

Morphological characterization of the USA *Arachis pinto* Krap. and Greg. collection

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Abstract *Arachis pinto* accessions were morphologically characterized using a list of descriptors prepared by IBPGR/ICRISAT. Data from stems, leaves, flowers, pegs, pods, and seeds were collected. Phenotypic correlations were calculated among descriptors, and diversity indices were computed to assess the genetic diversity among accessions. Principal component analysis was executed, and a cluster analysis was performed to group the germplasm. The germplasm presented great morphological variability with most of the descriptors showing polymorphism. From 595 correlations computed, 96 were statistically significant. Biologically meaningful correlations ($r \geq 0.71$) were found between leaf length and pod weight; leaf length and pod width; leaf length and seed weight; leaf length and seed width; and leaf length and seed length. Diversity values were variable among traits, but in general all morphological features expressed high genetic diversity. Total genetic diversity was estimated as 0.71. Principal component and cluster analysis was able to differentiated four distinct groups.

Keywords Tropical legume · Genetic resources · Diversity index · Biological correlations

Introduction

The genus *Arachis*, with diploid ($2n = 2x = 20$) and tetraploid ($2n = 4x = 40$) species, belongs to family Fabaceae, subfamily Papilionoideae, subtribe Stylosanthinae, tribe Aeschynomeneae. The genus has natural occurrence exclusively in South America, extending from east of the Andes Mountains, south of Amazonia, north of the Planície Platina and northwest of Argentina, until the Northeast of Brazil (Gregory et al. 1980; Krapovickas and Gregory 1994). Krapovickas and Gregory (1994) describe 69 species, with 39 exclusively from Brazil. Valls and Simpson (1994), however, assert that the genus has about 80 species. The genus can be divided into nine groups or sections; one of them with two series: *Arachis*, *Caulorrhizae*, *Erectoides*, *Extranervosae*, *Heteranthae*, *Procumbentes*, *Rhizomatosae* (Series *Prorhizomatosae* and *Rhizomatosae*), *Triectoides*, and *Triseminatae*.

The importance of *A. hypogaea* as a major food crop generated a need for a better understanding of the wild species of the genus, because of the potential to use them in breeding programs of the common peanut. Parallel to the interest in improvement of cultivated peanut, the acknowledgment of the forage potential of some wild species of the genus brought a general interest in these species. This interest generated an effort to recollect most of the available genetic diversity. According to Valls and Pizarro (1994) more than 30 expeditions were conducted between 1981 and 1993, which greatly impacted the number of accessions of perennial *Arachis* species stored in germplasm banks.

Arachis pinto is considered a multiple use legume, being grown for forage; ground cover in fruits orchards, forest, and low tillage systems; erosion control; and ornamental purposes. *A. pinto* is a herbaceous, perennial

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legume, with a low stoloniferous growth habit and axon-morphous roots, without enlargements. The stems are cylindrical, angular, and hollow. The leaves are alternate, compound, with four obovate leaflets. Stipules have the basal portion attached to the petiole. It shows indeterminate and continuous flowering. The inflorescence is axillary, in very short spikes, with four to five sessile flowers protected by two bracts. The corollas are yellow in the typical condition. The species is considered normally self-pollinated (Krapovickas and Gregory 1994). The small fruit of *A. pintoii* are located underground in an articulated legume form, with each articulation classified as an indehiscent capsule, which usually contains a single seed (Cook et al. 1990). The number of chromosomes is $2n = 2x = 20$ (Fernández and Krapovickas 1994).

Morphological characterization is used to assess and understand the genetic variability of germplasm collections. When working with a wild species of a genus of interest, this usually is the first chance to gather basic knowledge about the agronomic attributes of the species. Qualitative, highly heritable, morphological traits were originally used in early scientific investigations of genetic diversity; such as the ones performed by Mendel (1866) and De Vries (1900). In general, morphological studies did not involve sophisticated equipment or laborious procedures, and these monogenic or oligogenic morphological traits were simple, rapid, and inexpensive to score (Hawkes et al. 2000).

Improvements in computing and statistical tools provided the ability to utilize large data sets consisting of morphological measurements of multiple quantitatively inherited traits. The information generated from this type of morphological characterization research can be used to identify individual accessions based on a set of particular phenotype traits. Such data can also be useful to estimate genetic diversity of a germplasm collection, which will possibly impact the decision to enlarge the gene pool by further collection trips. Additionally, this activity can generate information about the genetic divergence among the accessions, which can be used to group accessions. Such grouping of the germplasm accessions can be used to assemble core collections, especially important in large germplasm banks.

Several statistical techniques may be used to assess genetic divergence. However, when a large number of accessions are present, multivariate analysis is most appropriate. The advantage of this technique is the ability to discriminate among accessions considering multiple variables at the same time. Among the multivariate techniques the most used in genetic studies are principal component and cluster analysis (Hawkes et al. 2000).

