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Phenotypic diversity and identification of wild *Arachis* accessions with useful agronomic and nutritional traits

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Abstract Wild relatives harbor novel sources of variation, which can be used to enhance the genetic base of a cultivar gene pool. A total of 269 accessions from 20 wild *Arachis* species belonging to six sections were evaluated for 41 morpho-agronomic traits and 89 selected accessions for oil, protein and total sugar content. Six plants from each accession were grown in an open *Arachis* house in large-cylindrical concrete structures during the 2004–2005 season at Patancheru, India. REML analysis showed significant differences between species and accessions for most of the traits studied. Hierarchical cluster analysis, based on the first five principal component scores accounted for 82.5% variation, resulting in four clusters. Variation in genome relationships and ploidy levels had no bearing on the clustering pattern which was predominated by life forms: clusters 1 and 2, contained mostly annuals and clusters 3 and 4 perennials. A large range of variations were noticed among species for some of the agronomic traits: days to flowering, pod and seed characteristics, specific leaf area (SLA) and for SPAD chlorophyll meter reading (SCMR). *Arachis duranensis* showed the maximum intraspecific variation as revealed by a high diversity index for 23 of the 41 traits which included: days to flowering, primary branches, plant width, pod length, pod width, SCMR and SLA. The other species with desirable traits were *A. pusilla* (earliest flowering) and *A. villosa* (high SCMR at 60 and 80 days after sowing). The latter species is cross compatible with cultivated groundnut, thus, is a good source to enhance the trait value in the cultigen's gene pool. The best 20 accessions with superior agronomic, nutritional quality and drought related trait combinations have been identified for their use in introgression of diverse and unique alleles from wild *Arachis* species into *A. hypogaea*.

Keywords *Arachis* species · Genepool · Clustering · Phenotypic diversity · SCMR

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Introduction

Crop wild relatives, have often been used to enhance the levels of resistance to biotic and abiotic stresses, and for improving yield and nutritional traits (Hajjar and Hodgkin 2007; Dwivedi et al. 2008; Upadhyaya 2008). The cultivated groundnut (also known as peanut) (*Arachis hypogaea* L.) has a narrow genetic base, probably because of the bottlenecks associated with its origin (Kochert et al. 1991; Jung et al. 2003; Seijo et al. 2004). More importantly, the level of

resistance to pests and diseases is either not present, or, exists only at very low levels. Wild *Arachis* species, in contrast, possess very high levels of resistance to pests and diseases, thus, offering a unique opportunity to exploit potentially useful alleles to broaden the genetic base of groundnut (Dwivedi et al. 2003). In addition, some of the perennial wild *Arachis* species are valued as high quality forage, and these have been used to develop forage cultivars in the USA as an alternative to alfalfa, because of their high levels of proteins and their resistance to pests and diseases (Prine et al. 1981, 1986; French et al. 1994).

The genetic variability in genus *Arachis* is locked into three distinct gene pools: primary gene pool–landraces of *A. hypogaea* and its wild form *A. monticola*; secondary gene pool–diploid species from section *Arachis* that are cross-compatible with *A. hypogaea*; and tertiary gene pool–species from section *Caulorrhizae*, *Erectoides*, *Extranervosae*, *Heteranthae*, *Procumbentes, Trierectoides*, *Triseminatae* and *Rhizomatosae*, which may be crossed with cultivated species using in vitro techniques (Mallikarjuna and Sastri 2002; Mallikarjuna 2003, 2005; Mallikarjuna and Hoisington 2009). The wild *Arachis* species, unlike cultivated groundnut, are difficult to maintain under field conditions due to their long generation time (from annual to perennial life cycle), pervasive nature (require more space to grow), deep, to very deep peg/pod penetration into the soil which make it difficult to recover all the pods from the soil while harvesting; and few seeds are produced, thus, requiring unique facilities to regenerate many of these species, as discussed in the next section.

Further, accessions from *Rhizomatosae* have to be maintained vegetatively as these do not produce seeds. Wild *Arachis* species have been extensively studied for resistance to many pests and diseases (reviewed in Dwivedi et al. 2003, 2005, 2008) and for thermal stress tolerance (Nautyal et al. 2008). Stalker (1990) was probably the first to characterize 73 wild species accessions from section *Arachis* for 56 morpho-reproductive traits, while Carvalho and Quesenberry (2009) reported morphological diversity among 34 *A. pintoi* accessions. In contrast, wild *Arachis* species have been extensively studied for molecular polymorphism, unraveling abundant molecular diversity among wild *Arachis* species (reviewed in Koppolu et al. 2010).

The aims of the present investigation were to (i) assess patterns of phenotypic diversity among *Arachis* species accessions belonging to six sections, (ii) assess inter-accession variability among *A. duranensis* accessions in section *Arachis*, (iii) study the nature and magnitude of character association among morpho-agronomic traits, and (iv) identify agronomically beneficial species/accessions for broadening the genetic base of groundnut cultigens.

Materials and methods

Two hundred and sixty-nine accessions from 20 wild *Arachis* species, belonging to sections *Arachis*, *Caulorrhizae*, *Erectoides*, *Heteranthae*, *Procumbentes* and *Triseminatae*, which differ in life forms, ploidy levels, and genome types were selected (Table 1). The number of accessions per species varied from 4 to 61; however, six plants in each accession were evaluated in an open Arachis house during the 2004–2005 season, the facilities built at ICRISAT Patancheru, India, for growing wild *Arachis* species, consist of large-cylindrical concrete structures (75 cm high, 90 cm inner ring diameter, 5 cm ring thickness) with ring-to-ring distance of 52.5 cm. These rings were filled with about 0.5 m^3 pasteurized (3 cycles of 1 h each at 82°C and 34.5×10^3 Pa (5 psi) soil mixture (soil, sand and farm yard manure in 3:2:1 ratio). Standard agronomic practices were adopted to control weeds, diseases and insect pests. Irrigation was provided as per requirement. Gypsum (calcium sulfate dihydrate) was applied at 10 g $ring^{-1}$ 50 days after sowing (DAS).

