SEEDCOAT DAMAGE IN NAVY BEANS, PHASEOLUS VULGARIS (L.), INDUCED BY MECHANICAL ABUSE

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M. W. Adams
Major professor

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ABSTRACT

SEEDCOAT DAMAGE IN NAVY BEANS, *PHASEOLUS VULGARIS* (L.), INDUCED BY MECHANICAL ABUSE

by Douglas Gordon Dorrell

A collection of bean varieties and types were tested for tolerance to seedcoat cracking due to mechanical abuse. Tolerance ranged from 24 to 96 percent at 12 percent moisture. Seedcoat cracking was found to increase with increased seed weight, decreased density and seed shape irregularity. Bias in tolerance ratings caused by seed weight was minimized by covariance adjustment.

Significant differences were noted in the calcium, anhydrous galacturonic acid and fiber contents of seedcoats of parents. Since none of these factors were significantly correlated with degree of tolerance they were not considered as criteria in selecting for tolerance.

Examination of seedcoat sections revealed that cracking was expressed as the separation of adjacent macro and osteosclerids.

Analysis of data from parental, F_1, F_2, and backcross populations revealed that tolerance to mechanical

damage is a complex trait probably controlled by numerous genes. Heritability estimates for this character ranged from 36 to 64 percent. The majority of the genetic variance was additive, however, there was a large non-additive component. Since there was no evidence of dominance this variation is best attributed to epistatic effects.

Tolerance to mechanical abuse was readily transfered to other genotypes and the resultant lines showed no deterioration in processing quality.

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By

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INTRODUCTION

Seed damage resulting from mechanical abuse of navy beans is a persistent problem despite progress in the development of improved varieties.

Damage is usually manifested as cotyledon splitting or coat cracking but is frequently accompanied by embryo injury and other cryptic damage. These conditions are translated into low germination and reduced seedling vigor for the grower and seed breakdown during canning and a high percentage of defects for the bean processor.

Seed damage is a particularly difficult problem to elucidate as it is strongly influenced by environmental conditions during maturation and during and after harvest. Because of this interaction and because the handling operations, which contribute to damage, occur in varying environments any improved variety will of necessity have to be adaptable.

Many of the older varieties, for example, Michelite, were quite tolerant of abuse. However, in the development of earlier varieties some tolerance was lost. Perhaps this was due to insufficient selection pressure for this character or a subtle association between tolerance and late maturity.

This study was undertaken to determine what range of tolerance to mechanical abuse was available to the breeder, and to combine the superior genotypes to produce a line with the best possible tolerance over a wide seed moisture range. In addition, tolerant and susceptible lines were studied for consistent differences in chemical, physical, or anatomical make up of the coat aimed at developing a rapid and accurate technique for the selection of tolerant lines. An understanding of the genetic control of tolerance was also sought to facilitate its transfer to other genotypes.

LITERATURE REVIEW

Studies dealing with the seedcoat of _Phaseolus_ are neither new nor unique. Originally emphasis was placed on testa development for academic and taxanomic purposes. Studies then shifted to investigations of the seedcoat as a barrier to water up-take. More recent studies have been the by-product of investigations dealing with the effect of mechanical damage on germination rates. Presently both processors and consumers are more quality oriented, and concerned with damage as it affects dry bean quality.

Anatomical studies

Among the more exact and comprehensive botanical studies were those of Haberlandt (8) and Pammel (18) who noted that the anatomy of the bean seedcoat differs little from the general pattern of the _Leguminoseae_. There are four basic layers, the cuticle, macrosclerids, osteosclerids and collapsed parenchyma cells. The studies of Reeve (22) and Sterling (25) very clearly elucidated the developmental pattern. Reportedly the outer integument arises from protodermal cells, whereas, the inner integument is derived from periclinal divisions of the outer integument (6).

Coat development is usually initiated at the striophiolar end with the lateral walls as a secondary site (22). Approximately four days after fertilization the outermost cells of the outer integument begin to undergo periclinal elongation and assume their characteristic rod shape. Anticlinal division continues in this region until the secondary walls begin to form. The inner integument develops independently but is considered unimportant as it begins to collapse after ten days. At this time the cuticle begins to form and active cell division in the macrosclerid portion of the seedcoat stops. Subsequent growth is extension rather than active growth. The next week brings a further breakdown of the inner integument leaving only a layer of collapsed aprenchymous tissue.

After one month the macrosclerids have become thickened with lignin laid down as longitudinal ridges constricting the lumen, particularly at the apical end. Thus, the typical fluting and star-shaped upper surface identify the macrosclerids.

The maturation sequence of the osteosclerids begins after the macrosclerids of the striophiolar region cease dividing. The cells undergo anticlinal division for a short period followed by differential mid-wall thickening. This coupled with continued end wall growth produces the typical bone-shaped osteosclerids.

