RESEARCH

Genetic Variability of Cultivated Rhizoma Peanut

Andrea L. Maas, William F. Anderson,* and Kenneth H. Quesenberry

ABSTRACT

Rhizoma peanut (RP; *Arachis glabrata* Benth.) is a vegetatively propagated tropical legume that combines high forage nutritive value and long-term persistence under a wide range of grazing and harvested hay systems. The objectives of this study were to measure the genetic relatedness among 15 RP accessions and assess purity of accessions maintained at different locations. A total of 22 RP clones were tested and included eight duplicate accessions sourced from the national *Arachis* collections and germplasm collections from Florida, Texas, and Georgia. Fourteen amplified fragment length polymorphism (AFLP) primer combinations produced a total of 951 bands, with an average of 67.92 ± 6.56 bands per primer combination. Analysis by unweighted pair group mean algorithm found genetic similarity coefficients (GSAs) that ranged from 0.21 to 1.0. STRUCTURE analysis found minimal population structure or admixture for the RP lines in this study. The duplicated accessions showed a range of genetic distances from no genetic drift (GSA 1.0) to a high degree of drift (GSA 0.28). Overall, AFLP markers provided sufficient polymorphism to successfully differentiate RP clones and determine when genetic drift occurred. The AFLPs substantiated genetic dissimilarity of newly released cultivars from standard accessions. Results also indicated the potential for genetic drift occurring from spontaneous crossing among field-grown accessions, which could lead to selection of improved cultivars.

A.L. Maas and W.F. Anderson, USDA-ARS, Coastal Plain Experiment Station, Tifton, GA 31794; K.H. Quesenberry, Dep. of Agronomy, Univ. of Florida, Gainesville, FL 32611-0500. Received 8 Oct. 2009. *Corresponding author (bill.anderson@ars.usda.gov).

Abbreviations: AFLP, amplified fragment length polymorphism; GSA, genetic similarity coefficient; PGRCU, USDA-ARS Plant Genetic Resource Conservation Unit; PI, plant introduction; RP, rhizoma peanut; UPGMA, unweighted pair group mean algorithm.

Rhizoma peanut (RP; *Arachis glabrata* Benth.) is a vegetatively propagated tetrapoid ($2n = 40$) tropical legume that combines both high nutritive value and long-term persistence under a wide range of grazing and harvested hay systems. Rhizoma peanut is planted primarily in the Gulf Coast region of the United States, with most of the acreage occurring in the USDA winter-hardiness growing zones 8b and higher of Florida and southern Georgia (Mallikarjuna, 2002; Williams et al., 2002). There are >26,000 acres of these cultivars planted in Florida, Georgia, and Alabama, with an estimated value of around $7 million (NRCS, 2008).

The species has been reported to survive as far north as Fort Valley, GA (32°33′ N, 83°54′ W), and Ardmore, OK (34° N, 97° W) (Terrill et al., 1996; Butler et al., 2006). There is an economic incentive to produce hay from RP for the horse (*Equus caballus* L.) industry. Currently, horse farms in the Southeast rely on alfalfa (*Medicago sativa* L.) from northern areas of the United States and Canada. With current RP cultivars, high economic returns can be expected once the field reaches maturity at approximately 3 yr (Lacy, 2006).

Though collections of RP were made as early as the 1930s, it was not promoted as forage until Plant Introduction (PI) 118457 and PI 262839 were evaluated by the USDA Soil Conservation Service

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(now the Natural Resources Conservation Service) and unofficially released in the 1960s as Arb and Arblick, respectively (Blickensderfer et al., 1964). Both of these accessions were not widely grown because they were slow to establish and had limited forage. An aggressive off-type was discovered between plots of Arb and PI 151982 in a nursery at the University of Florida, Gainesville. This off-type was assumed to be a hybrid of the two adjacent lines and was later evaluated for commercial use. This hybrid was released in 1981 as 'Florigraze' (Prine et al., 1986), currently the most widely grown cultivar for commercial hay production. 'Arbrook' (PI 262817), which was released in 1986 (Prine et al.,1990), establishes more aggressively in the spring and has greater drought tolerance. Florigraze and Arbrook make up the entire RP forage acreage in the United States.

