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

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Abstract

A collection of 108 accessions of *Sesbania sesban* (Fabacea, Papilionoideae) was grown in the field and classified using morphological attributes. Numerical analysis resulted in the division of the collection into 2 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 5 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* 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 focussed on the perennial species (Macklin and Evans 1990). Of the perennial species, *Sesbania sesban* has shown potential as a multi-purpose 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 characterisation 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_20) of 8.05, organic matter 1.96%, total N 0.01%, (Bray II) extractable P 4.57 mg/kg and exchangeable Ca 34.02 mg/kg, Mg 2.20 mg/kg, K 4.03 mg/kg and Na 0.27 mg/kg at 0–35 cm depth (Kamara and Haque 1988). The annual mean maximum and minimum temperatures (as a mean of 9

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years) of the area are approximately 27°C and 13°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 2 adjacent 5 m rows. Planting distance was 50 cm apart within rows, with rows 1.5 m apart. No fertiliser was applied during the trial period. A complete list of the morphological attributes measured is given in Table 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.

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 2. The results indicated a considerable correlation

Table 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 2-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 leaflets counted on the same leaf. 20 observations from 10 plants.
7	Leaflet surface below (LFSB)	(1) Smooth; (2) sparse hairs; (3) medium dense hairs; (4) dense hairs.
8	Leaflet surface margin (LFSM)	(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.

Table 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	–0.19*[1]																
4	MLFL	0.29																
5	MLFW	0.25			0.92													
6	MLFN	–0.29			–0.28	–0.39												
7	LFSB		0.49	0.40														
8	LFSM		0.59	0.24*				0.66										
9	MSTL	–0.34			–0.35	–0.36	0.36											
10	MSTW	–0.24*			–0.31	–0.32	0.38			0.92								
11	MKL	–0.36			–0.47	–0.47	0.36		–0.23*	0.74	0.68							
12	MKW	–0.33		0.25	–0.29	–0.29	0.22*			0.68	0.60	0.74						
13	FLCO	–0.24*		0.26														
14	MNF		0.37	0.22*	0.45	0.37		0.41	0.42		–0.20*	–0.34						
15	MPL							–0.22*	–0.24*									
16	MSEL	0.35			0.61	0.59	–0.48	–0.24*		–0.52	–0.45	–0.44	–0.39			0.37		
17	MSEW	0.24			0.44	0.38	–0.28			–0.25	–0.21*	–0.32			0.47	0.23*	0.45	
18	SECO	–0.39			–0.48	–0.46	0.36	0.31	0.34	0.48	0.36	0.44	0.48	0.20*		–0.25*	–0.67	–0.43

[1]Significant at 5% level; all others significant at 1% level.

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, 4 variables were omitted from the final run of the Principal Component Analyses (PCA). The first 2 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 3).

When the accessions were plotted against the first 2 components only 2 main groupings could be identified (Figure 1). The groups were formed

Table 3. Eigenvalues of the covariance matrix and eigenvectors of the first 5 principal components.

	PRIN1[1]	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[2]	0.972	0.203	0.017	−0.094	−0.034
MLFL	−0.191	0.878	0.109	−0.189	0.363
MPL	0.002	0.203	−0.892	0.344	−0.097
MNF	−0.001	0.312	0.308	0.620	−0.449
MSTL	0.088	−0.106	−0.087	0.322	0.679
GRHA	−0.032	0.040	0.016	−0.075	−0.135
STS	−0.019	0.029	0.144	0.342	0.019
STCO	−0.014	0.015	0.019	0.147	0.052
FLCO	0.002	−0.021	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	−0.021
MSEW	−0.008	0.019	−0.003	0.019	−0.029
MSEL	−0.041	0.072	−0.059	−0.041	−0.089
SECO	0.088	−0.169	0.159	0.331	0.400

Total variance = 78.57 [1]PRIN1 = the first principal component, etc. [2]Variable abbreviations as defined in Table 1.