Principal components analysis (PCA) has been widely used in genetic resource related research. The technique can be used with several objectives:

- Quantification of genetic divergence among germplasm accessions
- Selection of divergent parental genotypes to hybridize
- Variable reduction in sets of data with great number of parameters
- Variable exclusion based on its contribution to the total variance
- Calculation of a similarity index for the purpose of grouping accessions

The goal of this research was to morphologically characterize the *A. pintoii* germplasm accessions stored at the USDA-NPGS germplasm bank and to cluster the accessions based on similarity indices.

Materials and methods

Accessions of *A. pintoii* stored in the Southern Regional Plant Introduction Station of the National Plant Germplasm System (NPGS) located at Griffin, Georgia were transferred to the University of Florida in 2001 and 2002. A list of these accessions with information related to the respective PI numbers and sites of collection is presented in Table 1.

Morphological characterization of the accessions was accomplished by evaluating each individual accession for a list of morphological descriptors selected from the IBPGR/ICRISAT (1990, 1992) list of morphological descriptors for *Arachis* collected in a single year (2003). Data from leaves, stems, flowers, pegs, pods, and seeds were collected from plants of each accession. The list of morphological descriptors evaluated is presented in more detail in Table 2.

Two plots of four plants (2×2 m) were established at the Forage Research Unit of the Agronomy Department of the University of Florida in Gainesville-FL. Six random plants of each accession were selected from these plots and used as plant-part sources.

For each genotype, ten stems per plant were collected, the terminal part of each stem (first three internodes) was discarded and then five internodes were evaluated on each stem. For the other part categories, ten pieces were collected and evaluated. Continuous variables were measured with a 15 cm electronic digital caliper (Chicago Brand Industrial Inc., Fremont, CA), and categorical variables were scored under the dissecting microscope using the standards proposed by IBPGR/ICRISAT (1990, 1992). The mean, standard deviation, and range were calculated for quantitative descriptors, and the mode was determined for qualitative ones.

Genetic variability among the accessions with respect to the morphological descriptors was examined by calculating

Table 1 *Arachis pintoi* accessions characterized in this study

PI/CIAT number	Lat. (south)	Long. (west)	Altitude (m)
476132	16°08'	47°12'	690
497541	18°38'	44°04'	640
497574	13°23'	44°05'	450
604798	16°18'	46°58'	630
604799	16°19'	46°51'	580
604800	16°41'	46°29'	540
604801	16°42'	46°25'	560
604803	14°25'	44°22'	510
604804	14°20'	44°25'	560
604805	16°59'	45°57'	570
604807	13°18'	46°48'	510
604808	13°18'	46°42'	500
604809	13°02'	46°45'	610
604810	13°06'	46°45'	600
604811	13°51'	46°52'	490
604812	14°28'	46°29'	500
604813	14°27'	47°00'	480
604814	15°52'	39°08'	50
604815	15°49'	47°58'	1,080
604817	18°38'	44°04'	630
604856	16°53'	42°07'	360
604857	13°23'	44°05'	450
604858	15°26'	47°21'	700
604859	17°03'	42°21'	360
18745	16°05'	42°05'	280
20826	–	–	–
22150	15°07'	44°08'	510
22152	16°52'	46°35'	550
22159	15°17'	47°23'	650
22234	13°14'	46°44'	463
22256	16°10'	46°01'	580
22260	14°04'	47°18'	720
22265	–	–	–
22271	15°47'	47°56'	1,040

Simpson's (1949) and Shannon and Weaver (1949) diversity indices. These indices give a measure of phenotypic diversity and range from zero to one, where one represents great genetic diversity and zero the opposite or no genetic diversity. The indices correspond to the probability that two individuals randomly selected from a group of populations will have the same morphological feature. The formula to calculate both indices is presented below.

Shannon--Weaver diversity: $H = -\sum(p_i \log p_i) / \log p_i$

Simpson diversity index: $D = 1 - \sum(p_i^2)$

where $i = 1$ to n , and p is the proportion of the total morphotypes made up of the i th morphotype.

The data matrix was then analyzed with SAS software (SAS Institute Inc 1989). Phenotypic correlations among morphological descriptors were computed. Qualitative characteristics were transformed, and a principal component analysis was performed. Finally, a cluster analysis using the "complete linkage method" was prepared. Means of quantitative traits of each group were compared by using the Newman–Keuls procedure.

Results

Substantial morphological variability was observed among the accessions for all descriptors with the exception of pollen size and shape, and bristles on the superior and inferior leaf surface, which showed no polymorphism. The mean/mode, standard deviation, variance and range of each morphological descriptor are shown in Table 3.

Arachis pintoi flowers show typical characteristics of Papilionaceae legumes possessing five petals. They displayed one standard petal, two wing petals, and a keel that is actually formed by the fusion of two petals. The other flower structures are the calyx and the hypanthium. Most of the accessions (91%) have yellow flowers, with the 9% exception showing a lighter shade of yellow classified as lemon yellow. This was the case of accessions PI 604814, PI 604818, and CIAT 20826. Standard color is considered a very good genetic marker, and so it could be used in genetic studies with *A. pintoi*.

