Physiological and Genetic Analysis of Pod Shattering in Soybeans

By TAKEHIKO TSUCHIYA*

Hokkaido Prefectural Tokachi Agricultural Experiment Station
(Memuro, Kasai, Hokkaido, 082 Japan)

Mature pods of soybean (*Glycine max* (L.) Merrill) burst open along the dorsal and ventral sutures, and scatter their seeds under less humid conditions. The dehiscence of soybean pods is one of the major obstacles to harvesting by machines because the harvest loss increases due to scattering seeds. Therefore, a high degree of resistance to pod shattering is essential if soybeans are to be grown commercially with mechanization.

The objectives of the present study were: (1) to clarify the anatomical structure of pods related to shattering, (2) to investigate environmental factors inducing dehiscence, and establish an effective method of testing pod shattering, (3) to classify the varietal difference of pod shattering, (4) to estimate the heritability of pod shattering, (5) to estimate the minimum number of genes for the resistance to pod shattering, and (6) to introduce resistance genes into determinate-type varieties of Hokkaido with large seeds. The study was carried out at the Tokachi Agricultural Experiment Station during the years, 1970 to 1985[11-14].

Anatomical observation

Twenty-six varieties were used to clarify the relation between the degree of pod shattering and the shape and size of pods. Length (A), width (B), thickness (C), degree of curvature (D/A), and wall thickness (E) of pods were measured with each of 50 pods containing three seeds per pod (Fig. 1). The degree of pod shattering was measured after the pods were placed in a dryer (using forced circulation of heated air, 150 × 150 × 85 cm volume) kept at 60°C for three hr.

The varieties examined were distinctly divided by the degree of pod shattering into two groups, the susceptible one composed of varieties which showed the shattering of more than 78% of pods (average of 18 varieties: 94.1%), and the resistant one composed of varieties showing less than 19% of pod shattering (average of 8 varieties: 5.9%). However, as shown in Table 1, there was no significant difference in pod characteristics, except C/B, between the two groups. The value of C/B of the resistant varieties was a little higher than that of susceptibie ones. It seems that the size and shape of pods are not directly related to pod shattering.

In addition, the pods of Kitamusume (susceptible) and Toiku 208 (resistant) were selected for microscopic observation, because they showed a wide difference in the degree of pod shattering but were very similar in size and shape of pods. Twenty-one mature pods of Kitamusume and 17 of Toiku 208

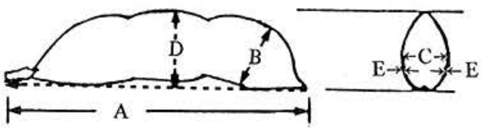

Fig. 1. Items of measuring the shape and size of pods

Present address:
* Hokkaido Prefectural Kamikawa Agricultural Experiment Station (Shibetsu, Hokkaido, 095 Japan)

Table 1. Comparison of pod characteristics between susceptible and resistant varieties to pod shattering

Pod characteristics	Means of		Significance by t test
	Susceptible varieties* (n = 18)	Resistant varieties* (n = 8)	
Length of pod (A) (mm)	51.8	47.1	n. s.
Width of pod (B) (mm)	11.2	10.5	n. s.
Thickness of pod (C) (mm)	8.0	8.1	n. s.
Thickness of wall (E) (1/10 mm)	1.44	1.51	n. s.
B/A	0.22	0.22	n. s.
C/B	0.72	0.77	5 %
Degree of curve (D/A)	0.28	0.26	n. s.
100 seeds weight (g)	28.0	24.1	n. s.

* Classified by the degree of shattering as follows:
Susceptible ; above 78%, Resistant ; below 19%.

were cross-sectioned in the dorsal sutures after 24 hr of soaking.

In general, the pod anatomy of the two varieties appeared very similar. The thickness of jointing area of the dorsal suture of pods was $568 \pm 20\ \mu$, and $606 \pm 27\ \mu$, and the thickness of sclenchyma layer of dorsal suture was $76 \pm 4\ \mu$, and $101 \pm 4\ \mu$, respectively, in Kitamusume and Toiku 208. The angle of the fibrous tissue appeared to be more acute in the susceptible than in the resistant variety. It was suggested that difference of these tissues between both varieties may be one of the factors related to pod shattering. Calson[1] stated that the direct cause of pod dehiscence must be the difference in the tension developed in cells of the inner sclenchyma layer due to moisture content loss.