Individual plant observations were recorded on 34 morpho-agronomic traits as per IBPGR descriptors (IBPGR 1990) which included days to flowering (DF); primary branches (PB), main stem height (cm) (MSH), main stem thickness (mm) (MST), plant width (cm) (PlW), hypanthium length (mm) (HL), standard petal length (mm) (SPL), standard petal width (mm) (SPW), peg length (mm) (PgL), pod basal segment length (mm) (PdBSL), pod apical segment length (mm) (PdASL), isthmus length (mm) (IL), main stem stipule length (mm) (MSSL), primary branch stipule length (mm) (PBSL), main stem stipule adnation length (mm) (MSSAL), primary branch stipule adnation length (mm) (PBSAL), main stem stipule adnation width (mm) (MSSAW),

Table 1 Description of wild *Arachis* species with the number of accessions within each species and their life forms, genome and ploidy levels used in the present study

Section	Species	Accessions	Life form	Ploidy	Chromosome number	Genome	Origin
Arachis	*A. batizocoi*	5	Annual	Diploid	20	B	Bolivia
Arachis	*A. cardenasii*	15	Perennial	Diploid	20	A	Bolivia
Arachis	*A. decora*	9	Annual	Aneuploid	18	A	Brazil
Arachis	*A. duranensis*	61	Annual	Diploid	20	A	Argentina and Bolivia
Arachis	*A. glandulifera*	4	Annual	Diploid	20	D	Bolivia
Arachis	*A. hoehnei*	5	Annual	Diploid	20	B	Brazil and Paraguay
Arachis	*A. kuhlmannii*	17	Perennial	Diploid	20	A	Brazil
Arachis	*A. monticola*	7	Annual	Tetraploid	40	AB	Argentina
Arachis	*A. stenosperma*	32	Annual	Diploid	20	A	Brazil
Arachis	*A. valida*	5	Annual	Diploid	20	B	Brazil
Arachis	*A.villosa*	9	Perennial	Diploid	20	A	Argentina and Uruguay
Caulorrhizae	*A. pintoi*	20	Perennial	Diploid	20	C	Brazil
Erectoides	*A. paraguariensis*	10	Perennial	Diploid	20	E	Brazil and Paraguay
Heteranthae	*A. dardani*	11	Annual	Diploid	20	H	Brazil
Heteranthae	*A. sylvestris*	15	Annual	Diploid	20	H	Brazil
Heteranthae	*A. pusilla*	21	Annual	Diploid	20	T	Brazil
Procumbentes	*A. appressipila*	7	Perennial	Diploid	20	P	Brazil
Procumbentes	*A. kretschmeri*	5	Perennial	Diploid	20	P	Brazil
Procumbentes	*A. matiensis*	5	Perennial	Diploid	20	P	Brazil and Bolivia
Triseminatae	*A. triseminata*	6	Perennial	Diploid	20	T	Brazil

primary branch stipule adnation width (mm) (PBSAW), main stem petiole length (mm) (MSPL), primary branch petiole length (mm) (PBPL), main stem rachis length (mm) (MSRL), primary branch rachis length (mm) (PBRL), main stem apical leaflet length (mm) (MSALL), primary branch apical leaflet length (mm) (PBALL), main stem apical leaflet width (mm) (MSALW), primary branch apical leaflet width (mm) (PBALW), basal main stem leaflet length (mm) (BMSLL), basal primary branch leaflet length (mm) (BPBLL), basal main stem leaflet width (mm) (BMSLW), basal primary branch leaflet width (mm) (BPBLW), SPAD (Soil Plant Analytical Development) chlorophyll meter reading (SCMR) at 60 (SCMR60) and 80 (SCMR80) and specific leaf area (SLA) at 60 (SLA60) and 80 (SLA80) DAS. Observations on days to emergence (DE), days to maturity (DM), pod length (mm) (PdL), pod width (mm) (PdW), seed length (mm) (SdL), seed width (mm) (SdW), and 100 seed weight (HSW) were recorded on plot basis. Data on SCMR was recorded using SPAD Chlorophyll meter (SPAD-502, Konica Minolta Sensing, INC, Japan; http://konicaminolta) at 1000°cd and 1270°cd (cumulative thermal time, CTT, measured in degree days, using 10°C as base temperature for groundnut) (Rao et al. 1992), which is equivalent to 60 and 80 DAS, respectively, in rainy season at Patancheru, India. The CTT was measured by the following formula:

$$\mathrm{CTT}\,(^{\circ}\mathrm{Cd}) = \sum_{P}^{H}\left(\frac{T_{\max}+T_{\min}}{2}\right) - T_{\mathrm{base}}$$

where T_{max} daily maximum temperature, T_{min} daily minimum temperature, T_{base} mean base temperature for peanut, P planting date, and H harvest date.

Two SCMR readings were recorded on each of the four leaflets of the tetra foliate leaf. One fully expanded second or third leaf from the apex of the main axis of each plant was used to record the SCMR (Nageswara Rao et al. 2001). Care was taken to ensure that the SPAD meter fully covered the leaf lamina and that the interference from veins and midribs was avoided. After recording the SCMR readings, the leaves were processed (soaked in water

for 2 h to bring to full turgor) for SLA measurement. The SLA was recorded using leaf area meter (LI-COR Area Mater Model 3000, LI-COR Inc, Lincoln, NE, USA) at 1000°cd and 1270°cd. The leaf area of the four leaflets was measured, and later on the leaves were oven dried at 80°C for at least 48 h to determine the leaf dry weight. SLA was calculated as follows,

$$\text{SLA} = \frac{\text{Leaf area } (\text{CM}^2)}{\text{Dry weight } (\text{g})}$$

Random seed sample of the selected 89 accessions, with enough seeds, was used for the estimation of oil by NMR spectrometry (Jambunathan et al. 1985), protein content by Kjeldal method with sulphuric acid-selenium digestion (Sahrawat et al. 2002) and total sugars by colorimetric method (Dubois et al. 1956).