Differences in standard petal length and width, wing length and width, keel length, and hypanthium length and width were also observed. Those features are directly related to overall flower size. Differences in hypanthium color were also found. The mode for this characteristic was absence of color, but some accessions presented a distinct purple color, which is probably associated with the presence of anthocyanin. One of the reported functions of anthocyanin is to attract insects: since it absorbs UV radiation that is attractive to insects (Mann 1987).

Large differences were observed in relation to stem internode length and diameter, and stem color. The average internode length was 29 mm, ranging from 18 to 52 mm. Average internode diameter was 3 mm, varying from 2 to 7 mm. As in the case of hypanthium color, it seems that stem internode color is determined by the presence of anthocyanin. However, here more variants in color were present than in the former feature, although scoring was just made in terms of presence and absence. Absence was the mode for this characteristic with 57% of the accessions showing no purple coloration, which is translated as a light green color. Among those that displayed stem coloration, yellowish, pinkish, light and deep purple were observed.

Table 2 Morphological descriptors applied to *Arachis pintoi* accessions

Morphological descriptor	Descriptor code	No. of structures measured	Unit	Equipment used
Flower/inflorescence	FPI	10	Number	Visual evaluation
Flower standard width	FSW	10	mm	Caliper
Flower standard length	FSL	10	mm	Caliper
Flower standard color	FSC	10	Color scale	Visual evaluation
Flower standard crescent	FScr	10	Present/absent	Dissect scope
Flower wing width	FWW	10	mm	Caliper
Flower wing length	FWL	10	mm	Caliper
Flower keel length	FKL	10	mm	Caliper
Flower hypanthium length	FHL	10	mm	Caliper
Flower hypanthium width	FHW	10	mm	Caliper
Flower hypanthium color	FHC	10	Present/absent	Dissect scope
Flower hypanthium hairiness	FHH	10	Present/absent	Dissect scope
Flower pollen size	FPSi	10	mm	Caliper
Flower pollen shape	FPS	10	IBPGR scale	Microscope
Stem internode length	SIL	50	mm	Caliper
Stem internode diameter	SID	50	mm	Caliper
Stem color	SC	50	Present/absent	Dissect scope
Stem hairiness	SH	10	Pres/abs/abun	Dissect scope
Stem bristles	SGH	10	Present/absent	Dissect scope
Leaflet shape	LS	10	Shape scale	Visual evaluation
Leaflet hairiness sup. surface	LHU	10	Present/absent	Dissect scope
Leaflet hairiness margin	LHM	10	Present/absent	Dissect scope
Leaflet hairiness inf. surface	LHL	10	Present/absent	Dissect scope
Leaflet bristles sup. surface	LGHU	10	Present/absent	Dissect scope
Leaflet bristles margin	LGHM	10	Present/absent	Dissect scope
Leaflet bristles inf. surface	LGHL	10	Present/absent	Dissect scope
Leaflet length	LL	10	mm	Caliper
Leaflet width	LW	10	mm	Caliper
Leaflet length/leaf width	LLLW	10	mm	Caliper
Leaf petiole length	LPL	10	mm	Caliper
Peg length	PegL	10	mm	Caliper
Peg width	PegW	10	mm	Caliper
Peg color	PegC	10	Present/absent	Visual evaluation
Peg hairiness	PegH	10	Present/absent	Visual evaluation
Pod weight	PodWe	10	Grams	Scale
Pod length	PodL	10	mm	Caliper
Pod width	PodW	10	mm	Caliper
Pod beak	PodB	10	IBPGR scale	Dissect scope
Pod reticulation	PodR	10	IBPGR scale	Dissect scope
Seed width	SW	10	mm	Caliper
Seed length	SL	10	mm	Caliper
Seed weight	SWe	10	Grams	Scale
Seed color	Scolor	10	ISCC-NBS color chart	Dissect scope
Pod weight/seed weight	PodweSwe	10	%	–

When examining leaflet characteristics, the features related to overall leaf size (leaflet length and width and petiole length) and shape were the ones that exhibited the

most variability. Average leaflet length was 25 mm with a range of 16–32 mm. Leaflet width varied from 9 to 21 mm, with an average value of 15 mm. Due to leaflet length and

