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Isozyme Variability in Mature Seeds of U. S. Peanut Cultivars and Collections[1]

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ABSTRACT

The mature seeds of 61 U. S. peanut (*Arachis hypogaea* L.) cultivars, one breeding and six exotic peanut lines representing three botanical types were surveyed for 25 enzyme systems using horizontal starch gel electrophoresis. The genotypes assayed showed no variation for most of the enzymes. For catalase and malate dehydrogenase, variability was present but not reproducibly within genotypes. Only three enzymes—glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), and phosphohexose isomerase (PHI)—were consistently polymorphic. Each of the three enzymes displayed two different banding patterns. With three exceptions, the distribution of the zymograms for GOT and PHI reflected the taxonomic relatedness of spanish and valencia botanical type peanuts which are members of the subspecies *A. hypogaea* L. ssp. *fastigiata* Waldron when compared with virginia botanical varieties which belong to the subspecies *hypogaea*. IDH showed only one banding pattern for the spanish- and valencia-type peanuts (one exception), whereas the virginia-type cultivars varied for this enzyme reflecting the narrow genetic base of most spanish cultivars and the broader germplasm base used for the development of virginia cultivars. The limited amount of variability appears to restrict the applicability of isozymes as genetic markers in the cultivated peanut.

Key Words: Isozymes, *Arachis*, genetic diversity, polymorphism.

The cultivated peanut species, *Arachis hypogaea* L., has been subdivided into two subspecies, ssp. *hypogaea* and ssp. *fastigiata* Waldron, according to morphological and physiological characteristics (9). Krapovickas (7, 8) and Gregory *et al* (5) further classified the peanut relating its variability to six geographical regions in South America. The Bolivian region was recognized as the center of origin of the cultivated peanut and the remaining regions as secondary centers of diversity. The subspecies *hypogaea* was divided into

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the two botanical varieties var. hypogaea (virginia botanical type) and var. *hirsuta* and the subspecies *fastigiata* Waldron into var. *fastigiata* (valencia botanical type) and var. *vulgaris* (spanish botanical type) (16). The commercial peanut crop in the United States is composed of four market types (6). The virginia botanical variety of the subspecies *hypogaea* is marketed as two different types, virginia and runner market types, with the distinction being based on fruit and seed size. The two botanical varieties of the subspecies *fastigiata* Waldron (valencia and spanish) are marketed as valencia and spanish types, respectively (Fig. 1).

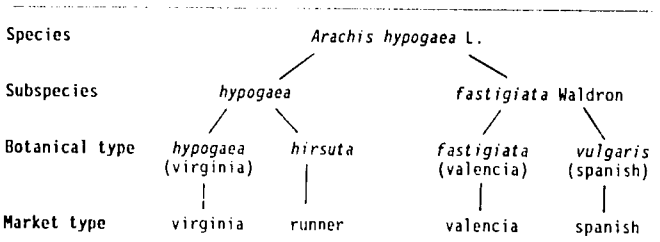

Fig. 1. Classification of intraspecific variation of *A. hypogaea*.

Information about the genetic relatedness among lines and cultivars is useful in choosing parents for the introgression of genes from wild species or for pure line cultivar development. Recently, Knauft and Gorbet (6) assessed the genetic diversity within the cultivated peanut using pedigree information. They pointed out that one of the assumptions made for the calculation of the coefficient of parentage—i.e., all lines used in crossing were homozygous and homogeneous—may have been particularly violated. Therefore, it would be useful to obtain additional estimates of genetic distance among peanut cultivars based on biochemical and morphological markers. Their use has the advantage that the assessment of genetic diversity can be extended to natural plant populations for which detailed pedigree data are not available. Cox *et al.* (4) compared coefficients of parentage (r) and similarity indices (s) based on 13 isozyme loci and seven morphological traits for combinations of 115 soybean cultivars and ancestral introductions and concluded that it is advisable to use a composite index including both r and s for the estimation of genetic relationships among cultivars because their biases cancel.