Environmental factors contributing to dehiscence, and the method of testing pod shattering

Observations made by soybean workers have indicated that low humidity, high temperature, rapid temperature changes, wetting and drying, etc. may induce pod shattering although any particular environmental condition has not yet defined.

The change of pod moisture content of six varieties was measured in the field condition. Although the maturity of these varieties varied each other, the pod moisture content gradually decreased as they matured, reaching 13 to 20% for all the varieties (Fig. 2). In addition, the moisture content of pods changed within a day due to humid night

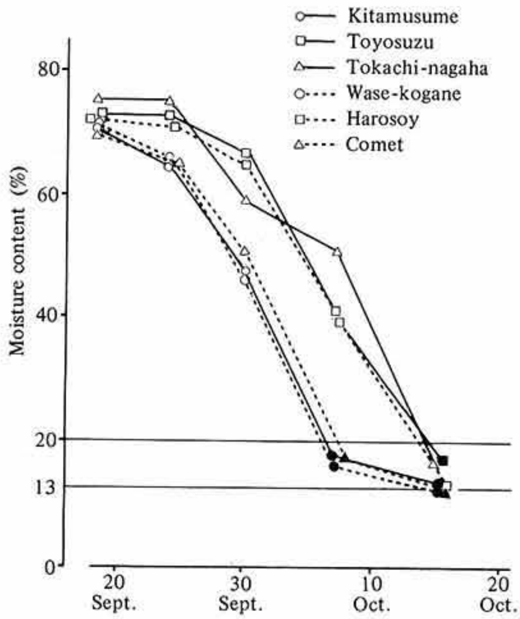

Fig. 2. Decrease of moisture content of soybean pods in field (1974)
Solid symbols: Moisture content of pods after maturity.
Empty symbols: Moisture content of pods before maturity.

and less humid daytime. This change was caused mainly by the change of moisture content of pod walls. As the pod shattering of susceptible varieties occurs under the less humid condition, the harvest of these varieties by machines is recommended to be done during the period of high humidity in early morning.

Fifty-two varieties were tested for pod shattering in the field condition during two years. The pod shattering was indicated by the date of incipient shattering (number of days from maturity to the date when the pod shattering began in each plant) and the degree of shattering (percentage of shattered pods to all pods of each plant on the 40th day after maturity).

The degree of pod shattering was slightly different between the two years, showing 34.2% and 22.4% on average of 52 varieties. Only 13 of 52 varieties showed the shattering over 2% in individual plants, and most of late maturing varieties showed only a few in the field. Negative correlation ($r=-0.723^{**}$) was obtained between percent shattering and date of maturity. However, it is supposed that the percent shattering in the field does not indicate the real degree of pod shattering of the varieties, because of humid environmental conditions in Hokkaido.

Therefore, the shattering and moisture content of pods were measured in the dryer set at 60°C. Fifty mature pods of each variety were tested. As shown in Figs. 3 and 4, varietal difference of the degree of pod shattering became clear as a result of the drying treatment. Susceptible varieties; i.e., Kitamusume, Toyosuzu and Tokachi-Nagaha, began to show the shattering at pod moisture content of 10 to 15%, and 90 to 100% of shattering at moisture content of 5%, while resistant varieties, i.e., Harosoy, Comet, and Wase-Kogane, gave only 10% of shattering at pod moisture content of 5%.

The pod shattering occurred in late varieties too, as the result of drying treatment. Even the pods that were 10 days before maturity showed the same degree of shattering as the mature ones. The degree of pod shattering caused by the drying treatment was the same between the two years.

Thus, it was clarified that the pod shattering is determined by both environmental and genetic factors, and that the moisture content of pods seems to be most influencial among environmental factors[3,8,11]. Therefore, to minimize errors in the test of pod shattering, the drying treatment must be employed.

Varietal difference of pod shattering

The wild soybean (*G. soja* Sieb. et Zucc.) easily scatters its seeds as soon as the pod matures, so that only shattered pods and immature pods remain on the plant. However, various degrees of pod shattering are observed in cultivated soybean.