The limited number of cultivars in production creates a potential vulnerability to RP hay production due to disease or insects. Two diseases, which cause minor yield reductions in cultivated peanut (*Arachis hypogaea* L.), have been recently discovered in production fields. In 2001, *peanut stunt virus* was first observed in fields of Florigraze (Blount et al., 2002), and has been recently observed in a large proportion of the University of Florida germplasm collection (Quesenberry, personal observation, 2008). At present, the disease has not killed production fields, but it has reduced dry matter yields or increased management costs (A.S. Blount, personal communication, 2007). Additionally, entry A176 within the RP germplasm lines at Tifton, GA, has tested positive for *peanut mottle virus*, which causes ring spots on immature leaves, as well as chlorosis and leaf drop in mature stages (Nischwitz et al., 2007).

Since traditional plant breeding efforts with RP have proved impractical, previous evaluations have been limited to observations of phenotypic traits of wild collected material or rare spontaneous crosses. Better understanding of the genetic diversity within the existing RP collection would be useful to ensure that future breeding efforts with these lines are centered on material that is the most widely divergent from existing cultivars. There is limited molecular diversity information currently available for RP in the literature. The primary emphasis has been on the development of genetic markers for cultivated peanut (Barkley et al., 2007). Much greater genetic diversity has been discovered using restriction fragment length polymorphisms and random amplified polymorphic DNA among wild accessions of the *Arachis* genus (Halward et al., 1991). One *A. glabrata* entry was part of genetic relationship studies with other *Arachis* species, including cultivated peanut (Gimenes et al., 2002, 2007). A high degree of polymorphism has been found within 15 *A. glabrata* accessions from Centro Internacional de Agricultura Tropical (CIAT), using isozymes (Maass and Ocampo, 1995). In a more recent study, Angelici et al. (2008) compared 54 natural populations of *A. glabrata* from Brazil with other wild *Arachis* species. They found greater heterozygosity among the *A. glabrata* tetraploids than the diploids of their study and were able to distinguish among all populations.

Another issue of importance is the measurement of potential genetic drift within stands of vegetatively propagated perennial forages. Genetic integrity of fields will be necessary as improved cultivars are developed and distributed. Molecular tools can be effective tools in the measurement of this drift. For example, the level of genetic drift in turfgrasses have been successfully characterized using molecular markers (Waltz et al., 2005). While the self-pollinating RP often flowers abundantly, making it an attractive groundcover, as in the case of the ornamental RP germplasms Brooksville 67 and Ecoturf, viable seed is rarely produced. However, the potential for even minimal seed set could create drift issues similar to those observed in vegetatively propagated grass. The objectives of this study were to (i) measure the genetic relatedness among duplicate entries of 15 RP lines that have been maintained in two national collections, and (ii) assess purity within selected lines.

MATERIALS AND METHODS

Plant Materials

Plant materials utilized for this study consisted of 15 RP accessions. Seven of the 15 entries were duplicates from the national *Arachis* collections maintained by the USDA-ARS Plant Genetic Resource Conservation Unit (PGRCU), Griffin, GA, and from the backup collection maintained by Texas A&M University, Stephenville (Table 1). Lines were chosen because they were either officially or unofficially released materials (UF Tito, Arbrook, Brooksville 67, UF Peace, Florigraze, Arb, Arblick, and Ecoturf), or currently under study (UGA experimental A23, A156, A160, PI 262821, PI 262819, and A76). Material from field-maintained research plots was supplied by Kenneth Quesenberry, University of Florida (UF Tito, UF Peace, Florigraze, Arbrook, Arblick, and Ecoturf); Wayne Hanna, University of Georgia (UGA Experimental); and from the USDA-ARS R. Hammons plots at Tifton, GA (A23GA, A156, A160, and A76). Greenhouse material maintained as part of the national germplasm collection system was provided by Roy Pittman, PGRCU, Griffin, GA (PI 262826, PI 262817, Brooksville 67, PI 118457 GA, PI 262839, PI 262794, and PI 262840), and Charles Simpson, Texas A&M University (PI 118457 TX, A23TX, PI 262821, and PI 262819). All plant material utilized for this study was established and maintained in 19-L pots in the greenhouse at Tifton, GA.