Although considerable secondary thickening has taken place up to this point, the cells still retain plasticity to accommodate fluctuation in seed size that accompanies maturity. Reeve (21) feels that this is made possible by the pentosan-cellulose matrix. As long as the tissue is hydrated, it is elastic, but once the cells begin to dry and shrink there is an increase in brittleness due either to an increase in crystallization or change in cellulosic orientation.

There is general agreement based upon studies of the seedcoat as a barrier to water uptake, that there is no visible anatomical difference to account for variation in permeability (24,28). This also may hold true for resistance to mechanical abuse. Atkin (1) however, found that with snap beans, lines tolerant to abuse had coats that adhered more tightly to the cotyledon than did susceptible lines.

Physical-environmental studies

The amount of coat damage is directly proportional to the amount of impact the seed receives (2,12). In addition, the moisture content of the seed is critical. For instance, Bainer (2) found that by dropping lima beans, variety Henderson, at 10.4 percent moisture, he could inflict coat damage in 38.1 percent of the seeds, but at 16.3 percent moisture the damage was reduced to only 1.7 percent. These findings were confirmed for navy beans (3,19,26). Perry (19) also found that damage decreased with increased temperature.

Chemical studies

Considerable research has been devoted to the chemical analysis of bean seeds *per se*; unfortunately, this is not true for specific tissues like the seedcoat.

The coat comprises 8 percent of the total seed weight and upon soaking will contain approximately 77 percent moisture, whereas the cotyledon without the coat, will contain only 54 percent moisture (20). The high water content of the coats has been ascribed to the hydrophilic nature of the cellulose and pectic material. Ott and Ball (17) report 19 percent polyuronide in the coats of Michigan navy beans, quite comparable to the 19-24 percent calcium pectate found by Snyder (23). She also reported that the coats contain 5-8 percent ash, of which 30 percent is calcium. One of the few papers dealing with differences in chemical composition affecting rate of cracking is that of Kannenberg and Allard (13). They found that mechanical harvesting of lima beans caused coat damage in 94 percent of the beans of white varieties but only 45 percent in coloured varieties. They attributed these differences to variations in seedcoat thickness (white = 81μ, coloured = 102μ) and lignin content (white = 1.1%, coloured = 15.1%). An enzymatic block in the shikimic acid pathway that prevented the formation of precursors of pigments and lignin was postulated.

Genetic studies

Differential response to abuse does exist among bean varieties. Barriga-Solorio (3) found that at 9.7 percent moisture Michelite had 18.9 percent coat damage while Sanilac had 27.8 percent. Other selections varied from 15.2 to 35.5 percent. Atkin (1) noted similar differences in snap beans.

Although there are strong environmental interactions that affect the expression of coat damage, there is genetic diversity for tolerance. What is necessary is a better understanding of susceptibility to enable breeders to select superior genotypes and manipulate them in breeding programs.

MATERIALS AND METHODS

Initial screening of beans to establish the range in tolerance

Navy beans were collected from diverse sources to insure that available genotypes would be adequately sampled. Fifty-four advanced generation selections were obtained from the Michigan program (F_6), 97 from the Ottawa program (F_{11}) and 71 white, field and horticultural beans from the Regional Plant Introduction Station (P.I.) Geneva, New York. Both the Ottawa and P.I. lines had been grown and selected for one generation at the Canada Department of Agriculture Research Station, Morden, Manitoba.

All entries were hand threshed to avoid uncontrolled damage. The beans (except for the P.I. lines) were screened so that they passed through a 22/64 inch but not a 12/64 inch circular screen. This insured that they were within acceptable commercial ranges. Any "off-types" due to disease or insect damage were removed.

The samples were then equilibrated to a standard moisture content in a germination chamber equipped with a circulating fan. A relative humidity of 50 to 55 percent was maintained with a saturated manganous chloride

($MnCl_2 \cdot 4H_2O$) solution at 20 C. After 14 days the moisture was stabilized at 12 $\pm$ 0.3 percent.

Two 100 seed samples from all entries were damaged by threshing simulation. The device used was a modification of the Barriga-Solorio (3) model where the beans were fed into the device singly and struck by a paddle rotating at approximately 900 r.p.m. This drove the beans against a deflection plate then into a collection tube. Under this system all beans received two impacts. Damage was subsequently assessed by soaking the beans in water for one minute, then rating them for presence of coat damage. In the initial trials the samples were also rated for cotyledon breakage.

The most tolerant and the most susceptible six percent were selected from each source. The resultant 32 lines were planted in 1966 and re-analysed under Michigan growing conditions. Based on this second cycle, the following six entries were selected as parents:

<u>tolerant</u>, lines 57, 70, 77.

<u>susceptible</u>, lines 64, 74, 75.

<u>Genetic analysis</u>

Standard tolerant x tolerant, tolerant x susceptible and susceptible x susceptible crosses were made in the fall of 1966. Backcrosses were made in the spring of 1967. Since the seedcoat is maternal tissue the F_1 lines were allowed to self in the greenhouse to produce F_2 seed for field planting. Phenotypic F_2 seed was avilable for testing in the fall of 1967. Following analysis, twenty F_3 families of the cross 64 x 77, forty of 57 x 74 and forty of 57 x 77 along with suitable checks and parents were sent to Florida for increase. A low yield was obtained, consequently, the sample size for F_3 analysis was reduced to fifty seeds per plant.