Seventy-six varieties (including 44 varieties from overseas) and 115 varieties (including 47 varieties from overseas) were tested for the degree of pod shattering by the above-mentioned method during the two-year test period. Based on the result, they were divided into three groups according to the degree of pod shattering. Most of Japanese varieties of the determinate growth type were susceptible while most of varieties of the indeterminate growth type introduced from U.S.A. and China were resistant to shattering. Varieties derived from crosses between Japanese and Chinese varieties were classified into the resistant or medium group. The resistant varieties showed a tendency to have a longer stem with more nodes on the main stem, lower pod-setting height and smaller seed size.

Estimates of heritability and correlation coefficients among characters in F_2 generation

Information is limited on the inheritance of pod shattering of soybean. Piper and Morse[9] reported in 1923 that in the cross between F.P.I. No. 22876 from Japan which showed a non-shattering character and

Table 2. Mean, range, variance, and coefficient of variation for the degree of shattering (%) in F_1 and F_2 generations

Cross	Population	Number of plants	Mean (%)	Range (%)	Variance	Coefficient of variation (%)
A	P_1 Comet	25	16. 2±1. 5	7— 31	57. 0	46. 6
	P_2 Toiku 127	22	99. 1±0. 4	94—100	3. 2	1. 8
	Mid-parents	—	57. 7	—	—	—
	F_1	20	69. 5±5. 3	20—100	552. 4	33. 8
	F_2	238	57. 1±2. 3	0—100	1276. 8	62. 6
B	P_1 Wase-kogane	31	9. 7±0. 9	0— 28	27. 6	54. 2
	P_2 Toiku 127	22	99. 1±0. 4	94—100	3. 2	1. 8
	Mid-parents	—	54. 4	—	—	—
	F_1	27	74. 4±3. 6	20—100	341. 0	24. 8
	F_2	224	41. 8±2. 1	0—100	1024. 3	76. 6
C	P_1 Kitamusume	32	97. 1±0. 7	88—100	14. 8	4. 0
	P_2 Thai 7012-56	31	8. 1±1. 2	0— 26	47. 6	85. 2
	Mid-parents	—	52. 6	—	—	—
	F_1	31	91. 0±3. 4	60—100	362. 4	20. 9
	F_2	295	33. 8±1. 8	0—100	989. 4	93. 1
D	P_1 Okuhara 1	4	98. 8±1. 3	95—100	6. 3	2. 5
	P_2 Lee	4	8. 8±2. 4	5— 15	22. 9	54. 4
	Mid-parents	—	53. 8	—	—	—
	F_1	2	80. 0±0	80	0	0
E	P_1 Okuhara 1	4	8. 8±2. 4	5— 15	22. 9	54. 4
	P_2 Harosoy	4	96. 3±2. 4	90—100	22. 9	5. 0
	Mid-parents	—	52. 6	—	—	—
	F_1	2	80. 0±0	80	0	0
F	P_1 Okuhara 1	7	100. 0±0	100	0	0
	P_2 Harosoy	5	6. 0±2. 4	0— 10	30. 0	91. 3
	Mid-parents	—	53. 0	—	—	—
	F_1	56	48. 6±2. 4	10— 90	311. 4	36. 3
G	P_1 Thai 7012-28	7	10. 0±3. 1	0— 20	66. 7	81. 6
	P_2 Kitami-shiro	7	90. 0±5. 3	60—100	200. 0	15. 7
	Mid-parents	—	50. 0	—	—	—
	F_1	16	79. 4±4. 3	30—100	299. 6	21. 8
H	P_1 Thai 7012-28	8	2. 5±1. 6	0— 10	21. 4	185. 2
	P_2 Toyosuzu	8	88. 8±3. 5	70—100	98. 2	11. 2
	Mid-parents	—	45. 7	—	—	—
	F_1	13	69. 5±4. 1	45— 90	222. 1	21. 4
I	P_1 Kitakomachi	33	92. 8±1. 1	75—100	42. 6	7. 0
	P_2 Harosoy	33	12. 1±1. 2	2— 34	50. 5	58. 7
	F_2	285	46. 3±1. 4	0—100	590. 5	52. 5
J	P_1 Wase-kogane	15	23. 9±2. 3	6— 39	78. 4	37. 0
	P_2 Tokei 629	15	93. 7±1. 3	84—100	25. 5	5. 4
	F_2	238	71. 6±1. 4	4—100	498. 8	31. 2
K	P_1 Wase-kogane	20	14. 0±2. 5	3— 52	128. 1	80. 8
	P_2 Toiku 191	20	95. 0±1. 4	76—100	38. 6	6. 5
	F_2	253	72. 8±1. 5	5—100	555. 1	32. 4

