 75 cm for all accessions.

There were significant differences in the leaf-stem ratio between the three and six months cutting frequency. At the three months interval 57% of the total DM comprised of leaves; 39% was stem and 4% unripe pods. When cut at six months only 36% of the total DM consisted of leaves, 58% of stem and 6% unripe pods. The leaf DM yield showed therefore no significant differences in cutting frequency but in height ($P < 0.05$) and accession number ($P < 0.001$) (Table 5.3). Although there were no statistical differences, variation was found between accessions in their response to cutting frequency when cut at the same height,

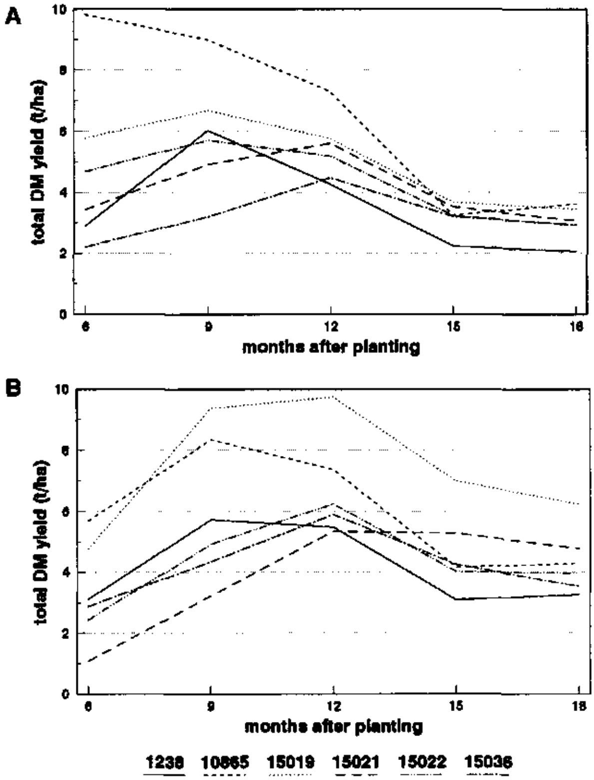

Fig. 5.1. Total dry matter yield of *S. sesban* accessions when cut every 3 months at 75 cm (a) and 150 cm (b).

with some producing more after coppicing every three months whilst others produced more regrowth when cut every six months. In general the average DM leaf production was higher from plants cut at 150 cm compared to those cut at 75 cm. Highest mean production was achieved from plants cut at 150 cm height and with three months cutting interval. The DM content in the leaves varied significantly ($P < 0.01$) with cutting frequency, but not with height. No significant differences were found in leaf DM content between accessions. The average leaf DM content was 26.4% at three months interval and 29.9% at the six months interval.

Table 5.3. Leaf DM yields (t/ha) for the regrowth cuts with three and six months cutting interval at 75 and 150 cm height.

Frequency (months)	Height (cm)	Accession Number						
		1238	10865	15019	15021	15022	15036	Mean
3	75	8.98	12.82	10.96	8.84	9.21	7.05	9.64
6		8.94	10.42	16.78	11.23	9.28	7 30	10.66
3	150	12.28	15.47	18.20	9.66	10.81	9.85	12.71
6		11.48	16.88	13.94	9.72	9.21	9.81	11.84
Mean		10.42	13.90	14.97	9.86	9.63	8.50	11.21

SED height 0.94 F prob. <0.05
SED accession no. 1.06 F prob. <0.001

There was large and significant variation in seed production between cutting frequencies (Table 5.4). Although more seed was produced when plants were cut at 150 cm height compared to those cut at 75 cm, these differences were not significant. Accession number 15036 produced the most seeds, both at the three and six months cutting interval. The accession 10865 was late flowering and therefore a very poor seed producer with a maximum of only 20 kg/ha produced over the full trial period when plants were cut every six months.

Table 5.4. Total seed production (t/ha) for the accessions cut at a three and six months interval.

Frequency (months)	Accession Number						
	1238	10865	15019	15021	15022	15036	Mean
3	0.10	0.01	0.19	0.23	0.21	0.43	0.20
6	0.24	0.02	0.79	0.95	0.77	1.56	0.72
Mean	0.17	0.02	0.49	0.59	0.49	1.00	0.46

SED cutting frequency 0.29 F prob. <0.05
SED accession no. 0.24 F prob. <0.001

After the 18 months trial period more plants had survived when cut every three months (Table 5.5). There were no significant differences observed in survival rates between cutting heights. Accession 10865 had much higher survival when plants were cut every three months cutting (95%) compared to those cut every six months (72%). Overall survival was highest in 15019 with less then 3% of the plant dying.

Table 5.5. Survival percentages after 18 months for the accessions cut at a three and six months interval.

Frequency (months)	Accession Number						
	1238	10865	15019	15021	15022	15036	Mean
3	85.7	95.6	97.8	87.8	95.6	96.4	93.1
6	69.3	72.2	97.8	85.6	95.1	96.7	86.1
Mean	77.5	83.9	97.8	86.7	95.3	96.5	89.6

SED cutting frequency	3.01	F prob. <0.05
SED accession no.	4.37	F prob. <0.001
SED cut freq x acc no	6.40	F prob. <0.05

At the end of the trial the remaining stubble wood was harvested at ground level and the fresh yield weighed. More wood was produced when trees were cut every six months and the average yield ranged from 45.9 t/ha for accession number 15019 to 32.2 t/ha for 1238 (mean 40.6 t/ha). This accounts for about 30% of the total biomass produced over the complete trial period.

Control trees

The mean yields of the control trees which were cut at the end of the trial are given in Table 5.6. The accession 10865 had highest leaf, stem and total DM production. The accession 15019 was by far the best seed producer (6.57 t/ha); it had also the highest amount of unripe pod DM produced. The lowest amount of seeds was produced by accession 1238 (2.73 t/ha). During the first year of establishment the seed production ranged from 0.5-2.1 t/ha which is about 30% of the total production during the trial period. On average stems constituted 75% of the total fresh yield, leaves 21% and unripe pods 4%. The mean leaf DM percentage was 34, which resulted in a DM leaf production ranging from 5.0-12.1 t/ha (average 8.3 t/ha). The remaining stems yielded 33.7 t/ha of fresh wood per accession when cut back to ground level, with no differences between accessions.

Table 5.6. Fresh yields (t/ha) of the control 18 months after sowing, when cut back to 75 cm height.

Accession Number	Yields (t/ha)				
	Total	Leaf	Wood	Unripe Pods	Seeds
1238	163.9	35.9	124.8	3.2	2.73
10865	179.1	34.2	138.0	6.9	2.86
15019	149.9	24.1	112.6	13.2	6.57
15021	74.7	13.9	56.4	4.4	3.02
15022	107.0	20.5	83.1	3.4	3.44
15036	80.2	15.7	68.5	4.0	3.69
Mean	127.1	24.0	97.2	5.9	3.72
SED	23.3	8.2	15.9	2.4	0.53
F prob.	<0.001	<0.001	0.002	<0.001	<0.001

Discussion

One of the major advantages of *S. sesban* over other fodder trees is its rapid early growth rates as exhibited by accession 10865 which produced almost 10 t/ha total DM after six months growth when cut back to 75 cm. After repeated cutting an average fresh yield of 77 t/ha was produced in one year. These yields are comparable with results obtained in earlier experiments (Gill and Patil, 1983;

Gutteridge and Akkasaeng, 1985). The mean total DM yield after repeated cutting was about 25 t/ha/year, but there exists considerable variation between accessions with highest yields of 26 and 42 t/ha total DM for accession 15019 when cut at a three and six months interval respectively. This accession was also the highest yielding variety, producing 20 t/ha total DM after five cuttings during the first year in an irrigated experiment in Hawaii (Evans and Rotar,1987). The leaf DM production was not significantly different between plants cut at different frequencies, but showed significant differences between cutting heights. Higher yields were achieved at the 150 cm cutting height. This is in agreement with results obtained by Galang *et al.* (1990) but is in contrast with the report of Mune Gowda and Krishnamurthy (1984). The latter authors found highest forage yields at a cutting height of 50 cm which decreased as cutting height increased in 50 cm intervals to 200 cm. The leaf DM production ranging from 9.6-18.2 t/ha/year at the three months cutting interval is high, giving a lot of high protein supplement for crop residues and dry season forages. The accession number 15019 produced the highest amount of leaf material, followed by 10865.

In some accessions survival was influenced by the cutting regime with higher mortality when trees were cut at the six months interval. Cutting height had no significant effect on survival rates. Galang *et al.* (1990) found that the cutting treatments did not effect survival, whereas Mune Gowda and Krishnamurthy (1984) reported a higher mortality percentage in longer stubble heights compared to the low ones. One of the causes for a high mortality for accessions cut every six months might have been the fact that only very few leaves remained on the trees after cutting. The general opinion is that the removal of all leaves should be avoided to prolong survival; leaving from 5 to 25 % of the foliage when pruning (Evans and Macklin, 1990).

Large variation was found in the seed production under repeated cutting, with more seeds produced from trees cut every six months. The late flowering accession 10865 produced low amounts of seed. When cut at 150 cm the other accessions were able to produce reasonable amounts of seed whilst also producing leaf material for livestock feed. This cutting height offers a reasonable management option, considering the fact that no commercial supply for *Sesbania* seeds exists and the availability of seeds might be a constraint for large scale introduction.

The control trees produced on average 8.3 t/ha DM leaf after 18 months growth. Mean leaf DM production after repeated cutting (including the standardization cut) was 12.7 t/ha. It is obvious that when the accessions were allowed to develop into trees their leaf yield per unit area of land and time was less then when subjected to frequent cutting. However, allowing the trees to grow and cut only after one and half years yielded apart from leaf material a considerable amount of seeds (2.8-6.6 t/ha and also firewood or construction wood (56-138 t/ha). This fresh wood yield is high compared to other trials over a one year period in which the seedlings were watered in the field upto the start of the rains (Onim and Otieno, 1993).

Conclusion

It can be said that large (genetic) variation was observed in the productivity of these six accessions. This high variability can be utilised in the selection of accessions according to their end use which could be either forage or wood. The accessions with numbers 15019 and 10865 had the highest forage production. Fresh wood production was highest in the accessions 10865 and 1238. Accession 15019 combined the high forage production with a very high seed production, which makes it an outstanding accession for future use. Feeding trials are being carried out in order to find out whether nutritional differences between these accessions exist.

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Chapter 6

The classification of a *Sesbania sesban* collection. I. Morphological attributes and their taxonomic significance

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Chapter 6

The classification of a *Sesbania sesban* collection. I. Morphological attributes and their taxonomic significance

Abstract

A collection of 108 accessions of *Sesbania sesban* (*Leguminosae, Papilionoideae*) was grown in the field and classified using morphological attributes. Numerical analysis resulted in the division of the collection into two major groups which corresponded to the different varieties that exist in the subspecies *sesban*. The varieties *sesban* and *bicolor* could be distinguished from variety *nubica* mainly on the basis of their leaflet number and size, seed size, seed colour and growth habit. The variety *nubica* could be further subdivided into five groups by both the average linkage and the Partitioning Around Medoids (PAM) algorithms. The average linkage algorithm differentiated accessions mainly on the basis of quantitative characters, whereas the PAM method used qualitative attributes for grouping accessions. This classification will assist in the selection of germplasm for further evaluation and or breeding.

Introduction

The genus *Sesbania* Scop. contains about 50 species, the majority of which are annuals. The greatest species diversity occurs in Africa with 33 species described (Gillett, 1963). Although the annual species have received some attention, recent research has focused on the perennial species (Macklin and Evans, 1990). Of the perennial species, *Sesbania sesban* has shown potential as a multipurpose tree in agroforestry systems. Its leaves and young twigs are used as high protein fodder for ruminants, while the thick branches and stem provide fuelwood and construction material. This species is also used to improve soil fertility and to reduce soil erosion (Heering and Gutteridge, 1992).