Statistical analysis was performed using the residual maximum likelihood (REML) procedure in Genstat 13 release (http://www.vsni.co.uk), to partition genotypic variance into between species and between accessions within species. The respective standard errors were estimated and used to determine the significance of variance components. Shannon and Weaver (1949) diversity index (H′) was used to measure and compare the phenotypic diversity for all the 41 traits. Mean data of 41 quantitative traits on 20 species was used to estimate phenotypic correlations between traits. The mean observations of traits were standardized by subtracting from each observation the mean value of the character and subsequently dividing by its standard deviation. This resulted in standardized values for each trait with average 0 and standardized deviation of one or less. The standardized values were used to perform principal component analysis (PCA) on Genstat 13 release. Cluster analysis (Ward 1963) was performed using scores of the first five principal components (PCs). The phenotypic correlations were estimated to show relationships between traits. The best 20 wild *Arachis* accessions possessing more than five desirable agronomic (DF, primary (lateral) branches, MST (mm), PdL, PdW, SdL, SdW and HSW), nutritional (oil, protein and sugar content) and drought related (SCMR and SLA, both at 60 DAS and 80 DAS) traits combination were identified and their cluster analysis (Ward, 1963) was performed using scores of the first five PCs.

Results and discussion

REML analysis showed a significant variance component due to species (δ^2s) and between accessions (δ^2g) for all 41 traits (Table 2). The species showed a large range of differences in means for 23 of the 41 traits (Table 3). Of particular interest are the range differences in flowering (29.7 days), maturity (106.8 days), MSH (35.2 cm), PlW (227.3 cm) and HSW (32.4 g). ICG 14898 and ICG 14906 of *A. pusilla* flowered in 13–14 days, the earliest flowering accessions among the 20 wild *Arachis* species studied, which is a week earlier than Chico (ICG 476), the earliest flowering cultivated groundnut germplasm (Bailey and Hammons 1975). Large range variations among species were also noticed for SCMR (21.0 and 24.3) and SLA (80.9 and 151.3 $\text{cm}^2\ \text{g}^{-1}$) between the two readings (60 and 80 DAS). *Arachis villosa* recorded the highest SCMR values (57), both at 60 and 80 DAS. High SCMR has been associated with low SLA or high SLN, and could be used as surrogate trait to identify genotypes with high water use efficiency in groundnut (Nageswara Rao et al. 2001; Bindu Madhava et al. 2003). In an extensive study involving 184 groundnut mini core collection accessions (Upadhyaya et al. 2002), evaluated for two (rainy and postrainy) seasons, Upadhyaya (2005) identified few accessions with SCMR ranging from 29 to 41 in the rainy season, and from 32 to 50 in the postrainy season. The SCMR between the two stages (60 and 80 days after sowing) was more variable in the postrainy than in the rainy season (Upadhyaya 2005). The high SCMR reported in *A. villosa* in the present study, provides an interesting source which could perhaps be exploited to develop improved genotypes with a high SCMR to enhance water use efficiency under drought stress conditions. However, it should be noted that transferring desirable traits from wild *Arachis* species into cultivated groundnut is a long drawn process (i.e. laborious, time consuming and some times frustrating), and to date, the only success reported, has been in transferring resistance to pests and diseases (reviewed in Dwivedi et al. 2003, 2008). The large variations in SLA between the two readings could probably be associated with the variation in the leaves sampled. *Arachis glandulifera* accessions had the largest average seed size (41 g 100 seed), while those of the *A. cardenasii* accessions, the smallest

Table 2 Estimates of variance of 41 quantitative traits in 20 wild *Arachis* species used in phenotypic diversity assessment

Trait	Between species		Between accession	
	δ^2s	SE	δ^2g	SE
Days to emergence (DE)	0.51	0.20	0.92	0.08
Days to flowering (DF)	472.87	150.27	164.91	15.31
Days to maturity (DM)	1434.80	494.90	729.30	65.60
Primary branches (PB)	0.65	0.26	1.39	0.14
Main stem height (cm) (MSH)	73.97	25.47	59.27	5.58
Main stem thickness (mm) (MST)	0.36	0.14	0.49	0.05
Plant width (cm) (PlW)	5270.60	1723.60	2234.10	217.70
Hypanthium length (mm) (HL)	161.17	59.00	220.55	20.89
Standard petal length (mm) (SPL)	8.29	2.57	2.21	0.21
Standard petal width (mm) (SPW)	13.16	4.05	3.17	0.30
Peg length (mm) (PgL)	72.61	26.84	94.22	9.33
Pod basal segment length (mm) (PdBSL)	3.50	1.36	2.30	0.32
Pod apical segment length (mm) (PdASL)	4.23	1.73	2.54	0.39
Pod length (mm) (PdL)	4.44	1.94	14.04	1.33
Pod width (mm) (PdW)	0.31	0.13	0.71	0.06
Isthmus length (mm) (IL)	483.00	189.60	202.70	44.60
Seed length (mm) (SdL)	3.78	1.77	14.71	1.37
Seed width (mm) (SdW)	0.50	0.17	0.23	0.021
100 Seed weight (HSW)	49.03	16.54	11.56	1.09
Main stem stipule length (mm) (MSSL)	23.32	8.05	17.47	1.78
Primary branch stipule length (mm) (PBSL)	19.32	6.33	7.63	0.80
Main stem stipule adnation length (mm) (MSSAL)	3.30	1.18	3.17	0.33
Primary branch stipule adnation length (mm) (PBSAL)	4.43	1.45	1.74	0.18
Main stem stipule adnation width (mm) (MSSAW)	1.01	0.38	1.36	0.15
Primary branch stipule adnation width (mm) (PBSAW)	0.54	0.23	1.30	0.14
Main stem petiole length (mm) (MSPL)	31.06	11.56	41.36	4.17
Primary branch petiole length (mm) (PBPL)	14.59	5.46	19.77	2.03
Main stem rachis length (mm) (MSRL)	2.83	1.05	3.55	0.36
Primary branch rachis length (mm) (PBRL)	1.22	0.46	1.72	0.19
Main stem apical leaflet length (mm) (MSALL)	47.21	15.90	27.98	2.80
Primary branch apical leaflet length (mm) (PBALL)	22.29	7.58	13.97	1.45
Main stem apical leaflet width (mm) (MSALW)	12.82	4.26	6.39	0.65
Primary branch apical leaflet width (mm) (PBALW)	6.64	2.26	4.20	0.44
Basal main stem leaflet length(mm) (BMSLL)	5.32	1.90	5.08	0.53
Basal primary branch leaflet length (mm) (BPBLL)	2.48	0.93	3.26	0.35
Basal main stem leaflet width (mm) (BMSLW)	32.47	11.04	21.14	2.14
Basal primary branch leaflet width (mm) (BPBLW)	18.37	6.22	10.70	1.12
SPAD chlorophyll meter reading at 60 (SCMR60) days after sowing	33.38	11.22	20.35	2.12
SPAD chlorophyll meter reading at 80 (SCMR80) days after sowing	34.83	11.91	21.93	2.43
Specific leaf area at 60 (SLA60) days after sowing	632.00	249.00	1204.00	118.40
Specific leaf area at 80 (SLA80) days after sowing	322.40	118.00	355.6	40.9