Chemical analysis

A random sample of 25 beans was removed from each selection to be tested. Coat removal was facilitated by scoring the surface with a razor then soaking in distilled water. After 30 minutes the testa could be removed with forceps. The excised coats were dried overnight at 105 C. and ground to pass through a 40-mesh screen.

The tissue was analysed for uronic acids, as a measure of pectin in the seedcoat by the Bitter (4) modification of the McComb carbazole method (15). Standard methods of desugaring, cation sequestering and saponification were employed. The pH was adjusted to 5.6 and the sample incubated with 10 mg. pectinase per 100 mg. tissue for 3 hours on a shaker at room temperature. The uronic acid was reported as mg. anhydrous uronic acid (AUA) per gram dry tissue.

The calcium content of the coats was determined as both total and enzymatically extractable calcium. The latter was considered to be that involved in pectin linkages, thus freed during digestion with pectinase, consequently, aliquots were removed from samples being analysed with uronic acids.

All samples were put into solution with 2N HCl then adjusted to a final concentration of 5 percent HCl and 1 percent La_2O_3. The latter was necessary to stabilize the calcium absorption band. The samples were analysed on a PERKIN-ELMER 303 Atomic Absorption unit at a wavelength of 4227 Å following standard operating procedures (5).

The seedcoats were analysed for total fiber (cellulose and lignin) using the acid detergent method of Van Soest (27). A sample size of 250 mg. was used.

Histological examination

The best method of obtaining adequate coat selections was to hydrate pieces of excised coats to twenty percent, fix in chrom-acetic acid then run through the standard infiltration and embedding schedule. It proved too difficult to infiltrate whole or even half seeds when they were mature. The sections were stained with Bierhorsts triple-stain (13). They were deparaffinated and stained with safranin-hematoxylin for 30 minutes, then counterstained with analine blue for 1 minute. Safranin provided background stain while analine blue was specific for cellulose.

Canning evaluation

The six parental lines, with Seafarer and Sanilac as checks, were evaluated for their canning quality. They were tested at two moisture levels, low (13%) and high (16%) and at two damage levels, normal field run with and without threshing simulation. The beans were processed and evaluated following the standard procedures used by the MSU Food Science Department. The beans were pre-soaked for one hour at 140 F. The cans were filled with 8 ounces of beans, topped with boiling brine, then cooked for 45 minutes at 240 F. The samples were scored for appearance, texture, defects and drained weight.

RESULTS AND DISCUSSION

1. Initial screening for lines tolerant and susceptible to mechanical abuse

Three unrelated sources of genetic material representing advanced generation navy bean selections and a range of horticultural types were subjected to mechanical damage and rated for their reaction. Substantial variability was encountered (Table 1).

Table 1. Response of bean selections to mechanical abuse at 20 C. and 12 percent moisture.

Source	No. tested	No. selected	Mean tolerance, %	range, %	s
P.I.	71	7	27.0	2-67	13.1
Michigan	54	12	56.1	39-73	8.4
Ottawa	97	13	38.2	16-68	11.8
Total	222	32			

Six lines that consistently represented the extremes in tolerance and susceptibility in 1965 and 1966 were selected and used as parents in the breeding program (Table 2).

Despite a change in evaluating methods there was good agreement between seasons (r = 0.95 **, 4 df).

Table 2. Damage response of lines selected for parental material grown in 1965 and 1966.

	tolerance, %	
Selection no.	1965 crop	1966 crop
57	58	96
77	64	94
70	47	89
64	10	24
75	16	48
74	17	58

2. Effect of seed moisture content on coat cracking

The moisture content of the beans at time of damaging was important. Less than 14% moisture was required to detect significant differences among lines but unless the moisture was above 10% the damage was expressed as cotyledon shattering rather than coat cracking. Since the majority of Michigan beans are handled and subjected to damage at moisture levels above 10%, selection should take place in practical ranges. Thus, 11-13% was chosen for all damage screening.

All lines would probably not react similarly to damage over a range in moisture content due to subtle differences in structure and/or amount of hydrophilic material in the coat and cotyledon. This assumption was tested by damaging the parental lines at moisture contents ranging from 7-17 percent. Each line had its own characteristic response curve, but within the 11-13 percent test range they maintained their reported ranking (Figure 1).

In most lines there was little improvement in tolerance from 7-9 percent moisture. This was probably due to the high rate of shattering masking true coat damage. Line 70 however, was an exception as it had significantly less shattering, 6.5 percent compared to an average of 11.5 percent for all other lines. A cross between lines 70 and 57 might produce progeny that have the attributes of both parents enabling them to tolerate mechanical abuse over a wide range of moisture.

