Mendelian and Non-Mendelian Inheritance of Three Isozymes in Peanut (*Arachis hypogaea L.*)[1]

Uta Grieshammer and Johnny C. Wynne*[2]

ABSTRACT

Because of the importance of peanut (*Arachis hypogaea* L.) as an oil, food, and feed source worldwide and the contributions of breeding and genetics to yield and quality improvement, it is desirable to understand the genetic structure of the plant. Isozymes have been used to gain an understanding of the genetic structure of several plant species. However, we found no literature on the inheritance of isozymes in peanut. The F_1 and F_2 seed of several crosses between cultivars and plant introduction lines of three botantical types of peanut were used to investigate the inheritance of three isozymes by horizontal starch gel electrophoresis: phosphohexose isomerase (PHI), isocitrate dehydrogenase (IDH), and glutamate oxaloacetate transaminase (GOT). Each of the three enzymes displayed two different banding patterns, the difference being the presence vs. the absence of either one (IDH) or two (PHI, GOT) bands. Chi-square analysis for goodness of fit of the observed F_2 segregation ratios to ratios expected from genetic models indicated that the polymorphisms for both PHI and IDH are controlled by single genes. Two loci, *Phi-1* and *Idh-1*, respectively, are proposed. Sixty-five of 71 F_1 progeny monitored for GOT showed the banding pattern of the male parent. The F_2 progeny segregated into the two parental types, but the ratios did not fit a simple genetic model. Possible explanations for the observed paternal inheritance of GOT include biparental transmission of plastids, prezygotic RNA synthesis and genomic imprinting.

Key Words: Electrophoresis, protein, PHI, IDH, GOT, groundnut, paternal inheritance.

Isozymes have found wide use as genetic markers for basic and applied research in several crop species. Many applications in plant breeding use unmapped markers, such as the estimation of outcrossing rates, the measurement of genetic variability, the introgression of genes from wild species, and the identification of haploid-derived plants from anther culture (26). Covering the entire genome with genetic markers increases the probability of finding linkages of markers with monogenes of economic interest and could provide insight into the organization of polygenic systems, thereby potentially facilitating the transfer of qualitative and quantitative traits between breeding lines. In a few crop species, such as maize (*Zea mays L.*) and tomato (*Lycopersicon esculentum L.*), extensive linkage maps based on isozyme and restriction fragment length polymorphisms are being developed (2, 9).

Because of the importance of peanut (*Arachis hypogaea* L.) as an oil, food, and feed source worldwide and the contributions of breeding and genetics to yield and quality improvement, it is desirable to understand the genetic structure of this plant species. A few isozyme systems have been established and used for the development of electrophoretic standard patterns of healthy peanuts to which zymograms from damaged, processed or mold-infected seeds might be compared for induced changes (18). Phylogenetic evaluations based on electrophoretic data of total seed proteins (11) and of six enzymes (3) have been conducted for the genus *Arachis*. Two enzymes, peroxidase (27) and lipoxygenase (19) have been subject to biochemical investigations. We analyzed 67 diverse peanut genotype for 25 enzyme systems (7). Only three enzymes—glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), and phophohexose transaminase (PHI)—were consistently polymorphic. We found no data in the literature on the inheritance of isozymes in peanut.

The objectives of this study were to determine the inheritance of these three polymorphic enzyme loci—phosphohexose isomerase, isocitrate dehydrogenase, and glutamate oxaloacetate transaminase for four peanut crosses.

