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Pedigreed Natural Crossing to Identify Peanut Testa Genotypes[1]

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ABSTRACT

Pedigreed natural crossing to produce marker-identified hybrids for specific uses has been exploited in USDA-ARS/Georgia cooperative peanut (*Arachis hypogaea* L.) germplasm enhancement projects since the discovery in 1959 of suitable genetic markers. The principal advantages of natural hybridization using dominant alternative alleles to identify the outcrosses are that (1) the production of F_1 hybrid plants is not dependent upon conventional manual emasculation, (2) the identification and harvest of plants exhibiting the markers can be performed by semi-skilled workers, and (3) the procedure is more economical than the standard crossing method.

We utilized pedigreed natural crosses to screen an extensive sample of white-testa peanut phenotypes from the world gene pool for the five-loci recessive genotype, $r_1\ r_1\ f_1\ f_1\ f_2\ f_2 d_1\ d_1\ d_2\ d_2$. Four accessions, 'Spanwhite', P. I. 299468, P. I. 408730, and P. I. 306228, were found to be recessive at all five of the loci which condition testa color. F_2 populations from marker-identified natural crosses of each of these lines to a tester genotype which was homozygously dominant at four of the testa-color loci fit the ratio of 225 tan:31 white expected from the cross of these genotypes.

Key Words: *Arachis hypogaea* L., Duplicate alleles, Flavonoid, Genetic marker, Genetic ratio 225:31, Groundnut, Outcrossing, Peanut flour, Qualitative inheritance, Seedcoat color.

Peanut (*Arachis hypogaea* L.) testa color is inherited as a qualitative trait. Previous studies (2, 6, 8) have provided genetic models to explain the inheritance of testa color. Color *development* is governed by duplicate genes (symbolized $D_1\ D_1\ D_2\ D_2$); a single dominant allele at either of these "D" loci suffices to produce testa color, *provided* that one or more dominant "color" genes also is present. One or more dominant alleles of the duplicate "color" genes (symbolized $F_1\ F_1\ F_2\ F_2$) produces tan-colored testa in the presence of dominant "D" alleles. Both sets of these duplicate genes interact to produce the phenotypic expressions of testa color; dominant alleles at either or both of the "F" loci produce the tan (pink, rose, or flesh) color in the presence of dominant alleles at either or both "D" loci. All genotypes which are homozygous recessive at both of the "D" loci or at both of the "F" loci have white testa. These four loci also interact with the R_1 locus to produce red testa when either "D" loci, either "F" loci, and one or both dominant R_1 alleles are present.

Hammons (2) showed that 14 true-breeding white-testa genotypes could result from all possible combinations of the five loci conditioning testa color. One of these genotypes, designated G-V, lacks color due to homozygous recessiveness at all five loci, i. e. $r_1\ r_1\ f_1\ f_1\ f_2\ f_2\ d_1\ d_1\ d_2\ d_2$. Identification of peanut lines having the G-V genotype would facilitate investigations of testa color and of genes linked to loci conditioning this trait. If the 14 true-breeding white-testa genotypes were crossed with a tan-testa genotype homozygously recessive at the r_1 locus and

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homozygously dominant at the F_1, F_2, D_1 and D_2 loci, only the cross with the G-V genotype would give an F_2 distribution of 225 tan to 31 white (Table 1).

Table 1. Genotypic constitution and testcross behavior of the 14 true-breeding white testa peanuts expected from the interaction of 5 basic loci.