If sufficient isozyme polymorphism is present in peanut germplasm, it could not only contribute to diversity studies but also could be used for increasing the understanding of the genetic structure of the allotetraploid cultivated peanut. The detection of linkages of isozyme alleles to genes of agronomic importance could potentially facilitate the transfer of economically useful traits from one line to another. In this study 61 U.S. peanut cultivars, 1 breeding line, and 6 plant introductions, two each collected from centers of diversity for the three major botanical varieties were screened for isozyme polymorphisms using 25 enzyme systems.

Materials and Methods

The germplasm screened using horizontal starch gel electrophoresis for 25 different enzymes consisted of 61 U.S. peanut cultivars, 1 breeding line, and 6 plant introductions. The year of release, the releasing agency, and the source of the seeds used in this study are listed in Table 1.

Table 1. Peanut germplasm of three botanical varieties screened for isozyme polymorphism.

Genotype	C/L/E[a]	Year released	Releasing agency[b]	Breeding method[c]	Source for this study[d]
			Spanish botanical type		
GFA Spanish	C	1941('55)	GA	LS	C.H.
Spantex	C	1948	TX	LS	NC-75-F
Dixie Spanish	C	1950	GA	PI	NC-F
Argentine	C	1951	OK	PI	NC-L-87
Spanette	C	1959	GA	LS	C.H.
Starr	C	1961	TX	HY	NC-75-F
Spancross	C	1970	GA-ARS;OK	IH	C.H.
Tifspan	C	1970	GA-ARS;OK	HY	NC-76-F
Spanhoma	C	1970	OK;GA	LS	NC-75-F
Comet	C	1970	OK;GA	HY/LS	NC-L-87
Chico	C	197[illegible]	GA-USDA,VA,OK	(MS/LS)	T.C.
Tamnut 74	C	1974	TX;GA	IH	O.S.
Toalson	C	1979	TX	HY	O.S.
Pronto	C	1980	OK;USDA-GA	HY	NC-L-87
Spanco	C	1981	OK	HY	T.C.
PI 261924	E				NC-L-87
PI 262000	E				NC-L-87
			Valencia botanical type		
N.M. Valencia A	C	1971	NM	LS	D.H.
Valencia McRan	C	1973	Borden	PI	C.H.
N.M. Valencia C	C	1979	NM	HY(PI)	D.H.
Georgia Red	C	1986	GA;USDA-GA	HY	C.H.
Tennessee Red	C				NC-L-87
NM Valencia	C				NC-L-87
PI 275751	E				NC-L-87
NC 17090	E				NC-L-87
			Virginia botanical type, virginia market type		
VA Bunch 67	C	1945	GA	LS	C.H.
VA Bunch G2	C	1952	GA	LS	C.H.
NC 1	C	1952	NC	HY	NC-F
NC 2	C	1952	NC	HY	NC-75-F
GA 119-20	C	1954	GA	HY	C.H.
VA 56R	C	1956	VA	LS	NC-78-F
NC 4	C	1959	NC	MU	NC-R-87
Florigiant	C	1961	FL	HY	NC-R-87
VA 61R	C	1962	VA	LS	T.C.
NC 5	C	1964	NC	HY	NC-R-87
Shulamith	C	1968	Israel (GA)	HY	NC-75-F
NC 17	C	1969	NC	HY	NC-R-87
VA 72R	C	1971	VA	HY	NC-75-F
Altika	C	1972	FL-Guyana	HY	D.K.
NC-Fla 14	C	1973	NC-FL	HY	NC-R-87
NC 6	C	1976	NC	HY	NC-R-87
GK 3	C	1976	Gold Kist	HY	D.K.
Avoca-11	C	1976	Reynolds	HY/LS	NC-75-F
Early Bunch	C	1977	NC	HY	NC-F
NC 7	C	1978	NC		NC-R-87
VA 81 Bunch	C	1981	VA	HY	NC-78-F
K29	C	1981	Keel	HY/LS	NC-76-F
NC BC	C	1982	NC	HY	NC-R-87
NC 9	C	1985	NC		NC-R-87
NC 10C	C	1988	NC		NC-R-87
NC 18411	L				NC-L-87
PI 262090	E				NC-L-87
Coll. no. 486	E				NC-L-87
			Virginia botanical type, runner market type		
Dixie runner	C	1943	FL	HY	D.K.
S.E. Runner 56-15	C	1947	GA	LS	C.H.
Early Runner	C	1951	FL	HY	D.K.
VA Runner G26	C	1952	GA	LS	C.H.
Florispan Runner	C	1953	FL	HY	D.K.
Florunner	C	1969	FL	HY	NC-L-87
Goldin-I	C	1976	Wilco	HY	O.S.
Tifrun	C	1977	GA-USDA	HY	C.H.
Sunbelt Runner	C	1982	GA-USDA	HY	C.H.
Sunrunner	C	1982	FL	HY	NC-L-87
GK 7	C	1984	Gold Kist	HY	NC-L-87
Langley	C	1986	TX	HY	NC-L-87
Okrun	C	1986	OK	HY	NC-L-87
Southern Runner	C	1986	FL	HY	NC-L-87
Tamrun 88	C	1988	TX	HY	O.S.

