Varietal Difference in Resistance to Purple Seed Stain of Soybean and the Method of Selecting for the Resistance

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Purple seed stain, caused by *Cercospora kikuchii* Matsumoto and Tomoyasu is one of the most prevalent diseases of soybean. It rarely reduces yields, but heavy infection sometimes retards seed germination[6]. Pods, stems and leaves are infected as well as seeds. But the purple discoloration on the seed which is one of the most typical and conspicuous symptoms reduces market value. Distributing widely, this disease occurs severely under humid conditions as particularly shown in upland fields converted from paddy fields.

Recently fungicide application to control this disease has become widely practiced in our country, resulting in less serious crop damage than previously recognized. But it is considered that fungicide effectiveness may be broken down by emergence of fungus strains resistant to fungicide in the future. Therefore, breeding for resistance to purple seed stain is significant for production of high quality soybean.

For that purpose, many kinds of experiments have been conducted at the Kariwano Branch of the Tohoku National Agricultural Experiment Station. In this paper, results of screening work for genetic resources of resistance to purple seed stain, inheritance of resistance to the disease and the effective method of selection for developing resistant varieties are reviewed.

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Screening of genetic resources for the resistance to purple seed stain

The screening was made with soybean varieties grown in the soybean variety preservation plot at Kariwano Branch[4]. Varieties with yellow or green seed coats which enable easy distinction of the symptom were used. As all varieties were cultivated in the plot without any treatment to promote or control infection, the damage was based on natural infection. After harvesting, purple discoloration of seeds was visually classified as "disease ratings" in conformity with the standard of determination. The disease ratings were determined by taking together both incidence of infection and degree of infection based on discolored area on the seed (Table 1).

Varieties differed obviously in their disease ratings. Furthermore, the average disease ratings of all tested varieties in each year showed a great variation among years (Table 2). It suggests that the infection with purple seed stain depends on environmental condition, so that it seems to be adequate to evaluate the resistance of each variety by average value of its disease ratings for several years. Of 736 varieties tested for more than two years, 110 varieties showed the values of less than 1.0 in their disease ratings. Most of them were late maturing varieties

Table 1. Determination of disease ratings for purple seed stain of soybean

Disease ratings	0	1	2	3	4	5
Incidence of infection (%)[a)]	5	1~5	6~30	31~60	61~90	91~100
Infection degree[b)]	Nothing	Slightness	Little	Medium	Much	Extreme

a) : By visual classification.
b) : Based on degree of discolored area on the seed.

Table 2. Average disease ratings of purple seed stain each year

	Years															
	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986
Number of tested varieties	277	271	244	291	312	235	366	416	494	369	277	154	66	196	150	148
Average disease ratings	0.8	1.7	1.7	2.0	1.6	1.6	2.1	2.6	2.7	3.0	1.9	0.7	1.7	1.8	1.9	1.5

whose sympton might be affected by weather conditions at the time of infection. The more favorable are the environmental conditions for the pathogen, the more severely they may be infected. Some of early or medium maturing varieties such as Wase-kin, Shimokawa, Hanayome, Hanayome-ibaraki 1 and Rikuu 9 showed low disease ratings, which seemed to be resistant to purple seed stain.

Average disease ratings of domestic varieties by origin are as follows; Hokkaido: 2.2, Tohoku: 1.9, Kanto: 1.5, Chubu: 1.7, Kinki·Chugoku·Shikoku: 0.8, Kyushu: 0.9. As for a remarkable tendency, northern varieties showed higher disease ratings than southern ones. On the other hand, average disease ratings of foreign varieties by origin are as follows; Korea: 2.7, China: 2.8, U.S.A.: 3.0. All of these disease ratings were obviously higher than average one of domestic varieties. A number of varieties have been reported as resistant to purple seed stain in U.S.A.[7)], but some of them were determined to be susceptible in this test. This indicates the possibility that different races of the pathogen may be distributed between Japan and U.S.A.

Varietal difference in resistance to purple seed stain

The field test of varietal difference in resistance to purple seed stain was conducted in 1982, 1984, 1985 and 1986 using 141 domestic varieties every year. In this test, the visual count of number of purple stained seeds per 100 seeds was recorded as the incidence of infection with purple seed stain. The incidence of infection in each variety varies from year to year, but significant positive correlations between years were obtained. The result suggests that the incidence of infection is easy to vary, but the trend of varietal difference in resistance is similar in each year.