Materials and Methods

Crosses including reciprocals between two cultivars and three plant introduction lines representing three different botanical types of peanut and differing in isozyme patterns were made for this study. NC 18411 and B_1 (PI 262090) parents of virginia type (ssp. *hypogaea* var. *hypogaea*) were chosen because of the presence of two slow bands for GOT (E.C. 2.6.1.1) and the absence of two slow bands for PHI (E.C. 5.3.1.9). The spanish-type (ssp. *fastigiata* var. *vulgaris*), Comet and C_1 (PI 261924) were used as parents because of absence of two slow bands for GOT and presence of two slow bands for PHI. The valencia type (ssp. *fastigiata* var. *fastigiata*) parents, Tennessee Red and A_1 (PI 275751) have the presence of the PHI bands and absence of the GOT bands. In addition, Tennessee Red does not have a single slow band for IDH (E.C. 1.1.1.41) that is present in the other parents. Seven F_1's from the cross between line NC 18411 and the cultivar Tennessee Red, 4 F_1's from the cross between NC 18411 and C_1, 5 F_1's from the cross between A_1 and NC 18411, and 3 F_1s from the cross between Tennessee Red and B_1 were selfed. The F_2 progeny seed of one or two F_1 plants were bulked into 4 (NC 18411 x Tennessee Red), 2 (NC 18411 x C_1), 3 (A_1 x NC 18411), and 2 (Tennessee Red x B_1) groups for analysis.

The embryonic axis and the cotyledon of each F_2 seed, and 25 remnant as well as 24 reciprocal F_1 seeds were assayed separately for PHI and GOT. Enzyme IDH was polymorphic for the cross Tennessee Red (female) x B_1 (male) only; therefore, only data from this cross were obtained for this enzyme. No B_1 x Tennessee Red F_1 seed was available. Additional F_1 seed from the following crosses were monitored for GOT: C_1 x B_1, cultivar Comet x NC 18411, B_1 x Comet, and NC 18411 x Comet.

Mature seeds were imbibed for 24 hours at room temperature prior to the extraction of the enzymes. Each embryonic axis and *ca.* 50 mg of a cotyledon per seed were macerated at 4C in 80 mL of the extraction buffer described by Arulsekar and Parfitt (1) (pH was changed from 8.0 to 7.5 and 2-mercaptoethanol was omitted). The proteins were separated by horizontal starch gel electrophoresis using the methods described by Stuber *et al.* (24). The staining solutions used for the detection of the isozymes were listed by Grieshammer and Wynne (7).

The frequencies of the different banding patterns for each of the F_2 seed groups were tested for goodness of fit to genetic hypotheses by means of chi-square analysis. Furthermore, the data for each cross were pooled and the pooled, sum, and heterogeneity chi-square were calculated.

Results and Discussion

Phosphohexose Isomerase

The enzyme PHI displayed two zones of activity for the genotypes analyzed in this study (Fig. 1a). The faster migrating

[1]Paper No. 12547 of the Journal Series of the N. C. Agricultural Research Service, Raleigh, NC 27695-7643. This publication was partially supported by the Peanut CRSP-USAID grant number DAN-4048-G-SS-2065-00. Recommendations do not represent an official position or policy of USAID.

[2]Former Graduate Research Assistant and Professor of Crop Science, North Carolina State University, Raleigh, NC 27695.

*Corresponding author.

Table 1. Chi-square tests for the 3:1 and 9:7 F_2 ratio of peanut seed segregating for presence vs. absence of the two slowest migrating isozymes of PHI.

Cross	χ^2	Observed Total	Present	Absent	df	χ^2 (3:1)	P	χ^2 (9:7)
NC 18411 x Tennessee Red	Pooled	147	117	30	1	1.653	.25-.10	32.541**
	Sum				4	3.014	.75-.50	
	Heterogeneity				3	1.363	.75-.50	
NC 18411 x C_1	Pooled	72	52	20	1	0.296	.75-.50	7.464**
	Sum				2	0.571	.75	
	Heterogeneity				1	0.274	.75-.50	
A_1 x NC 18411	Pooled	110	85	25	1	0.303	.75-.50	19.763**
	Sum				3	4.312	.25-.10	
	Heterogeneity				2	4.006	.25-.10	
Tennessee Red x B_1	Pooled	51	37	14	1	0.163	.75-.50	5.502*
	Sum				2	0.170	.95-.90	
	Heterogeneity				1	0.005	.95-.90	

*,**Significant at the 5 and 1% levels of probability, respectively.

zone consisted of two monomorphic bands, whereas the slower migrating zone showed one fast band fixed in all individuals (labeled 3 on Fig. la) and two slower bands either present or absent (null) (labeled 1 and 2 on Fig. la). All F_1 progeny of the four crosses showed the two slowest bands indicating dominance of the presence of the bands over their absence. Each F_2 group assayed segregated into the two parental types. Chi-square values for goodness of fit to a proposed monogenic 3:1 (present: absent) ratio were nonsignificant for all 11 groups assayed as well as for the data pooled over crosses (Table 1).

