PN-ABX-873

The use of a pre-shelling density sort in combination with the current seed-size-based market classes should significantly improve the user's ability to manage peanut lots for quality.

Acknowledgments

The authors appreciate the dedication and assistance of Fred Barnes, Dana Cheek, Melissa Tawzer, Gina Vickers, Christie Carr, Gerry Grimsley, Jo Ann Skeen, Tommy Olive, Lewis Carter Jr., and the support of Farmers Fertilizer and Milling, LMC Manufacturing, and the Georgia Peanut Comm.

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Use of Seed Protein Profiles to Characterize Peanut Cultivars[1]

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ABSTRACT

In the last 10 to 15 yr, the development of biotechnology and molecular techniques has allowed great advancements toward the identification of cultivars among plant species. In legumes, the success of cultivar identification depends on the species under investigation, the type and variability of genetic material found in cultivars, and the technology used for investigations. In this study, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to assess diversity of peanut (*Arachis hypogaea* L.) seed protein profiles. The objectives of this investigation were a) to assess diversity of protein profiles in peanuts for cultivar identification using SDS-PAGE and b) to determine the extent of variability of seed storage proteins (SSP) among samples of cultivars originating from different locations. The first study included 34 cultivars grown at Lewiston, NC and the second one included nine cultivars grown at six locations. The results of both studies indicated that it is possible to differentiate between subspecies but not to associate a particular profile with only one specific cultivar. Within subspecies, cultivars clustered in more than one group and most cultivars that grouped together were genetically related.

Key Words: *Arachis hypogaea*, peanut, cultivar, seed storage proteins, location effects.

Identification of cultivars among plant species is becoming increasingly important and developing reliable technologies for analysis is imperative. Correct cultivar identification benefits the farmer, who would thus receive a product that provides the expected agronomic characteristics, and benefits seed companies by assuring plant variety protection. Thus, it is important for the seed industry to have techniques which allow quick and reliable identification of cultivars without the necessity of long-term field experiments needed for morphological characterization. Several techniques involving the characterization of enzymes, total seed proteins, or DNA (e.g., by RFLP analysis or by RAPDs) have been successful in the cultivar identification of grain crops such as wheat (*Triticum aestivum* L.) (Jones *et al.*, 1982), perennial ryegrass (*Lolium perenne* L.) (Ferguson and Grabe, 1986), and maize (*Zea mays* L.) (Lee *et al.*, 1989; McDonald, 1991; Smith and Smith, 1991).

The technique of protein electrophoresis has been used in legumes to study seed variation in species and cultivars [e.g., profiles obtained for cultivars of *Phaseolus vulgaris* L. (Adriaanse *et al.*, 1969), subspecies of *Pisum* (Przybylska *et al.*, 1977), as well as wild species of *Arachis* (Klozova *et al.*, 1983)]. Although the diversity of sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) seed storage protein (SSP) profiles among peanut cultivars is much less than among wild peanut species (Bianchi-Hall *et al.*, 1990; Singh *et al.*, 1991), seed proteins may serve as a useful tool for germplasm identification. Shonkraii *et al.* (1985) reported a seed protein subunit in peanuts that correlates with blanchability, indicating the possibility of using seed proteins as molecular markers.

Attempts have been made to determine relationships of electrophoretic banding patterns in peanuts with nutritional characteristics (Cherry, 1975; Cherry *et al.*, 1971;

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Basha *et al.*, 1976). One of the earliest reports on seed proteins in peanuts included 16 cultivars grown in five environments (Cherry *et al.*, 1971). They concluded that a) great intravarietal variation was present in samples of cultivar VA 56R grown in Louisiana, and b) although the protein variation within and between different cultivars made it difficult to clearly distinguish genotypes, some major qualitative and quantitative protein banding differences distinguished several peanut types grown in Virginia, Georgia, Louisiana, and Texas from the ones grown in Oklahoma. The authors attributed the differences observed to variable expression of a structural gene for large molecular weight proteins caused by a drop in temperatures in the latter part of the growing season in Oklahoma. Before our investigations, little was known regarding the use of protein profiles for species and cultivar identification in peanuts, or about the variability of protein profiles of a single cultivar grown at different locations.

In general, the composition of seed proteins is stable (Boulter, 1981) and affected only slightly by environmental conditions or seasonal fluctuations (Lee and Ronalds, 1967; Adriaanse *et al.*, 1969). Although information is abundant about protein composition and quality in legume seeds, there is very little information about the effect of environment on proteins except for severe stress conditions. Young and Schadel (1984) found drought to cause cotyledon tissue damage and off-flavors in processed peanuts. Sulfur starvation of *Lupinus angustifolius* L. produced a lack of sulfur-containing amino acids (methionine and cysteine) (Blagrove *et al.*, 1976; Gillespie *et al.*, 1978).

The objectives of this study were to assess diversity of protein profiles in peanuts for cultivar identification using SDS-PAGE technique and to determine the extent of variability within cultivars originating from different locations.

Materials and Methods

Two related studies were conducted to characterize electrophoretic profiles of seed storage proteins (SSP) in peanut cultivars using SDS-PAGE. Protein extraction and electrophoretic procedures were the same as reported by Bianchi-Hall *et al.* (1993). For each seed lot, there were two extractions of raw proteins and at least two electrophoretic runs of each sample.

