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Inheritance of Seed Coat Cracking and Effective Selection Method for the Resistance in Soybean

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Abstract

Seed coat cracking of soybeans leads to the deterioration of seed quality for products derived from whole beans and a reduction of the market value. In also affects the storage ability of seed and decreases germination. Soybean cultivars differ in the incidence of seed coat cracking. The incidence increased by pod removal treatment and differences among cultivars could be readily detected. Using the pod removal treatment, heritability estimates for the incidence of seed coat cracking in early generations were relatively high. As a result of continuous selection for high and low incidence, heritability estimates for the trait increased and values greater than 0.9 were obtained in the F6 generation. Due to genotypic correlation, incidence of seed coat cracking was negatively correlated with maturity, plant height and 100 seed weight. Treatment with a growth regulator, ethychlozate, had a similar effect to that of pod removal treatment. Thus an efficient selection method to reduce the incidence of seed coat cracking was developed using ethychlozate.

Discipline: Plant breeding
Additional key words: ethychlozate, genotypic correlation, heritability, parent-offspring correlation coefficient, pod removal

Introduction

Seed coat of soybean [*Glycine max* (L.) Merr.] plays an important role in many aspects, but it sometimes shows cracks on the surface under natural conditions. Seed coat cracking results from splitting of the outer cellular layers[12], which begins approximately at the time of physiological maturity[15]. It results in the deterioration of the seed appearance as well as the processing suitability of products derived from whole beans such as natto. Seed coat cracking is considered to be one of the grade determinants in the grain grading system of Japan and can result in a lower market value for soybeans. Since high seed quality of soybean is required for food use, resistance to seed coat cracking is a major breeding objective. As cracked seed coats do not offer optimum protection for the embryo, seeds with cracked seed coats may be affected by adverse environmental conditions. Seed coat cracking also increases the incidence of infection with pathogens compared to intact seeds. Burchett et al. concluded that seedlots of cracked seed coats generally showed a lower level of germination than those of non-cracked seed coats[1]. Therefore, the problem of seed coat cracking is related to both utilization and agronomy.

Several studies on this trait have been previously conducted[9,11,14]. Liu recognized two types, Type I and Type II of seed coat cracking based on genetic studies[5]. He found that Type I was mainly controlled by two recessive genes, de_1 and de_2, and Type II was controlled by two complementary genes, de_3 and de_4. These types of seed coat cracking only occurred in a few cultivars regardless of environmental conditions.

Most cultivars occasionally exhibit seed coat cracking to some extent when exposed to adverse environmental conditions. In contrast to Type I and Type

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II, this type of seed coat cracking does not affect all the seeds. The adverse environmental conditions which can cause seed coat cracking are hot and dry weather during maturation when seeds desiccate rapidly[10]. Wolf et al. suggested that cracking could occur in the field as a result of alternate wetting and drying of mature seed[13]. Since the occurrence of seed coat cracking in cultivars can be readily affected by environmental conditions and shows continuous variations[6], it could be a quantitatively inherited trait like seed coat durability of field pea[7].

Sasaki and Nakamura reported that the occurrence of seed coat cracking could be increased by pod removal and cultivar differences were enhanced by this process[8]. Although the pod removal method may facilitate the evaluation of resistance to seed coat cracking, the method is laborious as it involves manual handling. Thus a more efficient method to estimate the resistance should be developed for practical selection.

The objectives of these studies were to (1) evaluate cultivar differences in the incidence of seed coat cracking using pod removal treatment, (2) determine the inheritance of seed coat cracking and (3) develop an effective selection method to obtain resistant cultivars.

Materials and methods

The variation in seed coat cracking of soybeans was investigated using 92 cultivars in 1982 and 33 cultivars in 1983 grown in field plots. To increase the incidence of seed coat cracking and improve the accuracy of the evaluation, plants from each cultivar were subjected to pod removal according to the method of Sasaki and Nakamura[8], in which half of the pods were removed by hand at 40 days after flowering. After harvest, preliminary data were collected on individual plants, including number of pods, seed yield, 100 seed weight and incidence of seed coat cracking. The visual count of seeds with seed coat cracking per plant was scored as a percentage.