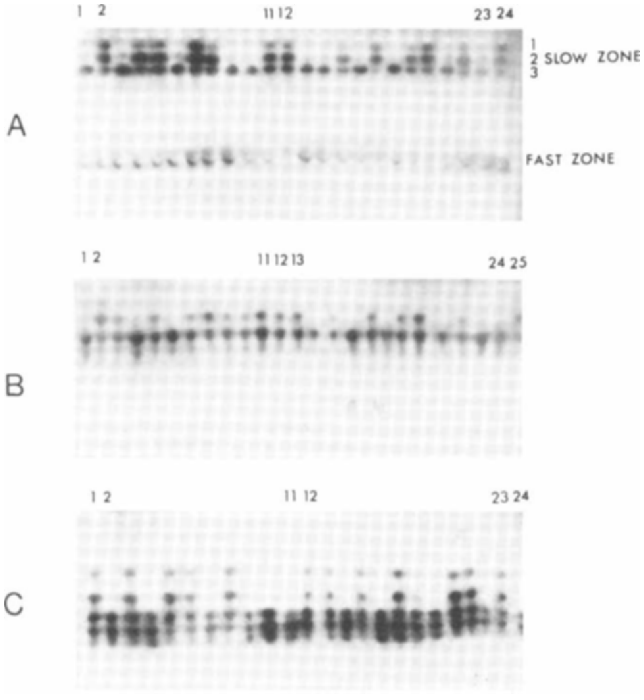

Fig. 1. Banding patterns of parent and F_1 and F_2 progeny seed. (A) PHI: lanes 1 and 23 = NC 18411, lanes 2 and 24 = A_1, lane 11 = A_1 x NC 18411 (F_1) lane 12 = NC 18411 x A_1 (F_1), remaining lanes = A_1 x NC 18411 (segregating F_2). (B) IDH: lanes 1 and 24 = Tennessee Red, lanes 2 and 25 = B_1, lanes 11-13 = Tennessee Red x B_1 (F_1), remaining lanes = Tennessee Red x B_1 (segregating F_2). (C) GOT: lanes 1 and 23 = NC 18411, lanes 2 and 24 = Tennessee Red, lane 11 = NC 18411 x Tennessee Red (F_1), lane 12 = Tennessee Red x NC 18411 (F_1), remaining lanes = NC 18411 x Tennessee Red (segregating F_2).

Because the cultivated peanut is considered to be a "diploidized" allotetraploid (8), it is expected that traits which are controlled by one gene in a diploid species are inherited digenically in peanut unless one locus on one of the genomes has mutated to nonfunctionality. A digenic model was considered for PHI by testing for goodness of fit to a 9:7 ratio which resulted in significant chi-square values. It is possible that the parents contained two different active alleles (rather than an active and a null allele) for the locus in question—represented by band 1 and band 3 (Fig. la).

The faster allele (band 3) possibly comigrated with the product of a second locus fixed for the genotypes assayed. Considering the relative intensities of band 1 and band 3, it appears that in heterozygotes (F_1, lanes 9 and 10; second lane of segregating F_2, Fig. la), band 1 is lighter than band 3, whereas in homozygotes for band 1 (parent A_1, lanes 2 and 24; fifth lane of segregating F_2, Fig. la), bands 1 and 3 show about the same intensity. If PHI is a dimeric enzyme as in maize (24), band 2 could be a heterodimer formed between the products of the polymorphic locus and the putative second locus and is, therefore, expected to be present in homozygotes for band 1 and heterozygotes.

Because we realized the possibility that two active alleles are segregating for PHI after the gels were discarded, rescoring could only be achieved from photographs taken from some of the gels. The results of the chi-square analysis for goodness of fit to the monogenic 1:2:1 ratio were nonsignificant for three of the four different crosses (Table 2). The difficulties encountered by scoring intensities of

bands from photographs could have led to the significant deviation from the proposed ratio for the cross NC 18411 x C_1. By scoring for presence vs. absence of bands 1 and 2, the homozygotes for band 1 and the heterozygotes were pooled in one class. Although we cannot determine whether the locus produces a single active or two active alleles, the data nevertheless support a single locus designated *Phi*-1.