Three g (+0.01 g) of whole seed were ground in 75 mL of borate buffer (0.050 M $Na_2B_4O_7 \cdot 10\ H_2O$ + 0.025 M $Na_2S_2O_5$ + 0.010 M L-cysteine, pH 8). After grinding, 18 mL of SDS (100 g L^{-1}) and 12 mL 2-mercaptoethanol were added to the extractions. Samples were stirred for 1 hr at room temperature. The supernatant was collected and centrifuged at 30,000xg for 30 min at room temperature. One mL was transferred to microtubes and stored at -40 C. No heat was applied to the samples other than that produced in the centrifuge. The protein supernatant was defrosted at ambient temperature and centrifuged for 3 min at 13,500xg in a Fisher microcentrifuge, Model 235A. Electrophoresis samples were prepared with 45 µL of raw extraction sample, 45 µL of tricine buffer (0.100 M, pH 7.8) + sucrose (100 g L^{-1}), 30 µL of 2-mercaptoethanol (2-ME), 20 µL of SDS (100 g L^{-1}), and 2 µL of bromophenol blue front marker dye (0.5%), and then mixed by vortex. The use of a one-step extraction procedure, using only SDS detergent and 2-ME in the extraction buffer, represents a departure from the traditional defatting/multi-step purification procedures. Preliminary experiments using peanut and soybean dry seed, comparing various seed extraction procedures with a one-step extraction, containing high levels of SDS and 2-ME or just high levels of SDS, indicated no observable differences in electrophoresable proteins between identical samples passed through each extraction, even with or without heating of the electrophoresis samples for up to 1 hr at 100 C (R. D. Keys, S. A. Modena, J. L. Dowty, R. Z. Baalbaki, and C. M. Bianchi-Hall, unpubl. data). Hence, the simple one-step extraction was used.

SDS-polyacrylamide vertical slab gels with a gradient of 12 to 21% (0.75-mm thickness) were used for electrophoresis. Gels were cast following the procedures indicated by Dowty (1987). Standard molecular weight mixture SDS-L70 from Sigma was used as the reference to estimate sample molecular weights. Ten µL of electrophoresis samples were loaded per well. The average concentration of proteins per well was 2.8 µg μL^{-1} (with a standard deviation of 1.45). The concentration of protein was determined by the Bradford assay, and by the Bio-Rad D-C protein assay in samples without 2ME because, during the course of this work, we observed a problem of strong background interference in samples containing 2ME. Twelve-cm length gels were run for 7 hr at 1.25-W power; 18-cm gels were run for 16 hr at 1.10-1.25 W.

A modification (Dowty, 1987) of the technique proposed by Stephano *et al.* (1986) was used for staining. Gels were rinsed in a prestaining solution of 44% methanol, 44% water, and 12% acetic acid three times with "rinse intervals" of at least 5 min. After the third rinse, the gels were immersed in four parts of a solution of 0.1 M Na picrate (pH 7.0-8.0) and soaked for 5 min. One part of a 0.2% solution of Coomassie blue was then added and the gels were left overnight in the staining solution. Successive rinses with a 10% acetic acid solution were performed for destaining until clarification of the background was achieved. The gels were photographed and densitometrically scanned. The R_f and the approximate MW were used to determine the position of the protein bands. The approximate MW of the bands was calculated from the photographs with the aid of standard curves calculated for individual gels. Regions of the protein patterns were named following the nomenclature in Krishna and Mitra (1987, 1988). The regions of greater concentration of arachin subunits are the acidic (38-49.9 kD) and basic (18-22.9 kD) arachins. Two other regions of lower protein concentrations were named intermediate (23-37.9 kD) and low molecular weight proteins (14-17.9 kD) (Bianchi-Hall *et al.*, 1993).

The first study was planned to determine if the variability of protein profiles observed previously in some wild and cultivated peanuts was present in a larger number of cultivars. To avoid effects of locations, all samples were obtained from plants grown at the Lewiston Research Station, NC and harvested in 1990. Seeds were harvested at full maturity per normal production practices. After collection, seeds were stored in paper bags in sealed 1-L glass canning jars at 10 C. Seeds were extracted and analyzed within 6 mo of collection and storage. The cultivars analyzed were Avoca 11, Early Bunch, Florigiant, GK 3, Japan Jumbo, Jenkins Jumbo, Keel 29, Sambyquarae, NC-Fla 14, NC 2, NC 4, NC 5, NC 6, NC 7, NC 8C, NC 9, NC 10C, NC 17, Robut 33-1, VA 56R, VA 81 Bunch (*A. hypogaea* subsp. *hypogaea*); and Argentine, Chico, Comet, J-11, Pearl, Pronto, Spanhoma, Spantex, Starr, Tamnut, Tifspan, TMV 2, and New Mexico Valencia C (*A. hypogaea* subsp. *fastigiata*). Their pedigrees and market class are listed in Table 1.

The second study was planned to evaluate the effect of locations on seed storage proteins (SSP). Two-replicate samples of nine cultivars grown at different locations were analyzed—Florigiant, Florunner, NC 7, NC 9, NC-V 11 (*A. hypogaea* subsp. *hypogaea*); and Pronto, Starr, Spanco, and MARC 1 (*A. hypogaea* subsp. *fastigiata*). Seeds were obtained from the 1991 Flavor Quality Research Test and originated as follows: Florunner (in Tifton, GA; Suffolk, VA; Lewiston, NC; Marianna, FL; Bryan, TX; Stephenville, TX); Florigiant, NC 7, and NC-V 11 (in Georgia; Virginia; North Carolina; Florida; and Bryan, TX); MARC 1 (in Georgia; Virginia; Florida; and Bryan, TX); NC 9 (in Georgia, Virginia, North Carolina, and Florida); and Pronto, Starr, and Spanco (in Georgia; Virginia; and Stephenville, TX). Due to the few seeds available, the extraction of the raw sample of seed storage proteins (SSP) in this study was performed on 1 g of seed, keeping the ratio 1 g seed : 25 mL extraction buffer.