The exact origin of *S. sesban* is unknown, but it is widely distributed and cultivated throughout tropical Africa and Asia. It has also been introduced in tropical America. *S. sesban* is the most widely collected species of the genus, however, collections have been mainly carried out in Africa and only a few accessions are of Asian origin. One of the largest germplasm collections is available at the Forage Genetic Resources Section of the International Livestock Centre for Africa (ILCA) in Addis Ababa, Ethiopia. The usefulness of a germplasm collection depends very much on the availability of documentation and information on the accessions and on the proper taxonomic identification of the germplasm. Numeric analysis has been used by several authors as an aid to understanding the variation within collections of particular taxa, genera or species (Burt *et al.*, 1971; Gramshaw *et al.*, 1987; Bishop *et al.*, 1988; Harding *et al.*, 1989; Pengelly *et al.*, 1992). This paper describes the morphological characteristics observed in the *S. sesban* collection and discusses the characterization and classification of the accessions into groups to aid future evaluation programs.

Materials and methods

The experiment was carried out at ILCA's seed multiplication site at Zwai in the Rift Valley of Ethiopia (8°00'N; 38°45'E) at an altitude of 1650 m above sea level. The soil at the site was loamy sand and classified as a vitric Andosol (King and Birchall, 1975) with a pH (H_2O) of 8.05, organic matter 1.96%, total N 0.01% (Bray II) extractable P 4.57 mg/kg, exchangeable Ca 34.02 mg/kg, Mg 2.20 mg/kg, K 4.03 mg/kg, Na 0.27 mg/kg at 0-35 cm depth (Kamara and Haque, 1988). The mean annual maximum and minimum temperatures of the area are approximately 27°C and 18°C, respectively. The study was implemented under flood irrigation to eliminate the effects of water stress.

Seedlings of 108 accessions of *S. sesban* were raised in the screenhouse and after one month transplanted to the field. Each accession was planted in two 5 m long rows. Planting distance was 50 cm apart within rows with rows 1.5 m apart. No fertilizer was applied during the trial period. A complete list of the morphological attributes measured is given in Table 6.1. The sample size had been calculated in a preliminary study, taking into consideration the minimal theoretical standard error of the mean from the existing variation between plants within an accession and between accessions.

Table 6.1. Morphological characters observed on the *S. sesban* collection.

Plant characteristics	
1 Growth habit (GRHA)	(1) erect, main stem vertical (2) semi erect main stem not vertical (3) shrubby.
2 Stem surface (STS)	(1) smooth (2) sparse hairs (3) medium dense hairs (4) dense hairs (5) aculeate.
3 Stem colour (STCO)	Several trees were observed when plants were two months old. (1) green (2) green/red (3) red.
Leaf characteristics	
4 Leaflet length (MLFL)	Measured in mm on the middle leaflet of a leaf at the longest point excluding the stalk. 20 observations from 10 plants.
5 Leaflet width (MLFW)	Measured in mm at the widest point of the same leaflet. 20 observations from 10 plants.
6 Leaflet number (MLFN)	Number of leaflet counted on the same leaf. 20 observations from 10 plants.
7 Leaflet surface (LFSB) below	(1) smooth (2) sparse hairs (3) medium dense hairs (4) dense hairs.
8 Leaflet surface (LFSM) margin	(1) smooth (2) sparse hairs (3) medium dense hairs (4) dense hairs (5) aculeate.
Flower characteristics	
9 Standard length (MSTL)	Measured in mm on freshly opened flowers at the longest point, including the claw. 20 observations from 10 plants.
10 Standard width (MSTW)	Measured in mm at the widest point. 20 observations from 10 plants.
11 Keel length (MKL)	Measured in mm at the longest point. 20 observations from 10 plants.
12 Keel width (MKW)	Measured in mm at the widest point. 20 observations from 10 plants.
13 Flower colour (FLCO)	Data taken from recently opened flowers of 5 trees. Since the standard is uniformly yellow, often speckled with purple of suffused purple (var *bicolor*), the percentage yellow was estimated and coded. (1) 0 - 25% (2) 25 - <50% (3) 50-75% (4) 75 -100%.
14 Number of flowers (MNF)	The number of flowers per inflorescence was counted. 30 observations from 15 plants.
Fruit characteristics	
15 Pod length (MPL)	Measured in cm on mature, fresh pods at the largest point not including the peduncle. 30 observations from 15 plants.
Seed characteristics	
16 Seed length (MSEL)	Measured in mm on 20 randomly selected seeds.
17 Seed width (MSEW)	Measured in mm.
18 Seed colour (SECO)	The colour of air dried seeds. (1) orange brown (2) brown mottled black (3) yellow green (4) light green mottled black (5) dark green mottled black (6) black mottled green (7) black.

The correlation between the observed characteristics was determined and principal component analysis was carried out using the PRINCOM procedure in the SAS program (SAS, 1987). Hierarchical clusters were formed using the centroid, average and single linkage methods and tree diagrams were drawn. The hierarchical classification allows groups to be established and the characteristics used in structuring these groups to be identified. Since SAS clustering analysis is not very appropriate for mixed ordinal and nominal attributes other algorithms were used on the same data and the results compared. The program DAISY (Kaufman and Rousseeuw, 1990) which is able to handle mixed measurements was used to compute a dissimilarity matrix. The matrix was then subjected to the Partitioning Around Medoids (PAM) program in which clusters are formed according to the k-medoid method (Kaufman and Rousseeuw, 1990). The established groups were further described by linear discriminant functions using the original attributes and the extent of misclassification (if any) identified. The discriminant functions were performed using the SAS procedure DISCRIM (option method = normal).

Results

Since significant associations among the variables directly influence the results of cluster analysis, the significant correlation coefficients of the 18 observed characters are presented in Table 6.2. The results indicated a considerable correlation between leaflet length (MLFL) and leaflet width (MLFW) and between the standard length (MSTL), standard width (MSTW), keel length (MKL) and keel width (MKW). Therefore, four variables were omitted from the final run of the Principal Component Analyses (PCA). The first two components of the PCA, using mean values of the 15 identified variables, explained 81% of the total variation. The first component was mainly related to the number of leaflets per leaf and the second to the length of the leaflets (Table 6.3).

Table 6.2. The significant correlation coefficients between descriptor pairs for the observed attributes in the *S. sesban* collection.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	GRHA																	
2	STS																	
3	STCO	-.19*[1]																
4	MLFL	0.29																
5	MLFW	0.25			0.92													
6	MLFN	-.29			-.28	-.39												
7	LFSB		0.49	0.40														
8	LFSM		0.59	0.24*				0.66										
9	MSTL	-.34			-.35	-.36	0.36											
10	MSTW	-.24*			-.31	-.32	0.38				0.92							
11	MKL	-.36			-.47	-.47	0.36		-.23*	0.74	0.68							
12	MKW	-.33		0.25	-.29	-.29	0.22*			0.68	0.60	0.74						
13	FLCO	-.24*		0.26														
14	MNF		0.37	0.22*	0.45	0.37		0.41	0.42		-.20*	-.34						
15	MPL							-.22*	-.24*									
16	MSEL	0.35			0.61	0.59	-.48	-.24*		-.52	-.45	-.44	-.39			0.37		
17	MSEW	0.24			0.44	0.38	-.28			-.25	-.21*	-.32			0.47	0.23*	0.45	
18	SECO	-.39			-.48	-.46	0.36	0.31	0.34	0.48	0.36	0.44	0.48	0.20*		-.25*	-.67	-.43

1 = Significant at 5% level; all others significant at 1% level.

Table 6.3. Eigenvalues of the covariance matrix and eigenvectors of the first five principal components.

	PRIN1*	PRIN2	PRIN3	PRIN4	PRIN5
Eigenvalues	50.02	13.32	5.92	3.25	2.16
Cumulative proportion of variation	0.64	0.81	0.88	0.92	0.95
Variable	Eigenvectors				
MLFN**	0.972	0.203	0.017	-.094	-.034
MLFL	-.191	0.878	0.109	-.189	0.363
MPL	0.002	0.203	-.892	0.344	-.097
MNF	-.001	0.312	0.308	0.620	-.449
MSTL	0.088	-.106	-.087	0.322	0.679
GRHA	-.032	0.040	0.016	-.075	-.135
STS	-.019	0.029	0.144	0.342	0.019
STCO	-.014	0.015	0.019	0.147	0.052
FLCO	0.002	-.021	0.047	0.044	0.057
LFSB	0.002	0.009	0.112	0.186	0.009
LFSM	0.006	0.016	0.156	0.237	-.021
MSEW	-.008	0.019	-.003	0.019	-.029
MSEL	-.041	0.072	-.059	-.041	-.089
SECO	0.088	-.169	0.159	0.331	0.400

Total variance = 78.57
* PRIN1 = the first principal component etc.
** Variable abbreviations as defined in Table 6.1.

Table 6.4. Basic univariate test statistics for the two variety classes.

Variable	Total sample SD*	Pooled within-class SD	Between class SD	Pr < F**
MLFL	3.55	2.43	3.66	0.0001
MLFN	6.92	5.70	5.57	0.0001
MSEL	0.53	0.30	0.62	0.0001
GRHA	0.71	0.62	0.49	0.0001
SECO	1.48	1.06	1.46	0.0001

* SD = Standard deviation
** Pr > F = level of significance

When the accessions were plotted against the first two components only two main groupings could be identified (Figure 6.1). The groups were formed on the basis of the different varieties within the subspecies *sesban*. The small group contained all accessions belonging to the varieties *sesban* and *bicolor* whereas the remainder belonged to the variety *nubica*.

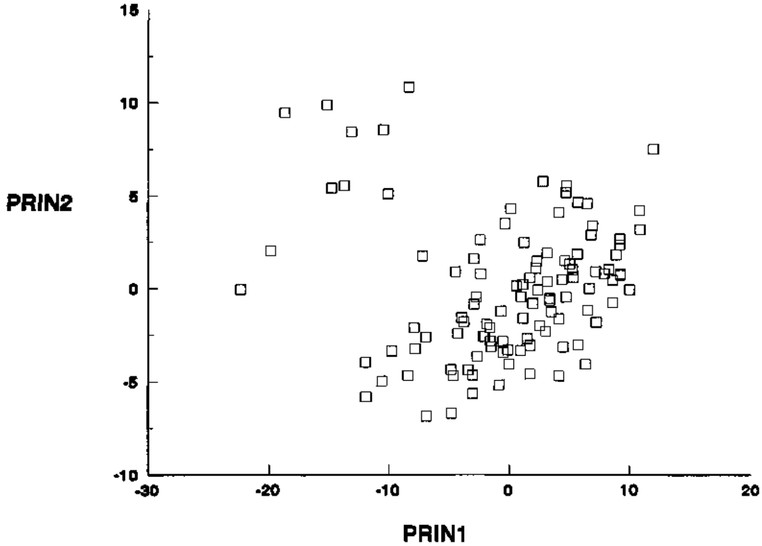

Fig. 6.1. Graph of the 108 accessions of *S. sesban* plotted against the first two principal components of the covariance matrix (explaining 81% of the variance). PRIN1 = the first principal component, PRIN2 = the second principal component.

Table 6.5. Linear discriminant functions for the two variety classes.

Variable	Class	
	var. *nubica*	var. *bicolor* and *sesban*
Constant	-130.28	-213.93
MLFL	1.63	3.74
MLFN	1.17	0.51
MSEL	45.69	62.37
GRHA	7.87	12.55
SECO	7.57	5.27

The results of the linear discriminant functions, using the most important and significant variables for these two main groups are given in Tables 6.4 and 6.5. The multivariate statistics, Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root were all significant, suggesting unequal class means in the population.

Further clustering was carried out to determine if subdivision of the groupings identified in the PCA into smaller units was feasible. Similar results were obtained for the average and centroid linkage algorithms, which produced better clustering than the single linkage method. Initially seven clusters were defined mainly on the basis of quantitative attributes (Figure 6.2).

The PAM program was only used to form clusters within the variety *nubica*. It was found that the division into five clusters was best. In this algorithm the division was mainly based on the observed ordinal characters. Discriminant analysis confirmed the division into five groups with very few misclassifications. A list with information regarding the origin and group allocation of the individual accessions used in this study is available on request from the ILCA Forage Genetic Resources Section.

