

Morphological characterization of a minicore subset in different botanical types of Groundnut (*Arachis hypogaea* L.)

C. MADHURA, KENCHANAGOUDAR, P.V., BASAMMA AND V. SUJAY

Received : March, 2011; Accepted : May, 2011

SUMMARY

Plant genetic resources are the most valuable and essential basic raw material to meet the current and future needs of crop improvement programmes. Although there is an increase in the number of germplasm accessions in gene banks, there is no corresponding increase in their use, indicating that the collections were not being used to their full potential. Not only is the limited use of germplasm a worrisome issue, but also the large-scale deployment of a very few genotypes complicates the whole situation even more. Extensive use of fewer and closely related parents in crop improvement is contrary to the purpose of collecting a large number of germplasm accessions, and could result in vulnerability of cultivars to pests and diseases. Developing core collection (about 10% of the entire collection) has been suggested as a method of enhancing the use of the germplasm. However, even this number could be large and unmanageable if the entire accession is several thousands; A methodology to reduce the size further and select a mini-core that is about 1% of the entire collection, yet represents full diversity of the species has been developed. The minicore subset consisting of 182 accessions of which 42 of *hypogaea* bunch, 39 of *hypogaea* runner, 63 of Spanish bunch and 38 of *fastigiata* along with nine cultivars, were evaluated for 10 morphological traits to characterize and to estimate phenotypic diversity using principal component analysis (PCA). PCA showed that all the ten characters except growth habit and pod constriction contributed for multivariate. Standard clustering procedure like euclidian distance is used to separate groups of similar accessions among 195 genotypes for qualitative characters. The hierarchical cluster analysis revealed eight distinct clusters at the distance value of 10 and it revealed that to maintain the diversity due consideration could be given to botanical types as well as clusters in selecting the diverse superior germplasm accessions for use as parents in crop improvement programmes Thus their morphological characters help in differentiating accessions of the same botanical type and their use in breeding will broaden the genetic base of the cultivars.

Madhura, C., Kenchanagoudar, P.V., Basamma and Sujay, V. (2011). Morphological characterization of a minicore subset in different botanical types of Groundnut (*Arachis hypogaea* L.). *Internat. J. Plant Sci.*, 6 (2): 280-284.

Key words : Principal component analysis, Germplasm, *Hypogaea*, Spanish, *Fastigiata*

Plant genetic resources are the most valuable, essential, and basic raw materials that allows identification of promising genes in the germplasm collection that can be incorporated in the breeding programmes to develop promising cultivars. The characterization of diversity in germplasm collection is important to plant breeders to utilize and to the gene bank curators to manage the collection efficiently and effectively for crop improvement programmes to meet the demands of increasing populations. Vavilov (1926) was the first geneticist to

realize the essential need for a broader genetic base for crop improvement. He and his colleagues collected germplasm of crops and their wild relatives globally. In the wake of new agricultural development in the early 1970s, the loss of traditional cultivars and landraces seemed to be the most urgent problem, and massive germplasm collecting efforts were made to address it. As Frankel and Brown (1984) indicated, germplasm could be used, for a wider range of characters, if a smaller number of well characterized accessions were to be given priority for use in crop improvement research. To pursue the same idea, Frankel (1984) proposed manageable sampling of the collection, A core collection contains a subset of accessions from the entire collection that captures most of available diversity of species (Brown 1989a). However, even this number could be large and unmanageable if the entire accession is several thousands. A methodology to reduce the size further and select a

Correspondence to:

C. MADHURA, Department of Genetics and Plant Breeding, University of Agricultural Sciences, DHARWAD (KARNATAKA) INDIA

Authors' affiliations:

KENCHANAGOUDAR, P.V. BASAMMA AND V. SUJAY, Department of Genetics and Plant Breeding, University of Agricultural Sciences, DHARWAD (KARNATAKA) INDIA

mini-core that is about 1% of the entire collection, yet represents full diversity of the species has been developed (Upadhyaya and Ortiz, 2001). The reduced collection size will also help in reducing expenses required to manage the genebank and can be evaluated extensively to select useful parents. Morphological characterization continues to be the backbone of a taxonomic system at any taxonomic level. Morphological characters can contribute much to the study of relationships between botanical types and subspecies in groundnut so the paper enumerates efforts to assess phenotypic diversity of various morphological traits to determine the importance of different descriptor traits and even to know the pattern of distribution of qualitative characters in different botanical types and contribution qualitative morphological diversity and differentiation of mini core.