Table 2. (continued)

Cross	Population	Number of plants	Mean (%)	Range (%)	Variance	Coefficient of variation (%)
L	P_1 Tokei 679	10	14.6±3.0	3— 33	89.6	64.8
	P_2 Kitahomare	10	99.0±0.5	95—100	2.7	1.7
	Mid-parents	—	56.8	—	—	—
	F_1	13	97.6±0.8	96—100	8.3	3.0
	F_2	166	87.1±1.8	0—100	544.6	26.8
M	P_1 Kitahomare	10	99.4±0.4	96—100	167.6	13.0
	P_2 Tokei 687	10	34.7±4.1	15— 55	1.8	3.9
	F_2	304	88.3±1.2	10—100	410.7	23.0
N	P_1 Tokei 687	11	51.5±4.2	32— 78	190.5	26.8
	P_2 Tokei 679	10	14.5±2.3	4— 23	51.2	49.3
	F_2	238	37.7±1.2	0— 94	360.8	50.4

Table 3. Estimates of heritability* for pod shattering and other characters

Characters	Crosses						
	A	B	C	I	J	K	Average
Degree of shattering	98	98	96	92	89	85	93
Date of bloom	96	97	78	85	—	—	(89)
Date of maturity	99	99	73	93	—	—	(91)
Plant height	98	98	79	97	96	94	94
Number of nodes on the main stem	96	91	43	92	94	86	84
Degree of indeterminate type of growth	93	94	41	77	—	—	(76)
Number of pods	71	72	58	58	87	77	71
Seed yield	60	60	39	61	89	79	65
100 grain weight	46	15	78	71	80	74	61
Average	84	80	65	81	(89)	(83)	

* Heritability $(\%) = \frac{\sigma^2 F_2 - \sigma^2 E}{\sigma^2 F_2} \times 100$

where $\sigma^2 F_2$=total variance in F_2 populations.

$\sigma^2 E_2$=enviromental variance which was calculated as the average variance of the parents.

Medium-Green variety, which shattered easily, the non-shattering character was dominant to the shattering character. Nagai[7] found in hybrid progenies of cultivated and wild soybeans that the shattering character was dominant to non-shattering. Ting[16] reported the shattering character of the wild type was dominant to the non-shattering character of cultivated soybean in F_1 generation. He also pointed out that segregation of shattering character was very complex in F_2 generation and needed further study. Caviness[2] indicated that only four major genes were involved in the shattering susceptibility in combinations among four varieties including wild soybean.

In the present study, F_1 and F_2 generations of 14 crosses between suscptible and resistant varieties were tested to study the inheritance of pod shattering. The variances of F_2 generation were large, and the possibility of selecting highly resistant plants for pod shattering was suggested in any crosses (Table 2). The degree of pod shattering of F_1 was located between those of the parents, and did not show the heterosis in all combinations. It was larger than the value of the mid-parents, i.e. rather near to the value of susceptible parent in eight of the nine crosses. It implies the susceptibility of pod shattering

Table 4. Correlation coefficient between the degree of pod shattering and other agronomic characters in F_2 population

Characters	Crosses					
	A (n=238)	B (n=224)	C (n=295)	I (n=285)	J (n=238)	K (n=253)
Date of bloom	0.26**	0.36**	−0.14*	0.07	—	—
Date of maturity	0.27**	0.22**	0.19**	−0.10	—	—
Plant height	0.03	0.25**	−0.17**	−0.15*	0.04	0.07
Number of nodes on the main stem	0.00	0.20**	0.04	−0.13*	0.00	0.08
Degree of indeterminate type of growth	−0.28**	−0.17*	0.02	−0.17**	—	—
Number of pods	0.01	0.15*	−0.32**	−0.13*	0.10	0.20**
Seed yield	−0.09	0.03	−0.13*	−0.13*	0.08	0.17*
100 grain weight	0.02	−0.27**	0.50**	0.05	0.01	0.05
Lowest pod height	—	—	—	−0.07	0.11	0.09

*, ** Significant at 5% and 1% levels, respectively.

partially dominant to the resistance.