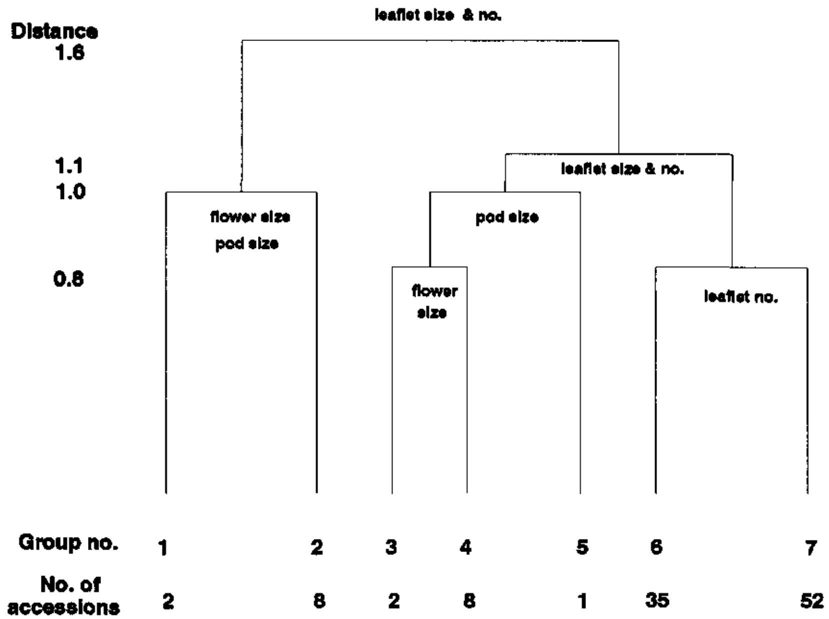

Fig. 6.2. Dendrogram of the morphological classification of the 108 *S. sesban* accessions, based on the average linkage algorithm. Height of the clusters indicates the normalized root mean square (RMS) distance between joined groups.

Discussion

In carrying out this classification an objective step-wise procedure was used from the study of the correlation matrix to the classification of the data set using Principal Component Analysis, the confirmation or modification of these groupings using clustering algorithms and finally to the construction of linear discriminant functions for the key groups that were identified.

The classification techniques used were successful in defining groups based on morphological characteristics. The average linkage algorithm distinguished groups mainly on the basis of quantitative attributes. Initially, seven clusters were defined and although the method of formation of the classification was agglomerative they will be discussed from the root downwards (Figure 6.2). The first dichotomy separated Groups 1 and 2 from the remainder primarily on the basis of their small leaflet number and very large leaflet size. The next dichotomy separated Groups 3, 4 and 5 from the remainder based on a smaller leaflet size and number. Group 1 was split from Group 2 on the basis of shorter pods and smaller standard length (and thus flower size), whereas Group 5 was separated from Groups 3 and 4 primarily because of its very large pods. Group 3 was separated from 4 because of larger flower size, while Groups 6 and 7 were split due to differences in leaflet number per leaf. Group 6 had relatively more leaflets per leaf than Group 7.

Groups 1 and 2 contained all the accessions belonging to the varieties *sesban* and *bicolor* whereas the remaining accessions belong to the variety *nubica*. These two groups were separated from the latter mainly on the basis of their leaflet number and size, seed size and colour and growth habit. This contrasts with the findings of Lewis (1988) who stated that the varieties *sesban* and *bicolor* are separated from variety *nubica* on rather weak and unstable characteristics. Evans (1990) included pod length as one of the contrasting characteristics between the varieties *sesban* and *nubica*, but this attribute did not contribute significantly in the discriminant function of this trial. Our classification however, confirms the conclusion of Evans (1990) that the variety *bicolor* is very similar to *sesban* and differs only in flower coloration. The cluster classification did not necessarily correspond to the area of origin. One discrepancy in the data base was noted in the accession number 15020 which is of the variety *sesban*, but has Kenya as the country of origin, where this variety does not occur (Gillett, 1963). This probably indicates that exchange of material has occurred and the material has not actually evolved in this ecological zone. This points out the need for complete passport data maintenance by genebanks whenever exchange of germplasm takes place.

The partitioning around medoids methods used only with accessions belonging to the var. *nubica* gave more weight to the qualitative characteristics. Five groups were distinguished of which Group 1 consisted of 15 accessions. The trees of this group had green-red or red stems with a very pubescent leaf and stem surface and almost completely yellow standards. Groups 2 and 3 had flowers with 50 - 75% yellow on their standard. The first group had 13 accessions with smooth stems and green-red coloured. Group 3 contained 13 accessions but with green coloured stems with sparse hairs on them. The remaining accessions formed two loose clusters of 26 and 31 accessions based on the indumentum on stem and leaflet margin.

Conclusion

This study identified some distinct groups within the collection. The var. *nubica* was divided into five groups by both clustering algorithms used, but on the basis of different classification variables. The average linkage method used leaflet size, leaflet number, pod size and flower size as distinguishing characters, whereas in the PAM method the stem and flower colour and the indumentum of leaves were the important attributes. In the maintenance and use of large genetic resources collections some group structure is required so that decisions can be made regarding the selection of material for evaluation and seed multiplication. The classification obtained can help in this regard.

The collection used in this study consists mainly of Ethiopian and Tanzanian material. However germplasm collected from the southern African countries of Botswana, Malawi, Namibia, Zambia and Zimbabwe (Ndungu and Boland, 1994) and requisitions from India recently added to the collection will be evaluated in the near future. This will enable us to determine how the germplasm from these countries differs from the existing material.

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Chapter 7

The classification of a *Sesbania sesban* collection II. Agronomic attributes and their relation to biomass estimation

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Chapter 7

The classification of a *Sesbania sesban* collection. II. Agronomic attributes and their relation to biomass estimation.

Abstract

A collection of *Sesbania sesban* accessions was grown out in the field and classified using real and standardized values of ten agronomic attributes. Clustering the accessions using the observed values of the attributes produced several groups which were mainly based on the dry matter yields after the first and second harvest. The cluster analysis on the standardized values of the descriptors provided ten similarity groups. These groups were identified and compared with an earlier morphological classification.

Some of the observed characters were used to establish their relationship with biomass yield of the trees. The data were therefore subjected to linear regression analysis. Predictive equations were obtained for the logarithmical transformed biomass yield using stem diameter at 30 cm from the ground level plus the plant height with R^2 values between 84-89 %.

Introduction

The objectives of a classification study are among others to facilitate an inventory of what is available in a collection and therefore to help determine which accessions are of value and should be multiplied for seed increase. This enables an efficient management and maintenance of the collection. It can further help a breeder in the selection of his material for the inclusion in his breeding program.

The numerical classification using morphological attributes of a collection of *Sesbania sesban* spp. *sesban* was successful in identifying groups within the species (Heering *et al.*, 1995). However, a morphological characterization does not always provide guidelines to the agronomic potential of the groups of accessions that are formed. Agronomic information should also be collected in order to get an understanding of the full range of variation available in the accessions as a guide to the existing desirable combinations of characters. Therefore, an attempt was made to establish a classification based on features of agronomic potential, using the same *Sesbania sesban* collection.

The study further tried to verify whether productive equations for the dry matter biomass yield as established for many forest species (Pardé, 1980) could also be constructed for this multipurpose tree species. This would allow a quick and non-destructive estimation of the biomass production which could be used in future evaluation and on-farm experiments. An earlier study on *Sesbania sesban* (Otieno *et al.*, 1991) showed that significant correlations exist between biomass yield and stem diameters measured at knee and breast height. Predictive equations for biomass yields with a R^2 value between 64-71% could be made using these measurements. This study tried to refine the biomass estimates by using diameter at 30 cm from ground level plus plant height in the equation for regression analyses. The results of both studies are reported in this paper.

Materials and methods

The details regarding the location of the site and its soil characteristics are described elsewhere (Heering *et al.*, 1995).

Classification

Two 5m long rows containing 10 plants were planted for each accession. One row was used for observations regarding flowering and seed production, whereas the other was used for the characters related to biomass production. The diameter and height measurements were carried out according to the procedure described by MacDicken *et al.* (1991). At six months after planting one row was cut back to 75cm height and the harvested material was weighed fresh in the field. A representative sub sample was taken from each row and sorted into leaf (leaves plus fine stem upto 5mm in diameter) and wood (stems > 5mm in diameter). Each sorted sample was weighed and oven dried at 70°C for 48 hours to determine the dry matter yields.

The complete list of the attributes used in the analysis is given in Table 7.1.

Table 7.1. Agronomic characters observed on the *Sesbania sesban* collection.

Character	Observation
1 Height at 6 months (HGT6)	Total stem length, measured in cm, 6 months after planting from the ground to the highest (apical) growing point (average of 5 trees)
2 Diameter at 6 months (DIAM)	Measured in mm at 30 cm height above the ground level (average of 5 trees)
3 Plant yield 1 (PYLD1)	Average dry matter yield per plant in g when cut back to 75 cm above ground level after 6 months growth
4 Leaf percentage (LFPC)	The amount of leaves as percentage of the total yield at the first cut
5 Regrowth height (HGTR)	Height of the trees in cm, measured 6 months after the first cut (average of 5 trees)
6 Plant yield 2 (PYLD2)	Average dry matter yield per plant in g when cut back 6 months after the first cut
7 Days to flowering (FLOW)	Number of days from planting until 50% of the plants reached flowering
8 Days to seeding (SEED)	Number of days from planting until 50% of the plants produced ripe pods
9 Seed set (SSET)	Rated as (1) very poor (2) poor (3) fair (4) good (5) very good
10 Thousand seed weight (TSWG)	Weight in g; measured for 6 samples with 100 dried seeds each

The procedures of the Statistical Analysis System (SAS, 1987) were followed in the data analysis. The correlation between the observed characteristics was determined and principal component and cluster analysis were carried out on both the observed and standardized values of the descriptors. In the latter case, the data were first standardized to a mean of 0 and a standard deviation of 1, using PROC STANDARD. The hierarchical cluster procedure according to the average linkage method as executed by PROC CLUSTER option AVERAGE was used. This agglomerative method begins with the calculation of a matrix of euclidean distances among group means and produces a dendrogram showing successive fusions of individuals which ends at the stage where all individuals belong to the same cluster.

Biomass estimation

For biomass estimation linear regression and analysis of variance were carried out with the SAS procedure, using the following equations:

$$\log y = a + b \log d^2h \qquad (1)$$

and

$$\log y = a + b \log d + c \log h \qquad (2)$$

in which y = biomass yield; d = diameter of the stem in mm at 30 cm above the ground level; h = height of the tree in cm; a,b and c = regression constants.

Results

Classification

One accession (ILCA 9256) was taken out of the analysis since all plants died after the first cut. The significant correlation coefficients of the agronomic characters are given in Table 7.2.

Table 7.2. The significant correlation coefficients between descriptor pairs for the observed agronomic attributes in the *S. sesban* collection.

	1	2	3	4	5	6	7	8	9
1 HGT6									
2 DIAM	0.78								
3 PYLD1	0.77	0.81							
4 LFPC	-.54	-.54	-.46						
5 HGTR	0.54	0.44	0.46	-.43					
6 PYLD2	0.32	0.44	0.38	-.27	0.73				
7 FLOW		-.23*	-.23*	0.30	-.22*				
8 SEED				0.27	-.25	-.24*	0.79		
9 SSET				-.27					
10 TSWG				-.30			-.20*		0.21*

* = significant at 5% level; all others significant at 1% level.

Principal component analysis (PCA) was carried out with all the observed characters. The first component in the principal component analysis which was mainly related to the yield after the first harvest explained 88% of the total variance. The second component referred to the yield of the regrowth harvest and accounted for another 9% of the total variance (Table 7.3).

Table 7.3. Eigenvectors of the first four principal components.

Variable	PRIN1	PRIN2	PRIN3	PRIN4
HGT6	0.141	-.043	0.848	0.317
DIAM	0.002	0.0	0.003	0.001
PYLD1	0.971	-.176	-.154	-.027
LFPC	-.010	-.003	-.057	0.023
HGTR	0.052	0.183	0.262	0.023
PYLD2	0.175	0.963	-.038	0.051
FLOW	-.020	-.028	-.150	0.723
SEED	-.013	-.051	-.208	0.596
SSET	0.0	0.0	0.006	-.003
TSWG	0.001	-.002	-.003	-.009
% Variation explained	88.4	8.5	1.4	1.1

When the accessions were plotted against the first two components two outliers, accessions with very high plant yield at the first harvest (ILCA 15024 and 15368) were noted. When these two accessions were left out of the PCA a better separation of the remaining ones occurred in which some groups could be visualized (Figure 7.1). Cluster analysis identified these groups as eight clusters which were mainly formed on the basis of their dry matter yields (Table 7.4).