AFLP and Marker Data Analysis

Plant DNA samples were isolated from fresh leaf tissues of a single potted RP plant for each identifier with DNeasy plant mini kit from QIAGEN, Inc. (Valencia, CA). The amplified fragment length polymorphism (AFLP) analyses were performed as described by Vos et al. (1995). Briefly, 100 ng of genomic DNA was double digested with *Eco*RI and *Mse*I restriction enzymes. The AFLP adapters for each enzyme were ligated to the restriction fragments. Ligated DNA was then preamplified with a primer

Table 1. List of *Arachis glabrata* materials, sources, country of origin, released or imported germplasm, and botanical variety if known.

Identifier	Plant identification	Structure identifier	Source	Country of origin	Imported†/ Released‡	Variety
UGA experimental	None	1	Univ. of Georgia	United States	NA	hagenbeckii
Florigraze	PI 421707	2	Univ. of Florida	United States	1981‡	
Arblick	PI 262839	3	Univ. of Florida	Paraguay	1960s	hagenbeckii
A156		4	USDA-ARS, Tifton, GA	Brazil	Pre-1960†	
A160		5	USDA-ARS, Tifton, GA	Brazil	Pre-1960†	
PI262821	PI 262821	6	Texas A&M Univ.	Paraguay	1959†	hagenbeckii
Brooksville 67	PI 262801	7	PGRCU§	Argentina	1960†/2003‡	hagenbeckii
Arbrook	PI 262817	8	Univ. of Florida	Paraguay	1986‡	
PI262817	PI 262817	9	PGRCU	Paraguay	1960†	
PI118457TX	PI 118457	10	Texas A&M Univ.	Brazil	1936†	
PI118457GA	PI 118457	11	PGRCU	Brazil	1936†	
A23GA	PI 338327	12	USDA-ARS, Tifton, GA	Uruguay	Pre-1960†	
A23TX	PI 338327	13	Texas A&M Univ.	Uruguay	Pre-1960†	
UF Peace	Disputed¶	14	Univ. of Florida	United States	2008‡	
A76	PI 262834	15	USDA-ARS, Tifton, GA	Paraguay	195†	
PI262794	PI 262794	16	PGRCU	Brazil	1960†	
Ecoturf	PI 262840	17	Univ. of Florida	Brazil	1990s	glabrata
PI262840	PI 262840	18	PGRCU	Brazil	1959†	glabrata
PI262839	PI 262839	19	PGRCU	Paraguay	1959†	hagenbeckii
PI262819	PI 262819	20	Texas A&M Univ.	Paraguay	1959†	glabrata
PI262826	PI 262826	21	PGRCU	Paraguay	1960†	glabrata
UF Tito	PI 262826	22	Univ. of Florida	Paraguay	2008‡	glabrata

†Imported.

‡Released.

§PRGCU = USDA-ARS Plant Genetic Resource Conservation Unit, Griffin, GA.