[a]Cultivar/breeding line/exotic.

[b]State Agricultural Experiment Stations in FL (Florida), GA (Georgia), NC (North Carolina), NM (New Mexico), OK (Oklahoma), TX (Texas), VA (Virginia); ARS (Agricultural Research Service) and USDA (U.S. Department of Agriculture).

[c]HY = hybridization followed by selection to attain stable line(s), LS = line selection in a land race, MS = mass selection, MU = mutation breeding, PI = introduction with some MS or LS, IH = interspecific hybrid.

[d]T.C. = Terry Coffelt (Virginia); C.H. = Corley Holbrook (Georgia); D.H. = David C. H. Hsi (New Mexico); D.K. = David Knauft (Florida); O.S. = Olin D. Smith (Texas); NC-L-87 = grown in Lewiston, NC, 1987; NC-R-87 = grown in Rocky Mount, NC, 1987; and NC-75-F, NC-76-F, NC-78-F = grown in NC, 1975, 1976 and 1978, respectively, and stored frozen until October 1988.

The source material were embryos of mature seeds imbibed for 24 hrs. at room temperature. Embryos of mature seed were used as the source tissue since better resolution was obtained in a preliminary study for embryos and cotyledons than for flower and leaf tissues. For the extraction of the enzymes each embryo was macerated at 4 C in 80 µL of extraction buffer modified from Arulsekar and Parfitt (1) (pH was changed from 8.0 to 7.5 and 2-mercaptoethanol was omitted). The procedures of starch gel preparation and electrophoresis published by Stuber *et al.* (14) were employed. The staining solutions used for the detection of the isozymes are referenced in Table 2. The 25 enzymes surveyed in this study are listed also in Table 2. Staining for an additional 30 enzyme systems in a preliminary study led to either smeared bands or no reaction. Each genotype was assayed at least three times on different gels for each enzyme. To confirm the polymorphic isozymes, all genotypes were assayed for GOT and PHI seven times and for IDH 11 times on different gels. The assays for the genotypes varying for IDH were further replicated to confirm the inconsistency.

Results and Discussion

Most enzymes were found to be monomorphic for the genotypes monitored in this study (Table 2), two enzymes (catalase and malate dehydrogenase) gave inconsistent results within genotypes, and three enzymes were polymorphic—glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), and phosphohexose isomerase (PHI). Each of the polymorphic enzymes displayed two different banding patterns, the difference being the presence vs. the absence of either one (IDH) or two (GOT, PHI) bands (Fig. 2). GOT and PHI showed consistent banding patterns for all genotypes, except Goldin-I for PHI whereas IDH zymograms for seven of the 68 genotypes (NC 2, Florispan Runner, Sunbelt Runner, Okrun, Florunner, Goldin-I, and N.M. Valencia) were not homogeneous. For individual embryos of each of these seven genotypes, the polymorphic band was either present or absent, with absence of the band ranging from one-third to one-half depending on genotype.