A soybean cultivar, Enrei, was crossed with Tohoku 68 to study the mode of inheritance of seed coat cracking in 1981. Enrei is resistant to seed coat cracking, while Tohoku 68 is susceptible and both of them have a yellow seed coat. F_1 seeds were planted in the greenhouse and harvested individually. Plants from F_2 or more advanced generations were grown 12 cm apart in rows at a spacing of 75 cm in the field. Pod removal treatment as described above was applied and selection for high and low incidence of seed coat cracking was conducted from F_3 to F_6 generations. After harvest, all the plants were threshed in a single plant thresher and the incidence of seed coat cracking per plant was recorded. As the seed coat is a maternal tissue, seed coat cracking in F_2 was scored by examining F_3 seeds derived from F_2 plants and the following generations were also treated in the same way. Based on these data, heritability estimates for seed coat cracking in early generations were calculated as follows:

$$h^2 = \Delta G/S,$$

where h^2 = heritability estimates, ΔG = change in population mean due to selection, S = selection difference[2]. Parent-offspring regression was also calculated as heritability estimates in the same generation. To predict the effectiveness of selection for seed coat cracking, parent-offspring correlation coefficients for the trait were calculated and compared among generations. In the F_6, heritability estimates were determined for several characters based on an analysis of variance and compared with those obtained for seed coat cracking. In addition, phenotypic, genotypic and environmental correlations of seed coat cracking with other characters were estimated in the same generation.

To develop a more efficient method to estimate the resistance, the use of growth regulators was examined. Several growth regulators were tested by foliar application and their effect on the increase of the occurrence of seed coat cracking was evaluated.

Regarding the incidence of seed coat cracking, the values were transformed into $\text{Arcsin}\sqrt{\%}$ and each parameter was calculated. These experiments were conducted at Tohoku National Agricultural Experiment Station as a series of studies on soybean breeding.

Results and discussion

1) Evaluation of seed coat cracking by pod removal treatment

Soybeans grown in the field showed consistent differences in the incidence of seed coat cracking among cultivars ranging from 0 to 32.2% in 1982 and 0 to 29.6% in 1983. However, their mean incidence was very low and the values were less than 1% in the majority of the cultivars. Some cultivars exhibited a marked increase in the incidence when exposed to adverse environmental conditions such as hot and dry weather. Under normal weather conditions, it may be difficult to estimate the resistance

Table 1. Influence of pod removal at 40 days after flowering on some characters[a)]

Treatment	No. of pods (plant^{-1})	Seed yield (g plant^{-1})	100 seed weight (g)	Seed coat cracking (%)
		1982		
Pod removal	17.0	7.0	24.2	9.3
Control	34.0	12.7	20.6	3.2
Pod rem./Cont.	0.50	0.55	1.17	2.91
		1983		
Pod removal	37.0	16.7	26.4	7.8
Control	65.0	26.9	23.5	3.3
Pod rem./Cont.	0.57	0.62	1.12	2.36

a): Data are the mean of 92 cultivars in 1982 and 33 cultivars in 1983.

of cultivars to seed coat cracking. The incidence increased when half of pods were removed, which resulted in the enlargement of the seeds of the remaining pods and the cultivar differences were more readily detected in both years (Table 1). The results agreed with those of Sasaki and Nakamura[8]. Therefore, pod removal treatment exhibited a similar effect on growth under adverse environmental conditions and enabled to estimate the intrinsic resistance of cultivars to seed coat cracking.

Table 2. Cultivar differences in incidence of seed coat cracking by using pod removal treatment

(%)

Cultivar	1982	1983	Mean[a)]
Enrei	0.0	0.0	0.0 a
Nanbushirome	0.8	0.0	0.4 a
Waseshiroge	0.9	0.0	0.5 a
Tamahomare	1.2	0.0	0.6 a
Hatsukari	0.6	0.7	0.7 a
Miyagishirome	0.0	1.4	0.7 a
Kitamusume	0.0	1.5	0.8 a
Norin 4	0.6	1.4	1.0 a
Shirosennari	1.4	1.2	1.3 a
Fukunagaha	0.0	3.5	1.8 a
Nakasennari	3.1	0.5	1.8 a
Nasushirome	1.7	2.7	2.2 a
Fusanari	1.6	3.4	2.5 a
Tachisuzunari	4.9	1.0	3.0 a
Shinanomejiro	3.5	2.4	3.0 a
Tokachinagaha	0.0	6.9	3.5 a
Norin 3	4.8	2.3	3.6 a
Mutsumejiro	4.9	4.7	4.8 a
Raikou	5.6	9.3	7.5 a
Karumai	8.3	7.6	8.0 a
Dewamusume	8.8	7.9	8.4 a
Shinmejiro	17.4	7.4	12.4 ab
Waseshiroge	15.9	10.6	13.3 abc
Toyosuzu	3.1	26.7	14.9 abcd
Karikachi	13.2	39.7	26.5 bcd
Okushirome	29.9	27.4	28.7 cd
Raiden	43.3	17.3	30.3 d
Yuuzuru	67.5	40.9	54.2 e
Mean	8.7	8.2	8.4

a): Means followed by the same letter are not significantly different (P>0.05) by Duncan's multiple range test.