¶Originally thought to be Plant Introduction (PI) 232839 (Arblick); however, plots were morphologically different from other sources of PI 232839.

combination to match the adaptor sequences. The *Eco*RI and *Mse*I primer sequences were 5′-GACTGCGTACCAATTC-3′ and 5′-GATGAGTCCTGAGTAA-3′, respectively. Preamplification conditions were 20 cycles of 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min. Fourteen pairs of selective AFLP primers (Table 2) with *Eco*RI primers labeled with infrared (IR) dye were used for selective amplification. Selective amplifications were performed using the following thermocycling profile: one cycle of 94°C for 30 s, 65°C for 30 s, and 72°C for 1 m, followed by 12 cycles of 94°C for 30 s, 65°C minus 0.7°C per cycle, and 72°C for 1 m, followed by 25 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. Polymerase chain reaction (PCR) reactions were conducted in either an MJ Research PTC-200 (Scientific Support, Inc., Hayward, CA) or a Gene Amp PCR system 9700 (Applied Biosystems, Foster City, CA). A total of 0.5 to 0.8 μL of the selectively amplified PCR products were loaded on a 6.5% (w/v) denaturing gel (LI-COR Inc, Lincoln, NE) and run in 1× TBE buffer at 1500 V, 40 W, and 40 mA at 45°C for 2 h in a LI-COR automated sequencer (LI-COR Inc, Lincoln, NE). IRDye 700–labeled DNA size standard (LI-COR Inc, Lincoln, NE) was loaded on first and last lanes of the gel for molecular weight reference. To ensure accurate scoring and reproducibility, all marker and genotype combinations were replicated twice.

Data Analysis

Polymorphic DNA bands were scored as present (1) or absent (0) for each entry by visual inspection. Data were compiled into a data matrix using Microsoft Excel (Microsoft Corporation, Redmond, WA) and analyzed using NTSYSpc (Numerical Taxonomy System) v 2.2 (Exeter Software, Setauket, NY). Genetic similarity coefficients of pair-wise comparisons among the RP lines were computed based on Jaccard's coefficients (Jaccard, 1908) within

Table 2. Number of total bands, polymorphic bands, percent polymorphic bands, and polymorphic information content (PIC) for each of fourteen amplified fragment length polymorphism selective primer pairs.

Selective amplification primer pairs†	Total bands	Polymorphic bands	% polymorphic bands	PIC
eACG-mCAT	66	34	51.51	0.04
eACT-mCAT	76	24	31.58	0.02
eACG-mCAC	58	18	31.03	0.02
eACT-mCAC	61	18	29.5	0.02
eACA-mCAC	67	41	61.19	0.07
eAGC-mCAC	60	32	53.33	0.05
eAAC-mCAC	82	35	42.68	0.03
eAGG-mCAC	65	28	43.08	0.03
eAGG-mCAT	69	20	28.98	0.01
eAAC-mCAT	84	37	44.05	0.03
eACC-mCAT	60	16	26.67	0.01
eAGC-mCAT	68	21	30.88	0.02
eACA-mCAT	59	10	16.95	0.004
eAAG-mCAC	76	23	30.26	0.02
Total	951	357		
Avg.	67.92 ± 8.56	25.5 ± 9.14	37.27	0.03 ± 0.02

†e = the preamplification primer sequence for *Eco*RI site (5′-GACTGCGTACCAATTC-3′) without any selective nucleotides; m = the preamplification primer sequence for *Mse*I site (5′-GATGAGTCCTGAGTAA-3′).

