Peanut Science (1983) 10, 36-40

Seedcoat Splitting in Peanut - Its Inheritance and Relationship with Seed Weight[1]

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ABSTRACT

Seedcoat splitting in peanut (*Arachis hypogaea* L.) can have direct effects on market quality, germination, and susceptibility of seeds to invasion by fungi. Six parental genotypes with differing degrees of seedcoat splitting were crossed with three non-splitting genotypes, and the F_1, F_2, and F_3 seedcoat generations were investigated to determine the inheritance of the seedcoat splitting trait and its effects on germination. Mature seeds were classified according to the number and extent of splits in the seedcoat. Individual seed weights and germination data were recorded.

In crosses involving genotypes with large differences in seed weight, the F_1 seedcoat generation tended to be closer to the smaller parent in splitting percentages. Highly significant phenotypic correlations were obtained between seed weight and seedcoat splitting which led to the conclusion that segregation for seed weight can confound or mask the seedcoat splitting trait.

In crosses of geneotypes with similar seed weight, the F_2 and F_3 seedcoat generations had mean seed weights below that of both parents. Natural selection for non-splitting seedcoats seemed to be involved in the decreased number of heavier seeds in those populations. Seeds with split seedcoat germinated less than non-split seeds under field and laboratory conditions. As a consequence of the lower germination percentage, the population means tended to skew toward the smaller seed weight, which was represented to a greater degree by the non-split seeds.

Genetic models were proposed for crosses in which the parents were similar in seed weight and significantly different in splitting percentage. F_2 populations revealed monogenic inheritance with additive effects in one cross, and also two unusual segregations which suggested duplicate additive and complementary gene action. Natural selection for non-split seeds appeared to bias the results. F_1 data, in some crosses, revealed some degree of dominance for the splitting trait, although subsequent generations presented seedcoat splitting as a recessive trait. It appears that the selective germination of split seeds affected the genetic ratios, and should be considered in further studies.

Key Words: Peanut genetics, Genetic models, Natural selection, Peanut breeding, Germination, Seed size.

Seedcoat splitting in peanut (*Arachis hypogaea* L.) can have direct effects on market quality, germination, and susceptibility of the seeds to invasion by fungi, including toxin-producing *Aspergillus flavus*. The important function of the seedcoat in protecting the seed from invasion by disease organisms after planting and during emergence has been demonstrated (2,3,6,7).

Seedcoat characteristics in peanut have been the object of numerous studies, especially in relation to color. Only two studies were found that dealt with seedcoat splitting and in both, seedcoat rupture and variegation were considered associated problems. A disharmony in the growth rates of the seedcoat and the embryo was proposed by Yona (10) and Ashri and Yona (1) as the cause of seedcoat

[1]Florida Agricultural Experiment Station Journal Series No. 4051. This research was supported in part by financial assistance from Empresa Brasileira de Pesquisas Agropecuarias (EMBRAPA).

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splitting and variegated seedcoat in peanut. Although a genetic model was provided, it was not consistent, suggesting that the inheritance of seedcoat splitting is affected by modifiers, and that the trait varies in penetrance and expressivity.

The objective of this research was to study the seedcoat splitting trait, its action and inheritance, as well as to determine the effects of seedcoat splitting on germination.

Materials and Methods

Nine parental genotypes selected for this study were obtained from the collection of breeding lines maintained by the Florida Agricultural Experiment Station at Gainesville, Florida, and are designated by the numbers and names in use in the Florida peanut improvement project. Splitting of the seedcoat was the criterion employed in the selection of the parents. Table 1 shows the parents and the percentage of seeds with split seedcoats.

Table 1. Percentage of seedcoat splitting in the parental genotypes.