Table 3 Minimum and maximum values and range difference of the 41 quantitative traits used in phenotypic diversity assessment among 20 wild *Arachis* species

Trait	Range and species information with minimum (Mn) and maximum (Mx) mean values				Range difference
	Species identity	Mn	Species identity	Mx	
Days to emergence (DE)	*A. kretschmeri*	6.6	*A. villosa*	9.4	2.8
Days to flowering (DF)	*A. dardani*	17.7	*A. stenosperma*	47.4	29.7
Days to maturity (DM)	*A. batizocoi*	167.2	*A. triseminata*	274.0	106.8
Primary branches (PB)	*A. paraguariensis*	4.2	*A. appressipila*	7.0	2.8
Main stem height (cm) (MSH)	*A. kuhlmannii*	5.2	*A. glandulifera*	40.4	35.2
Main stem thickness (mm) (MST)	*A. villosa*	3.7	*A. glandulifera*	5.8	2.1
Plant width (cm) (PlW)	*A. sylvestris*	71.4	*A. villosa*	298.7	227.3
Hypanthium length (mm) (HL)	*A. dardani*	32.1	*A. appressipila*	83.1	51.0
Standard petal length (mm) (SPL)	*A. dardani*	8.4	*A. paraguariensis*	19.2	10.8
Standard petal width (mm) (SPW)	*A. dardani*	10.3	*A. paraguariensis*	23.6	13.3
Peg length (mm) (PgL)	*A. dardani*	59.2	*A. triseminata*	252.2	193.0
Pod basal segment length (mm) (PdBSL)	*A. cardenasii*	9.1	*A. stenosperma*	17.6	8.5
Pod apical segment length (mm) (PdASL)	*A. cardenasii*	9.7	*A. stenosperma*	17.9	8.2
Pod length (mm) (PdL)	*A. cardenasii*	7.8	*A. glandulifera*	16.5	8.7
Pod width (mm) (PdW)	*A. glandulifera*	5.1	*A. monticola*	8.3	3.2
Isthmus length (mm) (IL)	*A. monticola*	7.3	*A. triseminata*	105.9	98.6
Seed length (mm) (SdL)	*A. decora*	4.5	*A. glandulifera*	13.8	9.3
Seed width (mm) (SdW)	*A. cardenasii*	4.7	*A. glandulifera*	8.1	3.4
100 Seed weight (HSW)	*A. cardenasii*	9.0	*A. glandulifera*	41.4	32.4
Main stem stipule length (mm) (MSSL)	*A. pintoi*	17.2	*A. paraguariensis*	34.2	17.0
Primary branch stipule length (mm) (PBSL)	*A. glandulifera*	13.4	*A. paraguariensis*	30.7	17.3
Main stem stipule adnation length (mm) (MSSAL)	*A. duranensis*	6	*A. paraguariensis*	13.6	7.6
Primary branch stipule adnation length (mm) (PBSAL)	*A. glandulifera*	4.5	*A. paraguariensis*	12.3	7.8
Main stem stipule adnation width (mm) (MSSAW)	*A. decora*	5.5	*A. paraguariensis*	9.1	3.6
Primary branch stipule adnation width (mm) (PBSAW)	*A. duranensis*	6.2	*A.paraguariensis*	9.3	3.1
Main stem petiole length (mm) (MSPL)	*A. dardani*	16.0	*A. batizocoi*	38.6	22.6
Primary branch petiole length (mm) (PBPL)	*A. kuhlmannii*	9.6	*A. monticola*	22.8	13.2
Main stem rachis length (mm) (MSRL)	*A. decora*	3.7	*A. glandulifera*	12.0	8.3
Primary branch rachis length (mm) (PBRL)	*A. hoehnei*	4.7	*A. batizocoi*	8.4	3.7
Main stem apical leaflet length (mm) (MSALL)	*A. villosa*	18.5	*A. paraguariensis*	40.2	21.7
Primary branch apical leaflet length (mm) (PBALL)	*A. duranensis*	16.5	*A. paraguariensis*	35.8	19.3
Main stem apical leaflet width (mm) (MSALW)	*A. decora*	8.8	*A. batizocoi*	23.4	14.6
Primary branch apical leaflet width (mm) (PBALW)	*A. decora*	10.2	*A. batizocoi*	21.3	11.1
Basal main stem leaflet length(mm) (BMSLL)	*A. villosa*	16.3	*A. glandulifera*	35.5	19.2
Basal primary branch leaflet length (mm) (BPBLL)	*A. duranensis*	14.6	*A. paraguariensis*	32.4	17.8
Basal main stem leaflet width (mm) (BMSLW)	*A. appressipila*	8.9	*A. glandulifera*	18.8	9.9
Basal primary branch leaflet Width (mm) (BPBLW)	*A. decora*	8.9	*A. batizocoi*	16.6	7.7
SPAD chlorophyll meter reading at 60 (SCMR60) days after sowing	*A. batizocoi*	35.8	*A. villosa*	56.8	21.0
SPAD chlorophyll meter reading at 80 (SCMR80) days after sowing	*A. dardani*	32.8	*A. villosa*	57.1	24.3
Specific leaf area at 60 (SLA60) days after sowing	*A. appressipila*	80.0	*A. monticola*	160.9	80.9
Specific leaf area at 80 (SLA) days after sowing	*A. paraguariensis*	97.6	*A. valida*	248.9	151.3

(9 g 100 seed). Although a much larger variation in seed weight was reported in cultivated groundnut (Upadhyaya et al. 2001), the large variation in seed weight among wild relatives may be another source to capture additional variability of seed weight, in groundnut cultigens. The preliminary work at ICRISAT has indicated that wild relatives, though with very small seeds, have contributed positive alleles for increased seed weight in some of the progenies derived from interspecific crosses (Upadhyaya 2008).