Results

For the first study, no differences were found between the two repetitions of each sample for the bands corresponding to the acidic or basic arachins. For the cultivars within the virginia market class, two distinct groups were found based on the variability of the protein profiles. Cultivars in Group I (Fig. 1A) have three main protein bands in the 38.0-49.9-kD region (acidic arachin) and one main band in the 22-kD region (basic arachins). Cultivars

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Table 1. Market type and pedigree of the peanut cultivars included in the three studies and some relevant parental lines (after Isleib and Wynne, 1992).

Cultivar	Pedigree	Study
Virginia Market Class		
Basse[a]	From Gambia; parent of GA 207-2 and GA 207-3	—
Avoca 11	Selection from NC 2	I
Early Bunch[b]	Virginia Station Jumbo /4/ F385-1-7-4, Pearl (F228) // F68 74 S_3-1-2, McSpan (F13, Small White Spanish) / Virginia Jumbo Runner (14), F249-42-3-1 /3/ Jenkins Jumbo, F406A /5/ F420, F231-51 (Dixie Runner sib) / F392-12-1-7 (Florigiant sib)	I
Florigiant[b]	Jenkins Jumbo // F230, Dixie Giant / Small White Spanish /3/ F334, Basse/Spanish 18-38, GA 207-3//F230-118-2-2 (same as F230)	I, II
GK 3	Florida Small Spanish / Dixie Giant, F231-51 /4/ F385-1-7-2, McSpan (F13, Small White Spanish) / Virginia Jumbo Runner (F14), F249-42-3-1 /3/ Jenkins Jumbo, F416 /5/ F392 (Florigiant sib)	I
Japan Jumbo	Plant introduction	I
Jenkins Jumbo	Selection from stocks obtained from R. B. Jenkins of Sumner, GA	I
Keel 29	Selection from Florigiant	I
Namboquarae	Plant introduction from Amazonia, Brazil	I
NC-Fla 14	Jenkins Jumbo / F334A-3-5-5-1 (Florispan derivative)	I
NC-V 11	Florigiant / NC 5 // Florigiant / PI 337396 (var. *fastigiata*)	I
NC 2	Basse / Spanish 18-38, GA 2076-2 // White's Runner	II
NC 4	Selection #4 from NC farmers cultivars, 1929, deemed typical Virginia Bunch	I
NC 5	NC 1 // C12, PI 121067 / NC Bunch	I
NC 6	NC Bunch / PI 121067, C12 // C37 (same as C12), GP-NC 343 (selection from NC Ac 4508) // VA 61R; resistant to SCR	I
NC 7	NC 5 // F393, F334-3-5-5-1 (Florispan derivative) / Jenkins Jumbo	I
NC 8C	NC 2 // A48, NC 4 / Spanish 2B, NC Ac 3913 /3/ Florigiant	I, II
NC 9	NC 2 / Florigiant	I
NC 10C	NC 8C / Florigiant	I, II
NC 17	Selection from F393, F334-3-5-5-1 (Florispan derivative) / Jenkins Jumbo	I
Robut-33-1	Introduction from India (likely virginia-spanish cross)	I
VA 56R	Selection from Atkins Runner	I
VA 81 Bunch	F392-8 (Florigiant sib) /3/ GA 119-20, Southeastern Runner / Dixie Giant, 210-4 // Virginia Runner	I
Virginia Runner Market Class		
Florunner[b]	Early Runner / Florispan	II
MARC I	Early Runner / Florispan, F439-17-2-1-1 (Early Bunch component)	II
Spanish Market Class		
Argentine	Selection from PI 121070 (var. *vulgaris*) from Chajari, Entre Rios, Argentina	I
Chico	PI 268661, line No. 370 from Krasnodar Territory, USSR, obtained in 1960 from Rhodesia	I
Comet	Selection from Starr	I
Florispan Runner[a]	Basse / Spanish 18-38, GA 207-3 // Dixie Giant / Small White Spanish	—
J-11	Introduction from India	I
Pearl	—	I
Pronto	Chico / Comet	I, II
Spanco	Chico / Comet	II
Spanhoma	Selection from Argentine	I
Spanish 18-38[a]	Selection from farmers' spanish stocks	—
Spantex	Selection from farmers' spanish stocks	I
Starr	Spantex / PI 161317 (var. *vulgaris* obtained in 1947 from Salto, Uruguay	I, II
Tammut (Tamnut 74)	Starr // TPL 647-2-5, Spantex / *Arachis monticola* Krapov. and Rigoni	I
Tifspan	Argentine (PI 121007-1) / Spanish 18-38	I
TMV 2	Introduction from India	I
Valencia Market Class		
NM Valencia C	Selection from PI 355987, irradiated Colorado Manfredi	I

[a] Parental lines not included in any of the studies.
[b] Multiline cultivars.