Genotype	Number of seeds observed	Percentage of splitting
Florunner (FR)	808	0.00
Tifspan	966	0.00
Florigiant (FG)	1,081	0.60
F459 B-2-2-4-6-2 (459)	685	0.00
F427 B-3-1-7-4 (427)	941	0.40
F393-8-2-1-4-1-M1 (393-8)	1,375	0.92
F439-17-2-1-1 (439)	671	2.00
PI 268883 (PI)	1,035	3.00
F393-9-5-4-2-1-7 (393-9)	1,220	29.00

Greenhouse crosses were made between the nine different strains following techniques discussed by Norden (9). In most cases, reciprocal crosses were also made. Fungicide-treated seeds were planted in individual 15-liter clay pots. Water, fertilizer, insecticides, and fungicides were applied as needed for growth and pest control.

The F_1 seeds were harvested at 60 to 70 days after pegging and then were allowed to dry for 15 days before determination of seedcoat splitting. These seeds were planted and allowed to self-pollinate naturally. As the number of seeds available from each cross and reciprocal was different, each population had a different number of rows randomly assigned into plots.

The F_2 seeds, as well as the F_3, and parental genotypes were funcigide-treated and planted in the field in June 1979 at the University of Florida Green Acres agronomy farm, Gainesville, in Arredondo fine sand. To avoid competition, the seeds were sown 60 cm apart in rows 6 m long with 91 cm between rows. Maturity was based on general appearance such as defoliation, general yellowing of foliage and decreased peg strength. Each plant was harvested separately.

After harvesting, the pods per plant were artifically dried for five days, hand-shelled and scored in relation to splitting of the testa.

Four different types of splitting were observed: a) splitting in the hilum region; b) splitting as a long crack from the radicle end to the distal end, which almost separates the two cotyledons; c) splitting in patches when a small piece of the seedcoat is lacking; and d) splitting in lines when the rupture of the seedcoat is in lines of different lengths, widths, and frequencies. This last type was the most frequent and was the only type considered in this research.

The seeds were individually classified by the number and extension of the splitting lines using the following criteria: a) normal - none, or one splitting line with an extension less than half the longitudinal height of the seed; b) slightly split - one to three splitting lines. If there was only one splitting line, the extension of it should be more than half the longitudinal height of the seed. If three splitting lines are present, their extensions should be less than half the seed longitudinal height; and c) split - from three to twelve (the observed maximum) splitting lines, extending more than half the seed length (Fig. 1).

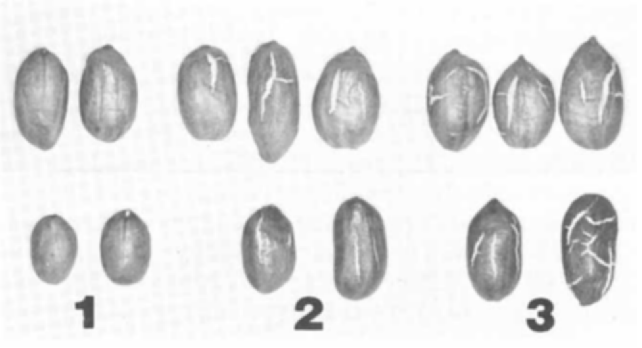

Fig. 1. Seedcoat splitting in parental lines 393-9 (Upper) and PI 268883 (Lower); 1 - normal (non-split); 2 - slightly split; 3 - severe split.

The genetic ratios for family segregations were analyzed by Chi-square (x^2) for goodness of fit. Chi-square values for total, pooled, and heterogeneity were also estimated according to Little and Hills (8).

It was observed under field conditions that split seeds had poorer germination than normal (non-split) seeds. Tests for independence were applied to the field germination data, and two subsequent tests were made under laboratory conditions. In the first test, seeds from genotypes known to have different percentages of split seeds were tested in a completely ramdomized design using a 2^2 x 3 factorial with four replications. The twelve treatments were all possible combinations of three genotypes (UF 714021, NC-FLA 14, and UF 519-3-1-2-1-3), two levels of fungicide (with and without) and two types of seeds (normal and split seeds of the same genotype). Covered plastic boxes with moist paper towels were used for the germination tests. Each box held 40 seeds. The plastic boxes were stored at room temperature (21 - 25 C), and the number of germinated seeds were recorded after 4,8, and 10 days. Seeds were counted as germinated if they had at least 15 mm of radicle elongation.