Estimates of heritability in the broad sense for pod shattering, and other agronomic characteristics are summarized in Table 3. Heritability of pod shattering had an average of 93% in the six crosses. It was larger than the value for seed yield, and about the same as those obtained for the date of bloom, the date of maturity, plant height, and number of nodes on the main stem. This result suggests the possibility of effective selection of the resistance to pod shattering in early generations.

Correlation coefficients between the degree of pod shattering and other agronomic characteristics of the six crosses are given in Table 4. Significant positive correlation at 1% level were obtained between the degree of pod shattering and the date of maturity in three of the four crosses. It suggests the possibility of selecting resistant plants to shattering with early maturity. The pod shattering was negatively correlated with the degree of indeterminate-type of growth in the crosses, A, B, and I, which used introduced varieties with indeterminate growth habit as a parent. Therefore, it must be noticed that the selection of non-shattering plants brings about an enhanced indeterminate growth habit.

Correlation coefficients between the degree of pod shattering and the number of pods, seeds yield, and 100 grain weight were different among the crosses. The lack of highly positive correlations between these characteristics in most of the crosses suggests the possibility of selecting high resistance to shattering without affecting seed yield. There was no significant correlation between the degree of pod shattering and the lowest pod height. It suggests the possibility of improving the shattering resistance without affecting the pod setting height.

Estimates of minimum number of genes for pod shattering

The minimum number of genes for the resistance to pod shattering was estimated with the maximum likelihood method by Ishige[5], and with the Castle-Wright method, in nine crosses between the susceptible and resistant varieties. Estimated number of genes was one or two pairs in eight of the nine crosses by the maximum likelihood method, and in all crosses by the Castle-Wright method (Table 5).

Additive effect was rather larger than dominant effect, while both were similar in crosses K, L and M. There were significant differences in the degree of pod shattering between both parents in all crosses, and the genetic variances were larger than the environmental variances in F_2 generation of all crosses. The precision of the estimated values was regarded relatively high because the

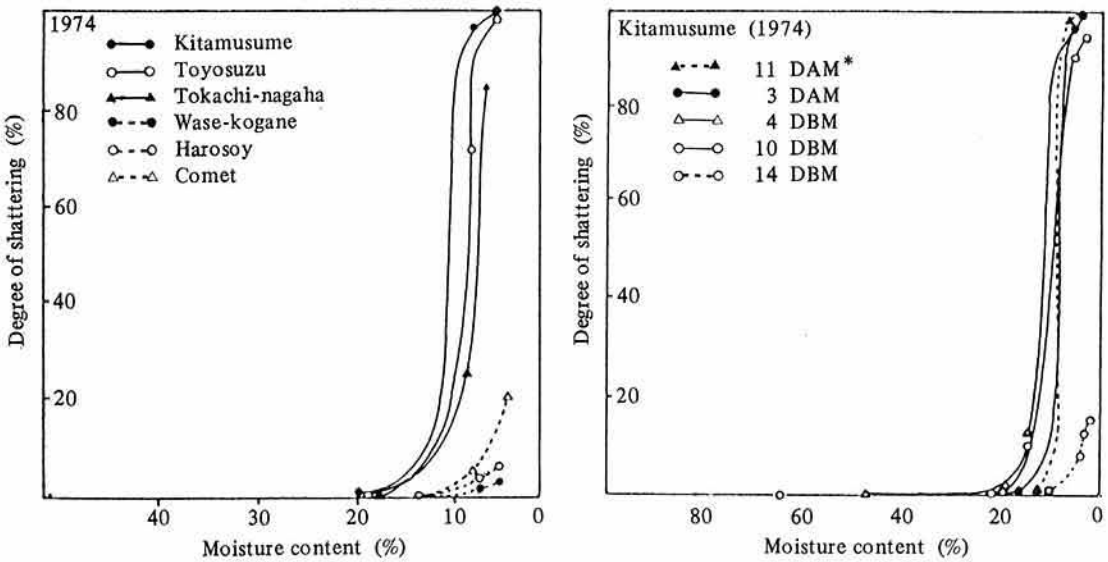

Fig. 3. The relation of pod moisture content to the degree of shattering observed after drying treatment
* DAM: Days after maturity, DBM: Days before maturity.