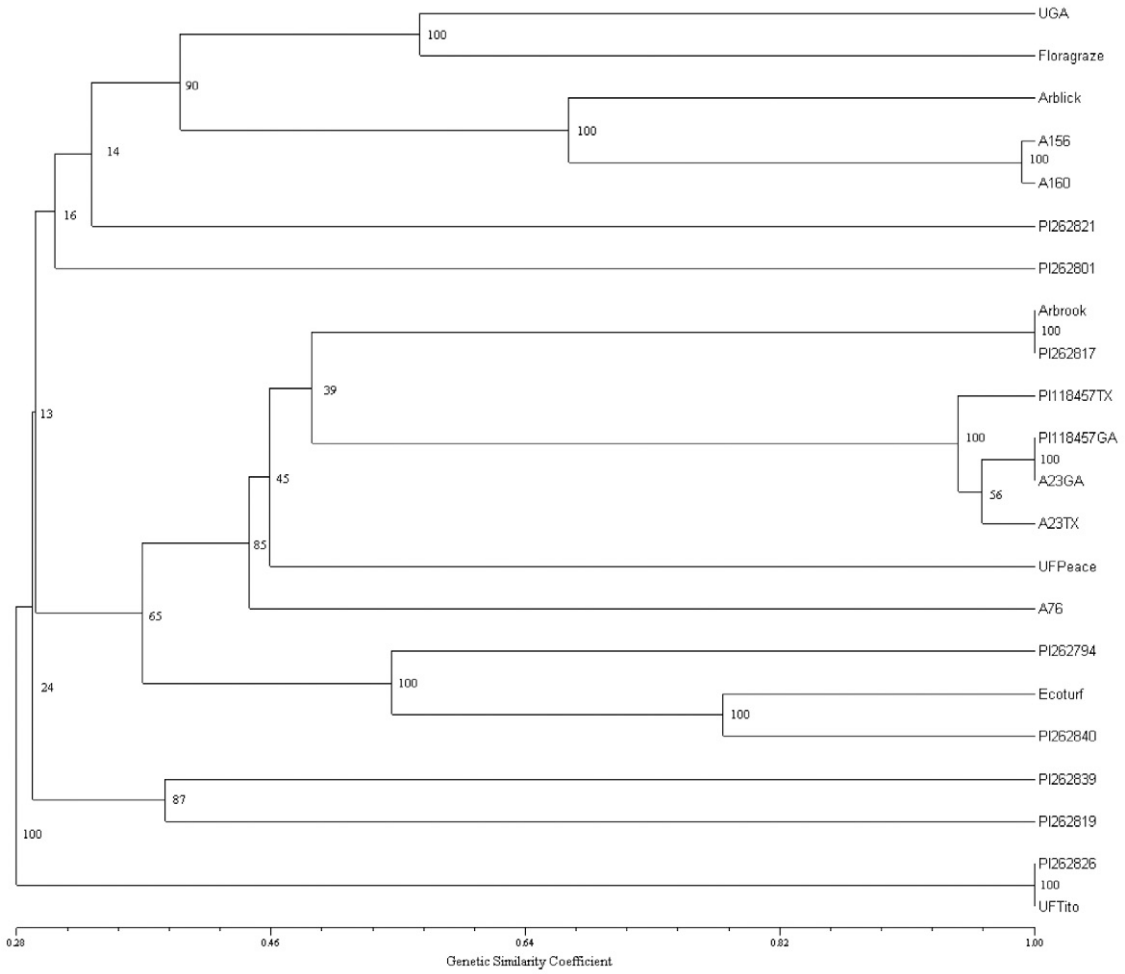

Figure 1. Dendrogram of 22 Arachis glabrata clonal lines maintained in two national collections and three research collections. Dendrogram was produced by unweighted pair group mean algorithm clustering methods based on the genetic similarity matrix (genetic similarity coefficient) derived from 951 markers. Bootstrap values are displayed. Line identifiers correspond to identifier listed in materials list (Table 1).

the SIMQUAL module. Cluster analysis was performed according to the unweighted pair group mean algorithm (UPGMA) within the SAHN module of the NTSYSpc program. Cophenetic correlation was calculated to measure goodness of fit using the MXCOMP module of NTSYS 2.2. Additionally, bootstrap analysis using 250 replications of the data matrix were performed with the FreeTree program according to the program manual to test for cluster robustness (Hampl et al., 2001).

STRUCTURE software Version 2.2 2007 (available at http://pritch.bsd.uchicago.edu/structure.html [verified 14 Apr. 2010]) was utilized to determine the number of structured groups (K clusters) (Pritchard et al., 2000; Falush et al., 2003, 2007). Statistics outlined by Evanno et al. (2005) were utilized in the analysis. Program settings used the admixture ancestry and correlated marker frequency models. The graphs of L(K), as defined by Evanno et al. (2005), along with its variance, and L′(K) were used to determine the number of clusters used for estimating admixtures. The length of burn-in was set at 10,000, followed by 30,000 iterations, and five replications were performed for proposed K values (1–6 tested). Polymorphic information content (PIC) indicating the ability to distinguish among genotypes with each primer combination was calculated as expected heterozygosity of polymorphic bands according to Powell et al. (1996).