Genotype designation and constitution		Expected testcross with tan (Spanish) testa, $r_1r_1F_1F_1F_2F_2D_1D_1D_2D_2$	
		F_1	F_2
G-I	$R_1R_1F_1F_1F_2F_2d_1d_1d_2d_2$	R	45R:15T:4W
G-II	$r_1r_1F_1F_1F_2F_2d_1d_1d_2d_2$	T	15T:1W
G-III	$r_1r_1f_1f_1f_2f_2D_1D_1D_2D_2$	T	15T:1W
G-IV	$R_1R_1f_1f_1f_2f_2d_1d_1d_2d_2$	R	675R:225T:124W
G-V	$r_1r_1f_1f_1f_2f_2d_1d_1d_2d_2$	T	225T:31W
G-VI	$R_1R_1f_1f_1F_2F_2d_1d_1d_2d_2$	R	45R:15T:4W
G-VII	$r_1r_1f_1f_1F_2F_2d_1d_1d_2d_2$	T	15T:1W
G-VIII	$R_1R_1F_1F_1f_2f_2d_1d_1d_2d_2$	R	45R:15T:4W
G-IX	$r_1r_1F_1F_1f_2f_2d_1d_1d_2d_2$	T	15T:1W
G-X	$R_1R_1f_1f_1f_2f_2D_1D_1D_2D_2$	R	45R:15T:4W
G-XI	$R_1R_1f_1f_1f_2f_2D_1D_1d_2d_2$	R	45R:15T:4W
G-XII	$R_1R_1f_1f_1f_2f_2d_1d_1D_2D_2$	R	45R:15T:4W
G-XIII	$r_1r_1f_1f_1f_2f_2D_1D_1d_2d_2$	T	15T:1W
G-XIV	$r_1r_1f_1f_1f_2f_2d_1d_1D_2D_2$	T	15T:1W

For progeny testa: R, T and W denote Red, Tan (Flesh, Pink), and White, respectively. Modified from Hammons (2).

Hammons (2, 3, 6) and Hammons and Leuck (7) made numerous studies of natural hybridization in the (normally) self-pollinated peanut. Since at least 24 species of bees occur in the insect complex associated with peanut flowering, their activity is scarcely a limiting factor in the extent of natural crossing in field nurseries (9).

Hammons (5) suggested the use of pedigreed natural crossing as a procedure for obtaining F_1's for breeding or genetic research, and postualted (2) that using the krinkleleaf trait to identify natural crosses of unknown "white-testa" genotypes to a tan "tester" genotype would be a suitable and economic procedure for detecting the G-V genotype of white-testa peanut. The simply inherited dominant character is ideally suited for detecting natural outcrossing because hybrid seedlings can be unmistakably identified shortly after emergence (4,5). The trait assorts independently of the five basic loci for testa color (6). Furthermore, when the duplicate dominant "F" and "D" alleles of the tan-testa krinkleleaf genotype are crossed with any of the 14 true-breeding white-testa genotypes, the F_1 plants produce seed with a colored testa (Table 1).

The current study was designed as a genetic survey of white testa germplasm using the krinkleleaf, tan-testa dominant marker to identify white testa peanuts of the r_1 r_1 f_1 f_1 f_2 f_2 d_1 d_1 d_2 d_2 genotype.

Materials and Methods

The parents, F_1 and F_2 populations were grown at the agronomy research farm near Tifton, Ga., under conditions similar to those used for commercial peanut production, but using marginal lands unsuited for standard cultivar trials. The double dominant tan-seeded, krinkleleaf r_1 r_1 F_1 F_1 F_2 F_2 D_1 D_1 D_2 D_2/*KrKr* marker (4) was used as the pollen donor in the natural crossing nursery. Thirty-two accessions, representing a portion of the white testa material in the world gene pool, were planted in 1979 at a 1:1 seed mixture of white:tan. The site was bordered by a noncultivated area that could provide nesting sites for solitary bees (9).

Krinkleleaf plants were discarded at digging. Seed from plants within each white-seeded female line were bulked. The great majority of the seed produced by the white-seeded female parent plants would result from self-pollination. In addition, three types of cross-pollinated seed would be expected to occur in low frequencies: sib-mated outcrosses between different plants within the same white-testa female line; outcrosses between different white-testa lines; and pedigreed natural crosses between the white-testa female and the krinkleleaf marker stock. Seed from the bulked white-seeded female lines were visually scanned to ensure that only the white-seeded phenotype was retained for planting.