The PCA using data on 41 traits, revealed that the first five PC scores explained 82.5% variation (data not given). Further, a hierarchical cluster analysis, based on the first five PC scores, at 75% dissimilarity, resulted in four clusters (Fig. 1). Cluster 1 and 2, contained most of the annual and diploid species, with the exception of *A. monticola*, a tetraploid with AB genome, and *A. pintoi* a diploid but with perennial life form, grouped, in cluster 2. Likewise, cluster 3, exclusively contained diploid species with perennial life forms except annual *A. hoehnei*. Cluster 4 consisted of two diploid perennial species with T or A genome. Interestingly, variation in genome relationships and ploidy levels had no bearing on clustering pattern which was predominated by life forms. This study consisted of 61 accessions of *A. duranensis*. The *A. duranensis*-based hierarchical dendogram (based on 10 PC and 81.4% variation) at ~70% dissimilarity, grouped the accessions into five major clusters, with the majority of the accessions falling into cluster 1, 2 and 5, which itself indicates sufficient intra-species variability for morpho-agronomic traits in *A. duranensis* (data and cluster diagram not given). Cluster 2 accessions (ICGs 8203, 8205, 8207, 8956, 13201, 13119, 13193, 13189, 13192, 13197, and 13242) represent those with high trait value (19 traits including pod/seed characteristics), while those in cluster five represent accessions (ICGs 8200, 8204, 8957, 11552, 11554, 11555, 11556, 13161, 13174, 13176, 13182, 13186, 13190, 13194, 13199, 13200, 13207, 13216, 13217, 13236, 13250 and 15179) with low trait value (20 traits including pod/seed characteristics). The cluster 3, has accessions (ICGs 8138, 8139, 13195 and 13218) with high SCMR, while accessions with low SCMR are grouped in cluster 2. Such information may be helpful to identify genetically diverse accessions within species with contrasting traits, to develop mapping populations for genomic studies that involve wild *Arachis* species.

The Shannon–Weaver diversity index (H′) was used as a measure of phenotypic diversity to identify species with the maximum diversity (Shannon and Weaver 1949). A high index means that the species

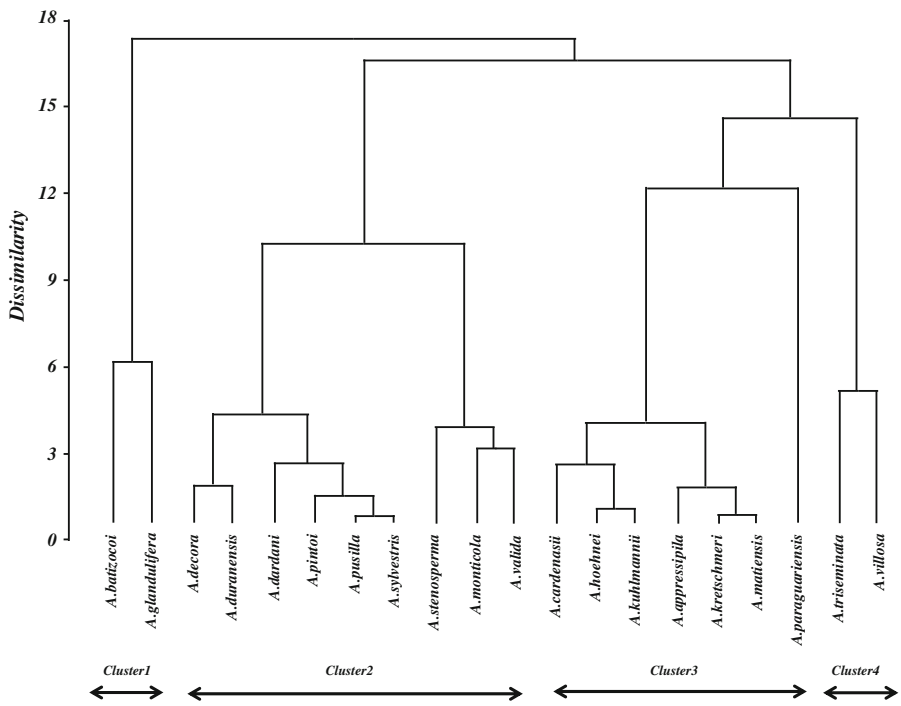

Fig. 1 Dendrogram based on scores of first five principal components (82.5% variation) of 20 wild *Arachis* species

Table 4 Mean and range in Shannon–Weaver Diversity index (H′) and species with highest and lowest H′ for 41 quantitative traits used in phenotypic diversity assessment among 20 wild *Arachis* species