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in this group were Avoca 11, Florigiant, Keel 29, NC 2, NC 9, NC 10C, Robut 33-1, and VA 81 Bunch. The cultivars in Group II (Fig. 1B) were characterized by four main protein

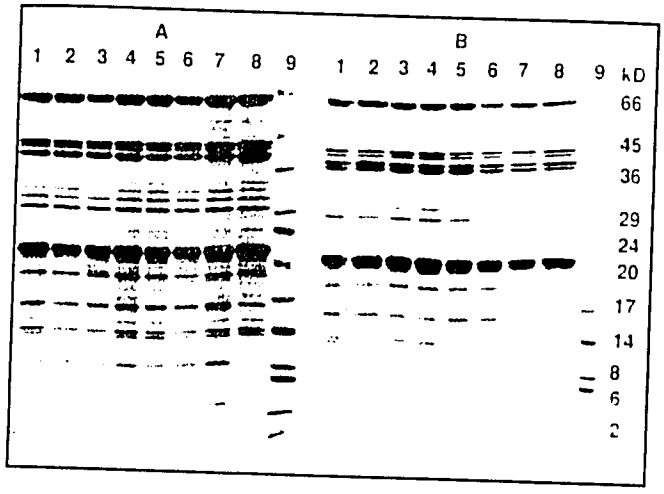

Fig. 1. SDS-PAGE protein profiles of peanut cultivars (*A. hypogaea* subsp. *hypogaea*) within the virginia market class. A. Group I: 1, Avoca 11; 2, NC 2; 3, NC 9; 4, Florigiant; 5, NC 10C; 6, Keel 29; 7, VA 81 Bunch; 8, Robut 33-1; and 9, molecular weight standards. B. Group II: 1, NC 7C; 2, NC 4; 3, NC 5; 4, NC 6; 5, NC 7; 6, Early Bunch; 7, VA 56R; 8, Nambyquarae; and 9, molecular weight standards.

bands in the 38.0-39.9-kD region and two bands in the area of 22 kD. Cultivars in this group were Early Bunch, Nambyquarae, NC 4, NC 5, NC 6, NC 7, NC 8C, and VA 56R. Also, the two spanish-type cultivars J-11 and TMV-2 had protein profiles to place them in this group (data not shown).

The SDS-PAGE protein profiles of most cultivars within the spanish market class were distinct from those in the virginia market class. Except for J-11 and TMV 2 (not shown), all spanish cultivars analyzed had four bands in the area of 38.0-49.9 kD (acidic arachins) and two bands in the area of 22 kD, but the intensity of the bands and the relative profile of the bands in each region, as shown in the electrophaerograms, were different for each group. Three groups were distinguished for spanish cultivars (correlative numbers were used for all groups). Group III was represented by Chico and Comet (Fig. 2). In this group, the two bands in the 22-kD region had both the same intensity and peak shape in the electrophaerograms. Group IV had a profile of acidic arachins which was slightly different from the ones in Group III. Also, the two bands in the 22-kD region had different intensities, and in the electrophaerograms the peak for the first band (22 kD) always contained relatively slightly less protein than the companion peak (21 kD) (see Fig. 5 for Pronto and Starr profiles, as examples). Cultivars in this group included Japan Jumbo, Jenkins Jumbo, Starr, Spanhoma, Tamnut (see Fig. 2) and Argentina, GK 3, NC-Fla 14, NC 17 and Pronto (data not shown).

Group V was separated from Group IV because of the different profiles of the bands in the intermediate region (23-38 kD) and in the intensity of the 17-kD band. The cultivars of Group V included New Mexico Valencia C, Pearl, Spantex, and Tifspan (Fig. 3).

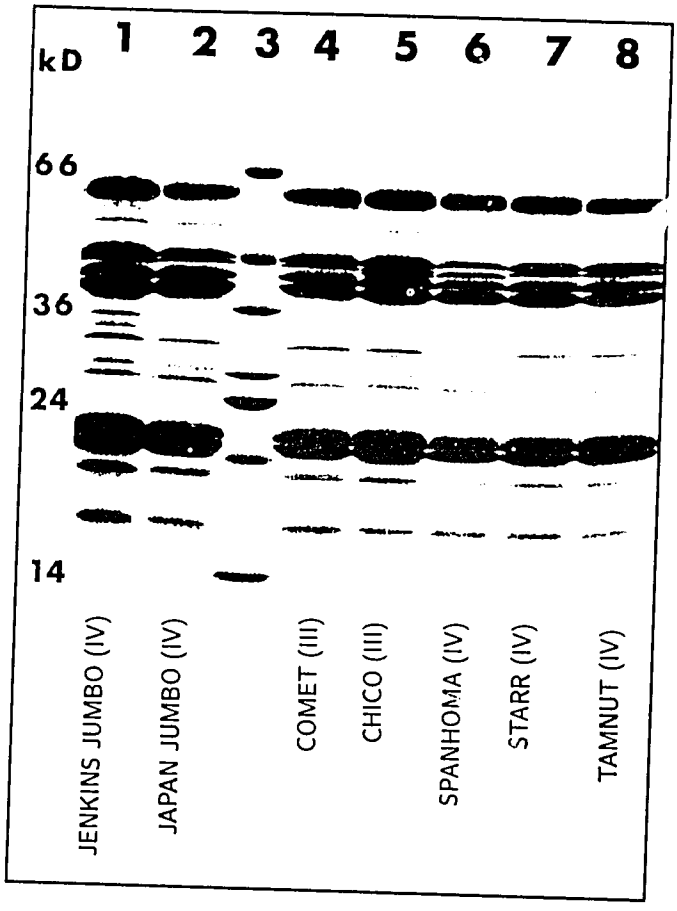

Fig. 2. SDS-PAGE protein profiles of peanut cultivars in Groups III (lanes 4 and 5) and Group IV (lanes 1, 2, and 6-8). Lane 3 represents molecular weight standards.

An attempt was made to further separate the profiles within each of the five groups to the cultivar level. The distribution of protein profiles of the intermediate molecular weight proteins (the area of the profiles that showed the greatest diversity in cultivated peanuts) was studied in detail. Although an attempt was made to obtain numerical data, the calculation of mathematical indexes with the purpose of classification was unreliable.