Numerous authors (7,10) have reported that beans soak at significantly different rates. If this applied to hydration in the 11-13 percent range, variation in moisture content could drastically bias cracking results. The parental lines were hydrated from an average of 7.6 percent to 16.0 percent in 17 days. The hydration rate among lines was found to vary significantly (Table 3).

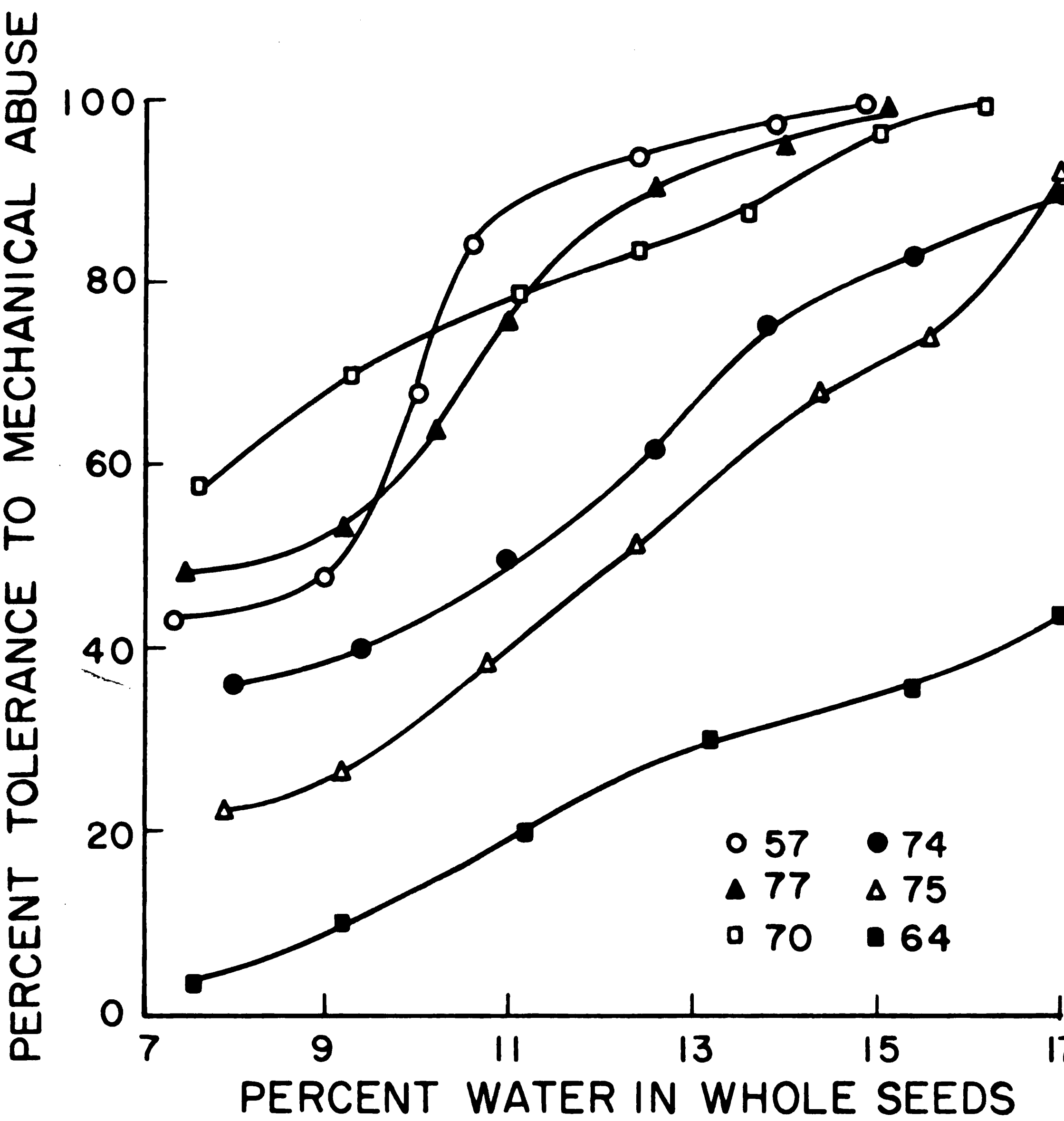

Figure 1 Response of parental lines to mechanical abuse at various moisture levels.

Table 3. Average rate of hydration for parental lines.

Days of hydration	Mean % H_2O	Range, %	s
0	7.6	7.2-7.9	0.35
4	10.0	9.2-10.8	0.66
8	12.2	10.9-13.3	0.93
12	14.2	12.9-14.9	0.82
16	15.7	14.7-16.6	0.73
5 days constant	15.9	15.5-16.2	0.28

To overcome this potential problem, all stored seeds with less than 10 percent moisture were hydrated in the following manner. The beans were exposed to a relative humidity of 70-80 percent for two days, then slowly equilibrated to 12 percent over a 12 day period at a relative humidity of 55 percent at 20 C. The differences among lines were non-significant.