MATERIALS AND METHODS

The experimental material comprised 182 accessions of groundnut minicore set, representing *hypogaea* bunch (42), *hypogaea* runner (39), Spanish bunch (63) and *fastigiata* (38) obtained from NRCG, Junagad. Besides these accessions, nine cultivars (GPBD-4, JL-24, Mutant-III, TGLPS-3, DSG-1, Gangapuri, ICGS-44, GAUG-10 and Kadiri-3) were evaluated in lattice square design (14 x 14) with two replications in the 2006 *Kharif* at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India. Morphological characters like growth habit and branching pattern were recorded at podding stage, stem pigmentation and hairiness on the main axis, leaf colour of fully expanded leaf let, flower colour from fresh fully opened flowers and pod characters like pod beak, constriction reticulation and testa colour from mature pods from each accession were recorded in all the four botanical types *viz.*, Virginia bunch, Virginia runner, Spanish bunch and Valencia in minicore according to a descriptor list (Anonymous, 1992). Primary colours of mature seeds were recorded within one month of harvest.

Principal component analysis (PCA) of data was performed to investigate the importance of different traits in explaining multivariate polymorphism. The mean performances of agronomic traits for each accession were standardized by subtracting from each observation the mean value of the character and then dividing by its respective standard deviation. This resulted in standardized values for each trait with average 0 and standard deviation of 1 or less. The standardized values are used to perform PCA using M Stat C.

Euclidian or straight line measure of distance is the most commonly used statistic for estimating genetic

distance (GD) between individuals by morphological data (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

The distribution pattern of the mini core and four botanical varieties for ten morphological characters are presented in Table 1. Four types of growth habits *viz.*, decumbent-1, 2, 3 and erect were observed in the mini core. Decumbent-1 was observed in sub species *hypogaea* and not in subspecies *fastigiata*. Decumbent-2 was predominant in subspecies *hypogaea* and Valencia while Spanish bunch was characterized by the predominance of decumbent-3 followed by erect (Table 1). Similar results were observed in the core collection of groundnut (*Arachis hypogaea*) (Mallikarjuna Swamy, 2001; Upadhyaya *et al.*, 2003; Yugandhar, 2005). Branching pattern is the most important criteria employed in the clarification of cultivated groundnut into subspecies. As expected sequential type was predominant in subspecies *fastigiata* (Valencia and Spanish bunch) and alternate type in subspecies *hypogaea* (Virginia runner and Virginia bunch) indicating predominance of alternate and sequential type branching pattern in the mini core. Similar results were reported in groundnut by Esquivel *et al.* (1993), Mallikarjuna Swamy (2001) and Yugandhar (2005).

The presence of stem colour and pubescence are important characteristics as descriptors for groundnut varieties. But both of these traits were highly variable. Colour of the stem varies with the presence or absence and intensity of pigments in the stem tissue, which is highly influenced by the environment particularly exposure to the sunlight. So recording of the typical colours becomes almost impossible. Therefore, stem colour was recorded as present and absent. In the current investigation stem pigmentation was observed in maximum number of accessions of Valencia types. Upadhyaya and Ortiz (2001), Mallikarjuna Swamy (2001), Upadhyaya and (2001) and Yugandhar (2005) also obtained similar results on the core collection.

Number and length of the stem pubescence and number of rows are also highly variable and even its distribution also varies. In the present study subglabrous type with one or two rows of hairs were observed to the maximum extent in Valencia followed by Spanish bunch and Virginia bunch glabrous type *hypogaea* type and in Virginia bunch and moderately hairy in *fastigiata* type. Similar results for sub glabrous and contrary to glabrous and moderately hairy were obtained by Mallikarjuna Swamy (2001) and Brigitte *et al.* (1993). Leaf characters are important in the subspecies classification of groundnut.