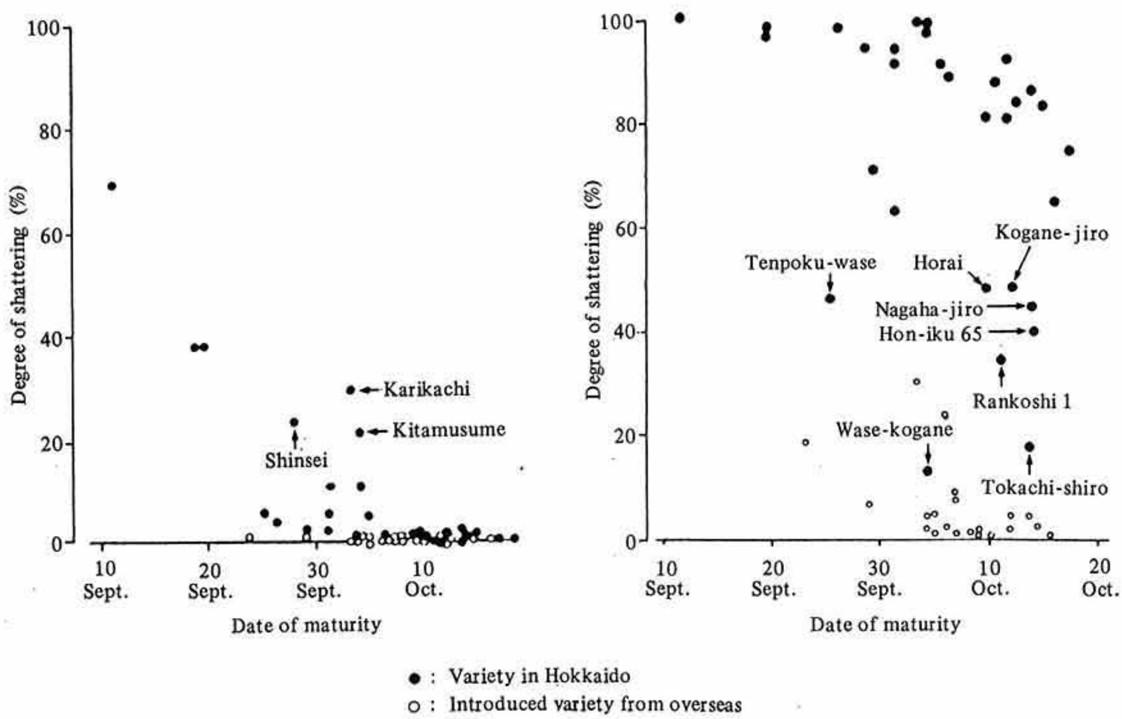

Fig. 4. The relation between the date of maturity and the degree of shattering under the field condition (left) and after the drying treatment (right)

Table 5. Estimation of number of genes for resistance to pod shattering

Cross	Number of genes*		Additive effect (D)	Dominance effect (H)	H/D	σ^{**}
	N_1	N_2				
A	1	1.2	40.66	18.45	0.45	0.33
B	1	1.5	40.81	11.84	0.29	0.25
C	1	1.0	42.16	25.80	0.61	0.15
I	2	1.5	20.94	−2.78	−0.13	0.17
J	2	1.4	18.09	9.80	0.54	0.21
K	3	1.7	13.94	12.79	0.92	0.23
L	2	1.7	20.08	18.87	0.94	0.13
M	2	1.6	16.78	15.75	0.94	0.28
N	1	0.7	20.64	−6.43	−0.31	0.60

1) $*N_1$: Number of genes estimated by maximum likelihood method.
N_2: Numer of genes estimated by Castle-Wright method.
$N_2=(\bar{P}_1-\bar{P}_2)/8(VF_2-VF_1)$ or $N_2=(\bar{P}_1-\bar{P}_2)/8(VF_2-0.5VP_1-0.5VP_2)$

2) $\sigma^{**}=2\sigma/|P_1-P_2|$

values of δ^{**}, i.e., the environmental variance standardized by the difference of the means of pod shattering degree between both parents, were very small.