3. Effect of seed weight on coat cracking

The screening phase of this project involved testing lines of considerable weight variation, consequently, it was deemed advisable to determine what effect, if any, weight had upon cracking. Entries from each of the three genetic sources were mechanically damaged and weighed. In all cases tolerance to mechanical abuse declined as the seed weight increased (Table 4).

Table 4. Relationships between weight and coat damage.

Source	No. entries	Mean wt. gms.	s wt.	Mean tolerance, %	s tolerance	r
P.I.	31	27.4	6.24	28.8	14.33	-0.585**
Michigan	54	22.8	1.98	56.0	8.36	-0.117ns
Ottawa	96	19.8	1.75	38.0	12.28	-0.278**

Since the correlations between these two variables was confounded with genetic variation it did not represent a valid relationship. To overcome this objection, the influence of weight differences within lines was then examined. An extreme situation was created by screening and separating seed from Sanilac, Seafarer and three parents, 57, 77, and 75 into two size groups, those that passed through a 20/64 screen but not through a 16/64 screen and those that passed through the 16/64 screen but were held on a 12/64 screen. The beans were damaged and variations attributable to seed source and seed size were found to be highly significant (Table 5).

In addition, a highly significant negative correlation was obtained between degrees of tolerance and with-in line weight variation (r = -57.1** 28df) supporting the original observation that tolerance increased as the weight decreased.

Table 5. **Analysis of variance of a split plot experiment involving the effect of seed source and seed weight on a degree of tolerance.**

Source of variation	df	MS	F
Blocks	2	2.04	
Genetic lines (A)	4	147.46	18.25**
Error (a)	8	8.08	
Seed size (B)	1	202.80	23.23**
AB	4	3.22	
Error (b)	10	8.73	
Total	29		

It should be re-emphasized that the preceding experiment involved extreme weight differences. The majority of seeds within a line approach the mean with only a few representing the extremes. Nevertheless, would the small variations that naturally occur between beans in a line affect cracking? Seeds from both P.I. and Michigan sources that had previously been subject to damage evaluation were divided into tolerant and susceptible groups and weighed. The tolerant seeds from the Michigan program were found to average 0.08 gms. heavier than the mean weight, whereas the tolerant seeds from the P.I. sources were 0.03 gms. lighter than their respective mean weight. With-in line weight variation could not be considered a factor influencing rate of cracking.

4. Effect of shape on coat cracking

The shape and volume of navy beans does not vary greatly, however, because of the diversity of the test material in this project both factors had to be considered.

Bean shapes ranged from nearly spherical to flattened, sharp-ended kidney, types. Considering resistance to external stress, a perfect sphere is the strongest rounded object and any shift from this would lead to progressive weakening. This assumption was tested for coat strength by rating 31 P.I. lines on a 1-5 basis (1 = sphere, 5 = flattened). Tolerance was found to decline as the shape became more irregular (r = 0.38*). When seed damage was divided into coat cracking and cotyledon shattering, shape affected cracking (r = 0.55**) much more strongly than shattering (r = 0.09 ns).

Apparently flatter beans have sharp ends which are particularly weak and susceptible to coat cracking. This may result from small areas of the seedcoat absorbing the complete stress load and not being able to transfer it to nearby cells.

5. Effect of seed density on cracking rate

There was no consistent relationship between seed density and degree of tolerance. However, if the susceptible fraction was again divided into those with cracked seedcoats and those with cotyledon shattering, patterns became evident (Table 6).

Table 6. Correlation of seed density with coat cracking and cotyledon shattering in Plant Introduction (P.I.) and Ottawa entries.

Source	No.	Cracking	Shattering
P.I.	31	-0.409*	0.266ns
Ottawa	96	-0.433**	0.170ns

Although there is increased cotyledon shattering at higher densities, it is not significant. But increased density does significantly reduce the amount of coat cracking. This may result from a mechanical relationship between the two structures. As the cotyledom becomes denser it also becomes less plastic, therefore, any external stress applied to the coat will be transmitted more directly to the cotyledon and not dissipated by coat movement and/or cracking.

6. <u>Effect of seed coat anatomy on cracking</u>

Differences in the seedcoat thickness should have a direct influence on resistance to cracking (2). However, after examining the six parental lines, it was concluded that variations in coat thickness were too small to affect tolerance (Table 7).

Damaged seedcoats were examined for cracks and areas that might indicate structural weakness. All cracking occurred between adjacent macro and osteosclerids with no evidence of intra-cellular breakage (Figure 2). This area may be weak because of a lack of fiber continuity between adjoining cells or incomplete pectin binding.

The parents exhibited considerable variation in amount of collapsed parechyma cells separating the osteosclerid layer from the cotyledon. The tolerant lines usually had a thinner layer which adhered quite tightly to the cotyledon, in fact, so tightly that coat removal was often difficult. Similar findings were reported by Atkin (1).