Standardization reduced the influence of the plant yield in the covariant matrix. The dendrogram formed after the hierarchical clustering of the standardized values of the original descriptors is presented in Figure 7.2. The cluster analysis on the standardized values of all the observed characters, including the morphological ones, emphasized the variety differences found within the *S. sesban* collection. A list with the details on origin and group allocation of the individual accessions is available on request from the ILCA Forage Genetic Resources Section.

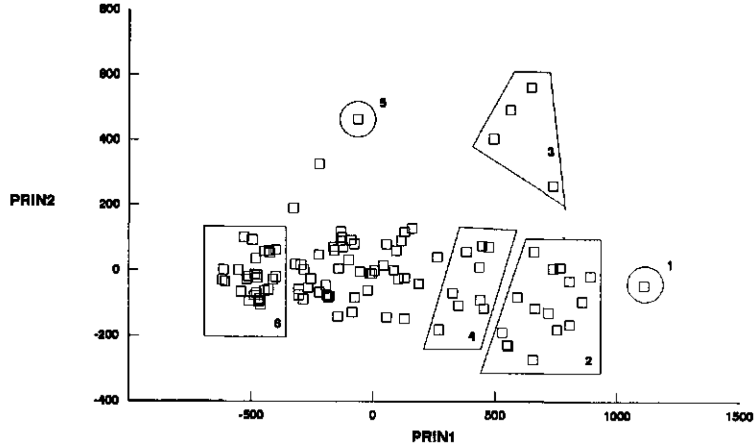

Fig. 7.1. Graph of 105 accessions of *S. sesban* plotted against the first two principal components of the covariance matrix using the real values of the attributes (explaining 97% of the variance). PRIN1 = the first principal component, PRIN2 = the second principal component.

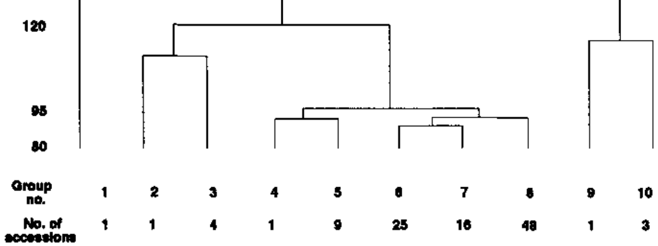

Fig. 7.2. Dendrogram of the agronomic classification of the 108 *S. sesban* accessions, based on the average linkage algorithm using standardized values. Height of the clusters indicates the normalized root mean square (RMS) distance between the joined groups.

Table 7.4. Means and standard deviation of the agronomic characters total dry matter plant yield after the first (PYLD1) and second (PYLD2) cut for the observed clusters using real values.

Cluster no.	No. of accessions	PYLD1 (g)	PYLD2 (g)
1	1	1690.1	336.6
2	15	1292.1 ± 101.7	202.0 ± 101.9
3	4	1103.9 ± 102.1	715.0 ± 104.3
4	9	984.3 ± 58.7	220.0 ± 90.6
5	1	448.8	636.3
6	28	124.0 ± 53.3	83.6 ± 55.4
7	2	275.2 ± 35.6	377.8 ± 73.6
8	45	498.4 ± 163.7	166.1 ± 82.0

Biomass estimation

The linear regression analysis for the models which included both height and diameter as variables in the equation gave comparable and significant R^2 values ranging from 0.84-0.89 for the different proportions of the biomass. These were higher than estimates based on equations with either height (R^2 values 0.67-0.78) or diameter (R_2 0.79-0.81). Further, the estimations for the wood and total biomass yields are slightly better then for the leaf biomass yields (Table 7.5).

Plots of the residuals (observed minus estimated values) and the dependent variables did not show any appreciable lack of fit (Figure 7.3). The regression lines with the estimated yields for model 1 are given in Figure 7.4.

Table 7.5. Predictive equations for the estimation of biomass yields in *S. sesban* accessions.

Model 1: log y = a + b log d^2h[a]

Dependent	a	b	R^2	EMS[b]	Prob > F
log tyld[c]	0.35	0.72	0.87	0.026	0.0001
log lyld	0.32	0.65	0.84	0.027	0.0001
log wyld	-0.50	0.86	0.87	0.040	0.0001

Model 2: log y = a + b log d + c log h

Dependent	a	b	c	R^2	EMS	Prob > F
log tyld	-0.90	1.20	1.25	0.88	0.025	0.0001
log lyld	0.02	1.24	0.77	0.84	0.027	0.0001
log wyld	-3.18	1.21	2.01	0.89	0.034	0.0001

a) d = diameter (cm); h = height (cm)
b) EMS = Error Mean Square
c) tyld = total dry matter yield (g); lyld = leaf dry matter yield (g); wyld = wood dry matter yield (g)

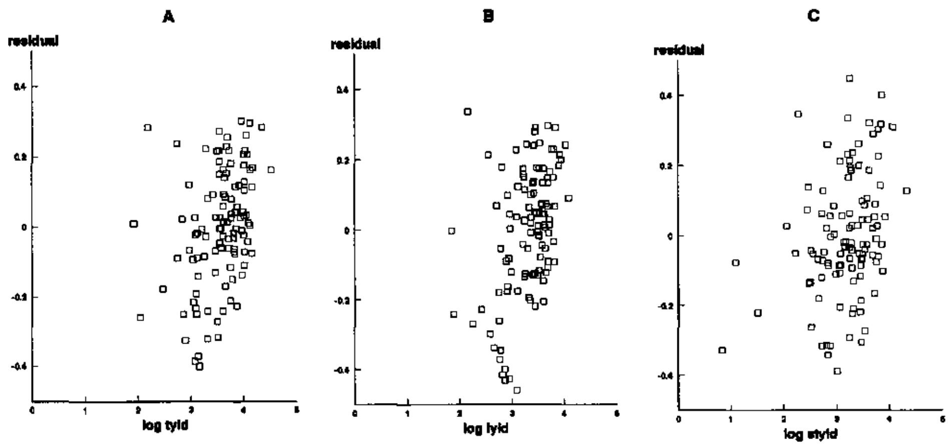

Fig. 7.3. Graphs of the residuals and the logarithm of the total (a), wood (b) and leaf (c) dry matter yields for *S. sesban* using model 2.

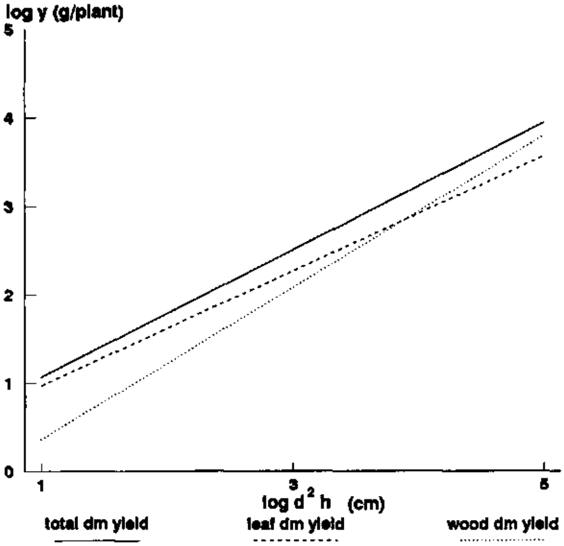

Fig. 7.4. Regression lines for the dry matter yields for *S. sesban* using model 1.

Discussion

Classification

The accessions showed a large variation in the dry matter yields per plant after the first and second cuts. This was reflected in the outcome of the PCA with the observed agronomic characters (Table 7.3) which was dominated by these two attributes. Several accessions had rapid early growth with a total dry matter production between 10-20 t/ha. However, as observed in earlier experiments (J.H. Heering, 1995) the yield of many accessions was markedly lower at the second cut. The accessions in group 3 are of interest in this respect because they also maintained high productivity at the regrowth cut (Figure 7.1).

The average linkage algorithm performed on the standardized values of the characteristics divided the collection in 10 clusters which will be discussed below. At the first dichotomy one accession (ILCA 15368) was split from the remainder because of its very high yield at the first harvest (Figure 7.2). At the second dichotomy four accessions were separated from the remaining group because of their very low yield at the first cut, consisting mainly of leaf material. One accession (ILCA 1229) was early flowering whereas the others were late flowering. Next, five accessions were detached because of their relatively high yield at the first cut and very high yield at the regrowth cut. The accession 15036 could be distinguished from the remaining four because of its fast growth rate after 6 months. At the next dichotomy a group with 10 accessions, all belonging to the varieties *sesban* or *bicolor*, was separated from the others, belonging to the variety *nubica* because of their higher thousand seed weights. Of these ten the accession number 15020 formed a separate group because of its very high yield after six months growth. The remaining 87 accessions were divided into two groups of 46 and 41 accessions respectively, of which the first group had a lower dry matter production. The latter group was divided into two clusters; one containing 16 accessions which are late flowering and seeding and another consisting of 25 early flowering accessions.

Biomass estimation

High and significant correlations were found between the plant dry matter yields after six months and height and diameter, justifying the inclusion of these characters in the biomass estimation equations. The dry matter biomass estimation gave good results for both equations. The equations which included both diameter and height provided a more accurate estimate of dry matter yield than those that used these parameters separately. Higher R^2 values were obtained for total and wood biomass production estimates than for the leaf biomass. This fact was also reported by Otieno *et al.* (1991). Now that this study has shown the relationship between height, diameter and biomass yield, the next step will be to develop biomass estimation tables that can be used for *S. sesban* at different sites and ecosystems. In this respect it is, however, important to note that leaf production can show major fluctuations over time and place and, as stated by Stewart *et al.* (1992), the relationship between height, diameter and biomass production should therefore mostly be used for the estimation of wood production in different environments.

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Chapter 8

Differences in *Sesbania sesban* accessions in relation to their phenolic concentration and HPLC fingerprints

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Chapter 8

Differences in *Sesbania sesban* accessions in relation to their phenolic concentration and HPLC fingerprints

Abstract

Phenolic concentration in the leaves from 103 different accessions of *Sesbania sesban* (L.) Merr. was determined and High Performance Liquid Chromatography (HPLC) was used to analyze the extractable phenolics. The chromatograms were subjected to cluster analysis. Large variation was observed between accessions in the soluble phenolic and insoluble proanthocyanidin concentration. The study further showed that the accessions could be grouped on the basis of their HPLC fingerprints. The results suggest that quantitative and qualitative selection for polyphenolics is possible. This type of information is relevant to future programs aimed at the improvement of the nutritive value in *Sesbania sesban.*

Introduction

A wide range of leguminous trees have been identified for use as ruminant feed in tropical areas. Some of the genera that have shown promise include *Leucaena, Gliricidia, Acacia, Erythrina, Calliandra* and *Sesbania* (NAS 1979, 1983, 1984; Macklin and Evans, 1990; Shelton *et al.*,1991). The genus *Sesbania* and the species *S. sesban* in particular has recently attracted considerable research interest because of its adaptability to alkaline and saline soils, tolerance to waterlogging, palatability and high nutritive value and its rapid early growth.

The evaluation and classification of a large number of accessions from the germplasm collection of the International Livestock Centre for Africa has shown considerable variation in several agronomic and morphological characteristics (Heering *et al.*, 1995a, 1995b). Laboratory analysis indicated that the crude protein concentration of the leaves ranges between 18-30% of dry matter (DM). Feeding trials revealed that the supplementation with *S. sesban* leaves had a positive effect on the growth rate of sheep (Reed *et al.*, 1990) and goats (Singh *et al.*, 1980). Although *S. sesban* generally has higher *in vitro* dry matter digestibilities and better apparent nutrient status than many other multipurpose trees (Akkasaeng *et al.*, 1989; Ahn *et al.*, 1989; Siaw *et al.*, 1993), the liveweight gains achieved in feeding experiments are often no better than for other tree forages. This may be attributed to the anti-nutritional factors in the *Sesbania* forage. Tothill *et al.* (1990) noted that the observed differences between accessions in nutritive value might be related to differences in polyphenolic concentration. Work of ILCA (1989) showed that differences in levels of soluble phenolics and insoluble proanthocyanidins in *S. sesban* were significant and negatively related to *in vitro* digestibility. Research on anti-nutritional factors has indicated that considerable variation exists in the polyphenol concentration between accessions of *S. sesban* (ILCA, 1989; Siaw *et al.* 1993; Nsahlai *et al.* 1994).