The second study was performed to characterize the consistency of protein profiles observed for a single cultivar grown at different locations. Sample repetitions did not show differences except for cultivars NC 9 and Florunner from Georgia (data not shown). For all other entries, including pure lines (NC-V 11, NC 7, NC 9, MARC 1, Pronto, Spanco, and Starr) (see Fig. 4) and the multilines Florigiant and Florunner, no differences were observed within cultivars grown at different locations. The variability among cultivars was observed again as in the previous study. Cultivars within the virginia market class were clearly differentiated from those of the spanish market class, and more than one type of protein profile was found for each market class (Fig. 5). NC 9 from the Virginia location did not separate as clearly in three acidic arachins as did the samples from the other locations; and NC 9 from Georgia presented a shoulder in the 22-kD band, as opposed to a sharp peak (band) in the samples from the other locations. The two repetitions (subsamples from the same seed lot) of

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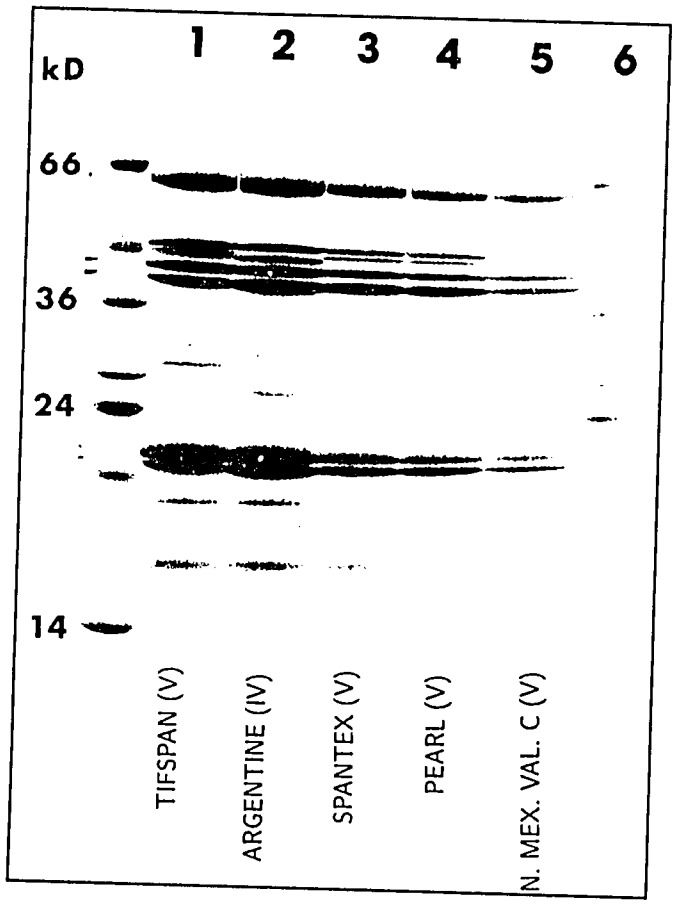

Fig. 3. SDS-PAGE protein profiles of peanut cultivars in Group IV (lane 2) and Group V (lanes 1 and 3-5). Lane 6 represents molecular weight standards.

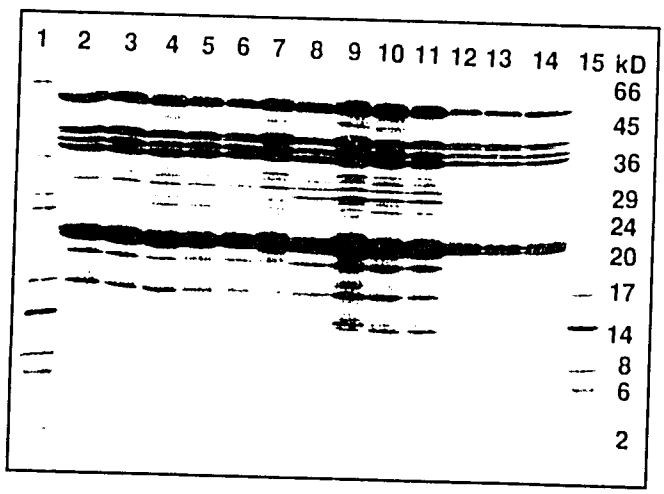

Fig. 4. SDS-PAGE protein profiles of four peanut cultivars (*A. hypogaea* subsp. *hypogaea*) grown in different locations (lanes 1 through 15): 1, molecular weight standards; 2-4, Pronto from Georgia, Virginia, and Texas; 5-7, Starr from Georgia, Virginia, and Texas; 8-11, Marc I from Georgia, Virginia, Florida, and Texas; 12-14, Spanco from Georgia, Virginia, and Texas; 15, molecular weight standards.

cultivar Florunner from Georgia had a different profile, with one being the same as those found for the samples of other locations. The electrophaerograms of Florunner samples from Bryan, TX presented two shoulders in the 22-kD band instead of a single shoulder as found for seeds from other locations. Patterns for the intermediate molecular weight proteins were consistent for all samples of cultivar Florunner, except the "odd" sample from Georgia.

One of the cultivars not included in the previous studies, MARC I, had a unique protein profile which was consistent throughout locations. Distinguishing bands for this cultivar were observed in the intermediate molecular weight region and in the area of 22 kD (Fig. 5). The unique characteristics of the protein profile of MARC I allowed for its separation from all other cultivars.