During the course of this study a number of related observations were made which led to the assumption that the amount of inward coat movement directly affects cracking. During abusing, the susceptible lines lacked sharp impact noise and appeared to absorb a great deal of the impact. This may, in part, be explained by a thicker spongy parenchymous layer and a lower cotyledon density allowing the coat to be forced inward thereby creating an uneven stress at the point of maximum coat deflection. If, as in the tolerant lines, the parenchmous layer is contiguous with the coat and a high density cotyledon, there may be less movement with

Table 7. Analysis of parental lines for chemical and physical components (average of three replications from a random sample of 1967 seed).

	Whole seed				Seedcoat dry wt. basis			
Parent	Wt./100, gm.	Vol./100, ml.	Density	H_2O uptake 3 hr., %	AUA, mg/gm	Fiber, %	Total Ca. mg/gm	Coat thickness μ
57	16.8	12.2	1.37	76	256	60.7	18	80
70	15.6	11.2	1.38	65	214	59.9	14	80
77	17.7	13.0	1.36	74	218	60.9	13	84
Average	16.7	12.1	1.37	72	229	60.5	15	81
64	28.8	24.0	1.20	12	157	54.5	25	83
74	19.6	15.2	1.29	78	234	59.8	21	83
75	20.0	14.8	1.36	90	186	56.8	14	77
Average	22.8	15.0	1.28	60	192	57.0	20	81

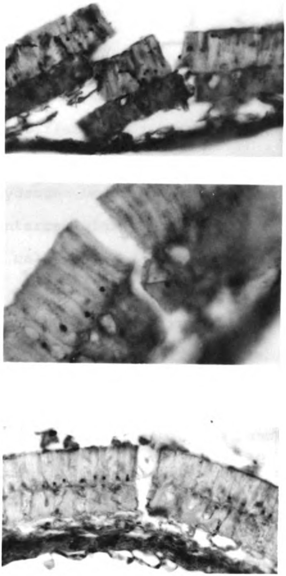

Figure 2. Types of seedcoat cracking (magnification a,c = 960 X, b = 2250 X).

the stress being transferred to and absorbed by the cotyledon rather than expressed as intercellular separation.

7. Effect of pectic substance content on coat cracking

The weakest portion of the seedcoat appears to be between macrosclerids where the pectic substances act as a binding material. Differences in amounts of polygalacturonic acid and types of linkage, for instance, esterification, cation bonding, hydrogen bonding, etc., might influence the strength of the intercellular connections.

The total calcium, anhydrous galacturonic acid and methanol contents of the seed coats of the parental lines were determined and the treatment variations for all except methanol content found to be significant (Table 8).

Table 8. Analysis of variance for total calcium, anhydrous galacturonic acid (AUA) and methanol in parental lines.

Source of Variation	df	Total Calcium MS	Total Calcium F	Total AUA MS	Total AUA F	Total Methanol MS	Total Methanol F
Treatment	5	46.36	20.60**	2449.00	4.79*	5.90	2.47ns
Error	6	2.25		511.67		2.43	
Total	11						

However, none of these factors correlated significantly with tolerance to mechanical abuse (total calcium r = 0.66, **AUA** r = 0.81, methanol r = 0.51 for 4df). In addition, when segragating lines of the cross 64x77, which represent the extreme range of both calcium and **AUA** were analysed no correlation with degree of tolerance was observed. Despite the obvious differences in chemical composition, and the observations that the coats of tolerant lines usually had a higher **AUA** and lower calcium content than susceptible lines, the differences were too small and too strongly influenced by environment to be used as selection criteria for assessing damage potential.

8. Effect of seedcoat fiber content on coat cracking

The parental lines were tested for acid detergent non-soluble fiber which corresponds to crude fiber under the proximate analysis system. Among-parent variation was found to be significant (F = 4.425* 12,5 df) with the tolerant lines averaging 60.5 percent fiber and the susceptible lines all lower with an average of 57.0 percent (Table 7). Although the range of fiber content in the parents was not great (54.5 - 60.9), it was highly correlated with degree of tolerance (r = 0.93** 4df). However, when segregating lines of the cross 64x77 were tested, the parental types were recovered but there was little correlation of fiber and cracking (r = 0.29 28df). Once again this points out the difficulty of utilizing small differences in chemical composition as a tool for evaluating segregating populations.

9. Genetic control of tolerance to mechanical abuse

It is evident from previous sections that seed weight influences the degree of damage. A more accurate estimate of gene controlled tolerance to abuse was obtained by removing the weight covariate, thereby making the tolerance variance independent of the weight variance. The following formula was applied:

$$y_i = y_i + b_{yx} (\bar{x}_1 - x_i) \quad \text{where } y_i = \text{percent tolerance}$$

x_i = weight, gm/100 seeds

b_{yx} = regression coefficient

All crosses except those involving parent 64, in which weight and shape played an integral part in low abuse tolerance, were corrected for weight bias. In cases where there was a significant correlation between weight and tolerance the covariance correction reduced the overall tolerance variance by shifting the extreme segregates toward the mean.