In the second test, one-year-old seeds were tested in a completely randomized design with a 2^2 factorial. Seeds of a mixture of genotypes stored in a cold chamber for one year were classified (split and normal), treated with fungicides, and germinated according to the procedure described in the first test. The germination percentages of the two experiments were statistically analyzed by using the arcsine transformed values of the percentages.

Phenotypic correlation coefficients between seed weight and the three degrees of splitting were also calculated after weighing the seeds of parents, F_2, and F_3 seedcoat generations.

Results and Discussion

Table 2 shows the mean percentage of split seeds of all parents, and the F_1 seedcoat populations, as well as the midparent values. Crosses involving genotypes with small amounts of splitting were not significantly different from parents or midparent values in splitting percentage, except for the cross 393-8 x FG, that showed over dominance of the splitting trait. In crosses of genotypes with large differences in seed weight, the F_1 seedcoat population tended to have splitting percentages closer to the parent with smaller percentages. These results, together with the highly significant phenotypic correlations between seed weight and seedcoat splitting, led to the conclusion that segregation for seed weight can confound or mask the expression of the seedcoat splitting trait (Table 3). In genotypes with high percent of splitting and crosses involving these parents, it appears that the heavier the seed, the more prone it is to splitting. This suggested linearity between seed weight and degree of splitting (non-split, slightly split, and split) seems to confirm the proposal of Ashri and Yona (1) that differential growth

Table 2. Mean percentage of split testae in parental and F_1 seedcoat populations and midparent values for different crosses.

Population	Splitting (%)	Population	Splitting (%)
PI	3.00 (a)[1]	PI	3.00 (a)
PI X FG	0.66 (a)	PI X FR	0.00 (a)
midparent	1.79 (a)	midparent	1.68 (a)
FG X PI	0.50 (a)	FR X PI	0.00 (a)
FG	0.60 (a)	FR	0.00 (a)
PI	3.00 (a)	PI	3.00 (c)
PI X Tifspan	1.94 (a)	PI X 393-9	36.41 (a)
midparent	1.55 (a)	midparent	10.07 (b)
Tifspan X PI	0.00 (a)	393-9	29.00 (a)
Tifspan	0.00 (a)		
		393-9	29.00 (a)
PI	3.00 (a)	393-9 X FG	36.22 (a)
midparent	1.80 (a)	Midparent	15.69 (b)
459 X PI	0.00 (a)	FG X 393-9	14.75 (b)
459	0.00 (a)	FG	0.60 (c)
393-9	29.00 (a)	393-9	29.00 (a)
393-9 X FR	1.85 (c)	393-9 X Tifspan	6.78 (c)
midparent	17.45 (b)	midparent	16.19 (b)
FR X 393-9	0.51 (c)	Tifspan X 393-9	3.09 (cd)
FR	0.00 (c)	Tifspan	0.00 (d)
393-9	29.00 (a)	393-9	29.00 (a)
393-9 X 459	8.89 (b)	393-9 X 439	0.00 (b)
midparent	18.58 (a)	midparent	19.46 (a)
459 X 393-9	5.26 (b)	439	2.00 (b)
459	0.00 (c)		
393-9	29.00 (a)	393-8	0.92 (b)
393-9 X 427	1.82 (c)	393-8 X FG	9.78 (a)
midparent	16.57 (b)	midparent	0.77 (b)
427	0.40 (c)	FG X 393-8	0.00 (b)
		FG	0.60 (b)
393-8	0.92 (a)		
393-8 X Tifspan	0.00 (a)	393-8	0.92 (a)
midparent	0.51 (a)	midparent	0.55 (a)
Tifspan X 393-8	0.00 (a)	FR X 393-8	0.00 (a)
Tifspan	0.00 (a)	FR	0.00 (a)

[1] Means within each population accompanied by the same letter are not significantly different at 5% probability.