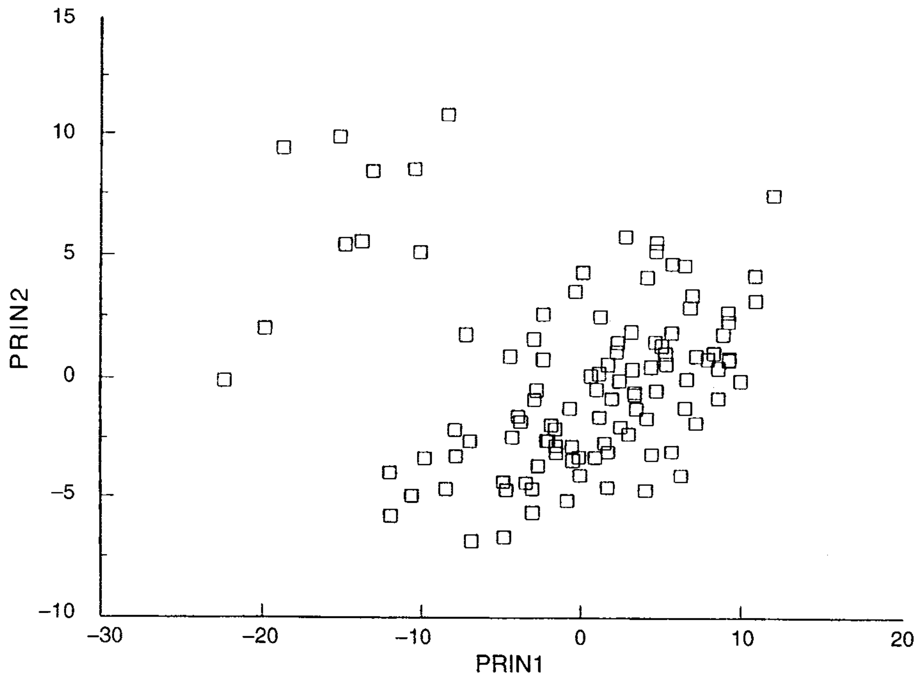

Figure 1. Graph of the 108 accessions of *S. sesban* plotted against the first 2 principal components of the covariance matrix (explaining 81% of the variance). PRIN1 = the first principal component, PRIN2 = the second principal component.

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*.

The results of the linear discriminant functions, using the most important and significant variables for these main two groups are given in Tables 4 and 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.

Table 4. Basic univariate test statistics for the 2 variety classes.

Variable	Total sample SD[1]	Pooled within class SD	Between-class SD	Significance level
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

[1]SD = Standard deviation.

Table 5. Linear discriminant functions for the 2 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

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, 7 clusters were defined mainly on the basis of quantitative attributes (Figure 2).

The PAM program was used only to form clusters within the variety *nubica*. It was found that the division into 5 clusters was best. In this algorithm, the division was mainly based on the

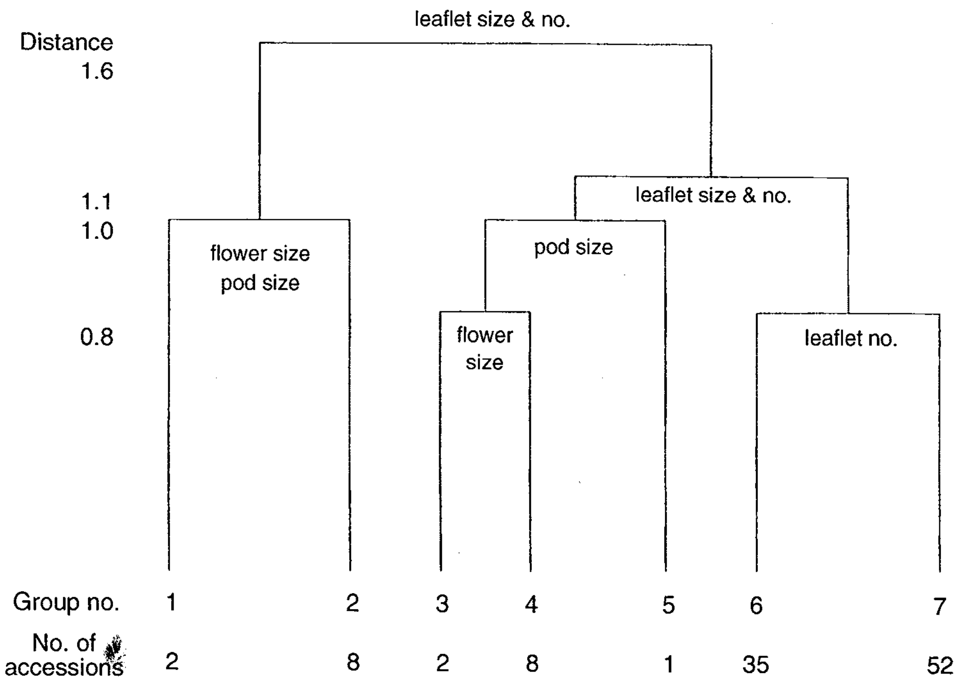

Figure 2. Dendrogram of the morphological classification of the 108 *S. sesban* accessions, based on the average linkage algorithm. Height of the clusters indicates the normalised root mean square (RMS) distance between joined groups.