Trait	Mean (H′)	Range and species information with lowest and highest H′			
		Species identity	H′ (Min.)	Species identity	H′ (Max.)
Days to emergence (DE)	0.362	*A. hoehnei* and *A. matiensis*	0.217	*A. paraguariensis*	0.556
Days to flowering (DF)	0.403	*A. pintoi*	0.196	*A. duranensis*	0.587
Days to maturity (DM)	0.382	*A. decora*	0.152	*A. pintoi*	0.556
Primary branches (PB)	0.457	*A. decora*	0.320	*A. duranensis*	0.560
Main stem height (cm) (MSH)	0.417	*A. triseminata*	0.196	*A. cardenasii*	0.569
Main stem thickness (mm) (MST)	0.461	*A. glandulifera*	0.244	*A. pusilla*	0.586
Plant width (cm) (PlW)	0.444	*A. batizocoi*	0.217	*A. duranensis*	0.598
Hypanthium length (mm) (HL)	0.475	*A. valida*	0.217	*A. duranensis*	0.605
Standard petal length (mm) (SPL)	0.459	*A. glandulifera*	0.244	*A. duranensis*	0.604
Standard petal width (mm) (SPW)	0.477	*A. kretschmeri*	0.217	*A. pusilla*	0.620
Peg length (mm) (PgL)	0.415	*A. pusilla*	0.178	*A. duranensis*	0.584
Pod basal segment length (mm) (PdBSL)	0.394	*A. sylvestris*	0.217	*A. duranensis*	0.633
Pod apical segment length (mm) (PdASL)	0.394	*A. triseminata*	0.196	*A. pusilla*	0.574
Pod length (mm) (PdL)	0.450	*A. cardenasii*	0.106	*A. duranensis*	0.589
Pod width (mm) (PdW)	0.476	*A. decora*	0.377	*A. stenosperma*	0.592
Isthmus length (mm) (IL)	0.370	*A. villosa*	0.152	*A. duranensis*	0.559
Seed length (mm) (SdL)	0.461	*A. appressipila*	0.260	*A. duranensis*	0.609
Seed width (mm) (SdW)	0.473	*A. batizocoi*, *A. hoehnei*, *A. kretschmeri*, and *A. valida*	0.413	*A. duranensis* *A. pintoi*	0.622
100 Seed weight (HSW)	0.434	*A. glandulifera*	0.244	*A. pintoi*	0.592
Main stem stipule length (mm) (MSSL)	0.482	*A. glandulifera*	0.301	*A. stenosperma*	0.610
Primary branch stipule length (mm) (PBSL)	0.446	*A. batizocoi* and *A. kretschmeri*	0.244	*A. duranensis*	0.614
Main stem stipule adnation length (mm) (MSSAL)	0.455	*A. matiensis*	0.217	*A. dardani*	0.583
Primary branch stipule adnation length (mm) (PBSAL)	0.493	*A. decora*	0.391	*A. duranensis*	0.623
Main stem stipule adnation width (mm) (MSSAW)	0.464	*A. glandulifera*	0.244	*A. duranensis*	0.601
Primary branch stipule adnation width (mm) (PBSAW)	0.479	*A. matiensis*	0.217	*A. duranensis*	0.610
Main stem petiole length (mm) (MSPL)	0.437	*A. decora*	0.287	*A. stenosperma*	0.625
Primary branch petiole length (mm) (PBPL)	0.435	*A. sylvestris*	0.217	*A. pintoi*	0.543
Main stem rachis length (mm) (MSRL)	0.446	*A. kretschmeri*	0.217	*A. cardenasii*	0.592
Primary branch rachis length (mm) (PBRL)	0.447	*A. triseminata*	0.196	*A. pusilla*	0.576
Main stem apical leaflet length (mm) (MSALL)	0.457	*A. kretschmeri*	0.217	*A. stenosperma*	0.624
Primary branch apical leaflet length (mm) (PBALL)	0.455	*A. batizocoi* and *A. kretschmeri*	0.244	*A. stenosperma*	0.613
Main stem apical leaflet width (mm) (MSALW)	0.464	*A. hoehnei*	0.217	*A. duranensis*	0.572
Primary branch apical leaflet width (mm) (PBALW)	0.446	*A. paraguariensis*	0.217	*A. duranensis*	0.594
Basal main stem leaflet length(mm) (BMSLL)	0.479	*A. villosa*	0.391	*A. duranensis*	0.608
Basal primary branch leaflet length (mm) (BPBLL)	0.464	*A. batizocoi* and *A. glandulifera*	0.244	*A. duranensis*	0.600
Basal main stem leaflet width (mm) (BMSLW)	0.470	*A. paraguariensis*	0.217	*A. kuhlmanni*	0.604

Table 4 continued

Trait	Mean (H′)	Range and species information with lowest and highest H′			
		Species identity	H′ (Min.)	Species identity	H′ (Max.)
Basal primary branch leaflet Width (mm) (BPBLW)	0.476	*A. hoehnei*	0.217	*A. duranensis*	0.601
SPAD chlorophyll meter reading at 60 (SCMR60) days after sowing	0.465	*A. valida*	0.217	*A. duranensis*	0.587
SPAD chlorophyll meter reading at 80 (SCMR80) days after sowing	0.478	*A. triseminata*	0.276	*A. duranensis*	0.605
Specific leaf area at 60 (SLA60) days after sowing	0.464	*A. villosa*	0.230	*A. pusilla*	0.597
Specific leaf area at 80 (SLA80) days after sowing	0.411	*A. pusilla*	0.086	*A. duranensis*	0.562

per se has a wide variability in a given trait. For example, *A. duranensis* accessions had shown maximum H′ in 23 of the 41 traits, thus, a good source to exploit between accession variability within a species (Table 4). *Arachis duranensis,* is cross compatible with cultivated groundnut, and has shown a high index of SCMR (both at 60 and 80 DAS), thus, a good source to enhance drought tolerance in cultivated groundnut. A high SCMR is related to increased drought tolerance in groundnut (Upadhyaya 2005).

Mean data of the 41 traits on 20 species were used to estimate phenotypic correlations (r) between traits. Phenotypic correlation coefficients in 206 of the 820 trait combinations were significant (P 0.05), ranging from 0.445 to 0.987 (data not presented). Biologically meaningful correlations, are those which have coefficients greater than 0.707, or smaller than −0.707 (Skinner et al. 1999). In this study, there were 66 trait combinations which have $r > 0.710$ (data not given). PdL, PdW, SdL, SdW and HSW were highly significantly correlated (0.763–0.987). In a similar study on 34 *A. pintoi* accessions, evaluated for a range of morpho-agronomic traits, Carvalho and Quesenberry (2009) also reported biologically meaningful correlations ($r > 0.70$) between leaf length and pod weight, PdW, seed weight, SdW and SdL. Further, a very high correlation (0.945) between SCMR60 and SCMR80 revealed that observation at either stage will provide a reliable estimate of SCMR. Many of the wild *Arachis* species included in this study were reported resistant to pests and diseases (reviewed in Dwivedi et al. 2003, 2005, 2008). Furthermore, several traits such as main stem thickness, HL, leaflet shape and length, leaf hairiness, SPL and petal markings, basal leaflet width, main stem thickness and hairiness, stipule adnation length and width, and PgL showed significant correlation and/or regression coefficients with damage by *Helicoverpa armigera*, *Spodoptera litura*, and leafhoppers, which can possibly be used for selection of resistance to these insect pests (Sharma et al. 2003).

Stalker (1990) was the first to report morphological diversity among 73 wild species accessions that belong to the section, *Arachis*, with substantial variation detected in leaflet size and shape, branching habit and flower size. More recently, Carvalho and Quesenberry (2009) characterized *A. pintoi* accessions from the section *Caulorrhizae*, which also showed greater variability in most of the morphological traits. The present study in contrast had involved the largest number of accessions (269 accessions from 20 species), belonging to six of the nine sections of the genus *Arachis* that were assessed for phenotypic diversity using 41 morpho-agronomic traits, with SPAD reading and SLA reported for the first time amongst such a large number of wild *Arachis* species accessions. When we examined the variable loadings of the first five PCs in the present study, we observed that 12 traits in PC1, nine in PC2, five in PC3, and three each in PC4 and PC5 contributed significant variation to the total phenotypic variability, with most of the variation predominantly originating from vegetative traits in PC1, PC2, PC4 and PC5, while SCMR (both at 60 and 80 DAS), pod length and SdL contributed more in PC3.