Table 2. Chi-square test for the 1:2:1 F_2 ratio of peanut seed segregating for two alleles (bands 1 and 3) of PHI.

Cross	Observed				df	χ^2	P
	Total	Homoz. band 1	Heteroz.	Homoz. band 3			
NC 18411 x Tennessee Red	18	7	6	5	2	2.444	0.50-0.25
NC 18411 x C_1	23	6	7	10	2	4.913	0.10-0.05
A_1 x NC 18411	44	15	20	9	2	2.000	0.50-0.25
Tennessee Red x B_1	36	10	19	7	2	0.611	0.75-0.50

Isocitrate Dehydrogenase

The enzyme IDH showed only one zone of activity for all individuals assayed (Fig. lb). The faster migrating band was fixed for all genotypes and the slower band was either present or absent (null). (Some additional weak bands appearing inconsistently were ignored.) In all eight Tennessee Red x B_1 F_1 progeny the slow band was present (absent in Tennessee Red), showing dominance of the presence of the band over its absence. The F_2 progeny of the three Tennessee Red x B_1 crosses segregated into the two observed phenotypes. The chi-square values for goodness of fit to the 3:1 (present:absent) ratio were nonsignificant for both groups (data no shown) and the pooled data, whereas the data did not fit to a 9:7 ratio (Table 3). One locus designated *Idh*-1 is proposed.

Independent Segregation of *Phi-1* and *Idh-1*

The combined data for PHI and IDH for the cross Tennessee Red x B_1 suggested independent segregation of the two loci *Phi-1* and *Idh-1* using the presence vs. absence scoring for the polymorphic bands of PHI. The chi-square value for goodness of fit to the expected 9:3:3:1 ratio for two dominant genes was nonsignificant (Table 4).

Table 3. Chi-square tests for the 3:1 and 9:7 F_2 ratio of peanut seed segregating for presence vs. absence of the slow migrating isozyme of IDH in the cross Tennessee Red x B_1.

χ^2	Total	Present	Absent	df	χ^2 (3:1)	P	χ^2 (9:7)
Pooled	51	35	16	1	1.105	.50-.25	3.172*
Sum				2	1.149	.75-.50	
Heterogeneity				1	0.039	.90-.75	

*Significant at the 10% level of probability.

Glutamate Oxaloacetate Transaminase

The enzyme GOT showed a maximum of five bands which were approximately equidistant from each other. As in PHI and IDH, GOT showed two different banding patterns for the seed assayed, with the two slowest bands being either present or absent (Fig. 1c). However, the F_1 seed displayed phenotypes not compatible with a Mendelian genetic model. Sixty-five out of 71 F_1 progeny examined showed the banding pattern of the male parent (Table 5) which suggests paternal inheritance. Since the F_2 seeds segregated into the two parental phenotypes, plastids passing only through the pollen can be ruled out as being the causal factor for the observed phenomenon.

Mouli and Patil (14) reported on paternal inheritance of x-ray-induced foliaceous stipule in the peanut. They had observed the expression of the recessive character in the F_1 when it was contributed by the male parent. Because the F_2 segregated into the two paternal types, they concluded that the paternal inheritance of foliaceous stipule in the peanut is controlled by nuclear genes. Their chi-square analysis of four different reciprocal crosses led them to propose a digenic model of inheritance with transmission through the pollen. If the paternal inheritance of GOT was due to nuclear genes, the haploid F_2 segregation ratios of 1:1 or 3:1 would be expected for the action of one or two genes, respectively. The chi-squares for the goodness of fit of the observed ratios to these expectations were partly (1:1) or always (3:1) significant (Table 6). Therefore, a model of nuclear paternal inheritance cannot be proposed from these data. Interestingly, the observed ratios fitted the 9:7 ratio expected for two nuclear dominant genes with complementary action.