observed ordinal characters. Discriminant analysis confirmed the division into 5 groups with very few misclassifications. A list with information regarding the origin and group allocation of the individual accessions used in this study can be found in Appendix 1.

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, 7 clusters were defined and although the method of formation of the classification was agglomerative it will be discussed from the root downwards (Figure 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 2 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 with the area of origin, partly due to a lack of information regarding the exact place of collection in the passport data. 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. The above mentioned 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 and yielded a completely different group allocation for the different accessions. 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 2 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 5 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.

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 (N'dungu 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.

Acknowledgements

The authors acknowledge Dr J. Hanson and Professor L. 't Mannetje for their comments on the manuscript. The authors are also indebted to the staff of the Zwai Station, in particular Mr M. van de Wouw, for their assistance in this study. Financial support for this project was provided by the German Bundesminister fuer Wirtschaftliche Zusammenarbeit (BMZ).

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Appendix 1. Origin and group allocation after cluster analysis on the morphological and agronomic characters for the *S. sesban* collection.

ILCA No.	Origin[1]	Latitude	Longitude	Altitude (m)	Annual rainfall (mm)	Group allocation		
						Morphological characters		Agronomic characters
						SAS[2]	PAM[3]	SAS
920	TZA				1200	6	1	8
988	RWA	02°17'S	030°14'E	1400		7	1	8
1177	TZA			360	895	6	5	7
1178	TZA	06°22'S	037°18'E	430	544	7	5	6
1179	TZA	06°21'S	037°15'E	680	544	6	5	8
1180	TZA			900	544	7	5	7
1188	TZA	08°05'S	037°47'E	1520	626	4	4	8
1189	TZA			1300	672	4	5	7
1190	TZA			1060	672	7	4	7
1191	TZA			1050	672	4	4	7
1192	TZA			1080	672	7	4	8
1193	TZA			1060	672	7	4	8
1194	TZA			1180	883	7	5	8
1195	TZA			1550	1154	7	5	8
1198	TZA			1380		7	4	8
1200	TZA			1350		7	5	8
1201	TZA			1810		7	2	6
1203	TZA			800		7	2	7
1208	TZA			800		4	5	8
1214	TZA			975		7	2	8
1215	TZA			780	977	4	2	8
1216	TZA			780	977	7	5	8
1221	TZA			1120		7	4	3
1228	TZA	01°51'S	031°39'E	1200	972	7	1	6
1229	TZA	01°11'S	031°44'E	1110	2040	4	4	9
1231	TZA			1090	2040	7	1	8
1232	TZA			1090	2040	7	1	8
1236	TZA			1300		7	4	8
1237	TZA			1280	972	6	1	3
1238	TZA			1280	972	6	1	8
1246	TZA			1100		6	4	10
1250	TZA			1080	1002	6	1	8
1256	TZA			1400		6	4	8
1259	TZA	04°02'S	035°46'E	1000	1074	6	5	7
1261	TZA			940	1074	7	4	7
1262	TZA			920	1074	7	4	8
1264	TZA			940	809	7	4	8
1265	TZA			910	809	7	5	6
1275	TZA			600		6	3	6
1276	TZA	04°19'S	037°30'E	600		7	5	8
1280	TZA	04°19'S	037°30'E	600		6	3	6
1281	TZA	04°22'S	038°02'E	400		7	5	8
1282	TZA	04°22'S	038°02'E	400		6	3	6
1283	TZA	04°22'S	038°02'E	400		7	5	7
1284	TZA	04°22'S	038°03'E	400		6	3	6
1285	TZA	04°38'S	038°45'E	400		7	3	6
1286	TZA	04°38'S	038°04'E	400		7	5	8
1287	TZA	04°46'S		400	611	6	3	6
1288	TZA	04°48'S	038°12'E	390	611	6	3	6
1289	TZA	04°55'S	038°17'E	385	611	6	5	6
1290	TZA			350	611	6	3	6
1291	TZA			220	1321	6	3	6
1292	TZA			235	1321	7	4	6
1293	TZA	04°33'S	037°41'E	625		6	3	7
1294	TZA	04°33'S	037°41'E	625		7	3	3
1295	TZA	04°33'S	037°41'E	625		7	5	6
1296	TZA	04°33'S	037°41'E	625		6	3	6
1297	TZA	04°33'S	037°41'E	625		7	5	6
1298	TZA	04°33'S	037°41'E	625		6	4	6
1299	TZA	04°33'S	037°41'E	625		6	5	6
1300	TZA	14°34'S	056°19'W	410	1230	7	5	6
1301	TZA	04°33'S	037°41'E	625		7	5	6
1302	TZA	04°33'S	037°41'E	840		7	5	6
1303	TZA	04°33'S	037°41'E	700	858	7	4	8