RESULTS

Fourteen AFLP selective amplification primer combinations produced a total of 951 bands among the 22 RP entries, with an average of 67.92 ± 6.56 bands per primer combination (Table 2). Of the 951 scored bands, 357 were polymorphic, with an average of 25.5 ± 9.14 polymorphic bands per primer combination. The primer combination eAAC-mCAT amplified the largest (84) number of bands and total polymorphic bands (37), while eACG-mCAC amplified the fewest (58) total number of bands and eACA-mCAT the fewest polymorphic bands (10). The average PIC value of the 14 primer combinations was 0.03 ± 0.02, ranging from 0.01 for eAGG-mCAT and eACC-mCAT to 0.07 for eACA-mCAC (Table 2).

From the UPGMA analysis, a dendrogram was generated (Fig. 1) utilizing the identifiers from Table 1 except for UGA experimental, which is shortened to UGA, and Brooksville 67, which is identified by PI 262801. The cophenetic correlation was calculated (r = 0.98) as a measure of goodness of fit of the similarity indices. The genetic diversity was relatively high among the plant material in this study. The genetic similarity coefficients

(GSAs), given in Fig. 1, are the proportion of matched markers between a given pair of entries among the 22 entries. These values ranged from 0.21 to 1.0. The lowest GSA (0.21) was between Arblick and PI 118457GA while the highest (1.0) was between three pairs PI 118457GA and A23GA, PI 262826 and UF Tito, and Arbrook and PI 262817. Bootstrap values of the GSA cluster analysis tended to be low for larger clusters, while values of smaller clusters were high. Twenty-one cluster values had range of 13 to 100, with 13 of the clusters >85.

The estimated log probability of data [L(K)] resulting from Bayesian cluster analysis generally improved from K = 1 to K = 3 (Table 3). At K = 3 the L(K) were at the lowest and the inferred ancestry coefficients (Fig. 2 and Table 1) generally correlated with groups supported by UPGMA bootstrap confidence levels (Fig. 1). The overall proportion of membership in each of the three clusters was 24.4% (A—red), 17.8% (B—green), and 57.8% (C—blue) in Clusters 1 through 3 respectively, but admixture was observed among all three clusters (Fig. 2 and Table 1). Results from the STRUCTURE analysis generally detected tight genetic relationships for Clusters A and B, but failed to identify much population structure for entries that comprised Cluster C.

Table 3. Mean estimated log probability of data [L(K)] under exhaustive sampling (mean over five runs), mean variance, standard deviation of L(K) of STRUCTURE software program, and mean difference for successive L(K) in model.

Value of K	Mean L(K)†	Mean variance	SD L(K)	Mean L′(K)‡
1	–5735	245	4.9	–
2	–4911	474	16.9	823
3	–4854	1164	33.3	57
4	–4893	1745	89.4	–39
5	–5187	2669	239.0	–293
6	–5614	3638	105.99	–428

†L(K) = log likelihood of the data from STRUCTURE software program.
‡L′(K) = L(K) – L(K – 1).

DISCUSSION

Nine hundred fifty-one fragments were detected using 14 pairs of primers, and collectively 357 (37.3%) were polymorphic. At 37.3% polymorphism, *A. glabrata* demonstrated a lower polymorphism detected with AFLP markers than the 68.1% found using microsatellites for *A. glabrata* (Angelici et al., 2008). The 37.3% was a much higher level of polymorphism with AFLP markers than has been found for *A. hypogaea,* which has been reported to have polymorphism at 6.7, 6.4, and 3.7% (He and Prakash, 1997; Gimenes et al., 2002; Herselman, 2003). In fact, using all 14 primers included in this study, Herselman (2003) found only 1.9% polymorphism for *A. hypogaea* compared with 37.3% in this study. Regardless of detection method, the high level of polymorphism in *A. glabrata* would suggest that sufficient variation exists for improved cultivar development; however, to date successful crosses have been limited (T. Stalker, personal communication, 2006).