There are several ways of explaining the paternal inheritance observed for the enzyme GOT. Differential expression in the F_1 can often be attributed to cytoplasmic genomes (chloroplast and mitochondrial). Mitochondrial DNA seems to be maternally inherited in all higher eukaryotes that have been studied (15) with one exception reported recently by Fairbanks *et al* .(5). They provided strong, though not conclusive, evidence that mitochondria are transmitted biparentally in alfalfa (*Medicago sativa*) based on the inheritance pattern of large mitochondrial RNAs. Plastids have been found to be predominantly maternally transmitted in angiosperms and paternally transmitted in gymnosperms (10, 20). Several angiosperm species show biparental plastid transmission so the hybridizations between individuals with genetically different plastids produce mixed

Table 4. Chi-square test for the 9:3:3:1 F_2 ratio of seed from the cross Tennessee Red x B, for the loci *Phi-1* and *Idh-1*.

Group	Observed				df	χ^2 (9:3:3:1)	P
	PHI(P)IDH(P)*	PHI(P)IDH(A)	PHI(A)IDH(P)	PHI(A)IDH(A)			
1 + 2	26	11	9	5	3	1.529	.75-.50

*P = slow bands are present, A = slow bands are absent.

Table 5. Banding patterns [presence (P) vs. absence (A) of the two slowest bands] of parents and their F_1 progeny for GOT.

Cross				Observed	
Female parent		Male parent		Presence	Absence
NC 18411	(P)	Tennessee Red	(A)		4
NC 18411	(P)	C_1	(A)		6
NC 18411	(P)	A_1	(A)	2	7
NC 18411	(P)	Comet	(A)		6
B_1	(P)	Comet	(A)		1
Tennessee Red	(A)	NC 18411	(P)	5	1
C_1	(A)	NC 18411	(P)	17	
A_1	(A)	NC 18411	(P)	8	
Tennessee Red	(A)	B_1	(P)	10	
C_1	(A)	B_1	(P)		3
Comet	(A)	NC 18411	(P)	1	

zygotes containing both types of plastids (cytoplasmic heterozygotes) (20).

The mode of plastid transmission in the peanut is not known. Recently Corriveau and Coleman (4) developed a diagnostic method to rapidly screen for plant species potentially capable of biparental inheritance of plastid DNA by using a DNA fluorochrome in conjunction with epifluorescence microscopy. They detected no plastid DNA aggregates in pollen obtained from *A. hypogaea*. But since they found an example of intraspecific variability in *Pisum sativum* (displaying both biparental and maternal inheritance of plastid DNA) the potential for biparentalism may exist within a plant species considered to be maternal. Furthermore, of all families studied the *Fabaceae* displayed the highest variation for the mode of plastid transmission among species. Smith *et al.* (22) found plastids in alfalfa to be inherited biparentally which was confirmed by Lee *et al.* (12) with evidence from the inheritance of restriction fragment length polymorphisms of the chloroplast DNA and ultrastructural investigations. Smith *et al.* (22) speculated that biparental transmission of plastids may be more common in the family *Fabaceae* than in other families of flowering plants based on evidence from other genera within the *Fabaceae*. If the inheritance observed for GOT in the peanut results from biparental plastid transmission and, if the presence of the two isozyme bands is assumed to be dominant over their absence, the lack of bands in the F_1 requires that the plastid transmission be heavily skewed towards the male parent; otherwise, the dominant phenotype should be seen in the tissues containing mixed and possibly sorted-out cells. Smith (21) and Smith *et al.* (22) reported a that strong paternal bias in plastid transmission exists in alfalfa.

Plastid shunting can be under nuclear control. In *Pelargonium*, the output of male and female plastids is controlled by the nuclear genotype of the female parent (10), whereas the plastid inheritance patterns in alfalfa are influenced by both maternal and paternal genotypes (21). If the shunting of the plastids in the peanut were due to the action of two dominant complementary genes in the zygote, a 9:7 segregation ratio as observed in the F_2 for GOT would be expected.