Table 3 Morphological characteristics of *Arachis pinto* germplasm accessions

Morphological descriptor	Mean/mode	Standard deviation	Variance	Range
FPI	3.14	0.33	0.11	2.00–3.80
FSW	15.31	1.63	2.67	11.99–18.85
FSL	11.58	1.10	1.22	9.84–14.45
FSC	Yellow	–	–	–
FScr	Present	–	–	Present/absent
FWW	6.27	0.69	0.47	4.47–7.95
FWL	8.07	0.69	0.48	6.92–9.80
FKL	5.09	0.23	0.05	4.45–5.55
FHL	70.66	12.58	158.28	52.39–104.79
FHW	0.97	0.10	0.01	0.77–1.12
FHC	Absent	–	–	Present/absent
FHH	Present	–	–	Present/absent
FPSi	4.00	0.00	0.00	–
FPS	Round	–	–	–
SIL	29.47	7.43	55.27	18.23–52.23
SID	2.95	0.84	0.71	2.05–7.15
SC	Absent	–	–	Present/absent
SH	Present	–	–	Present/absent
SGH	Absent	–	–	Present/absent
LS	Obovate	–	–	–
LHU	Absent	–	–	Present/absent
LHM	Absent	–	–	Present/absent
LHL	Present	–	–	Present/absent
LGHU	Absent	–	–	Present/absent
LGHM	Present	–	–	Present/absent
LGHL	Absent	–	–	Present/absent
LL	24.89	4.07	16.60	15.79–32.03
LW	15.14	2.79	7.80	9.45–20.78
LLLW	1.66	0.21	0.04	1.29–2.06
LPL	22.26	6.40	40.91	11.67–39.33
PegL	11.90	3.36	11.28	6.30–21.40
PegW	0.94	0.14	0.02	0.57–1.23
PegC	Present	–	–	Present/absent
PegH	Present	–	–	Present/absent
PodWe	0.13	0.07	0.01	0.07–0.25
PodL	9.33	4.52	20.40	7.55–14.25
PodW	4.70	2.22	4.91	4.53–6.91
PodB	Moderate	–	–	–
PodR	Slight	–	–	–
SW	3.89	1.86	3.45	3.55–5.46
SL	7.13	3.50	12.22	5.71–10.51
SWe	0.10	0.06	0.001	0.04–0.18
Scolor	Orange yellowish	–	–	–
PodweSwe	75.47	6.71	44.99	59.71–87.07

width variation, an array of leaflet sizes arise (Fig. 1). In terms of leaflet shape ten different types were present with obovate shape being the mode.

A. pinto leaves are compound with four leaflets. Usually the basal leaflets were smaller than the distal, and exhibited an elliptic shape. As observed in flower

and stem features, large differences were also present in leaves.

Pegs, pods, and seeds exhibited an array of variability among the accessions. One of the differences is the fact that accessions PI 604804, 604813, 604817, and CIAT 22152, 22159, 22234 did not produce any of the above plant structures under the conditions of these experiments. Additionally, differences were also present in the accessions that produced these structures. Average peg length was 12 mm, ranging from 6 to 21 mm. With respect to the other peg features, average peg width was 0.90 mm, and presence of peg color and hairiness were the mode.

Pod size and weight showed great divergence among the germplasm. These characteristics were most impacted by pod length and width. Large pod size and weight were reflected in large seed size and weight. Average seed weight was 0.12 g, with a range of 0.04–0.18 g.

Phenotypic correlations were calculated among morphological descriptors, and Pearson's correlation coefficient and significance test were also calculated. Pollen size and shape (FPSi and FPS) and bristles on the superior and inferior leaf surface (LGHU and LGHL) were not included because they did not show any variability. In addition, leaf length/leaf width ratio (LLLW) and pod weight/seed weight ratio (PodweSwe) were left out of the correlation matrix because they were derived from two other variables. Accessions PI 604804, 604813, 604817, and CIAT 22152, 22159, 22234 were excluded because they did not produce any pegs, pods, or seeds. In summary, 35 morphological descriptors and 28 germplasm accessions were used to calculate correlation coefficients. Therefore, with 26 degrees of freedom ($df = N - 2$ or $28 - 2$), any correlation coefficient with an absolute value greater than 0.361 had a P value of ≤ 0.05 .

A PCA was performed with the goal of discriminating among accessions and grouping even further. The goal of

PCA is to provide a reduced dimension model that would indicate measured differences among groups. It also can contribute to a better understanding of the set of variables by describing how much of the total variance is explained by each one. With this objective the PCA was performed with the matrix of morphological data generated by applying the list of descriptors presented in Table 2.

Variables pollen size and shape (FPSi and FPS) and leaf bristles superior and inferior surface (LGHU and LGHL) were not included because they did not show any variability. In addition, leaf length/leaf width ratio (LLLW) and pod weight/seed weight ratio (PodweSwe) were also left out because they are derived from two other variables. After the first analysis, the leaflet hairiness superior surface variable (LHU) was also excluded from the analysis because it contributed little to the exploration of total variance. The first nine principal components were used to execute a cluster analysis using the complete linkage clusters method. The dendrogram resulting from this analysis is presented in Fig. 2.

Genetic diversity was accessed using Shannon–Weaver and Simpson diversity indices. These indices are often used in ecological studies where species richness and composition of a particular community or ecosystem are evaluated. Recently, these indices have been applied to quantify genetic diversity of germplasm collections when phenotypic frequencies are collected. The greater the index value, the greater the genetic diversity. In Table 4 the values of the indices for each morphological descriptor are presented. The total genetic diversity was also calculated, and it is an indication of how different the accessions are in relation to the morphological features utilized in this research.