Table 1 : Frequency distribution of accessions with different characters morphological in minicore												
Sr. No.	Character	Descriptor	Minicore		Virginia bunch		Virginia runner		Spanish bunch		Valencia	
			Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
1.	Growth habit	Decumbent-1	11	5.76	1	2.27	10	23.81	0	0.0	0	0.0
		Decumbent-2	73	38.20	23	52.27	21	50.00	9	13.6	20	51.30
		Decumbent-3	59	30.90	11	27.27	4	9.76	31	46.97	13	33.30
		Erect	48	25.10	9	18.18	6	14.30	27	40.91	6	15.40
		Total	191	100	44	100	41	100	67	100	39	100.0
2.	Branching pattern	Alternate	74	38.70	29	65.91	33	78.60	20	15.15	2	5.13
		Sequential	96	50.30	11	25.00	5	12.20	48	71.64	32	82.00
		Irregular with flower on main stem	5	2.60	1	2.30	1	2.38	1	1.50	2	5.13
		Irregular without flower on main stem	16	8.40	3	6.82	2	4.80	8	20.50	3	7.70
		Total	191	100	44	100	41	100	67	100	39	100
3.	Pubescence	Glabarous	64	33.51	19	43.18	32	76.19	9	13.64	4	10.26
		Sub glabrous	87	45.50	21	47.73	5	12.20	38	56.70	23	58.97
		Moderately hairy	33	17.28	4	9.09	2	4.8	16	24.24	11	28.21
		Very hairy	7	3.70	0	0	2	4.8	4	6.06	1	2.6
		Total	191	100	44	100	41	100	67	100	39	100
4.	Stem pigmentation	Absence	150	78.5	43	97.73	35	85.37	60	89.55	12	30.77
		Presence	41	21.5	1	2.27	6	14.29	7	10.61	27	69.23
		Total	191	100	44	100	41	100	67	100	39	100
5.	Leaf colour	Yellowish green	55	28.8	4	9.09	5	11.9	37	56.06	10	25.64
		Light green	65	34.0	15	34.1	14	33.33	24	36.36	12	30.77
		Green	43	22.5	12	27.3	17	40.48	4	6.06	9	23.11
		Dark green	23	12.0	12	27.3	5	12.20	2	2.99	4	10.3
		Bluish green	5	2.62	1	2.3	0	0	0	0	4	10.3
		Total	191	100	44	100	41	100.00	67	100	39	100
6.	Flower colour	Orange	89	46.6	17	38.6	15	35.71	43	85.8	14	35.9
		Dark orange	90	47.1	23	52.3	23	56.10	24	65.15	20	51.3
		Garnet	12	6.3	4	9.1	3	7.14	0	0	5	12.8
		Total	191	100	44	100	42	100.00	67	100	39	100
7.	Pod beak	Absent	41	21.46	4	9.09	5	11.9	25	37.9	7	17.95
		Slight	68	35.60	15	34.09	13	31.7	25	37.3	15	38.46
		Moderate	50	26.14	12	27.27	17	40.48	9	13.36	12	30.77
		Prominent	25	13.09	12	27.27	6	14.29	3	4.55	4	10.26
		Very prominent	07	3.66	1	2.27	0	0	5	7.58	1	2.56
		Total	191	100	44	100	41	100	67	100	39	100
8.	Pod constriction	None	26	13.61	6	13.64	7	16.70	4	6.1	9	23.10
		Slight	80	41.89	14	31.82	17	40.48	28	42.4	21	53.85
		Moderate	59	30.90	16	36.36	9	21.95	26	38.8	8	20.50
		Deep	20	10.47	6	13.64	5	11.90	8	12.1	1	2.56
		Very deep	6	3.14	2	4.55	3	7.10	1	1.5	0	0
		Total	191	100	44	100	41	100	67	100	39	100

These leaf characters are however, highly variable. In minicore, light green and green colour were noticed almost in equal proportion but green color was found less in Spanish bunch but more of yellowish green was present. Similar result was obtained by Mallikarjuna Swamy (2001) for light green colour but contrary for green and yellowish

green could be due to differences in scoring. The range of flower colour in *Arachis* is not great. In the present study dark orange was noticed in maximum accessions belonging to subspecies *hypogaea* and Valencia and orange was highest in case of Spanish bunch type and garnet was found in very few accessions (Mallikarjuna

Swamy, 2001; Upadhyaya *et al.*, 2003; Yugandhar, 2005).

Pod and kernel traits are important as they largely determine consumer preference and marketability of the produce. In groundnut, the tip of the indehiscent fruit may end in an appearance called beak. The prominence of beak varies in different botanical types. In the present investigation, slight beak was more predominant in Valencia, Virginia runner and Virginia bunch but Spanish bunch was predominant in both slight beak and absence of beak. Pod constriction is an interesting characteristic as it affects the developing seed. The slight pod constriction and predominant in all the four botanical varieties besides moderate constriction and deep constriction was more predominant in Virginia bunch. Reticulation on the pod is a prominent characteristic. In general, most of the groundnut cultivars exhibit reticulation to different degrees. Reticulation contributes to the cleanness of pods at the time of harvest and has an impact on quality and marketability of the produce. In present study, moderate reticulation was predominant in all the four botanical types in addition, slight was predominant in Virginia runner, Spanish bunch and Valencia and prominent was predominant in Virginia bunch. Tan seed colour was predominant in all the four botanical types, followed by rose in case of Virginia bunch. Accessions with bright colours (light red, dark red and dark purple) were also observed in the minicore. Similar results were obtained on for pod traits by Mallikarjuna Swamy (2001), Upadhyaya *et al.* (2003) and Yugandhar (2005) in the core collection.