Discussion

Seed storage protein (SSP) profiles have been used in several cases to identify plant cultivars. Although this technique has been successful in *P. vulgaris* (Adriaanse *et al.*, 1969), *L. perenne* (Ferguson and Grabe, 1986) and *G. max* (Dowty, 1987), difficulties were encountered in its application to peanuts. Our results show that peanut cultivars may be differentiated by means of seed storage

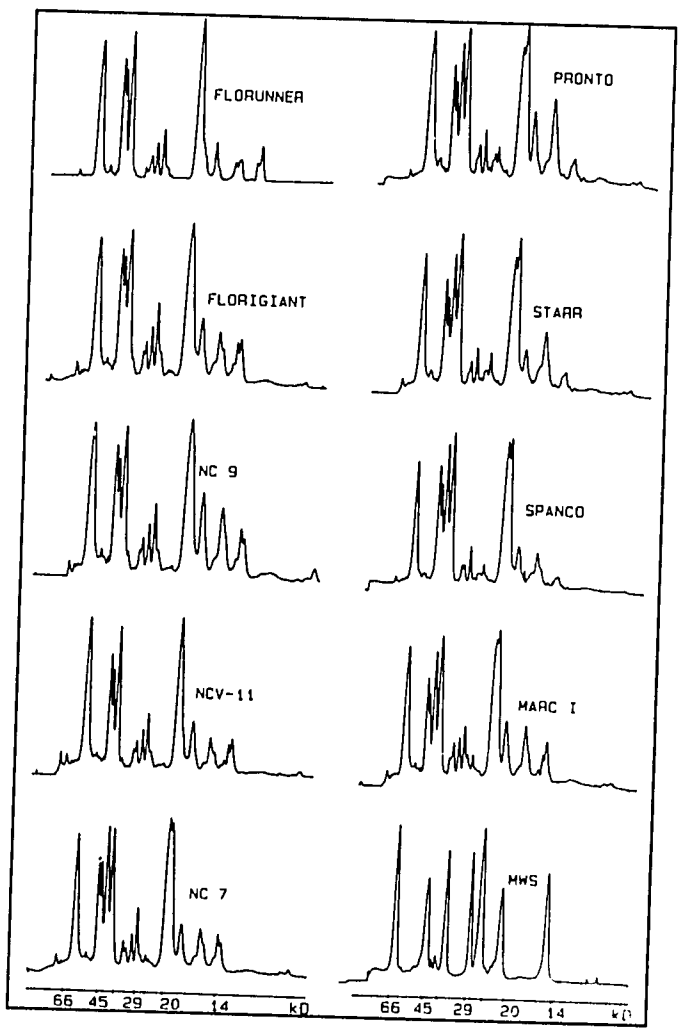

Fig. 5. Electrophaerograms of the nine peanut cultivars (*Arachis hypogaea*) included in Study II. *Arachis hypogaea* subsp. *hypogaea*: Florunner, Florigiant, NC 9, NC-V 11, NC 7, and MARC 1. *Arachis hypogaea* subsp. *fastigiata*: Pronto, Starr, and Spanco.

proteins (SSP) profiles at the subspecies level using major differences that appear in the areas of the acidic and basic arachins. However, to differentiate among cultivars within a subspecies, closer characterization of protein bands located in the intermediate (23 to 37.9 kD) region is necessary. At this level, several differences were observed among cultivars belonging to the same subspecies. The use of lower molecular weight standards (2 to 21 kD) did not help characterize bands appearing below the 14.2-kD band.

The uniformity of protein profiles within the virginia market class (subsp. *hypogaea*) and spanish (subsp. *fastigiata* var. *vulgaris*) market classes is believed to result from the low number of lines found in the pedigrees of superior cultivars (Figs. 6 and 7). Similar trends in genetic uniformity for isozymes were also observed by Cherry and Ory (1973). Of six enzymes they examined in peanuts, only one (esterase) distinguished spanish and virginia types and the variation did not allow the differentiation of cultivars. Grieshammer and Wynne (1990) reported 25 enzyme systems in 61 U.S. peanut cultivars, one breeding line, and six exotic peanut introductions. Only three enzymes—glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), and phosphohexose isomerase (PHI)—were consistently polymorphic. Each of the three enzymes displayed only two different banding patterns and most conformed to botanical types. They concluded that the apparent lack of variability seems to restrict the applicability of isozymes as genetic markers in the cultivated peanut. Shonkraii *et al.* (1985) referred to a 36-kD polypeptide related to blanchability in peanuts. It is probable that the same polypeptide is identified as the 38-kD band in our studies.

The first study in this investigation included the largest amount of cultivars and thus many different protein profiles were expected to be observed. It is interesting that most cultivars which associated in groups also had very close family relations. In Group I (Fig. 6), Avoca 11 is a selection form NC 2 which is also a parent of NC 9. Keel 29 is a

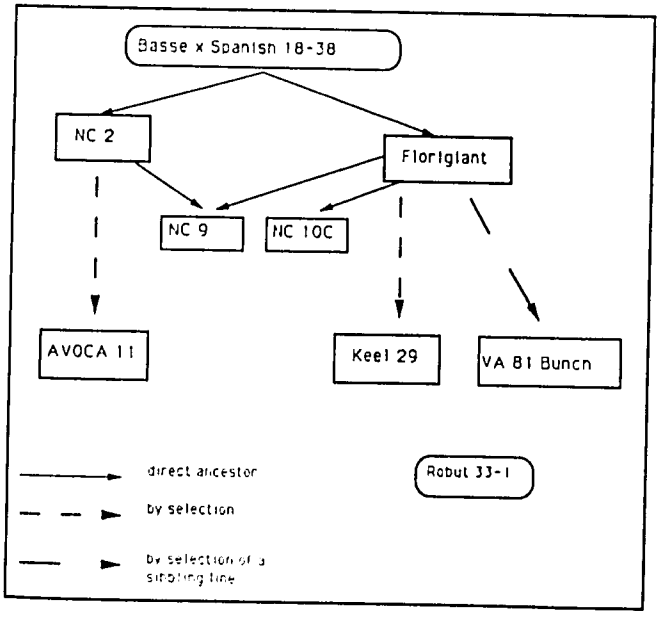

Fig. 6. Germplasm relationship of *A. hypogaea* cultivars presenting a seed sotrage protein profile corresponding to Group I.