The genetic variability among RP lines included in this study is substantial based on the high degree of polymorphism among AFLPs (Table 2 and Fig. 1). The STRUCTURE analysis, however, did not reveal either a high number of clusters or much admixture, suggesting that the material lacks any population structure and very low cross-contamination (Fig. 2). No apparent clustering by plant material collection origin or botanical variety was observed (Table 1 and Fig. 1 and 2). Additionally, the three ornamental selections (UGA experimental, Brooksville 67, and Ecoturf) did not cluster together despite distinct morphological differences from forage type materials. For example, the UGA experimental that forms a dense, low-growing mat clustered most closely to the widely grown tall forage cultivar Florigraze (Fig. 1).

Of the 15 separate lines tested, six lines were duplicated from the national germplasm collections (Table 1; Arbrook/PI 262817, UF Tito/PI 262826, Ecoturf/PI 262840, PI 118457TX/PI 118457GA, A23GA/A23TX, and Arblick/UF Peace/PI 262839). Additionally, the records for the R. Hammons collection (maintained at Tifton, GA) and the PGRCU list A160 and A156 the same as Florigraze (PI 421707). Of these seven possible pairings, only Arbrook/PI

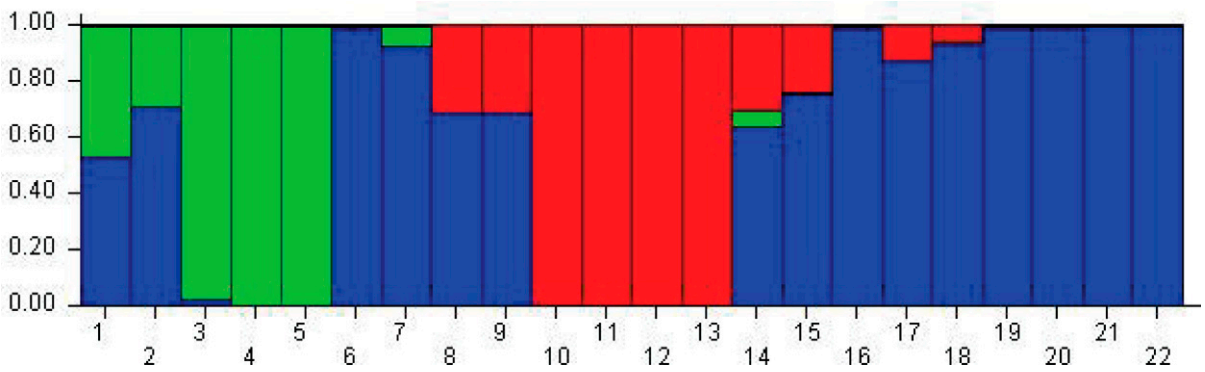

Figure 2. Graphic representation of the individual ancestry coefficients (percentage of alleles) of 22 *Arachis glabrata* clonal lines maintained in two national collections and three research collections that are attributed to one of three clusters (Clusters 1–3 represented by red [A], green [B], and blue [C], respectively) as determined by STRUCTURE software version 2.2 2007. Numbers correspond to structure identifier in materials list (Table 1).

262817 and 'UF Tito'/PI 262826 were genetically identical (1.0 GSA). The next closest pairing was A156 and A160 with a 0.99 GSA scoring, which could simply be a scoring misread. With only three out of seven possible pairs showing a GSA score of essentially 1.0, concern over genetic drift within RP lines may be warranted. Ecoturf/PI 262840 (0.78 GSA), Arblick/PI 262839 (0.29 GSA), and UF Peace/PI 262839 (0.29 GSA) pairings indicate that a great deal of drift may be possible with field-maintained plots. The original material of these pairs has been maintained both in the greenhouse as part of the national germplasm collection in Griffin, GA (PIs) and in the field at Gainesville, FL, where phenotypic changes have been observed (Quesenberry, personal observation, 2008). Some lines may be less susceptible to drift in the field, as in the case of A23GA (field source) and A23TX (greenhouse source) with only a small difference of 0.96 GSA.