Table 3. Mean seed weight of normal, slightly split, and split testae and correlation coefficient (r) between seed weight and splitting.

Populations	Normal	Slightly split	Split	r
Parents		Weight (g)		
FG	0.8846	0.8999	1.0164	0.5814**
427	0.8044	0.9203	1.2276	0.7353**
393-8	0.5375	0.6385	0.7293	0.8129**
439	0.5303	0.7807	0.7909	0.7575**
PI	0.9409	1.1495	------	0.6410**
393-9	0.9695	1.3195	1.4602	0.7192**
F_2 Seedcoat Populations				
FR X PI	0.5730	0.6781	0.8703	0.6410**
PI X FR	0.5921	0.6954	0.9284	0.5090**
Tifspan X PI	0.5555	0.6256	0.8872	0.6447**
PI X Tifspan	0.6467	0.7152	0.9034	0.5480**
459 X PI	0.8427	0.9319	1.2214	0.6361**
FG X 393-9	0.8547	1.1699	1.2856	0.7334**
FR X 393-9	0.6855	0.8337	0.9528	0.6337**
393-9 X FR	0.6855	0.8305	0.9818	0.7683**
Tifspan X 393-9	0.5512	0.8066	0.9251	0.5480**
393-9 X Tifspan	0.5684	0.7267	0.8751	0.7266**
393-9 X 459	0.7684	0.9099	1.1379	0.5046**
459 X 393-9	0.7410	0.9134	1.0867	0.7023**
393-9 X 439	0.6951	0.8159	0.8612	0.5822**
393-9 X 427	0.6626	0.8851	0.9267	0.4999**
FG X 393-8	0.7054	0.7988	0.9974	0.7170**
393-9 X FG	0.7036	0.8613	0.9036	0.4954**
FR X 393-8	0.5631	0.6235	0.6678	0.5053**
393-8 X Tifspan	0.4430	0.5036	0.5732	0.5730**

** Significant at 1% probability.

rates between the testa and the embryo were considered to be responsible for seedcoat splitting. In some crosses in which the parents differed widely in seed weight, even shell (pericarp) rupture was observed due to pressure caused by the seeds, along with the seedcoat.

The frequency distribution of seed weight among segregating populations showed a typical skewness toward the smaller parent (4). Skewness of the F_2 and F_3 frequency distributions may suggest the presence of partial dominance for genetic factors determining small seed weight.

Considering the parents, F_2, and F_3 plants, the split seed mean weight was always above that of the non-split seed of the same generation, and the frequency distributions of seeds with splitting seedcoats were generally limited to the parental range and skewed toward the smaller parent.

In crosses involving parents with similar seed weights, the F_2 and F_3 seedcoat generations had a mean seed weight below the values shown for both parents. The frequency of heavier seeds in segregating populations could decrease if dominance or partial dominance were involved, together with strong duplicate interactions. A more rapid decrease would occur if selection were involved. In fact, natural selection for non-split seeds could be observed under field and laboratory conditions, where split seeds germinated less than non-split seeds.

Field germination data of the parents, and the F_2 and F_3 seedcoat generations, analyzed by the test of independence with the Yates correction, showed significant differences in stand counts of split and non-split seeds in all the parents except 'Florigiant'. Chi-square values and probabilities are summarized in Table 4. F_2 and F_3 seedcoat populations also showed significant differences in final germination percentages between seeds with ruptured seedcoats and normal seeds. The F_2 seedcoat populations of crosses PI x FG, 393-8 x FG, and PI x Tifspan presented Chi-square values beneath the critical levels of significanace.

Table 4. Percentage of germination under field conditions of normal and split testae seeds, test for independence (Chi-square test applied with Yates correction) and significance.