In the present study, the estimates of the minimum number of genes for shattering varied little between two different methods, and it appeared that a small number of genes are responsible for the shattering. However, actual occurrence of pod shattering in field condition may be more complicated because the degree of shattering correlates with the variable moisture content of pods.

Introduction of resistance genes for pod shattering into Hokkaido cultivars

Our main objective was to introduce non-shattering genes into Hokkaido cultivars which have determinate growth habit, cool weather torelance, and large seeds.

Thai variety (SJ-2), American varieties (Harosoy, Lee, etc.), and Chinese varieties (Shika 4, Ohoju, etc.) were used for crossing. These non-shattering varieties from overseas were not adaptable to cultural conditions in Hokkaido, because of their late maturity, small seed size and susceptibility to lodging.

One-hundred and twenty hybridizations were made during the last 13 years, from 1973 to 1985. Individual selection and pedigree selection for shattering resistance were conducted from early generations by using the drying treatment proposed in this study. Three lines resistant to pod shattering, Toiku 207, Toiku 208, and Toiku 211, were successfully developed (Fig. 5). Toiku 207 is a selection from the cross between Thai 7012–28 and Tokei 423, and Toiku 208 is the hybrid between Kitamusume and Thai 7012–56. Thai 7012–28 and Thai 7012–56 are breeding lines with high resistance to shattering, selected from the cross between Karikachi and SJ-2.

Toiku 207 is excellent in seed quality, having yellow-hilum, and Toiku 208 matures relatively earlier than Kitamusume and has large seeds. Toiku 211 is a selection derived from the cross between Kogane-Jiro and Harosoy Dt_2, and is characterized by lodging resistance and high yielding ability.

As given in Table 6, these three lines showed much improvement in the resistance to pod shattering and in other agronomic characters, and look promising in Hokkaido. Thus, it was proven that the introduction of high-resistance genes to determinate Hokkaido varieties with a large seed-size was possible for an actual breeding program.

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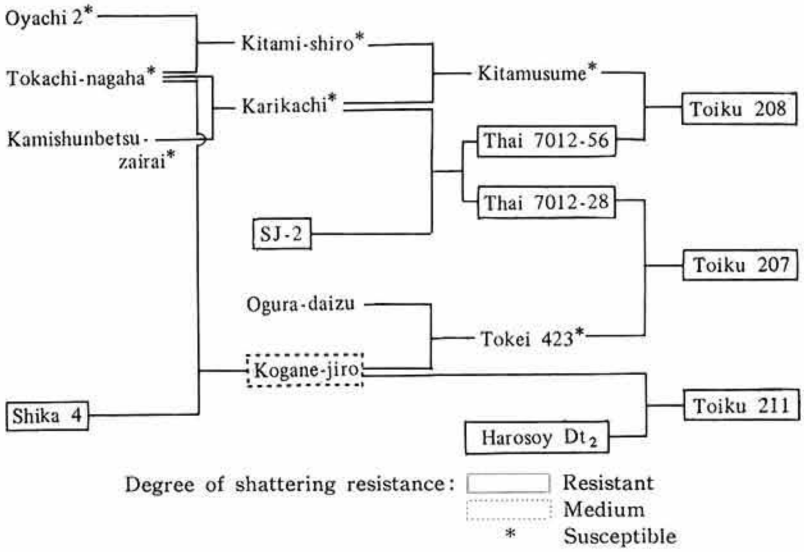

Fig. 5. Genealogy of promising lines Toiku 207, Toiku 208 and Toiku 211

Table 6. Results of yield test for promising Toiku-lines resistant to pod shattering (1982—1984)

	Degree of shattering (%)	Date of maturity	Plant height (cm)	Lowest pod height (cm)	Lodging score	Seed yield (kg/a)	100 grain weight (g)
Toiku 208	10	Oct. 1	73.7	17.2	1.3	28.7	28.9
Kitamusume	95	Oct. 4	71.6	17.4	1.9	29.4	27.4
Toiku 207	22	Oct. 3	65.4	15.8	1.2	27.4	23.9
Toiku 211	6	Oct. 7	65.6	9.7	0.9	29.0	19.2
Toyosuzu	89	Oct. 6	47.7	13.4	0.6	24.9	30.0
Wase-kogane	15	Oct. 4	85.0	12.3	2.8	25.1	21.7

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