In 1980, a random sample of 400 seed from each of the 32 white-testa "female" lines was planted and stands approximated 80%. Hybrids were identified as krinkeleaf seedlings, and these were transplanted to a spaced F_1 nursery. Following harvest, testa color phenotype was determined. Only those hybrids with tan testa were advanced to the F_2 in this study (2). An F_2 population from the single most productive F_1 plant for each cross combination was grown. After harvest, an individual pod from each F_2 plant was hand-shelled, and the testa phenotype was determined. Testa classification was based upon sound mature seeds. Standard chi-square computations measured goodness-of-fit of F_2 distributions to appropriate ratios.

The testa ("skins") and defatted flours from samples of all white-seeded lines were analyzed for flavonoids by high pressure liquid chromatography and UV spectrometry in cooperative investigations at the U.S.D.A. Southern Regional Research Center in New Orleans (1). These analytical tests were used to determine whether four white-testa lines which had segregation expected of the G-V genotype in crosses with the tan-krinkleleaf line also had similar flavonoid constitution.

Results and Discussion

The 80% emergence rate of the 400 seeds from each of the 32 white-testa lines previously grown in mixtures with the tan-krinkleleaf marker produced a field population of approximately 10,240 plants. This field population was visually screened for "krinkleleaf" plants, and 54 F_1 hybrids were isolated with a range of zero to five plants per cross combination. Thus, the frequency of detected outcrossing was calculated at 0.53%. This value compares favorably with natural crossing rates of 0.25 to 6.16% previously observed at this location (9). From the 54 plants which were identified as F_1 hybrids between white-testa females and the tan-krinkleleaf marker stock, nine individual plants which produced tan-testa seed were selected for further study. These cross combinations were evaluated by classifying individual F_2 plants for testa color. Segregation for four of these nine crosses was found to have an acceptable fit to the ratio of 225 tan-testa:31 white-testa expected when genotype G-V (r_1 r_1 f_1 f_1 f_2 f_2 d_1 d_1 d_2 d_2) is crossed with the r_1 r_1 F_1 F_1 F_2 F_2 D_1 D_1 D_2 D_2 genotype of the tan-krinkleleaf marker stock (Table 2).

The four white-testa female lines which appear to have the G-V genotype have varied backgrounds. The 'Spanwhite' peanut was isolated in the F_{12} generation of the pedigreed 'Spancross' cultivar (*A. hypogaea* cv. 'Argentine' x *A. monticola* Krap. *et.* Rig.); it is thought to have originated from natural outcrossing in the breeding nursery. The other three genotypes were accessioned from Africa. P. I. 299468 was collected in the Republic of South Africa (A. J. Oakes, Jr., USDA agricultural explorer, Col. No. 471). Two plants with white testa were found growing among plants with pink testa; seed from these two plants were introduced in 1964 as P. I. 299468. P. I. 306228 was obtained from Senegal (Col. 57-204); it previously had

Table 2. F_2 testa color segregation of candidate *krkr/r_1 r_1 f_1 f_1 f_2 f_2 d_1 d_1 d_2 d_2* white-seeded peanut genotypes in testcrosses with the tan-seeded krinkleleaf marker, *KrKr/r_1 r_1 F_1 F_1 F_2 F_2 D_1 D_1 D_2 D_2*, and chi-square tests for goodness of fit to the expected ratio of 225 tan:31 white.

Natural Cross	Female Parent Name/P.I.	Observed Tan	Observed White	x^2	P
1	Spanwhite	206	22	1.297	.26
2	P.I. 299468	141	16	0.543	.47
6	P.I. 408730	343	39	1.296	.26
23	P.I. 306228	179	18	1.635	.20
Total				4.770	.31
Pooled		869	95	4.603	.04*
Heterogeneity (total - pooled)				0.167	.98

* Significant at 0.05 probability level.

been introduced to Senegal from the Republic of South Africa as the cultivar 'Kaboka Tjina.' P. I. 408730, also accessioned from Senegal (Col. 57-161), had been obtained from Australia under the designation 'Argentine No. 131' (O. de Pins, pers. comm., 1982).