In the last few decades, chemotaxonomy has made extensive use of phenolic compounds to differentiate among plant species (Harborn, 1975). More recently, Lowry *et al.* (1984) used flavonol glycosides from leaves to identify various cultivars of *Leucaena leucocephala* and Van Sumere *et al.* (1985) used flower flavonoids to distinguish *Azalea* cultivars. These authors applied high performance liquid chromatography (HPLC) to obtain cultivar specific fingerprints. HPLC is a useful tool for rapid screening of plant material for tannins and other polyphenolics and is well suited for taxonomic purposes as it produces quantitative data and offers a high degree of discrimination not obtained through morphological systems (Morgan, 1989). Such quantitative HPLC data can be subjected to statistical analysis.

Plant breeders are interested in the use of phenolics as a genetic marker. Elite lines could be selected on the basis of phenolic profiles, if the different accessions in a collection have distinct phenolic profiles or can be grouped according to their fingerprints and the group structure can be understood in relation to other agronomic and nutritional characteristics. The objectives of this study were therefore, to assess the differences in phenolic concentration in the leaves of a large collection of accessions of *S. sesban*, to investigate whether leaf samples from different accessions have characteristic HPLC phenolic profiles and if so, to explore whether these HPLC fingerprints can be used to differentiate accessions or groups of accessions through cluster analysis. Groups formed through cluster analysis were related to groups formed through morphological and agronomic classification of the same accessions.

Materials and methods

The *S. sesban* accessions were grown at ILCA's seed multiplication station in the Rift Valley of Ethiopia as described in an earlier publication (Heering, 1995a). In total 103 accessions were used in this study. Leaf material was bulked from five randomly selected trees per accession after six months growth. The leaves were freeze dried and ground to pass a 1 mm sieve. Total soluble phenolics were determined gravimetrically after precipitation with trivalent ytterbium (Reed *et al.,* 1985). Determination of insoluble proanthocyanidins was conducted on the neutral detergent fiber. Samples (100 mg) were therefore extracted with 70% aqueous acetone and refluxed in neutral detergent solution. Samples of 5 ml butanol/HCl (HCl:n-butanol, 5:95, by vol.) were added to 10 mg NDF and heated at 95°C for 1 hour. The absorbance was then read at 550 nm in a spectrophotometer (Reed *et al.,* 1982).

For the HPLC analysis, 100 mg of leaf material was extracted with 5 ml 70% methanol at 10°C in an ultrasonic bath. The extract was filtered through a membrane filter and an aliquot (50 μl) was injected into the HPLC system. The column (25 cmx4.6 mm) was packed with μ-Bondapak C^{18} (Waters/Millipore). Glacial acetic acid:water (25:975, by vol.; solvent A) and methanol (solvent B) were used for gradient elution. A linear gradient was applied, starting with 100% solvent A and finishing with 100% solvent B over a 50 min period using a Gradient Master and Consta Metric II and III pumps (LDC/Milton Roy) and a flow of 1 ml min^{-1}. The effluent was monitored with a Spectro Monitor Absorption Detector (LDC) at 280 nm (Mueller-Harvey *et al.*, 1987). Preliminary observations on *S. sesban* chromatograms showed that the major peaks eluted between 20-30 minutes. Therefore all peaks with retention times within this range were counted, numbered and their peak heights determined. The number of major peaks, their retention time and peak height were used as characters in the average linkage cluster analysis using the Statistical Analysis System (SAS, 1987).

Results

Significant differences ($P<0.001$) were found between accessions in soluble phenolic and insoluble proanthocyanidin concentration. The total soluble phenolic concentration in the accessions under study ranged from 2.9-31.2% in DM (mean = 12.0; sd = 5.7). The absorbance readings at 550nm of the insoluble proanthocyanidins ranged from 0.6-103.8 per g NDF (mean = 15.0; sd = 20.6). After cluster analysis in which these two characteristics were used nine clusters could be identified (Table 8.1). A low but significant correlation was found between the soluble phenolic concentration in the leaves and stem colour ($r=0.23$; $P<0.05$) and between insoluble proanthocyanidins and this character ($r=0.19$; $P<0.05$). There were no other significant correlations found between polyphenolic concentration and the earlier observed morphological characteristics.

Table 8.1. Group formation after cluster analysis based on the concentrations of total soluble phenolic and insoluble proanthocyanidin in the leaves of *Sesbania sesban* accessions.

Group No.	No. of accessions	Soluble phenolics (%DM)		Insoluble proanthocyanidins (A550/g NDF)	
		mean	SD*	mean	SD
1	11	7.9	1.8	20.1	4.8
2	57	8.9	4.2	4.6	3.1
3	4	12.0	7.7	71.8	2.7
4	2	16.3	2.2	46.7	1.2
5	23	17.0	3.3	10.6	4.0
6	2	17.9	1.3	33.1	1.8
7	2	20.5	2.4	98.6	5.3
8	1	23.0		61.1	
9	1	31.2		77.8	

*=Standard Deviation

Table 8.2. Group allocation of *Sesbania sesban* accessions based on their phenolic HPLC fingerprints.

Group	Accession numbers	Total
IA	988, 1177, 1178, 1179, 1180, 1229, 1232, 1238, 1246, 1250, 1263, 2000, 2007, 2012, 9164, 14014, 15019, 15021, 15022, 15036, 15368, 15525	22
IB	1200, 1214, 1231, 1259, 1264, 1265, 1275, 1276, 1280, 1281, 1282, 1284, 1285, 1287, 1288, 1289, 1290, 1291, 1293, 1294, 1295, 1296, 1297, 1298, 1299, 1300, 1302, 1303, 10865, 13887, 15364	31
IC	920, 1192, 1221, 1228, 1256	5
ID	1237	1
II	1261, 1262, 1292, 1301, 1304, 9265, 10375, 10639, 13144, 13261, 13491	11
III	1188, 1194, 1195, 1201, 10379	5
IV	1191, 1215, 1216	3
V	15020, 15037, 16841, 16842, 16843	5
VI	2066, 2069	2
VII	1189, 1198, 1193, 1203	4
VIII	9043, 13444	2
IX	1190, 1208, 2024, 2057, 13516	5
X	15023, 15024, 15025, 15077	4
None	2076, 10521, 15018	3

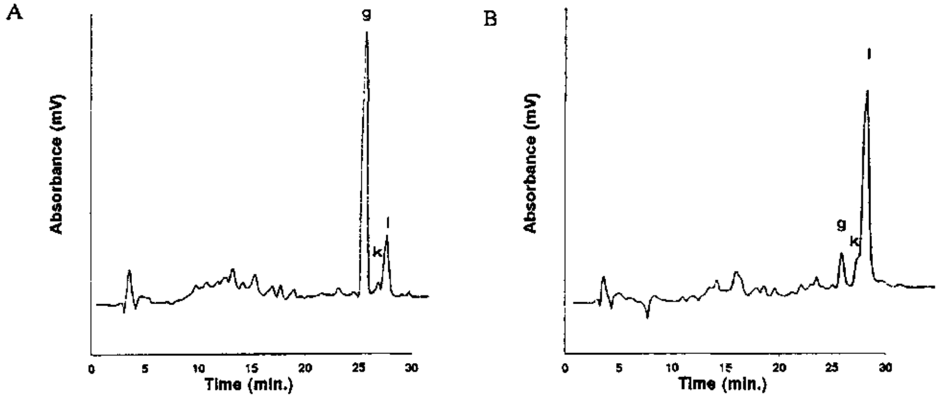

Fig. 8.1. Phenolic HPLC fingerprints of leaf extracts from accession number 15036, belonging to Group IA (A) and 1289, from Group IB (B).

Principle component analysis in which the first component (which explained 56% of the variation) was related to the total number of peaks and the second component (explaining 30% of the variation) was related to the height of the major peak, revealed that the majority of the accessions formed one large group. The accessions belonging to this group I had relatively simple profiles and contained only two prominent peaks. Cluster analysis divided this large group in four smaller sections, mainly due to quantitative differences. Table 8.2 summarizes the group allocation of the accession used in the experiment. Group IA with 22 accessions was formed which had peak g as their major component, whereas another Group (IB) consisting of 31 accessions was composed with peak I as their major component (Figure 8.1). Although quantitative differences must be used with caution, five accessions were separated on the basis of the height of peak g. Accession number 1237 had such a high concentration of component g that it formed a separate cluster. Within these groups only minor qualitative differences were observed. Component k was

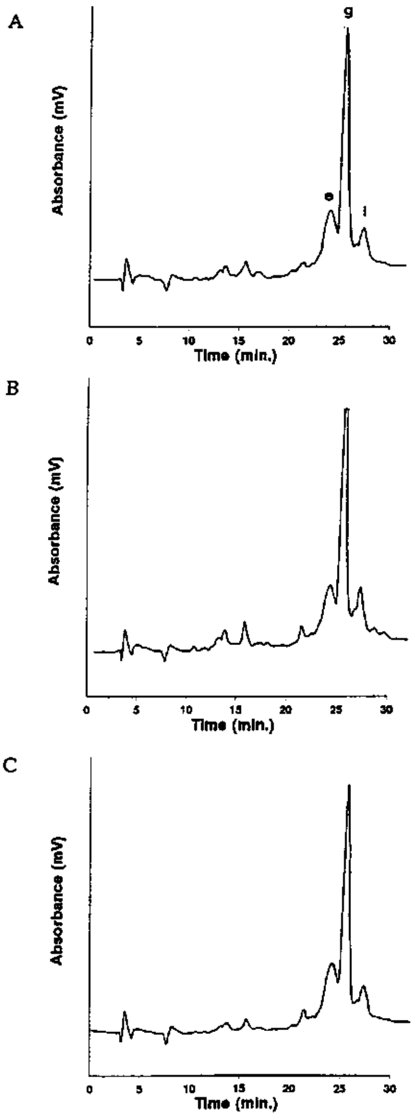

Fig. 2. Phenolic HPLC fingerprints of leaf extracts from accession number 10639 (A); 13144 (B) and 13261 (C), all belonging to Group II.

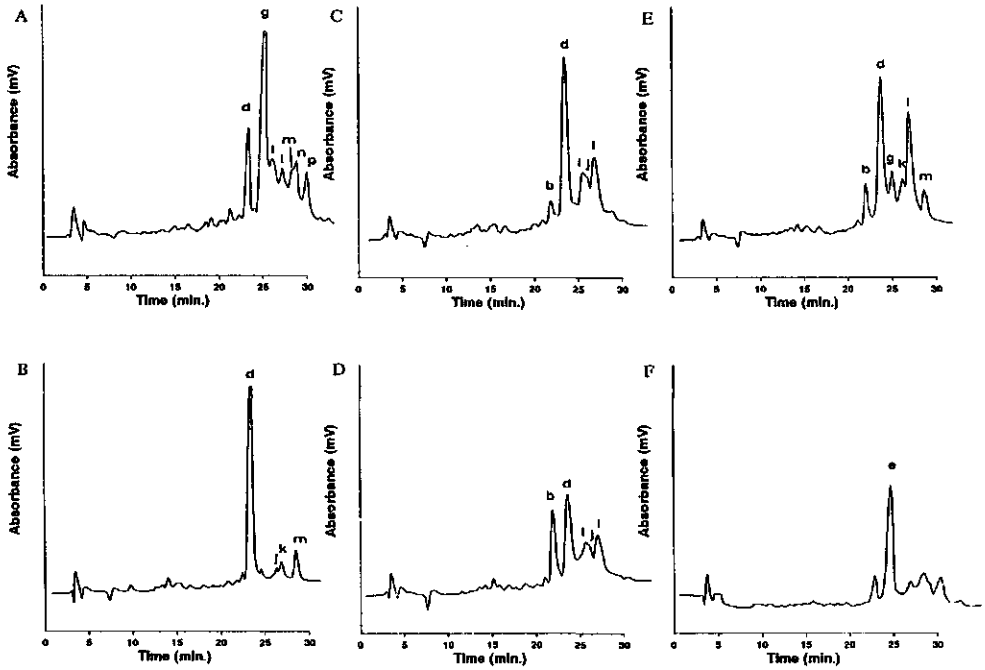

Fig. 8.3. Phenolic HPLC fingerprints of leaf extracts from accession number 1194, from Group III (A); 1215, from Group IV (B); 16842, from Group V (C); 2069, from Group VI (D); 13444, from Group VIII (E) and 1190, from Group IX (F).

present in most accessions, though sometimes only visible as a shoulder.