All crosses involved one parent that was considerably more tolerant to abuse (85 - 100%) than the other. Because of this and the imposed boundary to the upper range due to percentage data, the segregates were skewed toward the 100 percent limit. This non-normal distribution prevented the parental variances from being proportional and accurately compared. To improve linearity of data, numerous transformations were attempted. The most suitable appeared to be a square root transformation where the percent tolerance was first subtracted from 100 (Table 9). Somewhat similar results were obtained with arcsin transformations. Once again parent 64 was not adjusted since both extremes were represented and the F_2 distribution appeared normal.

In most cases the F_1 and F_2 means fell very close to the mid-parent values, consequently, there was little evidence that the genes controlling tolerance exhibited dominance. No differences were found between reciprocal crosses so F_2 populations were reported as composites. Crosses that involved extreme differences, for example 64 x 77 (Table 10), had only a small number of segregates attaining parental extremes and no cases of transgressive segregation. This indicated that at least two genes were involved in the expression of tolerance. Because of the relatively narrow range (Table 11) between parents in other crosses and the size of the parental variance, it is difficult to make further statements about gene number.

The variance from parental, F_1, F_2 and backcross populations of crosses 64 x 77 and 57 x 70 were partitioned into additive (D), non-additive (H) and environmental (E)

Table 9. Frequency distribution and means of tolerance to mechanical abuse for parental, F_1, F_2 and backcross populations of the cross 57 x 70.

a) Tolerance, percent

Lower class limit	70	75	80	85	90	95	No.	Mean
57					16	10	26	93.4
BC_1			3	2	4	1	10	88.6
F_1			1	5	5	1	12	89.5
F_2	2	4	20	40	45	12	123	88.3
BC_2		4	7	6	5		22	84.6
70			5	12	7		24	87.1

b) Tolerance $\sqrt{100 - x_2}$ transformation

Upper class limit	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5	
57					5	6	13	2	2.27
BC_1			3	1	3	1	2		3.30
F_1			1	3	4	1	3		2.92
F_2	2	4	18	22	40	21	12	4	3.09
BC_2		4	8	4	5	1			3.71
70			4	5	11	4			3.44

Table 10. Frequency distribution and means of tolerance to mechanical abuse for parental, F_1, F_2, and backcross populations of the cross 64 x 77 and parental and F_2 populations of the cross 57 x 64 (non-transformed data).

Lower Class Limit	Tolerance, %																		No.	Mean
	15	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95		
Cross 64 x 77																				
64	2	5	10	6															23	20.9
BC_1				2	3	3	1	2	3	2									16	41.4
F_1								1	2	5	3	1							12	57.5
F_2		1	4	5	8	11	19	14	27	26	23	27	23	15	5	5	2		217	57.3
BC_2								1	2	3	2	2	4	2	2	3			19	68.1
77														1	3	2	14	11	31	93.5
Cross 57 x 64																				
64	2	5	10	6															23	20.9
F_2			1	1	3	6	4	8	5	10	5	3	4	2	0	1			53	52.0
57															3	13	12	5	33	90.3

Table 11. Frequency distribution and means of tolerance to mechanical abuse for parental and F_2 populations of crosses 57 x 74 and 57 x 77 (weight transformed).

	Tolerance, %										
Lower class limit	55	60	65	70	75	80	85	90	95	No.	Mean
Cross 57 x 74											
57						3	13	12	5	33	90.3
F_2	10	8	17	26	38	57	66	25	9	258	80.4
74	5	8	7	7	2					29	65.3
Cross 57 x 77											
57						6	23	26	2	57	89.4
F_2			3	11	32	64	48	15	3	176	82.9
77		2	4	7	3	3	1			20	73.8

variances (Tables 12, 13). Heritability estimates were also made for degree of tolerance to mechanical abuse.

Cross 57 x 70 had a tolerance heritability of 35 percent with additive, non-additive and error variances of 18.34, 14.08 and 13.73 respectively. When the square root transformation was applied, heritability increased to 41 percent with a decline in the non-additive component. This was probably due to increased linearity. Taking into consideration the partial overlap of the parental populations and the relatively large error variance, these estimates are considered representative.

Cross 64 x 77 had similar additive and non-additive trends (D = 311.5, H = 209.3), but in this case heritability was higher (narrow sense, 64%). The relatively large non-additive genetic component suggested inter-allelic interaction (dominance). However, there was no indication of dominance in either the F_1 and F_2 means or distribution. An alternate explanation was non-allelic interaction (epistasis). The non-additive variance could not be directly separated into dominance and epistatic components since the variances had been partitioned by the Mather method which excludes epistasis. The cross was re-analysed with a model utilizing generation means from the parents, F_1, F_2 and backcrosses (16). The model was fitted by least squares and a highly significant additive and epistatic variance noted (Table 14).

Table 12. Means and variance of tolerance to mechanical abuse for parental, F_1, F_2 and backcross populations of the cross 57 x 70 (weight transformed).