Discussion

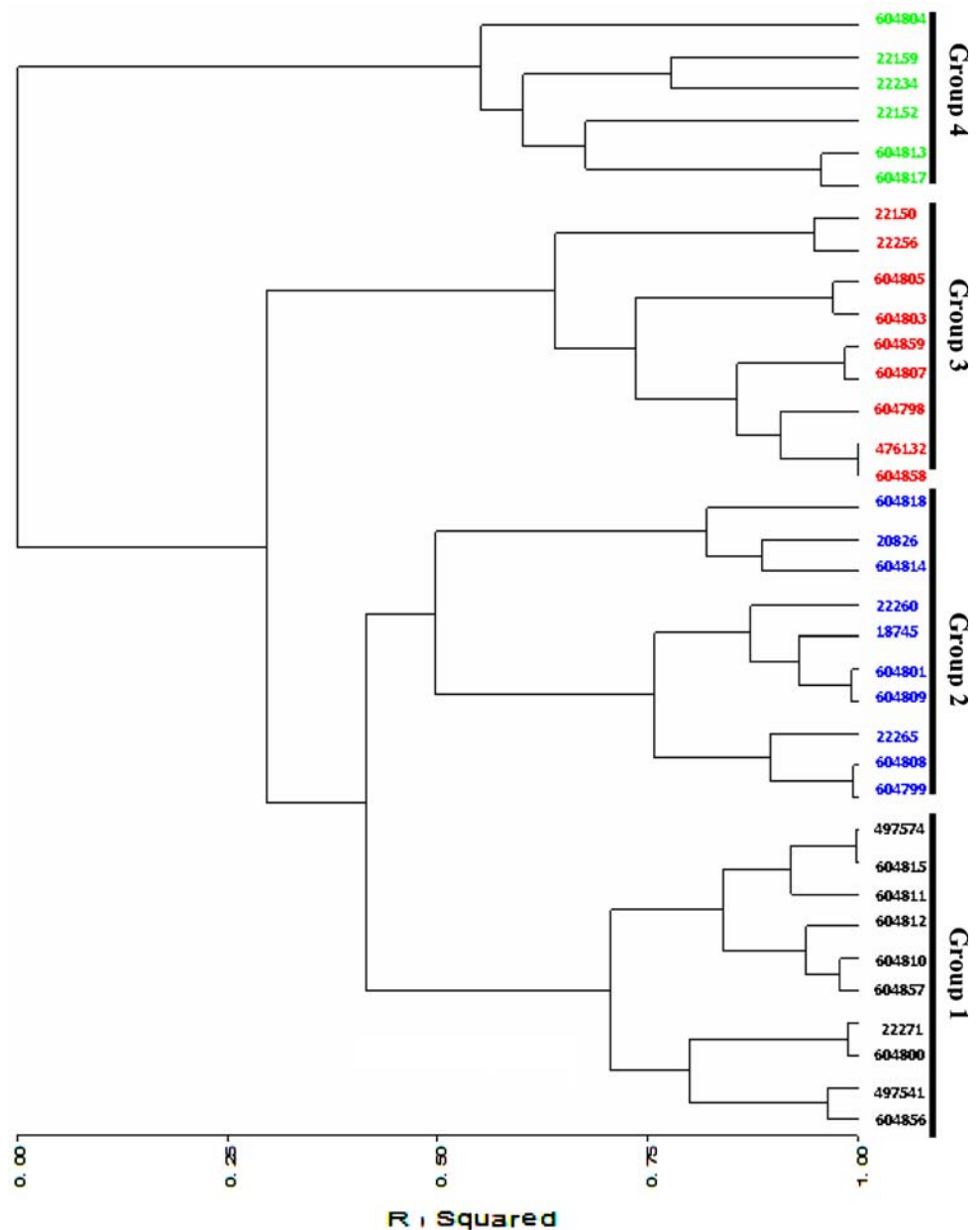
Examining the 595 correlations computed, 96 were considered significant, presenting a correlation coefficient higher than 0.361. These 96 significant correlations could be divided into eight different groups:

1. correlations between flower features dimensions;
2. correlations between leaflet features dimensions;
3. correlations between flower features dimensions and leaflet dimensions;
4. correlations between stem diameter and leaflet dimensions;
5. correlations between flower features dimensions and pod and seed dimensions;
6. correlations between stem diameter and pod and seed dimensions;
7. correlations between leaflet features dimensions and pod and seed dimensions;



Fig. 1 Differences in *Arachis pintoi* leaflet shapes and sizes

Fig. 2 Dendrogram of 35 *Arachis pintoi* accessions based on morphological descriptors and the first nine principal components



8. correlations between pod features dimensions and seed dimensions

Correlations in Groups 1, 2, and 8 are not very meaningful and could be explained by the fact that part proportions should be maintained within each plant organ or structure. So, flowers with large standards length will also have large standard width, wing length and width, and keel length. For the same reason we should expect that pods with large measures will also have large seed if an adequate development had occurred, because the seeds are enclosed by the pod.

Correlations in Groups 3 and 4 would have agronomic importance if leaflet size is translated into higher vegetative mass production. Likewise leaf/stem ratio could be

important assuming leaves have higher nutritive values than stem. These are very important features in forage species. These correlations suggest that flower size and stem diameter could be included as selection indices in *A. pintoi* forage selection and breeding programs. The same idea could be applied to Groups 5, 6, and 7, where stem, flower, and leaflet size appear to impact pod and seed size.

Although 96 correlation coefficients were considered significant ($r = 0.361$), Skinner et al. (1999) suggested that only correlation coefficient with absolute values higher than 0.71 should be considered biologically meaningful. They explain that only in these situation is more than 50% of the variance of one trait is predicted by the other.