The best 20 wild *Arachis* accessions, possessing more than five desirable agronomic traits (DF, primary (lateral) branches, main stem thickness (mm), pod length, PdW, SdL, SdW and HSW),

Table 5 Top 20 accessions of wild *Arachis* identified for their superior agronomic and nutritional quality traits

ICG	Species	DF	PB	MSH	MST	PDL	PDW	SDL	SDW	HSW	SCMR60	SCMR80	SLA60	SLA80	Oil (%)	Protein[a] (%)	Sugar[a] (%)
8144	*A. villosa*	38.8	4.0	14.3	3.3	16.5	8.3	12.7	6.3	22.9	59.0	58.7	73.8	113.6	52.0	51.8	7.0
8197	*A. monticola*	26.3	6.0	34.0	4.2	15.2	8.4	12.1	6.8	26.9	39.2	41.2	162.1	125.5	50.0	58.0	6.5
13160	*A. batizocoi*	20.0	5.0	37.7	4.3	15.8	7.3	12.5	5.4	16.3	38.6	39.9	118.2	157.3	49.0	59.0	6.6
13173	*A. stenosperma*	53.5	6.0	3.8	5.0	18.0	6.4	13.6	5.4	19.8	50.1	53.2	140.1	114.7	47.9	60.0	5.8
13177	*A. monticola*	26.4	6.8	25.2	3.8	14.4	8.5	12.2	6.4	25.0	46.9	44.8	159.0	159.2	51.0	56.0	5.9
13178	*A. monticola*	25.8	5.8	33.8	3.8	17.3	9.7	15.4	7.0	36.6	39.6	41.2	173.5	166.2	50.0	53.0	7.3
13189	*A. duranensis*	29.5	5.0	7.7	4.0	14.4	6.9	11.9	5.7	18.3	49.6	50.3	170.9	155.0	53.0	61.0	7.3
13211	*A. pusilla*	17.0	5.8	55.0	3.9	16.0	8.4	12.5	5.9	20.8	43.0	39.0	122.4	141.6	55.0	60.0	5.5
13221	*A. pusilla*	28.2	6.4	5.6	4.0	11.8	7.0	8.9	5.0	13.6	48.8	47.3	125.6	112.9	53.0	59.0	6.5
13223	*A. stenosperma*	62.0	5.0	4.3	5.4	20.0	7.2	14.4	5.3	22.1	44.2	49.3	169.8	137.7	45.4	61.0	6.1
13227	*A. dardani*	19.2	6.6	13.4	4.6	10.8	6.0	7.6	4.5	7.2	30.3	35.2	211.8	149.3	52.0	66.0	5.0
13244	*A. stenosperma*	42.3	5.4	4.2	5.7	18.7	7.2	16.2	5.6	25.4	48.3	51.5	160.2	119.0	44.7	64.0	5.5
14866	*A. kuhlmannii*	34.2	6.0	4.4	6.2	14.0	7.3	11.4	5.7	18.1	53.4	47.8	140.5	117.1	47.9	63.0	6.3
14868	*A. stenosperma*	20.7	7.8	9.0	5.5	18.7	7.5	14.0	5.9	21.2	47.8	49.8	97.5	157.9	50.0	63.0	6.2
14872	*A. stenosperma*	54.5	5.5	3.8	5.6	21.0	7.7	14.8	5.5	20.7	51.6	48.4	152.0	140.4	51.0	62.0	6.2
14874	*A. stenosperma*	48.5	6.2	6.1	6.1	19.5	7.0	14.0	6.8	22.5	56.7	51.0	112.3	102.2	50.0	64.0	6.1
14884	*A. stenosperma*	61.0	5.2	3.6	5.5	19.2	6.5	14.5	5.3	23.1	47.7	44.4	114.8	135.0	46.7	62.0	6.1
15142	*A. pusilla*	45.2	5.0	3.8	5.0	13.0	7.9	8.6	4.9	9.9	40.5	40.8	127.6	95.4	50.0	67.0	4.9
15144	*A. kuhlmannii*	30.2	7.8	10.8	6.2	13.5	7.0	11.2	5.4	13.6	48.7	46.3	133.2	132.2	53.0	53.0	6.4
15149	*A. paraguariensis*	20.0	3.6	24.2	5.5	13.2	6.4	9.5	5.0	11.4	54.8	57.3	67.0	83.5	55.0	56.8	5.7
	Trial mean	29.3	5.7	11.3	4.5	12.1	6.2	9.9	4.9	15.4	43.4	44.8	129.9	158.8	50.9	60.4	5.7
	SE±	0.92	0.07	0.62	0.06	0.3	0.1	0.2	0.1	0.39	0.43	0.45	2.58	11.99	0.31	0.39	0.09
	CV %	8.5	3.4	14.7	3.3	6.4	5	5.6	5.1	6.6	2.7	2.7	5.4	20.1	5.7	6.2	14.3

DF days to flowering, *PB* primary (lateral) branches, *MSH* main stem height (cm), *MST* main stem thickness (mm), *PDL* pod length (mm), *PDW* pod width (mm), *SDL* seed length (mm), *SDW* seed width (mm), *HSW* 100-seed weight (g), *SCMR 60, SPAD* chlorophyll meter reading at 60 days, *SCMR 80*, *SPAD* chlorophyll meter reading at 80 days, *SLA60* specific leaf area at 60 days, *SLA80* specific leaf area at 80 days