Further, a cluster (II) was formed comprising 11 accessions with 3 major peaks, which apart from the peaks g and l also included peak e. Within this group three accessions (accessions 10639, 13144 and 13261) had very similar profiles with peak g as the highest peak (Figure 8.2). The accessions 9265, 10375 and 13491 had peak l as their highest peak instead of peak g.

The remaining 33 accessions had more complex profiles and formed three loose clusters which were hard to explain and they were therefore grouped visually. Within most of these groups only minor differences occurred. Five accessions made up Group III which apart from components g and l contained the peaks d, i, m, n and p. Variation within this group was mainly in the amounts at which the components e, j and m were present (Figure 8.3A). A large number of accessions could be identified which had peak d as their highest peak. The three accessions belonging to Group IV were lacking peaks g and l, but contained component m at a relatively high level and j and k in small amounts (Figure 8.3B). The remaining accessions also possessed peak b as one of their components. The accessions in Group V all belonged to variety *sesban* and contained apart from b and d the compounds i, j and l as their major components (Figure 8.3C). The three accessions of Egyptian origin in this group had almost identical profiles. The accessions in Group VI also possessed these five components but at very different quantities (Figure 8.3D). Group VII with four accessions were lacking peak i, but contained an additional peak n instead. The accessions 9043 and 13444 in Group VIII contained the components b, d, g, k, l and m (Figure 8.3E).

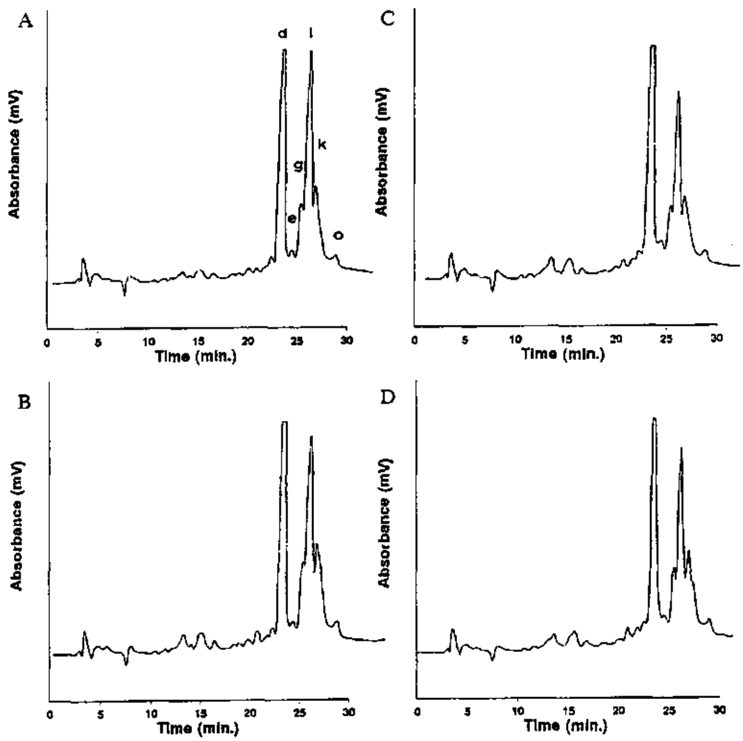

Figure 8.4. Phenolic HPLC fingerprints of leaf extracts from accession number 15023 (A); 15024 (B); 15025 (C) and 15077 (D), all from Group X.

Group IX was formed with five accessions which all had peak e as their major component (Figure 8.3F). However, within this group considerable variation existed. Group X contained four accessions, of which one belonged to var. *bicolor* and the remaining three belonged to var. *sesban*. They had very similar profiles with the components d and i as major and very high peaks, but they also contained the compounds e, g, k and an unique peak o (Figure 8.4). The remaining accessions could, due to various qualitative and quantitative differences not be allocated to any of the previously described groups.

Discussion

The concentration of soluble phenolics and insoluble proanthocyanidins of the accessions under study were somewhat lower than reported in earlier publications (Tothill *et al.,* 1990; Nsahlai *et al.,* 1994). The results nevertheless confirmed that a large variation existed between accessions. This large variation suggests that selection for low levels of polyphenolics and high digestibility should be possible. The relationship of the polyphenolic concentration with morphological attributes showed a significant correlation between stem colour and amounts of soluble phenolics and insoluble proanthocyanidins in the leaves. The correlation coefficients however had such low values that the stem colour can not be used as the only criterion in the selection of accessions which are low in polyphenolics.
The study further showed that different groups of accessions could be formed on the basis of their HPLC fingerprints. The cluster analysis did not always provide clear cut results and additional visual grouping on similarity and uniqueness of the chromatograms was necessary. The HPLC fingerprints of extractable phenolics differed greatly between groups of accessions. The accessions belonging to var. *nubica* possessed qualitatively and quantitatively different profiles compared to those belonging to the var. *sesban* and *bicolor*. Within groups the profiles were sometimes very similar and unless refined methods for component separation were applied it would be difficult to use the fingerprints reliably for the identification of accessions. This is in agreement with results from Plumb *et al.* (in press) who reported that the profiles from mature leaves of the *S. sesban* accessions 15021,

15022 and 15036 were very similar and could not be distinguished from each other.

No clear relations could be observed between the origin of the different accessions and their fingerprints.

In addition to genetic differences between accessions, the environment also effects phenolic composition. Earlier experiments showed that the concentration of soluble phenolics and insoluble proanthocyanidins varied with season (Woodward, 1988) and site (Le Houérou, 1980). In *Sesbania* however, it was found that the soluble phenolic and proanthocyanidin concentrations across accessions did not vary significantly from one site to another (ILCA, 1989). Our observations suggest that HPLC fingerprints from the same accession grown at different sites might have some quantitative differences, but are qualitatively stable. More research would be required to determine environmental effects on polyphenolic composition. A better understanding of the factors influencing leaf phenolic composition would assist in the selection of accessions with improved nutritive value. *Sesbania* genotypes with relatively stable phenolic composition across environments need to be identified.

The HPLC peaks have not been identified individually in this study, but observations on their uv-spectra suggested that the major peaks were flavonol glycosides. Co-chromatography with standard compounds after hydrolysis and observations on the uv-spectra identified the flavonol quercitin as one of the compounds in the leaf extract. In order to study the effects of the different polyphenols on feeding value it would be necessary to look at the effects of the individual compounds. Alternatively, representatives of the different groups could be used in feeding experiments to find out whether there is any relation between animal response and HPLC profiles.

Selection of accessions on both agronomic characters and nutritive value is desirable. This information on group formation could be used with the information on feeding value and in combination with agronomic data to select improved accessions.

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Chapter 9

General discussion

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Multipurpose tree introduction

A multipurpose trees development and introduction program should mainly be aimed at selecting germplasm adapted to low input systems such as marginal or degraded lands where agroforestry can play an important role in the regeneration. Thus in selecting germplasm of woody species special attention should be given to species which show tolerance to particular constraints such as low soil fertility, soil acidity, drought, pests, diseases etc. Apart from showing tolerance to these constraints the species should be capable of producing adequate amounts of fodder, mulch, wood or food which is of a high quality. The main challenge therefore is to identify, select and evaluate multipurpose tree species and accessions within species which are able to enhance the productivity, profitability and sustainability of farming enterprises across agro-ecological zones and land-use systems.

The first step in this process is the acquisition of a wide range of germplasm from which adapted species and accessions can be selected. If the material is not readily available, additional collection of germplasm from areas of the natural distribution of the species is required. In general, the plant genetic resources centers have so far given highest priority to the collection, conservation and characterization of germplasm. Only recently more attention has been given to the systematic evaluation of the accessions and to strategic research which supports germplasm management. With regard to the multipurpose trees, only species such as *Leucaena leucocephala* or *Gliricidia sepium* have received thorough investigation and research in these areas. For most species however, including *Sesbania sp.*, knowledge of the extent of genetic variation, both within and between species, is very limited whereas information regarding reproductive biology and hybridization is completely lacking. This research was carried out in order to promote the usefulness of *S. sesban* germplasm and to provide greater knowledge about the taxonomy, genetics, interspecific relationships and variation in this plant species.

Breeding system and interspecific relations

The three perennial *Sesbania* species used in this study are considered to be largely outcrossing (Chapter 4). For genetic resources management practices this means that seed increase should be done in isolation in order to maintain the genetic integrity of the different accessions. Since selfing is clearly possible it would be interesting to find out the percentage of outcrossing under natural conditions. The determination of the outbreeding percentage however, requires the identification of a stable morphological or biochemical marker. As distinctive differences between individual accessions or groups of accessions in their polyphenolic HPLC fingerprints exist (Chapter 8), it has been suggested to consider the use of these profiles as biochemical markers.

Considerable variation occurred in morphological, agronomical and chemical characters within *Sesbania sesban*, which means that there is a great potential for selection on desired specific characteristics. Selection and evaluation would initially be the preferred method to identify suitable genotypes. On the other hand *S. sesban* also possesses a number of characteristics which would make it suitable for plant breeding. The trees are able to produce flowers and ripe pods within the first year after planting and seed yields are generally good and can be very high under optimal conditions (Chapter 5). Further, the pollen of *S. sesban* is quite tolerant to orthodox storage conditions enabling controlled crosses to be performed in accessions that flower in different seasons of the year (Owuor and Owino, 1993). Finally, techniques for rapid clonal propagation of selected genotypes have already been developed (Hanson, 1993).

The karyotype analysis yielded a somatic chromosome number of 2n = 12 and comparable compliments for the three species under study. This together with the successful hybridization of the species leads to the conclusion that *S. sesban, S. goetzei* and *S. keniensis* are closely related (Chapter 3). Therefore these three species must be considered the 'effective gene pool' for genetic improvement (Brewbaker, 1990) and future collection missions should also include the related species for seed preservation. When collections are carried out in areas where any of these three species grow together, special attention should be given to the identification of natural hybrids. Lewis, (1988) mentioned in his treatment of the genus *Sesbania* that it seemed possible that interspecific hybrids could be common. So far however, they have not been reported. Experience with *Leucaena* has shown that it is possible to use natural and artificial interspecific hybrids for the selection of improved growth forms, resistance to psyllids (*Heteropsylla cubana*) and tolerance to acid soils and cold (Shelton and Brewbaker, 1994). It would also be feasible to carry out hybridization programs with *Sesbania* in the future, if these are considered necessary for the improvement of particular traits of the trees.

Classification and evaluation

The result from the classification and clustering in this study provide a structure for selecting *S. sesban* accessions for agronomic, genetic and breeding studies, where a range of diversity is desired. A major application of this research is to aid in the development of a future core collection of wide variability from the large *S. sesban* collection. Such a core collection could be established by selecting a limited number of entries to represent the ecological and morphological diversity of the germplasm. This would allow the genebank manager to use the available resources more efficiently by maintaining a sufficient quantity of seed of the core entries to respond to the various requests and keep minimal amounts of seed of the other accessions of the genepool. The results obtained in this study could serve as a first step towards defining such a core collection.

The observed characteristics in this study could at the same time provide the basis for the establishment of a descriptor list for *S. sesban* germplasm. Apart from providing a classification of accessions based on reliable data, such a systematic and standardized description can help in the differentiation between accessions and varieties. This classification for instance identified the major morphological characteristics which define the distinction between the variety *nubica* and the variety *sesban* (Chapter 6). It can also assist in the identification of accessions with particular desired characteristics. This study illustrated the possibility of a qualitative and quantitative selection of accessions based on polyphenolic concentration in the leaves (Chapter 8). Furthermore, a systematic description allows the determination of relationships between specific attributes. The existence of a positive correlation between red stem color and a relatively high polyphenolic concentration in the leaves of *S. sesban* trees which was found in this study could serve as an example of this fact. It could also be used to establish relationships between geographical groups of accessions. This however requires adequate information regarding the collection site of each accession (passport data) and stresses the importance of proper documentation of germplasm collections. Finally, a methodical description of the germplasm collection makes it possible to estimate the available variation within the collection.