The extent of isozyme polymorphism found among the U.S. peanut cultivars was considered not to be sufficient to calculate meaningful similarity indices for the assessment of genetic relatedness among lines. Therefore, it was not possible to study the statistical correlation between the coefficients of parentage obtained by Knauft and Gorbet (6) from pedigree information and a measure of genetic distance based on isozyme markers in order to assess the utility of the two different similarity measures for peanuts. But, the general trend of genetic variability within and among botanical and market types of the cultivated peanuts shown from pedigree data (6) was reflected by the polymorphic isozyme systems, GOT, IDH, and PHI. The two banding patterns of GOT and of PHI conformed to the botanical types with three

Table 2. Enzyme systems monitored.

Enzyme	EC number	Zymogram[a]	Staining[b] procedure reference
Acid phosphatase	3.1.3.2	M	14
Aconitase	4.2.1.3	M	14
Adenylate kinase	2.7.4.3	M	14
Alanine aminopeptidase		M	3
Alcohol dehydrogenase	1.1.1.1	M	14
Aldolase	4.1.2.13	M	15
Arginine aminopeptidase	3.4.11.6	M	14
Aromatic amino acid transaminase	2.6.1.5	M	11
Catalase	1.11.1.6	I	3
Diaphorase	1.6.2.2	M	14
Esterase	3.1.1.1	M	14
Galactose dehydrogenase	1.1.1.48	M	15
Glutamate dehydrogenase	1.4.1.3	M	14
Glutamate oxaloacetate transaminase	2.6.1.1	P	14
Glyceraldehyde 3-phosphate dehydrogenase	1.2.1.12	M	12
Glycerol 3-phosphate dehydrogenase	1.1.1.8	M	12
3-Hydroxybutyrate dehydrogenase	1.1.1.30	M	11
Isocitrate dehydrogenase	1.1.1.42	P	14
Leucine aminopeptidase	3.4.11.1	M	3
Malate dehydrogenase	1.1.1.37	I	14
Menadione reductase	1.6.99.2	M	3
Phosphoglucomutase	2.7.5.1	M	14
Phosphohexose isomerase	5.3.1.9	P	14
Shikimate dehydrogenase	1.1.1.25	M	14
Superoxide dismutase	1.15.1.1	M	--
Triosephosphate isomerase	5.3.1.1	M	14

[a]P = polymorphic, M = monomorphic and I = inconsistent for genotypes assayed.

[b]For more details, see Grieshammer, U. (1989), Isozymes in peanuts: Variability among U.S. cultivars and Mendelian and non-Mendelian inheritance, Master's thesis, N. C. State Univ., Raleigh.