Another conceivable mechanism which could explain the observed paternal inheritance is the occurrence of prezygotic RNA synthesis. It is known that pollen grains have a high metabolic activity during their development from a microspore to the end of the fertilization process. It has been demonstrated that several genes in different plant species, including isozyme genes, are expressed, i. e., transcribed and translated, in pollen (6). If a gene is transcribed during the male gametophytic phase, the nucleus of the male gamete which will finally unite with the egg cell to from the zygote still contains some of the heteronuclear RNA (hnRNA) formed during pollen development. It is conceivable that this RNA could be processed to mRNA, transported into the cytosol and translated into protein during seed development

Table 6. Chi-square tests for the 1:1, 3:1, and 9:7 F_2 ratio of peanut seed segregating for presence (P) vs. absence (A) of the two slowest migrating isozymes of GOT.

Cross	χ^2	Observed			df	χ^2		
		Total	P	A		(1:1)	(3:1)	(9:7)
NC 18411 x Tennessee Red	Pooled	146	81	65	1	1.753	29.671**	0.036
	Sum				4	2.915	31.221**	1.218
	Heterogeneity				3	1.162	1.552	1.182
NC 18411 x C_1	Pooled	71	45	26	1	5.085*	5.113*	1.465
	Sum				2	5.455	5.607	1.843
	Heterogeneity				1	0.371	0.501	0.382
A_1 x NC 18411	Pooled	110	70	40	1	8.182**	7.576**	2.442
	Sum				3	8.538*	8.050*	2.797
	Heterogeneity				2	0.356	0.475	0.362
Tennessee Red x B_1	Pooled	51	31	20	1	2.373	5.498*	0.425
	Sum				2	6.044*	10.393**	4.161
	Heterogeneity				1	3.672	4.896*	3.730

*,**Significant at the 5 and 1% levels of probability, respectively.

and early seedling stages. If the genes coding for or influencing the expression of the two GOT isozymes investigated in this study are expressed exclusively during the male gametophytic development, and if the hnRNA is produced abundantly and in a sufficiently stable form, these characters could be passed on by the pollen through "delayed" expression. Supportive evidence for this hypothesis stems from our unpublished observation that the two polymorphic slow bands of GOT were not found in leaves of mature plants. The exceptions from paternal inheritance observed for six of the 71 F_1s assayed could have been caused by varying environmental factors, during pollen and seed development influencing RNA stability.

Finally, genomic imprinting—as postulated for mammals—cannot be excluded as being operational in plants (23). The molecular mechanism for the differential expression of paternally and maternally derived genes is not known but some recent advances indicate the involvement of methylation of cytosine nucleotides in parental imprinting in mice (25). Methylation of DNA has been suggested to constitute a regulatory mechanism for inhibiting the expression of genes in several plant species (13, 16, 17, 28). Methylation of certain genes could be dependent on their parental origin in plants which could explain the differential expression of the GOT isozymes in the F_1 and the segregation of the trait in the F_2.

This study was not designed to distinguish between these and other possible explanations for the differential expression of GOT isozymes in the F_1 and F_2 generation because paternal inheritance was not expected to be observed. A detailed investigation at the ultrastructural, genetic and molecular level is necessary to determine the mechanisms governing paternal inheritance in the peanut.