Table 4 Simpson and Shannon–Weaver diversity indices for *Arachis pintoii* morphological descriptors

Morphological descriptor	Simpson	Shannon–Weaver
Flower/inflorescence	0.60	0.34
Flower standard width	0.81	0.89
Flower standard length	0.68	0.80
Flower standard color	0.18	0.47
Flower standard crescent	0.24	0.58
Flower wing width	0.56	0.33
Flower wing length	0.60	0.33
Flower keel length	0.54	0.74
Flower hypanthium length	0.73	0.89
Flower hypanthium width	0.73	0.46
Flower hypanthium color	0.50	1.00
Flower hypanthium hairiness	0.24	0.58
Stem internode length	0.50	0.58
Stem internode diameter	0.65	0.45
Stem color	0.47	0.96
Stem hairiness	0.63	0.96
Stem bristles	0.36	0.80
Leaf shape	0.83	0.83
Leaflet hairiness inf. surface	0.50	1.00
Leaflet hairiness margins	0.48	0.98
Leaf bristles margins	0.36	0.80
Leaf length	0.64	0.46
Leaf width	0.58	0.34
Leaf length/leaf width	0.67	1.00
Leaf petiole length	0.74	0.84
Peg length	0.59	0.50
Peg width	0.46	0.71
Peg color	0.45	0.92
Peg hairiness	0.45	0.92
Pod weight	0.69	0.45
Pod length	0.47	0.74
Pod width	0.74	0.89
Pod beak	0.73	0.87
Pod reticulation	0.64	0.97
Seed width	0.69	0.48
Seed length	0.75	0.88
Seed weight	0.64	0.37
Seed color	0.82	0.89
Pod weight/seed weight	0.57	0.87
Total	0.58	0.71

Reexamining the correlation coefficient table under this new criterion only 29 correlation coefficients out of 595 would be considered biologically meaningful. In this situation only Groups 1, 2, 7, and 8 remain in place. As stated before, Groups 1, 2, and 8, do not have great biological importance, since they could be explained by the fact that part proportions should be maintained within each plant

organ or structure. Therefore, only correlations relative to Group 7 may have meaningful biological importance. Examining this information more carefully, we can observe that meaningful correlations were found between leaf length (LL) and Pod weight (Podwe), leaf length (LL) and pod width (PodW), leaf length (LL) and seed weight (Swe), leaf length (LL) and seed width (SW), and finally leaf length (LL) and seed length (SL). Thus, leaf length could be used as a selection criterion in programs where increased seed size/weight is one of the objectives.

One of the main problems with forage legumes species is slow establishment, which sometimes is associated with small seed size. Having a large seed could represent more stored reserves and higher seedling vigor, which would reduce the establishment time. These suggestions based on the above correlations should be verified in future research to evaluate the use of these characteristics as selection indices.

To support this hypothesis we could examine the findings of Skinner et al. (1999) who worked with the Australian annual *Medicago* collection (20997 accessions) measuring 27 traits. They reported that the traits seeds per gram and winter and spring herbage yield were correlated (0.42 and 0.29). They also stated that seeds per gram and seedling vigor were correlated (0.44). Upadhyaya (2003a) also found correlations between 100 seed weight and yield (0.32) in the ICRISAT *Arachis hypogaea* core collection.

Diversity values were variable among traits, but in general all morphological features expressed high genetic diversity. According to Simpson's index, leaf shape (0.83), seed color (0.82), flower standard width (0.81), and seed length (0.75) were the descriptors with greatest diversity. In opposition, the lowest diversity values were related to flower standard color (0.18), flower standard crescent (0.24), and flower hypanthium hairiness (0.24). The total Simpson's index to all morphological descriptors was 0.58.

Shannon–Weaver's diversity values were in general higher than Simpson's. Descriptors with higher values for the Shannon–Weaver index were leaf hairiness inferior surface (1.00), flower hypanthium color (1.00), leaf hairiness margins, and pod reticulation (0.97). Total genetic diversity was estimated as 0.71.

Shannon–Weaver diversity index values observed in this work are much higher than the ones observed by Upadhyaya et al. (2002), who applied 38 agromorphological descriptors to the whole *A. hypogaea* ICRISAT collection (13342 accessions). These authors found a total genetic diversity value of 0.50. Leaflet length (0.62) and shelling percentage (0.62) were the traits showing most variation.

These values are also higher than those reported by Upadhyaya (2003a) who evaluated a core collection prepared using the results of the previous work. He obtained a total diversity index of 0.44 in 32 agromorphological traits.