Prospective use of *S. sesban*

Although *S. sesban* plays an important role as forage and green manure in a number of regions in the world, it could be said that in general the species is relatively underutilized in many tropical and sub tropical areas. There seems to be scope for a greater use of *S. sesban*. In identifying the potential for increasing the use of *S. sesban* however, one has to consider several important aspects. Some of the factors to take into account are the advantages and disadvantages of the species and its products, the ecological zone and agricultural system in which it could be used, the current use in the traditional farming system and finally, the farmers aspiration and acceptance of the species and the technology which accompanies its introduction. Ultimately, it will be the economic and social advantages for a farmer which dictate the acceptance of the species.

The positive attributes *S. sesban* possesses can be summarized by stating that the species is:

- easy to establish and fast growing
- able to fix high levels of nitrogen
- producing high yields of forage which is palatable and high in protein and other nutrients
- tolerant to saline and alkaline soils and can therefore be grown at unproductive sites
- extremely tolerant to waterlogging

- tolerant to cool temperatures and so able to grow at high altitudes
- showing a great genetic variation
- indigenous to Africa and thus well adapted to several conditions of this region.

Possible drawbacks to the increasing use of *S. sesban* have also been reported. They could be summarized as follows:

- the infestation with the beetle *Mesoplatys ochroptera* and its larvae has occasionally caused severe defoliation of the trees
- poor regrowth of some accessions after cutting, particularly under semi-arid conditions
- alleged susceptibility at particular sites to nematodes
- excessive intercrop shading when used in alley cropping
- anti-nutritional factors in the leaves might have a negative effect on animal performance.

It is extremely important that these negative aspects are taken into account when selection and evaluation programs are carried out.

When introducing a species like *S. sesban* one has to realize that a widely known species is not necessarily adapted to every possible site or ecosystem. *S. sesban* has particular promise for the highland ecological zone as it is one of the few agroforestry species that can produce relatively high amounts of forage under cool temperatures. The highland zone in sub-Saharan Africa is defined as all those areas in the semi-arid, subhumid and humid zones where the mean daily temperature during the growing period is below 20 °C (ILCA, 1987). The highlands are further characterized by a high population density and by the highest livestock density of sub-Saharan Africa. The most common farming systems are the smallholder crop-livestock farms. Because these farms are largely crop based and in general do not have access to grazing lands their livestock production depends heavily on feed that is produced on-farm and on available crop residues. Leguminous multipurpose trees like *S. sesban* therefore have great promise in the highlands as a supplement for these low quality feeds.

It could further be introduced in the higher rainfall areas of the semi-arid zone and in some subhumid areas. This region with medium high rainfall offers great opportunity for expanding agricultural production in sub-Saharan Africa (Winrock, 1992). *Sesbania* could play a role in areas with higher population density where its foliage could either be used as a green manure in existing cropping systems or as a supplement to crop residues for ruminants in mixed crop-livestock systems.

Sesbania could be used for alley cropping or as a species for improved fallow. So far, most alley farming has been carried out with the tree legumes *Leucaena* and *Gliricidia*. The wide use of only one or two species however involves great risks of infestation with pests and diseases. Species diversification adds to the system's stability. The introduction of *S. sesban* in those areas where its forage yields are comparable to the commonly used species could contribute to that aspect.

It could also be useful in places prone to seasonal waterlogging or flooding. Where saline and alkaline conditions restrict the agricultural productivity, *S. sesban* could be used to reclaim some of these sites. When introducing the species, special attention should be given to those areas where *Sesbania* is currently used in the traditional system and ways for improvement and intensification should be determined.

Future research

Although considerable research efforts have recently been focused on *S. sesban* and its related species, there are still several areas that require more insight. Some of the subjects which in the author's opinion require further study are discussed below.

If continued serious research attention has to be given to the use of *Sesbania* species, systematic germplasm collections should also be carried out in those areas of its natural distribution that have not been covered so far. With regard to the *S. sesban* complex, hardly any material is available of the subspecies *punctata* and very little information exists about its agricultural value. Also the varieties *sesban* and *bicolor* which are widely distributed in Asia are underrepresented in the existing germplasm collections. The systematic collection of *Sesbania* germplasm in Asia has not been reported so far, but might be worthwhile, considering its use in Asian farming systems. A future large scale collection of germplasm in Southern African countries will probably depend on the outcome of the ongoing screening and evaluation of material that has recently been collected in this region (Ndungu and Boland, 1994). The evaluation and selection of the existing *S. sesban* collection should especially be focussed on identifying accessions with a great longevity, good coppicing ability and high resistance against pests and diseases. With regard to pests, special attention should be given to the beetle *Mesoplatys ochroptera* and its larvae and to nematodes, because they can have a negative effect on the production and survival of the trees.

More research is needed to fine-tune the appropriate management systems to the different agro-ecological settings in order to maximize the foliage yields. For alley farming and fallow planting strategic research is necessary to determine the effects of *S. sesban* on the fertility of the soil. This includes the quantification of nitrogen fixation and studies regarding the rooting behavior and nutrient cycling dynamics.

Feeding studies on *S. sesban* have mostly been limited to its use in cut and carry systems. Because of the great interest to the use of the trees in extensive farming systems (particularly in Australia) the effects of direct grazing on the regrowth and longevity need to be examined. As described in the introduction, the use of *S. sesban* as a fodder should be restricted to ruminants because of the deleterious effects that have been observed in monogastric animals. However, even when fed to ruminants as a high proportion of the ration and over long periods of time the forage might have an adverse effect on animal production and health. Work on anti-nutritional factors should therefore try to identify the compound or compounds which cause these negative effects on animal performance. Once identified, there is a need to find out whether the anti-nutritional factors can be controlled or reduced through selection or by management practices.

On-farm research and development activities should be expanded and carried out in a wide range of agro-ecological and social-economic conditions. This way it will be possible to assess the productivity of *S. sesban* under on farm management conditions and to determine its acceptability and adaptability by farmers.

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Summary

Botanical and agronomic evaluation of a collection of *S. sesban* and related perennial species

Background

One of the major constraints for increasing livestock productivity in sub-Saharan Africa is the shortage and poor quality of the available feed resources. The increased utilization of leguminous and other tree and shrub species, fed as a high quality supplement to the low quality natural pastures and crop residues could improve the forage supplies of ruminants in pastoral and crop-livestock systems. Most research on leguminous trees has been confined to the two species *Leucaena leucocephala* and *Gliricidia sepium*. The infestation of *L. leucocephala* with the psyllid *Heteropsylla cubana* however, has stimulated an effort to obtain species diversification. Several other species are therefore being evaluated for use in agroforestry systems.

Some of the perennial *Sesbania* species possess several desirable characteristics which make them suitable for use as multipurpose trees in farming systems. Because of this, the three species *S. goetzei, S. keniensis* and *S. sesban* in particular, have recently attracted considerable interest. They are all indigenous to Africa, but *S. sesban* is the most widely distributed and most important species, with a large amount of accessions collected. As described in Chapter 2 it shows a rapid early growth, grows well in various ecological environments and can produce high yields of leaf material after repeated cutting which contains a high protein concentration.

This study was initiated to provide information on these three species regarding their chromosome numbers, breeding system and interspecific relations. Knowledge of these areas is required in the establishment of proper seed multiplication techniques for the development and future utilization of the species and in their regeneration for genetic resources conservation. The thesis further focuses on the evaluation and classification of a *S. sesban* collection on agronomic, morphological and nutritional characteristics. Such information is necessary for the maintenance and use of large genetic resources collections. It can provide the basis for the selection of specific accessions for agronomic, genetic or breeding purposes. Finally, the study demonstrated the multi disciplinary research approach which is needed for the development of multipurpose trees germplasm.

Breeding systems and interspecific relations

Chapter 3 reports on the karyotype analysis and interspecific relations in the three species. The somatic chromosome number in *S. sesban*, *S. goetzei* and *S. keniensis* was found to be $2n = 12$. The karyotypes were quite similar for the three species. Interspecific hybridization was carried out in all possible combinations and resulted in pod and viable seed formation for all crosses except for those in which *S. keniensis* was used as the female parent. This leads to the conclusion that the three species are phylogenetically closely related. Since there is an overlap in the distribution of the species, natural hybrids may occur, but they have not been reported so far.

The reproductive biology of the three species is described in Chapter 4 and data regarding the phenology of flowering and seeding are presented. The pollination experiment showed that all three species were self compatible and no evidence was found for stigmatic or stylar incompatibility. *S. sesban* and *S. goetzei* were also found to be cross compatible and the percentage of fully developed seeds per pod was greater in pods formed after cross pollination compared to self pollination. It was concluded that outcrossing is probably the common method of reproduction under natural conditions.

Evaluation and classification

Chapter 5 reports on the evaluation for forage, wood and seed production of six *S. sesban* accessions grown over an eighteen months period, under irrigated conditions. Two cutting frequencies (3 and 6 months) and heights (75 cm and 150 cm) were applied. The trees showed a rapid early growth, with almost 10 t/ha total

DM produced after six months for the highest yielding accession. Total DM yield was higher at the six months cutting frequency compared to the three months cutting interval. Plant survival was better at the three months cutting interval. In general leaf DM production was higher at the higher cutting level. After 18 months undisturbed growth seed yields between 2.7-6.6 t/ha were obtained.

The results of the classification of a collection of 108 accessions of *S. sesban* on morphological and agronomic attributes are reported in Chapters 6 and 7, respectively. The classification based on morphological attributes resulted in the formation in two groups which corresponded to the different varieties that exist in the subspecies *sesban*. The varieties *sesban* and *bicolor* formed one group and the variety *nubica* the other. The characteristics that contributed to this division were leaflet size and number, seed size and colour and growth habit. Within the variety *nubica* five groups were identified by both clustering algorithms that were used. The average linkage algorithm used the quantitative characters leaflet size, leaflet number, pod size and flower size as distinguishing attributes, whereas in the Partitioning Around Medoids method the qualitative characters stem and flower colour and indumentum were used for grouping accessions.

Clustering the accessions by means of agronomic characteristics produced several groups which were mainly based upon dry matter yields after cutting. Standardization of the data reduced the influence of the plant yield in the cluster analysis. The accessions belonging to the varieties *bicolor* and *sesban* were separated from those belonging to var. *nubica* because of their higher seed weights. High and significant correlations were found between the plant dry matter yields after six months growth and the characters height of the trees and diameter of the stems at 30 cm above the ground level. Predictive biomass production equations were obtained with the use of these characters which had R^2 values between 84-89 %. Establishment of these equations could provide a quick and non destructive estimation of the biomass which could be used in future evaluation and on-farm experiments.

The same *S. sesban* collection was used to determine the differences between accessions in their concentration of polyphenolic compounds in the leaves and High Performance Liquid Chromatography (HPLC) fingerprints. The results of this experiment are discussed in Chapter 8. Large variation was found between the accessions in the concentration of soluble phenolics and insoluble proanthocyanidins in the leaves. Individual accessions or groups of accessions could be identified on the basis of qualitative and quantitative differences in their HPLC chromatograms.

Consequences for the use of *S. sesban*

The study showed that considerable variation exists within the *S. sesban* collection in morphological, agronomic and nutritional characters and that it is possible to identify different groups on the basis of these attributes. The observed characters could serve as a first step in the establishment of a descriptor list for *S. sesban.* The obtained information could at the same time be used in the development of a core collection and provide a structure for selecting accessions for agronomic or breeding studies. Apart from providing a great potential for selection, the species possesses several characteristics such as early flowering, good seed yield, ability to produce seeds after self and cross pollination and the ability to produce viable seeds after hybridization with *S. goetzei* or *S. keniensis,* which makes it also suitable for plant breeding.

It could be stated that the species is at the moment relatively underutilized and there seems to be scope for a greater use of it, particularly in alley farming or for improved fallow. Based on its ecological adaptation and agronomic potential *S. sesban* has definite potential for the highlands and could further be introduced to the higher rainfall areas of the semi-arid zone and in some subhumid areas. It could be very useful to reclaim some of the areas with saline and alkaline soils and could also be grown in places prone to seasonal waterlogging and flooding.

Areas which require future research attention are the collection of germplasm, the evaluation and selection in different ecological conditions of accessions with a great longevity, good coppicing ability and resistance against pest and diseases, the identification of anti-nutritional factors and the further adjustment of the management techniques to the systems in which the species is being used.