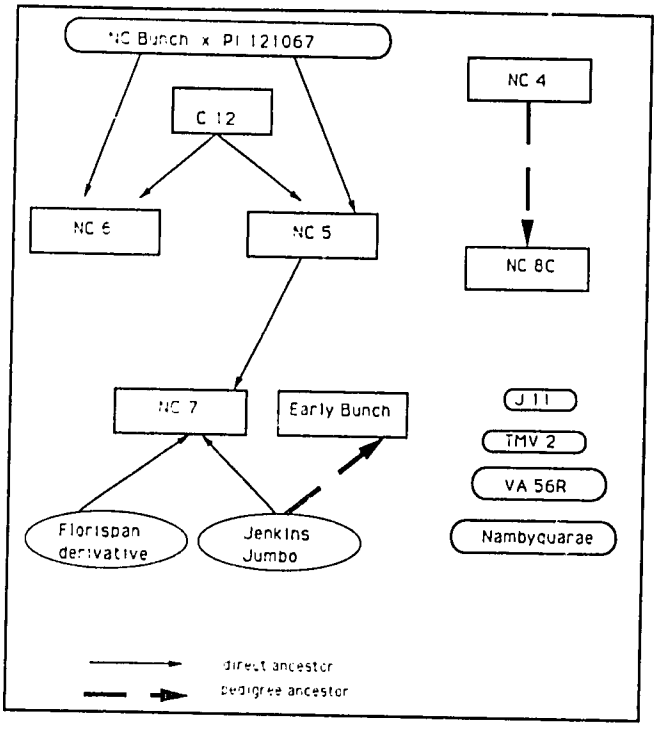

Fig. 7. Germplasm relationship of *A. hypogaea* cultivars presenting a seed storage protein profile corresponding to Group II.

selection from Florigiant which is also a parent of NC 9 and NC 10C. One of the parents of VA 81 Bunch was a sibling line of Florigiant. Both NC 2 and Florigiant have in their pedigrees GA 207, a cross between Basse and Spanish 18-38. Basse and Spanish 18-38 are represented in 75% of the runner cultivars released in the USA and are among the most frequently used germplasm sources in virginia-type cultivars (Knauft and Gorbet, 1989). The only other cultivar that fell into Group I was Robut 33-1, for which pedigree information is unavailable.

Group II (Fig. 7) included cultivars NC 5 and NC 6 which have common ancestors NC Bunch, PI 121067, and C 12. NC 7 has NC 5 in its pedigree. Cultivars NC 4, a selection form North Carolina farmer's field in 1929 (typical Virginia Bunch), and NC 8C, which has NC 4 as an ancestor, are also in this group. Other cultivars included Early Bunch, which shares the ancestor Jenkins Jumbo with NC 7; VA 56R, a selection from Atkins Runner; Nambyquarae; and TMV 2 and J-11.

Cultivars in Groups III, IV, and V belong mostly to the spanish market class; and there are some similarities between their respective pedigrees. Although apparently not genetically related, cultivars Chico, a selection from the USSR, and Comet, a selection from Starr, had very similar profiles and both fell in Group III (Fig. 2). In Group IV, Spantex is related to Starr (Spantex x PI 161317) and Tamnut. Pronto originated from the cross of Comet x Chico. Argentine, a selection from PI 121070, is in the pedigree of Tifspan and Spanhoma. In addition to the spanish-type cultivars, four virginia market class cultivars were found in Group IV, including Jenkins Jumbo as a cultivar and ancestor to NC-Fla 14, NC 17, and GK 3. GK 3 is a multiline, which also has Pearl (another cultivar in this

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group) in its pedigree. Other cultivars with similar profiles were New Mexico Valencia C and Japan Jumbo. Although there remains a strong degree of relatedness among the peanut cultivars in the U.S. their genetic base has been widened considerably since 1976 (Knauft and Gorbet, 1989).

The analysis of the protein profiles of peanut cultivars included in the second study showed consistency of results across locations for both pure lines and multilines. We previously found that multiline cultivars Florigiant and Florunner presented variability across locations (Bianchi-Hall *et al.*, 1991). The different protein profiles observed in multiline cultivars are probably due to segregation resulting from natural selection effects at the different locations. Both cultivars were originally composed of equal proportions of four pure sister lines, tracing to two separate F_2 plants with similar phenotypic characteristics and released as multilines because of their yield stability and market acceptability (Norden *et al.*, 1986). They share closer genetic backgrounds to each other (coancestry r = 0.44) than to NC 7 (r Florunner, NC 7 = 0.13; r Florigiant, NC 7 = 0.22) (Knauft and Gorbet, 1989). The natural selection imposed on the component lines in different environments is believed to account for the variation in the protein profiles observed in Florigiant and Florunner. Samples of other cultivars were highly consistent across environments even though the seed storage proteins (SSP) profile for Pronto in the second study from Georgia, Texas, and Virginia were different from the earlier one observed for samples harvested in North Carolina as reported in the first study. Pronto has been maintained at North Carolina State University for more than 10 yr; and the growing conditions in the field plots could have presented an opportunity for outcrossing, and/or natural selection in an environment different from the one in which it was developed (T. G. Isleib, pers. commun., 1991). No seeds from Pronto originating in NC were included in the second study.