Source	No.	Mean tolerance, %	Variance	$\sqrt{100-x_i}$ Mean tolerance %	Variance
57	26	93.4	11.76	2.27	0.186
BC_1	10	88.6	18.00	3.30	0.427
F_1	12	89.5	15.00	2.92	0.375
F_2	123	88.3	26.42	3.09	0.560
BC_2	22	84.6	25.67	3.71	0.465
70	24	87.1	14.67	3.41	0.241
D = 18.34		h^2 = 35%		D = 0.456	h^2 = 41%
H = 14.08				H = 0.026	
E = 13.73				E = 0.267	

Table 13. Means and variances of tolerance to mechanical abuse for parental, F_1, F_2 and backcross populations of the cross 64 x 77 (non-transformed).

Source	No.	Mean tolerance, %	Variance
64	23	20.9	20.18
BC_1	16	43.4	143.56
F_1	12	57.5	31.54
F_2	217	57.3	243.27
BC_2	19	68.1	187.23
77	31	93.5	43.88

D = 311.50
H = 209.28
E = 31.87

h^2 = 64%

Table 14. Analysis of variance of generation means of the cross 64 x 77.

Source of variation	df	Mean square
Additive	1	305.1**
Dominance	1	1.4
Epistasis	3	19.0**

**indicates significance at the 1% level.

It is particularly enlightening to note the non-significant dominance effect. Apparently the non-additive variance was almost entirely epistatic in nature. However it is not known whether this interaction occurs at the level of primary gene function or among the numerous components of tolerance (6). In any case, this investigation has shown that it was possible to successfully transfer tolerance from one geneotype to another.

10. <u>Effect of tolerance to mechanical abuse on processing quality</u>

An important consideration in the development of damage tolerant lines was the maintenance of processing quality. A selection would be of little value if it resisted mechanical abuse but processed poorly. To avoid this problem, the parental lines and two check varieties, Sanilac and Seafarer, were tested for rate of water uptake and general processing quality. Differences in hydration were quite pronounced during the first three hours, however, after 18 hours the differences were not significant (Table 9). In addition, the tolerant lines reacted very well under actual processing conditions with an average of 70% uptake compared to 70% for the check varieties. An uptake of 80% or greater is sought for processing.

Table 15. Relative water uptake of Sanilac, Seafarer and parental lines after 1, 3, and 18 hours in distilled H_2O at 30 C.

	Percent uptake after		
Lines	1 hr.	3 hr.	18 hr.
Tolerant	40	75	90
Susceptible	28 (41)*	61 (85)	80 (98)
Check Varieties	64	90	97

*figures in brackets represent lines 74 and 75 as line 64 had a large number of hard seeds, 35 percent after 3 hours.

Samples that had been processed after receiving mechanical damage at 13 percent moisture were subjectively rated for consistency of the liquor medium, texture of the beans and for the presence of defects, primarily loose seedcoats. All tolerant lines were judged acceptable (Table 15).

Table 16. Relative quality of navy beans mechanically abused at 13 percent moisture and processed at 245° F for 45 minutes.

Ranking	Material
1	Seafarer
2	57
3	70
4	77
5	Sanilac
6	75*
7	74*
8	64*

*These lines were considered unacceptable because of coat separation and cotyledon breakdown.

CONCLUSIONS

Tolerance to mechanical damage in navy bean lines essentially isogenic for physical characters can be most effectively evaluated by direct cracking analysis under controlled conditions. If non-related genotypes are involved, physical differences such as weight, shape and density must be considered.

Seedcoat cracking was found to increase as seed weight increased, as seed density decreased and as shape became more irregular.

Tolerance bias due to weight differences is very important but can be reduced by making the tolerance and weight variances independent with a covariance correction.

An examination of seedcoat sections revealed that cracking occurred between adjacent macro and osteosclerids with no evidence of intercellular breakage.

Significant differences were observed in the calcium, anhydrous galacturonic acid and fiber contents of the parental seedcoats. However, for all practical purposes these can be ignored. The differences were too small, too strongly influenced by environment, the analysis too slow for routine determination and most importantly there was too strong an interaction among components. The chemical components appeared to exert their effect as a complex rather than individually and directly.

It is speculated, based on density and anatomical observations, that the amount of inward coat movement following impact directly influenced coat cracking. If the cotyledon density was low and the parenchymous layer thick, the seedcoat may have been forced inward beyond a critical point, thereby, creating a stress that could only be dissapated by cellular separation.

The genetic study indicated that degree of tolerance is a complex trait controlled by numerous genes. Heritability estimates ranged from 36 to 64 percent depending upon the cross and method of partitioning the variance. Most of the genetic variance appeared to be additive although there was a large non-additive component. Since population means and distributions showed no evidence of dominance in the F_1, or F_2 generations, the large non-additive component of variance is best attributed either to interactions of non-allelic genes, or to interaction of the somatic components of the complex trait.

All damage-tolerant lines processed well and produced a quality product.

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