Population	Germination % normal	Germination % split	χ^2	P
Parents				
439	47.8	30.0	6.39	.05-.01
393-9	48.3	0.0	7.35	.01
427	60.0	33.3	4.77	.05-.01
FG	75.0	50.0	2.86	.10-.05
393-8	61.8	37.5	4.25	.05-.01
PI	42.0	22.0	6.54	.05-.01
F_2 Seedcoat Generation				
393-9 X 459	34.3	0.0	3.95	.05-.01
459 X 393-9	32.4	0.0	4.78	.05-.01
393-9 X FR	60.0	25.0	3.88	.05-.01
FR X 393-9	32.4	0.0	4.14	.05-.01
393-9 X Tifspan	31.4	0.0	7.12	.01
Tifspan X 393-9	58.9	0.0	6.22	.05-.01
FG X 393-9	42.8	9.1	5.17	.05-.01
393-9 X 427	36.8	0.0	3.95	.05-.01
PI X FG	36.8	0.0	2.78	.10-.05
FG X PI	39.5	0.0	3.88	.05-.01
PI X Tifspan	45.0	9.0	3.04	.10-.05
PI X 393-9	38.7	9.1	3.92	.05-.01
393-8 X FG	34.2	0.0	2.71	.10-.05
FG X 393-8	31.6	0.0	3.92	.05-.01
FR X 393-8	28.9	0.0	3.86	.05-.01
393-8 X Tifspan	30.5	0.0	3.88	.05-.01
459 X PI	36.7	0.0	4.12	.05-.01
F_3 Seedcoat Generation				
393-9 X 439	35.7	0.0	7.18	.01
393-9 X 427	31.9	7.9	7.04	.01
393-9 X 459	35.1	0.0	6.77	.01
459 X 393-9	34.9	3.7	6.52	.05-.01
459 X PI	40.6	4.3	7.03	.01

Following the field germination results, two laboratory germination tests were made. In the first experiment the following genotypes with the respective splitting percentages were used: UF 714021, 5.1%; NC-FLA 14, 28.7%;

and UF 519-3-1-2-1-3, 2.8%. The germination data were statistically analyzed, after an arcsine percentage transformation (Table 5). Only the more severely split genotype, NC-FLA 14, presented a statistically significant difference between the two levels of fungicide and type of seeds at 1% probability. In this genotype, split seeds without fungicide gave poorer germination in comparison with all other treatment combinations. There were no significant differences in germination of split and normal seeds of NC-FLA 14 when the seeds were treated with fungicide. Also, there was no significant difference between normal seeds, whether or not they were fungicide-treated. These results confirm, at least for genotypes with large percentages of split seedcoats, the earlier reports of Carter (5,6) and Bell (2), in which they emphasized the importance of an undamaged seedcoat to good emergence in the field and laboratory conditions.

Table 5. Actual and transformed (in parentheses) germination percentages of three genotypes, and one-year mixed genotypes in laboratory germination tests.

Genotype	Seed Type	Fungicide with	Fungicide without
UF 714021	Split	91 (76)ns	79 (64)ns
	Normal	89 (73)ns	89 (74)ns
NC-FLA 14	Split	84 (73)b[1]	16 (24)a
	Normal	81 (65)b	69 (57)b
UF 519-3-1-2-1-3	Split	87 (70)ns[2]	81 (66)ns
	Normal	79 (63)ns	75 (60)ns
Old Mixed genotypes	Split	18 (25)c	0.5 (3)a
	Normal	34 (36)d	15 (23)b

[1] Means accompanied by the same letter are not significantly different at 1% probability.

[2] Nonsignificant at 5% probability.

Researchers have shown that an intact testa is necessary for resistance to invasion by *Aspergillus flavus* and other pathogenic microorganisms (2,3,7). The loss of resistance to microorganisms, invasion, and colonization can be better observed in old seeds. In the second experiment in which one-year-old mixed genotypes were utilized, the seeds with split testae gave lower germination than normal ones even when fungicide was applied (Table 5). This may indicate that split seeds were already infected by fungi, which confirms the loss of resistance of seeds with ruptured testae. In fact, the growth of fungi was observed in split seeds, even before the seeds had started the germination process.