ILCA No.	Origin[1]	Latitude	Longitude	Altitude (m)	Annual rainfall (mm)	Group allocation: Morphological characters SAS[2]	Group allocation: Morphological characters PAM[3]	Group allocation: Agronomic characters SAS
1304	TZA	04°33′S	037°41′E	710	858	7	4	3
2000	ETH	08°21′N	039°05′E	1750		7	1	6
2007	ETH			1320		7	4	8
2012	ETH			2100		4	1	10
2021	ETH	05°22′N	039°32′E	1420	700	7	4	8
2024	ETH	05°28′N	039°29′E	1470	700	7	4	7
2055	ETH	10°58′N	036°25′E	1700	1500	3	2	8
2057	ETH	10°59′N	036°23′E	1740	1500	6	2	8
2066	ETH	10°58′N	036°22′E	1700	1500	5	2	8
2069	ETH	11°08′N	036°03′E	1200	1200	6	2	8
2076	ETH	09°57′N	038°19′E	1400	1000	4	4	8
9043	ETH	07°05′N	038°30′E	1680	970	7	4	7
9164	ETH	07°56′N	038°43′E	1550	700	7	5	7
9265						7	5	—
10375	ETH	06°06′N	037°37′E	1200	900	7	5	8
10379	ETH	06°25′N	037°22′E	1470	900	7	2	8
10381	ETH					6	2	8
10521	ETH	06°50′N	037°45′E	1925	1300	6	5	8
10639	ETH	07°45′N	036°34′E	1640	1700	7	1	8
10865						7	4	7
13144	KEN	00°35′N	034°34′E	1450	1900	6	2	10
13261	KEN	00°01′S	034°44′E	1200	1300	6	2	8
13444	ETH	05°13′N	039°46′E	1650		3	4	8
13491	ETH	06°08′N	037°35′E	1860	1500	6	5	8
13516	ETH	06°24′N	037°06′E	1150	1500	7	5	8
13887	NER	12°15′N	002°23′E	270		6	5	8
14014	ETH					7	1	8
15018	TWN					2	—	5
15019	ZAR					6	3	6
15020	KEN					2	—	5
15021	UGA					7	1	8
15022	RWA					7	1	8
15023						2	—	5
15024	IND					2	—	4
15025						2	—	5
15036	UGA					6	4	2
15037	EGY					2	—	5
15077						2	—	5
15364	KEN	00°44′S	036°26′E	1890	615	6	2	7
15368	KEN	00°22′S	036°05′E	1730	860	6	5	1
15525						6	1	7
16841	EGY	25°16′N	032°30′E			1	—	5
16842	EGY	25°41′N	032°24′E			1	—	5
16843	EGY	25°44′N	032°39′E			2	1	5

[1] EGY = Egypt; ETH = Ethiopia; IND = India; KEN = Kenya; NER = Niger; RWA = Rwanda; TZA = Tanzania; TWA = Taiwan; UGA = Uganda; ZAR = Zaire.
[2] SAS = group allocation after cluster analysis using the average linkage algorithm in SAS.
[3] PAM = group allocation after cluster analysis using the partitioning around medoids algorithm.

(Received for publication November 14, 1994; accepted August 29, 1995)