[a] Protein and sugar estimates are based on defatted samples

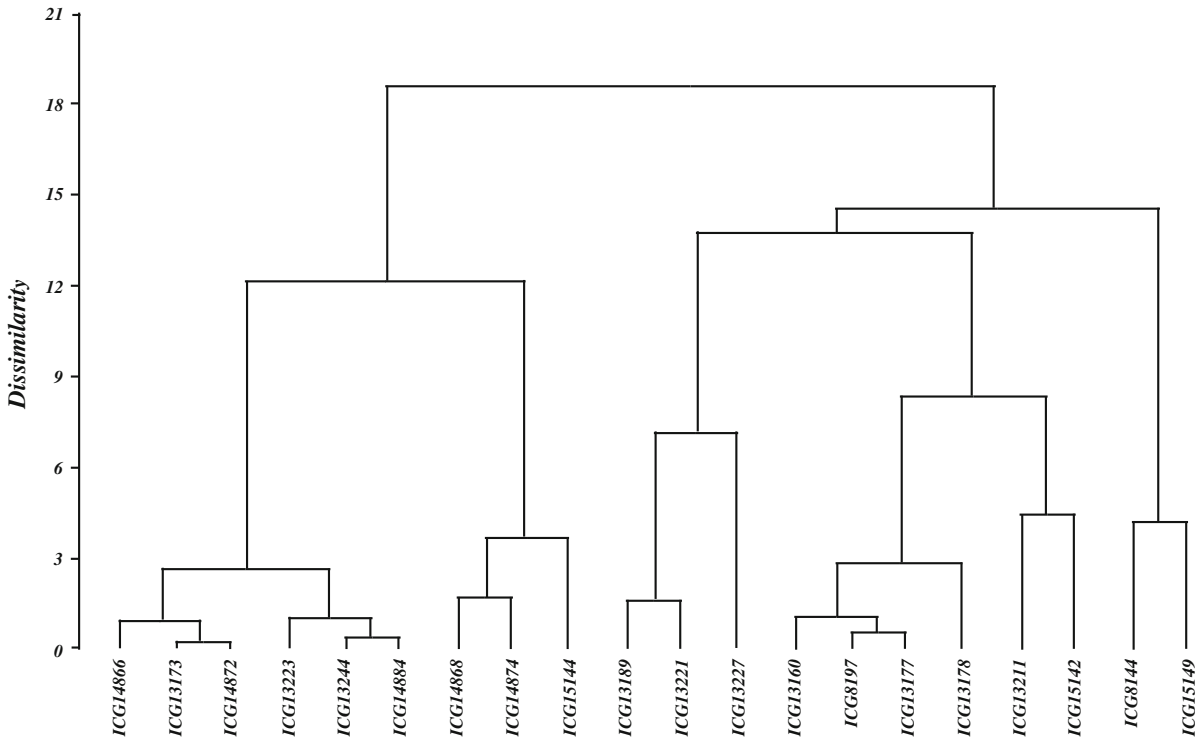

Fig. 2 Dendrogram of 20 selected wild *Arachis* accessions for multiple traits based on scores of first five principal components (84.6% variation)

nutritional (oil, protein and sugar content) and drought related (SCMR and SLA, both at 60 DAS and 80 DAS) trait combinations were identified (Table 5). Seven of these accessions belong to *A. stenosperma*, three each to *A. monticola* and *A. pusilla*, two to *A. kuhlmannii* and one each to *A. villosa, A. batizocoi, A. duranensis, A. dardani* and *A. paraguariensis*. All, except *A. monticola* (AABB), *A. batizocoi* (BB), *A. pusilla* (TT), *A. dardani* (HH) and *A. paraguariensis* (EE) are diploids with AA genome. All except *A. pusilla*, *A. dardani* and *A. paraguariensis* are cross compatible with *A. hypogaea*. Biotechnological tools can be applied to tap the rare alleles of non-crossable species such as *A. pusilla*, *A. dardani* and *A. paraguariensis*. Individual trait performance of a number of accessions, exceeded the trial mean significantly, with a number of accessions superior for a range of agronomically important traits: an *A. villosa* accession (ICG 8144) high in SCMR, low SLA, high sugar content; *A. stenosperma* accessions (ICG 13223, 13244, 14868, 14872, 14874, 14884) superior in pod length and width and/or SdL and width; *A. pusilla* accession (ICG 13211) earliest to flower; *A. monticola* (ICG 13178) and *A. duranensis* (ICG 13189) accessions high in sugar content; another *A. pusilla* accession (ICG 15142) and an accession from *A. dardani* (ICG 13227) high in protein content. For oil content, the variability was in the range of 45–55%, similar to those reported in groundnut cultigens (Upadhyaya et al. 2003). The previous studies reported ICG 8144, ICG 14868, ICG 14872 and ICG 15144 as resistant to late leaf spot and ICG 13173, 13189, 14868, 14872 and 15144 to rust (Pande and Narayana Rao 2001); ICG 8144 to tobacco streak virus (Kalyani et al. 2007); ICG 13173 and ICG 14872 to rosette virus (Subrahmanyam et al. 2001); and ICG 13160 and 15149 to nematode *M. javanica* race 3 (Sharma et al. 1999).

A hierarchical dendogram of the 20 selected wild *Arachis* accessions (with a range of desirable agronomic, nutritional quality and drought related traits, Table 5), based on the scores of the first five PCs of 16 agronomic, nutritional and drought related traits (Table 5) capturing 84.6% variation, grouped these accessions into five clusters (Fig. 2). The clustering

pattern was based on species and accessions of the same species that were clustered together, or in a cluster with less divergence like *A. dardani* (one accessions), *A. monticola* (three accessions), *A. pusilla* (two accessions). 'A' genome species accessions were clustered together with the exception of accessions, ICG 8144 (*A. villosa*) and ICG 13189 (*A. duranensis*), and other genomes (T, H and E), were grouped in different clusters. This clustering pattern was similar to the one reported earlier (Koppolu et al. 2010) based on molecular diversity as detected by SSR polymorphism, with a few exceptions such as low divergence of the *T* genome species accessions ICG 13221 (*A. pusilla*), and *H* genome species accession ICG 13227 (*A. dardani*) with A genome species in the present investigation, as opposed to more divergence, based on molecular data. This difference could be due to predominance of the variations not captured in these accessions, or lower coverage of the genome with the screened SSR markers, which needs further investigation. However, it may be worth involving the diverse accessions from different clusters that possess a combination of agronomic, nutritional and drought related traits, in a breeding program that would enhance tolerance to drought, and improve nutritional quality.

Most of the accessions that possess a combination of agronomically desirable traits are diploids, it would first be required to develop amphidiploids or autotetraploids, followed by backcrossing to cultivated species, such a pre-breeding activity is being followed at ICRISAT to broaden the genetic base of groundnut cultigens (Mallikarjuna et al. 2010).

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