Table 5 Morphological characteristics of *Arachis pintoi* accession groups obtained by the cluster analysis

Characteristics	Group 1	Group 2	Group 3	Group 4
Quantitative descriptors^a				
Flower/inflorescence	3.14a ^c	3.03a	3.34a	3.00a
Flower standard width	14.17b	15.15ab	16.23a	16.07a
Flower standard length	10.80b	11.32ab	12.40a	12.06a
Flower wing width	5.72c	6.13bc	6.81a	6.62ab
Flower wing length	7.53b	7.85b	8.64a	8.49a
Flower keel length	4.98a	5.02a	5.21a	5.20a
Flower hypanthium length	65.00b	64.00b	79.00a	78.00a
Flower hypanthium width	0.94b	0.91b	1.08a	0.94b
Stem internode length	26.40a	32.27a	28.13a	31.94a
Stem internode diameter	2.72a	2.79a	3.14a	3.34a
Leaflet length	20.68b	26.61a	27.80a	24.69a
Leaflet width	13.01b	16.73a	17.23a	12.91b
Leaf petiole length	16.13c	27.44a	23.89ab	21.42b
Peg length	9.85b	13.66a	12.14ab	0.00c
Peg width	0.97a	0.89a	0.96a	0.00b
Pod weight	0.11b	0.18a	0.17a	0.00c
Pod length	10.23b	12.07a	11.53a	0.00c
Pod width	5.26c	5.66b	6.12a	0.00d
Seed width	4.17c	4.80b	5.15a	0.00d
Seed length	7.33b	9.56a	8.96a	0.00c
Seed weight	0.08b	0.14a	0.13a	0.00c
Qualitative descriptors^b				
Flower standard color	Yellow	Yellow	Yellow	Yellow
Flower standard crescent	Present	Present	Present	Absent
Flower hypanthium color	Absent	Present	Absent	Absent
Flower hypanthium hairiness	Present	Present	Present	Present
Stem color	Absent	Absent	Absent	Present
Stem hairiness	Present	Absent	Abundant	Present
Stem bristles	Absent	Absent	Absent	Absent
Leaflet shape	Obovate	Obovate	Narrow elliptic	Obovate
Leaflet hairiness sup. surface	Absent	Absent	Absent	Absent
Leaflet hairiness margin	Absent	Absent	Present	Present
Leaflet hairiness inf. surface	Present	Absent	Present	Absent
Leaflet bristles margin	Present	Present	Present	Present
Peg color	Present	Present	Absent	–
Peg hairiness	Present	Present	Absent	–
Pod beak	Moderate	Slight	Moderate	–
Pod reticulation	Moderate	Moderate	Slight	–
Seed color	Yellow brownish	Orange yellowish	Orange yellowish	–

^a Mean^b Mode^c Differences between means of different groups were tested by Student Newman–Keuls test. Means followed by same letter are not different at $P = 0.05$

He concluded that the core collection had significant variation for the morphological and agronomic traits evaluated.

Several authors have reported that a considerable amount of variation has been observed in *A. hypogaea* (cultivated

peanut) for agromorphological traits. However, little variation has been detected at the DNA level using techniques such as RAPDs, AFLPs, and RFLPs compared to the variation observed in its wild relatives (Halward et al. 1991;

Kochert et al. 1991; Moretzsohn et al. 2004). These authors considered three possible reasons for the low level of variation observed in cultivated peanut: (1) barriers to gene flow from related diploid species to domesticated peanut as a consequence of the polyploidization event, (2) recent polyploidization, from one or a few individual(s) of each diploid parental species, combined with self-pollination, or (3) use of few elite breeding lines and little exotic germplasm in breeding programs, resulting in a narrow genetic base. This information could explain the higher values of genetic diversity observed in this work when compared to the work done by Upadhyaya et al. (2002) and Upadhyaya (2003a) evaluating the genetic diversity of the cultivated peanut.

The first five principal components (PCs) were responsible for 67.7% of the total variation. Values similar to these were reported by Stalker (1990), Upadhyaya et al. (2002), Upadhyaya (2003a, b), who worked with wild species of *Arachis*, groundnut, and chickpea germplasm collections, respectively, to explain their results. The first PC explained 30.0% of the variation, the second accounted for 15.2%, the third for 10.1%, the fourth for 6.6%, and the fifth explained 5.8% of the total variation.

Examining the variable loadings of the first five PCs we observed that the characteristics of pegs, pods, and seeds are the ones with highest contribution to PC1. Therefore, PC1 could be termed the “sexual reproduction axis”. Performing the same exam to PC2 we can conclude that the features related with flower and leaf dimensions were the ones with highest loadings. Therefore, PC2 could be called the “vegetative axis”. Finally, examining the loading of PC3 we note that features related to the shape, color, and hairiness of morphological structures were the ones with the most contribution. Those are all qualitative features and because of that we could call PC3 the “qualitative axis”.

Therefore, we could state that the PCA was able to discriminate and separate the accessions in terms of these three dimensions, represented by “sexual reproduction”, “vegetative”, and “qualitative” axes. All the 35 morphological features used in the PCA presented high loading values, reinforcing the importance of each one as an *A. pinto* descriptor.

From the dendrogram we can differentiate four distinct groups of accessions (Fig. 2). Group 1 was composed of accessions PI 497541, 497574, 604800, 604810, 604811, 604812, 604815, 604856, 604857, and CIAT 22271. Group 2 was formed by PI 604799, 604801, 604808, 604809, 604814, 604818, CIAT 18745, 20826, 22260, and 22265. Group 3 was composed of PI 476132, 604798, 604803, 604805, 604807, 604858, 604859, CIAT 22150, and 22256. Finally, Group 4 was composed by PI 604804, 604813, 604817, CIAT 22152, 22159, and 22234.