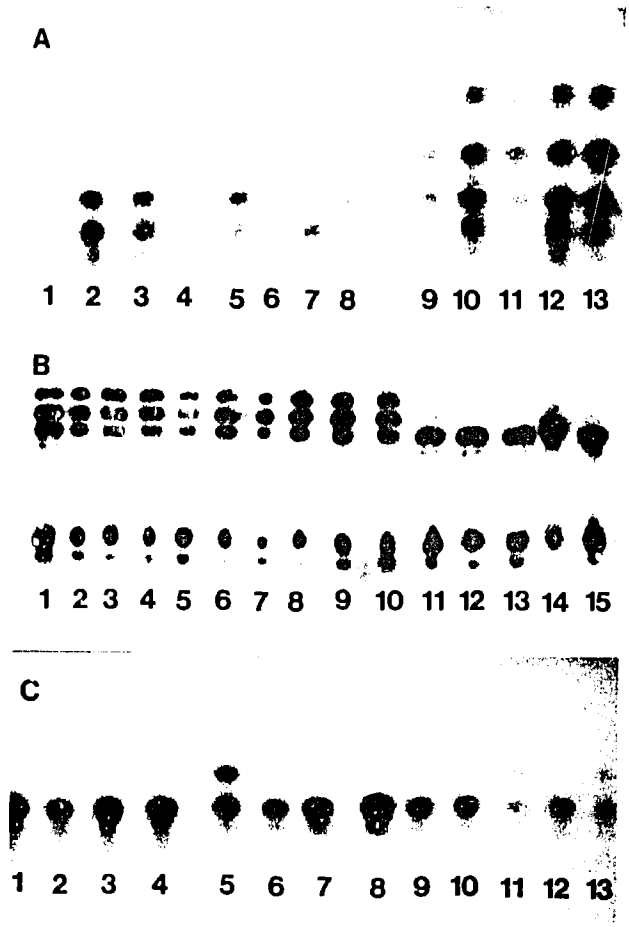

Figure 2. Zymograms of polymorphic enzymes.
A: GOT; lanes 1-4 = spanish botanical types (1 = Comet, 2 = Argentine, 3 = PI 261924, 4 = PI 262000); lanes 5-8 = valencia botanical types (5 = Tennessee Red, 6 = NM Valencia, 7 = PI 275751, 8 = NC 17090); lanes 9-13 = virginia botanical types (9 = NC 18411, 10 = NC 4, 11 = NC 9, 12 = PI 262090, 13 = Coll. no. 486).
B: PHI; lanes 1-5 = spanish botanical types (1 = Comet, 2 = Argentine, 3 and 4 = PI 261924, 5 = PI 262000); lanes 6-10 = valencia botanical types (6 = Tennessee Red, 7 = NM Valencia, 8 and 9 = PI 275751, 10 = NC 17090); lanes 11-15 = virginia botanical types (11 = NC 13411, 12 = NC 4, 13 = NC 9, 14 = PI 262090, 15 = Coll. no. 486).
C: IDH; lanes 1, 6, 7, 8 = spanish botanical types (1 - PI 261924, 6 = Comet, 7 = Argentine, 8 = PI 262000); lanes 2, 3, 9, 10 = valencia botanical types (2 = Tennessee Red, 3 = PI 275751, 9 = NM Valencia, 10 = NC 17090); lanes 4, 5, 11, 12, 13 = virginia botanical types (4 = NC 18411, 5 = PI 262090, 11 = NC 4, 12 = NC 9, 13 = Coll. no. 486).

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exceptions. The two polymorphic bands of PHI were present in spanish- and valencia-type peanuts and absent in virginia botanical types with the exception of the cultivar Southern Runner (virginia type with the two PHI bands present). Embryos of Goldin-I were not homogeneous for either banding pattern of PHI. The two polymorphic GOT bands were absent in spanish and valencia botanical types and present in virginia types except for the exotic line NC 17090 (valencia type with two GOT bands present). This distribution of banding patterns reflects the distinction between the two subspecies of the cultivated peanut *A hypogaea* L. (ssp. *fastigiata* Waldron contains the spanish and valencia botanical varieties and ssp. *hypogaea* contains the virginia-type peanuts) as well as the relationships derived from pedigree information. Knauft and Gorbet (6) had concluded from the coefficients of parentage they had calculated for 41 peanut cultivars that most spanish cultivars are unrelated to virginia cultivars released in the United States (they did not include valencia-type peanuts in their study).

The two banding patterns of IDH did not conform to the botanical types. Although the polymorphic band was always absent from the spanish and valencia genotypes with the exception of the inconsistent results of the cultivar N. M. Valencia, both banding patterns were well represented among the virginia botanical type cultivars and lines. The virginia market types contained seven one-band phenotypes, 20 two-band phenotypes, and one inconsistent phenotype (NC 2), and among the runner market types five one-band phenotypes, five two-band phenotypes, and five inconsistent phenotypes were found. This pattern of variability reflects the findings of Knauft and Gorbet (6) who reported that the pedigrees of the spanish type cultivars are less complex than those of virginia and runner cultivars. They found that Spancross and Tifspan are unrelated to the other spanish cultivars, while Starr and Comet, a selection from Starr, are both closely related to the remaining spanish cultivars included in their study. Virginia and runner market-type cultivars were developed by using a broader germplasm base.

The apparent lack of variability seems to restrict the applicability of isozymes as genetic markers in the cultivated peanut. A large amount of variability is present in widely used lines of corn (13), whereas cultivars of other plant species, in particular self-pollinated crop peanuts, have been found to be remarkably uniform with respect to isozyme markers (2, 10). Because the wild *Arachis* species show more isozyme variation (H. T. Stalker, personal comm.), applications utilizing unmapped genetic markers can still be readily adopted for peanut.

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