Much of the variation may also arise from mistakes in germplasm record keeping. For example, the dendrogram (Fig. 1) and STRUCTURE analysis (Fig. 2) indicated that A160 and A156, which were recorded as Florigraze, are substantially separated genetically from Florigraze (0.38 GSA), and in fact are more closely related to Arblick with a 0.67 GSA score. An example of faulty record keeping combined with possible genetic drift can explain the lack of genetic difference between A23 GA and PI 118457GA (1.0 GSA). The records from R. Hammons were incomplete for A23 GA. Hammons listed it with "No PI" but it was assigned PI 338327 by PGRCU. However, these data indicate that, in fact, A23 GA was identical to PI 118457GA. Furthermore, though A23 GA and PI 118457GA were vegetatively duplicated in Texas (A23 TX and PI 118457TX), there were recorded genetic differences (GSA 0.96 and 0.95, respectively) with the clones maintained in Georgia. This may indicate genetic drift occurring in the greenhouse in Texas. Issues related to genetic drift in between locations were not limited to the Texas-maintained material, as even greater genetic distances existed between other duplicate PIs maintained at different locations. Ecoturf from the field planting at UF clustered with its greenhouse counterpart PI 262840 but only had a GSA of 0.77. Arblick is reported to be PI 262839, originally from Paraguay. However, the Arblick from University of Florida was genetically dissimilar (0.29 GSA) to the PI 262839 at PGRCU. Also, UF Peace, which was reported to be PI 262839, exhibited phenotypic differences from other plots of Arblick in this study and was shown to be genetically distinct from both Florida field-grown Arblick (0.31 GSA) and PGRCU greenhouse-grown PI 262839 (0.29 GSA). This variability within this accession may result from hybrid seed production and germination within plots. Although early work found no seed set in a study at Florida (Williams, 1994), Venuto et al. (1997a) studied seed set among a number of *A. glabrata* lines in Louisiana. Arblick, Arb, Arbrook, and Florigraze all produced seed over 3 yr from field plots, which ranged in germination from 26 to 51%. It appears that seed set occurs under varying environments, and cross-pollination from bees could cause genetic drift to occur (Leuck and Hammons, 1965). This study showed that naturally occurring outcrosses could be easily distinguished using AFLPs.

Also, this study shows that presumptions on parentage of crosses can be validated using AFLPs. The cultivar Florigraze originated from a volunteer plant found between two PIs in Florida and was assumed to be a cross of the two PIs (Prine et al., 1986). This study showed that PI 118457, which is thought to be one of Florigraze's parents, was genetically distinct from Florigraze, possibly dissociating PI 118457 as a parent. The other presumed parent (PI 151982) is no longer maintained in any of the major collections and thus could not be evaluated in this study. This information and the fact that natural selfing of the highly heterozygous RP produces heterogeneous progeny (Simpson et al., 1993) suggest that Florigraze may be a natural self. Also, naturally produced progeny from Florigraze have been shown to vary significantly for forage characteristics such as crude protein, neutral detergent fiber, and plant height (Venuto et al., 1997b).

In conclusion, AFLP markers provided sufficient polymorphic markers to successfully differentiate RP materials utilized in this study. The degree of genetic variation in RP also suggests that the release of material currently under consideration for cultivar or germplasm release (UF Peace, UF Tito, Ecoturf, UGA experimental, A156 or A160, PI 262819, and PI 262821) could provide improved diversity to buffer against commercial crop losses. Additionally, the potential for genetic drift of a cultivar through either cross-pollination or self-induced seed production may occur for specific genotypes and certain environmental conditions. This creates challenges to maintaining purity but can also create opportunities for genetic improvement.

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