Extracting proteins from several seeds appeared to average single seed differences within a sample. This investigation indicated electrophoretic variants or biotypes of a single cultivar could be recognized and monitored. A similar situation exists for wheat biotypes for gliadin composition (Cooke, 1984). As long as variant electrophoretic patterns are recognized and catalogued, they present no serious problems to the use of protein profiles for cultivar identification.

SDS-PAGE is not an effective tool for separating individual peanut genotypes, but it is just one technique available for analyzing variation in peanuts. Other fingerprint techniques, such as isozymes or RFLPs (Grieshammer and Wynne, 1990; Kochert *et al.*, 1991), have not been any more useful for identifying peanut cultivars. The limited number of molecular markers, coupled with a large number of cultivars with similar pedigrees, will make positive identification of single cultivars very difficult in the future.

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A Note on Use of Seed Protein Markers for Identification of Aflatoxin Resistance in Peanut[1]

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ABSTRACT

Fungi in the genus *Aspergillus* produce aflatoxins which are a group of toxic secondary metabolites. Fungal invasion of peanut seed and subsequent aflatoxin production can occur before or during harvest as well as during storage. Because storage proteins comprise a large percentage of the peanut seed, this study attempted to associate protein markers with previously reported aflatoxin-resistant genotypes. Variation was observed among 24 genotypes for electrophoretic banding patterns, but it was not possible to correlate the presence or absence of specific bands with aflatoxin resistance.

Key Words: *Arachis hypogaea*, groundnut, *Aspergillus* spp., aflatoxins, seed storage proteins.

Fungi in the genus *Aspergillus* produce aflatoxins as secondary metabolites which are highly poisonous, teratogenic, and carcinogenic (Wogan and Pong, 1970; Heathcote and Hibbert, 1978). Among the most widely distributed *Aspergillus* species that infect oil seed and cereal crops are *A. flavus* Link ex Fries and *A. parasiticus* Speare. Different strains of these species produce varying types and levels of aflatoxins (Diener *et al.*, 1982).

The invasion of peanut by *Aspergillus* occurs any time during seed development, harvest, and storage. Suppression of toxin accumulation can be accomplished if plants have preharvest resistance to infection, dry seed resistance to pathogen invasion during storage, or if seeds inhibit toxin production when the pathogen is present. Variability among peanut cultivars for resistance to *Aspergillus* spp. has been reported by Mixon and Rogers (1973), Davidson *et al.* (1983), Mixon (1983a,b), Blankenship *et al.* (1985), Mehan *et al.* (1988), Azaizeh *et al.* (1989), Pettit *et al.* (1989), Vasudeva Rao *et al.* (1989), Szerszen and Pettit (1990), Utomo (1990), Holbrook *et al.* (1992), and Waliyar *et al.* (1994) (Table 1).

Infection by *Aspergillus* fungi and toxin production are processes that occur in the peanut seed. Screening genotypes for aflatoxin resistance in the field or greenhouse is time-consuming and expensive, and alternative approaches to identify resistant genotypes are needed. Because storage proteins comprise approximately 70% of the total nitrogen in peanut seeds, and many differences in electrophoretic profiles have been observed among *Arachis* species (Bianchi-Hall *et al.*, 1992), it may be possible to identify protein markers in resistant peanut genotypes. The objective of this study was to associate seed storage protein markers in peanut with aflatoxin resistance.

Table 1. Aflatoxin reaction reported in the literature for genotypes studied and 30-kD band observed.

Genotype	Seed source	*Asp.* spp.[a] reaction	Citation	30 kD band[b]
Ah 7223	NCSU	R	Mehan *et al.*, 1988	-
AR-1	USDA	R	Mixon, 1983b	-
AR-2	USDA	R	Mixon, 1983b	-
AR-3	USDA	R	Mixon, 1983b	-
AR-4	USDA	R	Mixon, 1983b	+
C55-437	NCSU	R	Mehan *et al.*, 1988	-
Faizpur	NCSU	R	Mehan *et al.*, 1988	-
GFA-1	NCSU	R	Mixon, 1983a	+
GFA-2	NCSU	R	Mixon, 1983a	+
J-11	NCSU	R	Mehan *et al.*, 1988; Szerszen & Pettit, 1990	-
Monir 240-30	NCSU	R	Mehan, 1989; Utomo, 1990	+
PI 337409	USDA	R	Szerszen & Pettit, 1990; Mixon & Rogers, 1973	-
PI 337394(F)	NCSU	R	Mehan *et al.*, 1988; Mixon & Rogers, 1973	-
Sunbelt Runner	NCSU	R	Davidson *et al.*, 1983	+
U4-47-7	NCSU	R	Mehan *et al.*, 1988	-
UF 71-513	NCSU	R	Mehan *et al.*, 1988	-
Var. 27	NCSU	R	Mehan *et al.*, 1988	-
Florunner	NCSU	S	Szerszen & Pettit, 1990; Mixon & Rogers, 1973; Mixon, 1983b	+
NC 7	NCSU	S	—	+
(NC 7 x AR-4) 89-03	NCSU	R	Utomo, 1990	+
(NC 7 x AR-4) 89-05	NCSU	R	Utomo, 1990	+
(NC 7 x AR-4) 89-08	NCSU	R	Utomo, 1990	+
(NC 7 x GFA-2) 89-59	NCSU	R	Utomo, 1990	-

[a]*Aspergillus* reaction rating: R = resistant, S = susceptible.
[b]+ = present, - = absent.

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