Literature Cited

1. Arulsekar, S., D. E. Parfitt. 1986. Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio, and fig. HortSci. 21:928-933.
2. Bernatzky, R., and S. D. Tanksley. 1986. Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887-898.
3. Cherry, J. P. 1975. Comparative studies of seed proteins and enzymes of species and collections of *Arachis* by gel electrophoresis. Peanut Sci. 2:57-65.
4. Corriveau, J. L., and A. W. Coleman. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Amer. J. Bot. 75:1443-1458.
5. Fairbanks, D. J., S. E. Smith, and J. K. Brown. 1988. Inheritance of large mitochondrial RNA's in alfalfa. Theor. Appl. Genet. 76:619-622.
6. Frova, C., G. Binelli, and E. Ottaviano. 1987. Isozyme and HSP gene expression during male gametophyte development in maize. Isozymes: Current Topics in Biological and Medical Research 15:97-120.
7. Grieshammer, U., and J. C. Wynne. 1990. Isozyme variability in mature seeds of U.S. peanut cultivars and collections. Peanut Sci. 17:72.
8. Hammons, R. O. 1973. Genetics of *Arachis hypogaea*, pp. 135-173. Peanuts: Culture and Uses. Amer. Peanut Res. Educ. Assoc., Inc., Stillwater, OK.
9. Hoisington, D. A. 1989. Working linkage maps. Maize Genet. Coop. Newslett. 63:141-151.
10. Kirk, J. T. O., and R. A. E. Tilney-Bassett. 1978. The Plastids: Their Chemistry, Structure, Growth and Inheritance. Elsevier, North-Holland Biomedical Press, Amsterdam.
11. Klozova, E., J. Svachulova J. Smartt, E. Hadoc, V. Turkova, and V. Hadacova. 1983. The comparison of seed protein patterns within the genus *Arachis* by polyacrylamide gel electrophoresis. Biol. Plant. 25:266-273.
12. Lee, D. J., T. K. Blake, and S. E. Smith. 1988. Biparental inheritance of chloroplast DNA and the existence of heteroplasmic cells in alfalfa. Theor. Appl. Genet. 76:545-549.
13. Matzke, M. A., M. Primig, J. Trnovsky, and A. J. M. Matzke. 1989. Reversible methylation and inactivation of marker genes in sequentially transformed tobacco plants. EMBO. J. 8:643-649.
14. Mouli, C., and S. H. Patil. 1975. Paternal inheritance of x-ray induced foliaceous stipule in the peanut. J. Hered. 66:28-30.
15. Neale, D. B., and R. R. Sederoff. 1989. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor. Appl. Genet. 77:212-216.
16. Ngernprasirtsiri, J., H. Kobayashi, and T. Akazawa. 1988. DNA methylation occurred around lowly expressed genes of plastid DNA during tomato fruit development. Plant Physiol. 88:16-20.
17. Ngernprasirtsiri, J., H. Kobayashi, and T. Akazawa. 1988. DNA methylation as a mechanism of transcriptional regulation in nonphotosynthetic plastids of plant cells. Proc. Natl. Acad. Sci. USA 85:4750-4754.
18. Ory, R. L., and J. P. Cherry 1972. Proteins from peanut cultivars (*Arachis hypogaea*) grown in different areas. V. Biochemical observations on electrophoretic patterns of proteins and enzymes. J. Amer. Peanut Res. Educ. Assoc., Inc. 4:21-31.
19. Sanders, T. H., H. E. Pattee, and J. A. Singleton. 1975. Lipoxygenase isozymes of peanut. Lipids 10:681-685.
20. Sears, B. B. 1980. Elimination of plastids during spermatogenesis and fertilization in the plant kingdom. Plasmid 4:233-255.
21. Smith, S. E. 1989. Influence of parental genotype on plastid inheritance in *Medicago sativa*. J. Hered. 80:214-217.
22. Smith, S. E., E. T. Bingham, and R. W. Fulton. 1986. Transmission of chlorophyll deficiencies in *Medicago sativa*. Evidence for biparental inheritance of plastids. J. Hered. 77:35-38.
23. Solter, D. 1987. Inertia of the embryonic genome in mammals. Trends Genet. 3:23-27.
24. Stuber, C. W., J. F. Wendel, M. M. Goodman, and J. S. C. Smith. 1988. Techiques and scoring procedures for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). North Car. Agric. Res. Serv. Tech. Bull. 286. pp. 1-95.
25. Swain, J. L., A. Stewart, and P. Leder. 1987. Parental legacy determines methylation and expression of an autosomal transgene: A molecular mechanism of parental imprinting. Cell 50:719-727.
26. Tanksley, S. D. 1983. Molecular markers in plant breeding. Plant Mol. Biol. Rep. 1:3-8.
27. van Huystee, R. B., and R. N. Chibbar. 1987. Peanut peroxidase: A model system for peroxidase analysis. Isozymes: Current Topics in Biological and Medical Research 13:155-179.
28. Watson, J. C., L. S. Kaufman, and W. F. Thompson. 1987. Developmental regulation of cytosine methylation in the nuclear ribosomal RNA genes of *Pisum sativum*. J. Mol. Biol. 193:15-26.

Accepted October 22, 1990