Morphological characteristics of each of the four groups created by the cluster analysis are presented in Table 5.

Group 1 was composed by accessions with small leaves, flowers, pods, pegs, and seeds. Therefore, we could name this group as the “small type” group. Group 2 was formed by accessions with intermediate size features and could be called as the “intermediate type” group. Group 3 was formed by accessions with large leaves, flowers, pegs, pods, and seeds. It was named as the “large type” group due to their large features. Finally, Group 4 was composed of accessions which did not produce any pegs, pods, and seeds. Their leaves, flowers, and stems characteristics were relatively similar to the ones displayed by the members of Group 3, which presented large sizes for these structures. So, based on the fact that this group did not produce seeds, this group could be called the “vegetative type” group. Low or even none seed production are not uncommon among accessions of *A. pinto*. In Brazil the cultivar ‘Belmonte’ is exclusively vegetatively propagated. Carvalho and Quesenberry (2003) reported low seed productions to several accessions cultivated in Florida.

References

- Carvalho MA, Quesenberry KH (2003) *Arachis pinto* seed production in Florida. In: Proceedings of the 35th APRES annual meeting, Clearwater, FL, APRES
- Cook BG, Williams RJ, Wilson GPM (1990) Register of Australian plant cultivars. B. Legumes. 21. *Arachis* (a) *Arachis pinto* Krap. & Greg. nom. nud. (Pinto peanut) cv. Amarillo. Austral J Exp Agricult 30:445–446
- De Vries H (1900) Sur la loi de disjunction des hybrides. Comptes Rendus de l'Academie des Sciences (Paris) 130:845–847
- IBPGR/ICRISAT (1990) Report of a workshop on genetic resources of wild *Arachis* species: including preliminary descriptors for *Arachis*. In: International crop network series 2. IBPGR/ICRISAT, Rome
- IBPGR/ICRISAT (1992) Descriptors for groundnut. IBPGR/ICRISAT, Rome
- Fernández A, Krapovickas A (1994) Chromosomes and evolution of *Arachis* (Leguminosae). Bonplandia 8:187–220
- Gregory WC, Krapovickas A, Gregory MP (1980) Structure, variation, evolution, and classification in *Arachis*. In: Summerfield RJ, Bunting AH (eds) Advances in legume science. Royal Botanic Gardens, Kew, pp 469–481
- Halward TM, Stalker HT, Larue EA, Kochert G (1991) Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. Genome 34:1013–1020
- Hawkes JG, Maxted N, Ford-Lloyd BV (2000) The ex situ conservation of plant genetic resources. Kluwer, Dordrecht
- Krapovickas A, Gregory WC (1994) Taxonomy of the genus *Arachis* (Leguminosae). Bonplandia 8:1–186
- Kochert G, Halward TM, Branch WD, Simpson CE (1991) RFLP variability in peanut (*Arachis hypogaea*) cultivars and wild species. Theor Appl Genet 81:565–570
- Mann J (1987) Secondary metabolism. Oxford University Press, London
- Mendel JG (1866) Versuche über Pflanzen-Hybriden. Verhandlungen des naturforschenden Vereines Brünn, Bd. IV, Abhandlungen

- Moretzsohn MC, Hopkins MS, Mitchell SE, Kresovich S, Valls JFM, Ferreira ME (2004) Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. *BMC Pl Biol* 4:1–10
- SAS Institute Inc (1989) SAS/STAT user's guide, version 6, 4th edn. SAS Institution Inc, Cary
- Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois Press, Urbana
- Simpson EH (1949) Measurement of diversity. *Nature* 163:688
- Skinner DZ, Bauchan GR, Auricht G, Hughes S (1999) A Method for the efficient management and utilization of large germplasm collections. *Crop Sci* 39:1237–1242
- Stalker HT (1990) A morphological appraisal of wild species in section *Arachis* of peanuts. *Peanut Sci* 17:117–122
- Upadhyaya HD, Bramel PJ, Ortiz R, Singh S (2002) Geographical patterns of diversity for morphological and agronomic traits in the groundnut germplasm collection. *Euphytica* 128:191–204
- Upadhyaya HD (2003a) Phenotypic diversity in groundnut (*Arachis hypogaea* L.) core collection assessed by morphological and agronomical evaluations. *Genet Resour Crop Evol* 5:539–550
- Upadhyaya HD (2003b) Geographical patterns of variation for morphological and agronomic characteristics in the chickpea germplasm collection. *Euphytica* 123:343–352
- Valls JFM, Pizarro EA (1994) Collection of wild *Arachis* germplasm. In: Kerridge PC, Hardy B (eds) *Biology and agronomy of forage Arachis*. CIAT, Cali, pp 19–27
- Valls JFM, Simpson CE (1994) The taxonomy, natural distribution and attributes of *Arachis*. In: Kerridge PC, Hardy B (eds) *Biology and agronomy of forage Arachis*. CIAT, Cali, pp 1–18