Principal component analysis (PCA) for ten morphological descriptors was performed. The percentage of variation explained by the principal components (PCs) and the eigen values provided are presented in Table 2. Out of ten, first five PCs contributed significantly and explained 70.326 per cent variation. The principal

Table 2 : Total variance explained by PCA analysis in qualitative characters

Components	Initial eigen values		
	Total	% of variance	Cumulative per cent
1	339.012	18.718	18.718
2	312.771	17.266	35.987
3	271.715	15.002	50.999
4	183.639	10.139	61.129
5	166.579	9.197	70.326
6	134.767	7.441	77.767
7	126.685	6.939	84.706
8	117.368	6.480	91.187
9	90.989	5.024	96.21
10	88.632	3.789	100.00

component analysis indicated contribution of eight *viz.*, petal colour, pod beak, pod reliculation, testa colour, stem pigmentation, branching pattern, leaf colour and pubescence out of ten characters (Table 3) by the PCs, indicating their importance as groundnut descriptors. Upadhyaya *et al.* (2003) and Mallikarjun Swamy (2001)

Table 3 : Component matrix revealed by PCA analysis in qualitative characters

Characters	Components				
	1	2	3	4	5
Growth habit	0.000	0.009	0.103	-0.126	0.323
Branching pattern	0.156	0.273	0.515	0.477	-0.213
Stem pigmentation	0.286	0.623	0.001	0.228	0.550
Pubescence	0.245	0.243	0.432	-0.122	0.581
Petal colour	0.401	-0.027	-0.446	-0.372	0.099
Leaf colour	0.158	0.100	0.483	0.619	0.043
Pod beak	0.408	-0.396	0.057	0.132	-0.199
Pod reticulation	0.403	-0.354	0.000	0.259	0.185
Pod constriction	0.265	-0.388	0.282	-0.280	-0.307
Testa colour	0.446	0.383	-0.134	-0.061	0.175

also reported high loading values for the above said traits.

The hierarchical cluster analysis for qualitative characters revealed eight distinct clusters at the distance value of 10.0. Different clusters represented predominantly by a particular botanical type (Table 4). This indicated relevance of classification into botanical types as an important approach to capture the morphological diversity. The cluster I, II and VIII were dominated by Spanish bunch types, cluster III by Virginia bunch and Virginia runner type. Cluster IV was dominated by Valencia, V by Virginia runner, cluster VI was having only one genotype of Valencia and VII with two genotypes each from Spanish bunch and Valencia. Qualitative characters were also found more useful in differentiating each botanical type into more subgroups and their contribution to diversity. Cluster I was predominantly having slight pod constriction. Cluster II by sequential type of growth habit and moderate pod constriction. Cluster III by dark orange type of petal colour and light green leaf colour, cluster IV by sequential type of branching pattern with Decumbent-2 type of growth habit and slight pod constriction and cluster V was predominantly having the accessions without any pod reticulation. All the clusters of I, II, III and V were predominantly having tan testa colour except cluster IV having maximum genotypes

Table 4 : Cluster composition based on similarity for qualitative characters

Sr. No.	Cluster	Total	Virginia bunch		Virginia runner		Spanish bunch		Valencia		Total Frequency
			No	Freq. (%)	No	Freq. (%)	No	Freq. (%)	No	Freq. (%)	
1.	I	72	13	18.10	13	18.10	35	48.60	11	15.28	100
2.	II	22	6	27.27	2	9.09	12	54.55	2	9.09	100
3.	III	30	10	33.33	10	33.33	7	23.33	3	10.00	100
4.	IV	38	7	18.42	7	18.42	6	15.79	18	47.37	100
5.	V	21	6	28.57	8	38.09	5	23.81	2	9.52	100
6.	VI	1	0	0.00	0	0.00	0	0.00	1	100.00	100
7.	VII	2	0	0.00	0	0.00	1	50.00	1	50.00	100
8.	VIII	5	1	20.00	1	20.00	2	40.00	1	20.00	100

showing dark purple colour (Table 4). The checks GPBD-4, DSG-1, GAUG-10 were present in same cluster (V). Among clustered TGLPS-3 and GPBD-4 and JL-24 and GPBD-4 were having higher diversity. To maintain the diversity due consideration could be given to botanical types as well as clusters in selecting the accessions for

hybridization programme. The study on morphological characterization revealed that distinct morphological features existing in different subspecies which could be exploited for developing cultivars with unique features that are required in the new regime.

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