The 2-year evaluation yielded consistent cultivar differences in the incidence of seed coat cracking (Table 2). Enrei exhibited the lowest incidence in both years of evaluation, which suggested that it can be used as a resistant donor.

2) Inheritance of seed coat cracking

In the progenies of crosses, the incidence of seed coat cracking ranged from 0 to 29.7% in F_2 plants and 0 to 34.1% in F_3 lines. Pod removal treatment was not conducted in F_2 plants and most of them exhibited a low level of seed coat cracking. Conse-

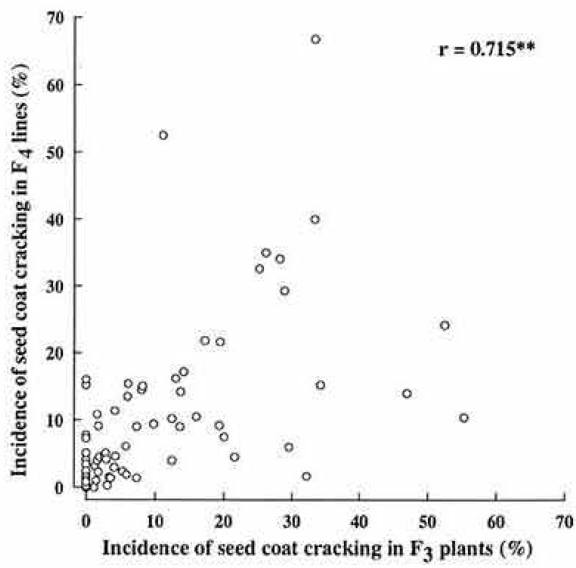

Fig. 1. Relationship between incidence of seed coat cracking in F_3 plants and that in F_4 lines by pod removal treatment

quently the incidence of seed coat cracking in F_2 plants and F_3 lines derived from F_2 plants showed a low parent-offspring correlation. However, the F_3 and F_4 lines subjected to the pod removal treatment in both generations, showed a parent – offspring correlation coefficient of 0.715**(Fig. 1). Heritability estimates for the incidence of seed coat cracking showed a similar tendency, in that the values were much lower in F_2 ($h^2 = 0.05$) than in F_3 ($h^2 = 0.76$). The increase of heritability may be due to the pod removal treatment which attenuated the influence of environmental conditions and improved the accuracy of evaluation. Effective selection for reduced seed coat cracking in the early generations seems thus to be possible by using the pod removal treatment.

As a result of continuous selection for reduced and increased seed coat cracking, the parent – offspring correlation coefficients for this trait increased with later generations (Fig. 2). Heritability for the incidence of seed coat cracking estimated by the analysis of variance in the F_6 generation was high. Among the other agronomic characters, heritability estimates for maturity, seed filling period, plant height and 100 seed weight were relatively high (Table 3). Selection was conducted based on the incidence of seed coat cracking. The data showed that the trait was under moderately strong genetic control and selection for reduced seed coat cracking may be highly effective.

Genotypic correlations of seed coat cracking with other characters were slightly higher than phenotypic ones but they were generally consistent (Table 4). Maturity, plant height and 100 seed weight were negatively correlated with seed coat cracking. It is thus suggested that resistance to seed coat cracking was genetically related to late maturity, tall plant height and large seed size. Of these traits, maturity and plant height were similar in both parents, while seed size of the resistant parent, Enrei, was larger than that of the susceptible parent, Tohoku 68. Thus the relationship between the incidence of seed coat cracking and seed size may be due to the parent combination. The resistance to seed coat cracking was generally considered to be correlated with late maturity and tall plant height. Late maturing cultivars