As a consequence of this poorer germination of seeds with ruptured seedcoats, the population mean tended to skew toward the smaller seed weight, which is represented, in great part, by the non-split seeds.

The seedcoat splitting characteristic, due to its close relationship with seed weight, is largely influenced by environmental factors and interactions with the maternal plant. Segregation for seed weight could mask the splitting trait, especially in cases with parents that have significantly different seed weights. Thus, genetic models were proposed only in crosses in which the parents were similar in seed weight and significantly different in percentage of seedcoat splitting.

Individual plants were classified as equal to one or the other parent when its splitting percentage was the same as the parent in consideration. Plants with intermediate splitting percentages were considered as segregating. The percentage of splitting in each plant was determined after each mature seed produced by the plant was classified as to normal or split (the two grades, slightly split and split, were added for convenience).

The F_1 population of the cross 393-9 x FG indicated complete dominance of the splitting trait, while the reciprocal cross showed partial dominance. The F_2 population in the reciprocal cross segregated in a ratio not significantly different from 1:2:1, ($x^2 = 0.0968$, $P = 0.99 - 0.95$). No seeds were obtained from the normal cross in the F_2 segregation.

The F_2 generation of cross 393-9 x 427 showed a good fit to 3:12:1, ($x^2 = 0.4285$, $P = 0.90 - 0.50$). This unusual ratio can be explained if it can be proposed that splitting in line 393-9 is controlled by the duplicate recessive factors *s1s1s2s2*. The genotype of line 427 would be *S1S1S2S2*. Following this proposition, genotype *S1S1S2-* would have splitting percentages equal to the 427 parent, and *s1s1s2s2* would be equal to the 393-9 parent. The remaining genotypes would be intermediate in splitting percentage.

Unusual segregation was also obtained in the cross 393-9 x 459 and reciprocal. The F_1 populations of the normal and the reciprocal crosses showed partial dominance of the splitting trait. The F_2 generation of the reciprocal (459 x 393-9) segregated in a 9:4:3 ratio (Table 6). The F_3 generation of both crosses confirmed the segregation found in the F_2. This unusual ratio can be explained if complete dominance at both gene pairs is present, but one gene, when homozygous recessive, is epistatic to the other. Other ratios were tentatively applied (as 9:3:4 and 11:4:1), but Chi-square values were greater than the calculated 9:4:3, and the genotypes proposed did not fit with the intermediate class found in the F_1. The small number of families did not permit speculation about more complex genetic models.

Although the different ratios obtained from the crosses in this study may suggest that the seedcoat splitting trait varies in penetrance and/or expressivity, a more reasonable explanation should be the differential germination of the split and non-split seeds.

Natural selection for non-split seeds, as discussed in the germination test results, seems to have influenced the genetic ratios obtained. Crosses that showed some degree of dominance in the F_1 generation for the splitting trait, such as 393-9 x FG and 393-9 x 459, presented F_2 and F_3 ratios in which splitting was recessive to non-splitting. It appears that the seedcoat splitting trait has a dominant character, but natural selection for non-split seeds biased

Table 6. Seedcoat splitting segregation in cross 393-9 x 459 and goodness of fit to 9:4:3. F_1 population is intermediate.

Generation	Families (number)	Number of seeds	Splitting % 0	Seg.	29	x^2	P
F_2 393-9 x 459	32	868	19	12	1	6.2222	.05-.01
F_2 459 X 393-9	24	820	15	5	4	0.3889	.90-.50
Total (df = 4)						6.6111	.50-.10
Pooled	56	1,688	34	17	5	3.7222	.50-.10
Heterogeneity (Total - Pooled)						2.8889	.50-.10
F_3 393-9 X 459	31	1,985	20	6	5	0.8818	.90-50
F_3 459 X 393-9	28	827	18	6	4	0.7618	.90-.50

the results of subsequent generations. This fact is of considerable importance and should be considered in studies involving parents having different levels of seedcoat splitting.

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Accepted April 16, 1983