Samenvatting

Botanische en agronomische evaluatie van een collectie *S. sesban* en verwante meerjarige soorten

Achtergrond

Een van de grootste beperkingen voor het verhogen van de produktiviteit van het vee in sub-Sahara Afrika is het tekort aan en de slechte kwaliteit van het beschikbare ruwvoer. Vlinderbloemige bomen en struiken zouden gebruikt kunnen worden als een kwalitatief hoogwaardig supplement bij het natuurlijk grasland en de gewasresten die grotendeels het dieet bepalen. Dit zou de ruwvoer voorziening van de herkauwers in de pastorale systemen en in gemengde bedrijfssystemen aanzienlijk kunnen verbeteren. Tot dusverre heeft het onderzoek aan vlinderbloemige bomen en stuiken zich vooral gericht op twee soorten, nl. *Leucaena leucocephala* en *Gliricidia sepium*. De plotselinge uitbraak van een insectenplaag, veroorzaakt door *Heteropsylla cubana*, leidde echter tot aanzienlijke dalingen in de opbrengst van de oogst van *L. leucocephala*. Dit toonde de gevaren van een krappe soortenlijst aan en heeft er mede toe geleid dat men op zoek is gegaan naar de identificatie van andere soorten die in agroforestry systemen gebruikt kunnen worden.

Een van de families waarin verschillende soorten voorkomen die geschikt zouden kunnen zijn voor gebruik als multipurpose tree is *Sesbania*. *S. sesban, S. goetzei* en *S. keniensis* zijn alle drie Afrikaanse inheemse soorten, maar de eerst genoemde is de belangrijkste, met het grootste verspreidingsgebied, waarvan er al vele accessies verzameld zijn. De eigenschappen van *S. sesban* zijn in Hoofdstuk 2 beschreven. De belangrijkste zijn: een zeer snelle groei, het bezit de mogelijkheid om onder vele ecologische omstandigheden te groeien en het is in staat om een grote hoeveelheid bladmateriaal te produceren, dat een hoog eiwit gehalte bevat.

Het doel van dit onderzoek was om informatie te verschaffen over het aantal chromosomen dat deze verschillende soorten bezitten en de verwantschap tussen de soorten en hun bestuivingswijzen vast te stellen. Deze kennis is noodzakelijk voor de ontwikkeling van de juiste zaadvermeerderingstechnieken, voor het toepassen van de juiste methode bij zaadcollectie en voor het uitvoeren van veredelingsprogramma's. Deze studie beschrijft bovendien de evaluatie en classificatie van een *S. sesban* collectie aan de hand van agronomische, morfologische en nutritionele eigenschappen. Deze informatie is noodzakelijk voor een efficiënt beheer van een genenbank en kan het selecteren van accessies met specifieke eigenschappen vereenvoudigen. Tenslotte is geprobeerd de noodzaak van een multi-disciplinaire benadering bij de ontwikkeling van multipurpose trees duidelijk te maken.

Bestuivingswijze en verwantschap tussen de soorten

Hoofdstuk 3 beschrijft de karyotype analyse en de verwantschap tussen de soorten. De drie soorten bleken een chromosomenaantal van $2n=12$ te bezitten en de karyotypes vertoonden grote overeenkomst. Dit zou kunnen betekenen dat het mogelijk is om hybriden tussen de drie soorten te produceren. Het resultaat van de kruisingen tussen de soorten was dat er in alle gevallen, behalve die waarin *S. keniensis* als vrouwelijke ouderplant werd gebruikt, peulen en kiemkrachtig zaad werd geproduceerd. Dit duidt op een nauwe fylogenetische verwantschap tussen de soorten. Omdat de soorten in dezelfde ecologische omgeving voorkomen en de verspreidingsgebieden zelfs overlappen, zou het mogelijk kunnen zijn om natuurlijke hybriden aan te treffen, maar deze zijn tot dus verre niet gemeld.

De biologie van de bloei wordt in Hoofdstuk 4 uiteengezet. Het resultaat van de bestuivingsproeven was, dat er zaadzetting mogelijk is na zelfbestuiving in alle drie soorten. Er waren geen aanwijzingen voor het optreden van incompatibiliteits reacties in de stijl of stamper. Zowel *S. sesban* als *S. keniensis* waren tot kruisbevruchting in staat, hetgeen tot een groter aantal volledig ontwikkelde zaden per peul leidde, in vergelijking met zelfbevruchting. De conclusie is dan ook dat onder natuurlijke omstandigheden de vruchtzetting plaats vindt door middel van kruisbestuivingen.

Evaluatie en classificatie

Hoofdstuk 5 geeft de resultaten van de evaluatie naar de blad-, hout- en zaadproduktiecapaciteit van zes *S. sesban* accessies, die over een periode van 18 maanden onder irrigatie verbouwd werden. Twee oogstintervallen (3 en 6 maanden) en twee snijhoogtes (75 cm en 150 cm) werden vergeleken met ongestoorde groei over een periode van 18 maanden. De bomen hadden een snelle vroege groei en de accessie met de hoogste opbrengst produceerde bijna 10 t/ha totale droge stof (DS) na zes maanden groei. De totale DS-opbrengst was, in vergelijking met een oogstinterval van 3 maanden, hoger bij een 6 maands oogstinterval. Omdat er bij een oogstinterval van 3 maanden meer blad aan de planten over bleef na de oogst, hadden deze een hoger overlevingspercentage. Over het algemeen leverde de grotere snijhoogte de grootste opbrengst aan blad DS. Na een periode van 18 maanden ongestoorde groei hadden de controle bomen een zaadproductie van tussen de 2.7 en 6.6 t/ha geleverd.

De resultaten van de classificatie van een *S. sesban* collectie, bestaande uit 108 accessies op grond van morfologische en agronomische eigenschappen worden in Hoofdstuk 6 en 7 weergegeven. Bij de classificatie, gebaseerd op morfologische kenmerken ontstonden 2 groepen die overeenkwamen met de verschillende variëteiten die er in de sub-soort *sesban* bestaan. Zo werd er een groep gevormd door de twee variëteiten *sesban* en *bicolor* en een groep door de var. *nubica*. De vorming van de twee groepen werd hoofdzakelijk veroorzaakt door verschillen in de kenmerken blaadjesgrootte en -aantal, zaadgrootte en -kleur en groeivorm. Binnen de var. *nubica* was het zowel bij gebruik van het 'average linkage' algoritme als bij het 'Partitioning Around Medoids' algoritme mogelijk om vijf groepen te onderscheiden. Bij de eerste methode was de indeling vooral gebaseerd op kwantitatieve kenmerken zoals de grootte en het aantal blaadjes per blad, de lengte van de peulen en de grootte van de bloem. De tweede methode maakte gebruik van kwalitatieve kenmerken zoals de kleur van de stam en van de bloem en de aanwezigheid van beharing op blad en stam.

De clustering van de accessies aan de hand van agronomische kenmerken leverde een aantal groepen op die hoofdzakelijk gebaseerd waren op verschillen in droge stof opbrengst na oogsten. Standaardisering van de data verminderde de invloed van de plant opbrengst in de cluster analyse. De accessies die behoren tot de variëteiten *sesban* en *bicolor* werden ook nu gescheiden van de var. *nubica*, en wel op basis van verschillen in zaadgewicht, dat hoger is voor de eerstgenoemden. Er werd een hoge en significante correlatie gevonden tussen de droge stof opbrengst van de bomen na zes maanden groei en de kenmerken hoogte van de bomen en diameter van de stam op 30 cm van het bodemoppervlak. Door gebruik te maken van deze twee kenmerken werden er vergelijkingen opgesteld die een voorspelling geven van de biomassa produktie met r^2 waarden tussen de 84-89 %. Het verder ontwikkelen van deze vergelijkingen zou een snelle, niet-destructieve methode kunnen opleveren voor het schatten van de biomassa, die gebruikt kan worden in toekomstige evaluatie en on-farm experimenten.

De *S. sesban* collectie werd ook gebruikt om na te gaan of er verschillen bestaan tussen accessies in de concentratie van polyfenolen in de bladeren en in de door High Performance Liquid Chromatography (HPLC) verkregen chromatogrammen. De resultaten van dit experiment worden in Hoofdstuk 8 besproken. Er bestonden grote verschillen in de concentratie aan oplosbare fenolen en onoplosbare proanthocyaniden in de bladeren tussen de verschillende accessies. Het bleek mogelijk te zijn om de afzonderlijke accessies of groepen van accessies te onderscheiden op grond van kwantitatieve en kwalitatieve verschillen in de HPLC chromatogrammen. Met behulp van deze methode is het mogelijk om accessies, die bepaalde gewenste nutritionele eigenschappen t.a.v hun fenolen samenstelling bezitten, te selecteren.

Consequenties voor het gebruik van *S. sesban*

Deze studie heeft aangetoond dat er aanzienlijke verschillen bestaan in morfologische, agronomische en nutritionele eigenschappen tussen de accessies die deel uitmaken van deze *S. sesban* collectie. Tevens bleek het mogelijk om aan de hand van de gemeten kenmerken d.m.v. cluster analyse verschillende groepen in de collectie te onderscheiden. De gemeten kenmerken zouden de basis kunnen vormen voor een te ontwikkelen beschrijvingslijst van de soort, zoals die in genenbank management gebruikt wordt. Aan de hand hiervan zouden accessies geselecteerd kunnen worden voor agronomisch of veredelingsonderzoek. De verzamelde informatie zou tevens het begin kunnen zijn voor het opzetten van en basis collectie (core collection) van de soort. De gevonden variatie toont aan dat er grote mogelijkheden bestaan om d.m.v. selectie de soort te verbeteren. *S. sesban* heeft bovendien verschillende eigenschappen die het geschikt maken voor gebruik in veredelingsprogramma's zoals: een vroege bloei, een hoge zaad opbrengst, de mogelijkheid om zowel na zelf- als kruisbestuiving zaad te produceren en de mogelijkheid tot het produceren van hybriden met de soorten *S. goetzei* en *S. keniensis*.

Men zou kunnen zeggen dat de soort op dit moment nog onvoldoende benut wordt en er kan meer gebruik van gemaakt worden, vooral in alley farming en in systemen voor een verbeterde braak. Gelet op de ecologische omstandigheden en agronomische eigenschappen is het vooral voor gebruik in de Afrikaanse hooglanden een geschikte soort. Bovendien kan het in de semi-aride streken met een hogere neerslag en in een aantal sub-humide gebieden geïntroduceerd worden. Het biedt de mogelijkheid om verzilte en alkalische gronden opnieuw in produktie te brengen en kan ook geplant worden op plaatsen die regelmatig overstromen.

Toekomstig onderzoek zou zich vooral moeten richten op de volgende gebieden: het verzamelen van zaad in de natuurlijke verspreidingsgebieden waarin dit nog niet heeft plaatsgevonden; het evalueren en selecteren, onder verschillende agro-ecologische omstandigheden, van accessies met een verhoogde levensduur, een goede bestendigheid tegen geregeld oogsten en een grote resistentie tegen ziekten en plagen; de identificatie van anti-nutritionele factoren en tenslotte het beter afstemmen van de management technieken op de toegepaste produktiesystemen.

Curriculum vitae

Jurgen Heere (Hero) Heering was born on August 29, 1959 in Vaassen. He completed the Atheneum B secondary school at the Christelijk Lyceum in Apeldoorn and started his university studies in Zoötechniek (Animal husbandry) at the Agricultural University in Wageningen in 1980. He spent a seven months practical training period at the Mid-Country Livestock Development Centre (MLDC) in Mahaberiyatenne, Sri Lanka and a six months period as an undergraduate associate at the International Livestock Centre for Africa (ILCA) in Ethiopia. He graduated in 1987 after passing his exams in tropical animal production, grassland science (major subjects), animal nutrition and extension education (minor subjects). In 1988 he was contracted by ILCA as the manager of the Zwai Seed Multiplication Station, in the Rift Valley of Ethiopia. From April 1989 to June 1994 he was employed by the Dutch Ministry of Foreign Affairs (DGIS) and stationed as bilateral associate expert at the Forage Genetic Resources Section of ILCA in Addis Ababa, Ethiopia. From June 1994 - December 1994 he spent his sabbatical leave at the Department of Agronomy of the Agricultural University, Wageningen where he finished his thesis. Since February 1995 he is employed as Range Management Adviser for DHV Consultants BV and based at the Social Forestry Project, Malakand/Dir, Saidu Sharif, Pakistan.