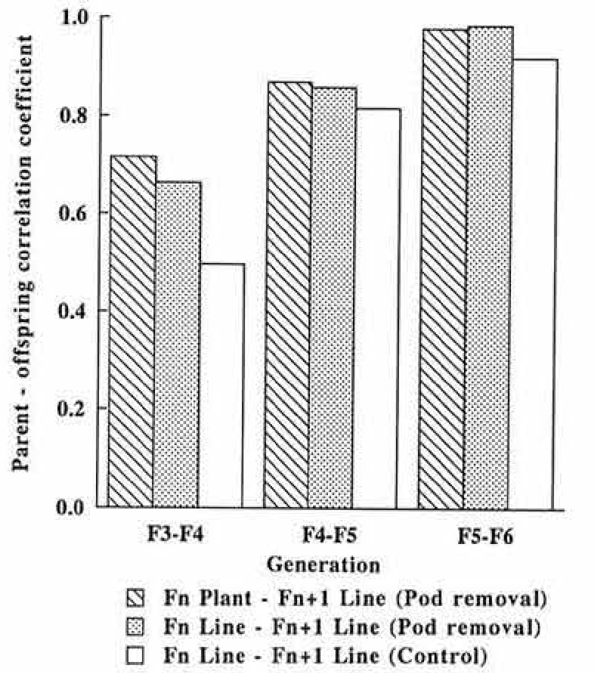

Fig. 2. Parent – offspring correlation coefficients of seed coat cracking with change of generation

Table 3. Heritability estimates for some characters in F_6 lines[a)]

	Seed coat cracking	Maturity	Seed filling period[b)]	Plant height	No. of pods	Seed yield	100 seed weight
Heritability	0.967	0.872	0.718	0.753	0.147	0.122	0.755

a): Estimated by the analysis of variance in the F_6 generation.
b): Duration from R1 to R8 defined by Fehr, W.R. et al.[3)] (1971).

Table 4. Estimates of phenotypic, genotypic and environmental correlations of seed coat cracking with other characters in F_6 lines

Correlations	Maturity	Seed filling period	Plant height	No. of pods	Seed yield	100 seed weight
Phenotypic	– 0.502**	– 0.264	– 0.456**	0.216	– 0.057	– 0.288*
Genotypic	– 0.560	– 0.323	– 0.572	0.439	– 0.356	– 0.382
Environmental	0.149	0.041	0.297	0.307	0.387	0.423

*,** Significant at $P<0.05$ and 0.01, respectively.

Table 5. Effect of rates and time of treatment with ethychlozate on seed coat cracking

(%)

Cultivar	Control	Pod removal	Application of ethychlozate*					
			100 ppm			200 ppm		
			20	30	20 + 30 (days)	20	30	20 + 30 (days)
Waseshiroge	6.7	28.9	29.8	8.6	29.2	25.5	23.7	26.5
Enrei	0.5	0.2	1.9	0.9	0.8	0.9	0.3	1.6
Okushirome	34.3	66.1	61.6	66.3	80.9	67.3	42.9	85.4
Suzuyutaka	13.4	29.8	18.5	25.3	31.3	24.6	24.5	40.2
Nattoshoryu	0.9	16.7	9.7	12.2	24.2	7.0	19.1	33.9
Miyagishirome	3.9	4.7	6.8	2.9	3.7	5.1	7.1	6.0
Mean	10.0	24.4	21.4	19.4	28.4	21.7	19.6	32.3

* Upper row: Application rates of ethychlozate,
Lower row: Application times expressed by the number of days after flowering.

are usually tall and the seeds may desiccate slowly because the temperature during maturation is relatively low. Therefore, it appears that maturity or plant height is not genetically related to seed coat durability in itself.

3) Development of effective selection method

Heritability for the incidence of seed coat cracking was relatively high when the pod removal treatment was applied, indicating that the selection for reduced seed coat cracking was effective by using the pod removal treatment. Although pod removal could facilitate the evaluation of resistance to seed coat cracking, this method is laborious and restricts the number of test materials that can be handled. Thus a more efficient method to estimate the resistance was developed for practical selection.

An artificial growth regulator, ethychlozate (ethyl-5-chloro-3(1H)-indazolylacetate), displays a physiological activity comparable to that of auxin and is used as a fruit thinning agent for citrus[4]. It promoted pod abortion by foliar application and increase of seed coat cracking of remaining seeds when applied to soybean. The results showed that ethychlozate treatment had a similar effect to pod removal treatment. There was no difference in effectiveness between application rates for early or medium maturing cultivars, but 200 ppm application was more effective than 100 ppm for late maturing cultivars such as Miyagishirome. Two applications at 20 and 30 days after flowering were more effective than other applications tested (Table 5). These results show that application of ethychlozate at the rate of 200 ppm at 20 and 30 days after flowering is the most effective treatment for evaluating seed coat cracking. It is a more efficient method to evaluate the resistance to seed coat cracking than manual pod removal. As the main agronomic characters except for seed quality and maturity were not influenced by the ethychlozate treatment, it was considered that this method is suitable for selection to reduce the incidence of seed coat cracking.

Since the heritability estimate for seed coat cracking in early generations is relatively high, selection using ethychlozate treatment may be effective from early generations. Maturity and plant height should be considered in a practical selection.

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