Each of the four accessions that met the genetic criterion for five-locus recessive white testa are botanically *Arachis hypogaea fastigiata vulgaris* (spanish). The plants are erect (bunch) in growth habit. The two principal N + 1 order vegetative (V) axes produce N + 2 reproductive (R) axes in large sequential runs, interrupted by shorter runs of N + 2 V axes. Inflorescences occur in some mainstem leaf axils. Slight differences in mainstem height and leaflet length:width ratios were noted for the four accessions, but these differences may have been due to environmental variations. Pods of each of the lines are two-segmented, and the white testa has a yellowish appearance.

Since the four accessions exhibited the same (G-V) breeding behaviour and also appeared to be very similar agronomically, we attempted to further characterize them by analyzing their flavonoid composition. Flavonoids detected by high pressure liquid chromatography were principally sugar derivatives of the isorhamnetin and quercetin aglycones. Classification of the four candidate genotypes into one group based upon the presence of common flavonoid constituents in their flours (1) provides additional evidence that they are genetically similar (Table 3).

We noted that the F_2 population from the tan-krinkleleaf cross with P. I. 306228 also had an acceptable fit to the 15 tan:1 white ratio expected for genotypes II, III, VII, IX, XIII, or XIV in such a testcross. However, the probability for goodness of fit (P = .10) to this ratio was not as good as that for the 225:31 ratio for genotype V (P = .20). We believe that the preponderance of evidence supports the inclusion of P. I. 306228 with the other three candidate G-V genotypes.

For another progeny, P. I. 313151 x krinkleleaf, the F_2 population of 56 tan:3 white testa plants was too small to differentiate between the 15:1 (P = 0.49) and the 225:31 (P = 0.10) ratios. P. I. 313151 differs from the four candidate genotypes in white testa coloration (bright vs yellow), and in having rhamnetin and an unknown aglycone as the major flour flavonoids. Thus, the existing evidence is too meager for accepting P. I. 313151 as genotype V.

Table 3. Flavonoid compounds isolated from flours of four peanut lines which appear to have the G-V genotype conditioning white testa color.[1]

Genotype identity	Major flavonoids[2] Kind	Major flavonoids[2] Ratio	Minor flavonoids[2]
Spanwhite	I : Q	1.6 : 1	UNK. 2, R (1.5)
P.I. 299468	I : Q	1 : 1.4	UNK. 2, R (0.5)
P.I. 408730	I : Q	1.6 : 1	UNK. 2, R (1)
P.I. 306228	I : Q	1.4 : 1	UNK. 2, R

[1] Data courtesy Dr. D. J. Daigle, USDA-ARS, Southern Reg. Res. Cen., New Orleans, LA.

[2] Flavonoids: R = Rhamnetin, Q = Quercetin, I = Isorhamnetin, UNK. 2 = Unknown aglycone.

Since the peanut is a partial outbreeder, other crosses may be detected in natural cross nurseries. At least one red testa plant appeared in natural cross progenies 2, 6, and 23, probably indicating natural pollination by a genotype other than the krinkleleaf marker. Since reciprocal crosses were not tested, there was no basis for estimating maternal effects in the populations. The krinkleleaf trait segregated 3 *Kr*:1 *kr*, and there was no indication that this trait was linked with any of the genes conditioning testa color.

Occurrence of the expected 225 tan:31 white phenotypic ratio is *prima facie* evidence of the genotypic constitution of the white testa parents. Traditional crossing experiments can now be conducted upon these few potential genotypes for confirmation.

Hammons (2) was the first to employ testcrosses with the krinkleleaf marker to confirm the F_2 behavior for a white-testa peanut, Genotype X. Later, he showed that screening for F_1 hybrids could be done inexpensively on land unsuited for yield trials (5).

In peanut, production of hybrid seed is both labor and capital intensive. Therefore, it would be desirable from a practical standpoint to keep the number of cross pollinations to a minimum. By limiting the number of seed screened to 400 per mother line, we obtained one to five F_1 plants per successful combination.

We dispensed with greenhouse or growth chamber culture of parental lines and with conventional manual emasculation. Hybrids were isolated, pedigreed, harvested, shelled, and classified by sub-professional workers. These advantages are obvious for initially screening a large number of genotypes to select the most probable candidates for a more intensive study.

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