Fig. 1 shows the relation between the date of maturity and incidence of seed infection expressed with the average value of four test years. The degree of varietal difference in incidence of infection varied with maturity groups, and was especially great in medium maturing varieties. Weather conditions during the maturity stage of these varieties seemed to be highly favorable for the pathogen. Koyama and Yunoki reported that the average temperature favorable for increase in seed infection might be $18\pm3°C$[2)], which agrees well with the temperature at the time

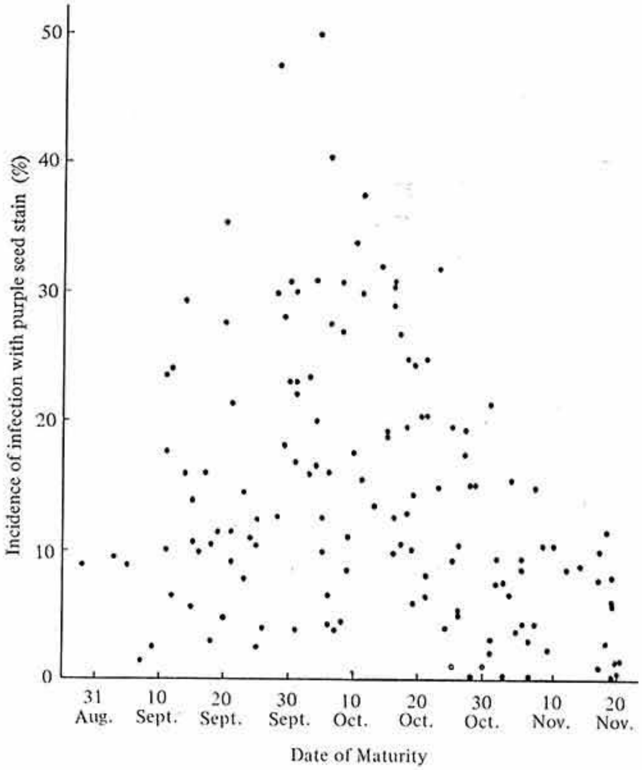

Fig. 1. Relation between maturity and incidence of infection with purple seed stain

Table 3. Classification of varieties in respective maturity classes based on incidence of infection with purple seed stain

Incidence of infection (%)	Date of maturity				
	Sept. 11～20	Sept. 21～30	Oct. 1～10	Oct. 11～20	Oct. 21～30
0～ 4.9	Orihime Hokkai-hadaka	Oodate 1 Bon-minori	Tamamusume Nourin 2 Hanayome-ibaraki 1		Asajiro Mejiro 1 Shin 1
5.0～ 9.9	Hatsukogane Hasiri-mame Higo-musume	Kariha-takiya Kogane-daizu	Asahi 60 Akita-ani Hatsukari	Dekisugi 1 Shiro-hachikoku	Kakushin 1 Akasaya Nangun-takedate
10.0～14.9	Kitami-shiro Matsuura Wase-suzunari	Wase-shiroge Tokachi-nagaha Nourin 1	Shiro-sennari Ugo-daizu	Dekisugi Ani Tachi-suzunari	Miyagi-shirome Yagi-shirohana
15.0～19.9	Wase-midori Shin 3 Suzuhime	Nourin 5	Ouu 13 Tachi-kogane Kokeshi-jiro	Oku-shirome Nourin 4 Fusanari	Tsuru-no-tamago 1 Iwate 2 Tamahikari
20.0～24.9	Ooyachi 2 Karikachi	Kitamusume Yougetsu 1	Karumai Ibaragi-mame 7 Shirome-nagaha	Iwate 1 Nasu-shirome Suzuyutaka	Shiroge 9 Geden-shirazu 1 Toukichi 1
25.0～29.9	Miyashiro Himeyutaka	Toyosuzu Nezumi-saya	Nourin 3 Shinmejiro	Tanrei Itsutsuba	
30.0～	Nagaha-jiro	Yuuzuru	Raiden Raikou Fukunagaha	Nanbu-shirome Enrei Yama-shiratama	Nemashirazu

of the peak of incidence in Fig. 1. On the other hand, extremely early or late muturing varieties gave low incidence of infection and small varietal difference. In these varieties, weather conditions, especially temperature at the time of infection seemed not to be favorable for the pathogen. From the results, it is desirable to evaluate the resistance to purple seed stain by maturity class.

Classification of varieties in respective maturity classes based on incidence of infection with purple seed stain is shown in Table 3. Extremely early or late maturing varieties which were difficult to be evaluated accurately are excluded from the table. Koyama and Sasaki found that Bonminori, Nourin 2, Akasaya and so on possessed a high degree of resistance to purple seed stain when assessed with artificial inoculation[3]. Some of them are classified into the groups with low incidence of infection in Table 3.

All of the varieties of the glabrous pubescence type or the sparse pubescence type such as Hokkai-hadaka, Tamamusume and Hanayome-ibaraki 1 showed low incidence of infection. This fact indicated the possibility of relation between the resistance and density of pubescence. But some varieties with dense pubescence type also showed low incidence of infection, so there should be other factors concerning the resistance.

Inheritance of resistance to purple seed stain

Varietal difference in resitance to purple seed stain has been recognized in many regions, but detailed studies about inheritance of resistance were limited. In the present study, F_2, F_3 and F_4 generations from the crosses T207 × Karikei 114 and T207 × Karikei 123 were tested to study inheritance of resistance. T207 is the pure line of P. I. 80837[1], which was recognized to be resistant to purple seed stain by artificial inoculation in U.S.A.[5]. Karikei 114 and Karikei 123 were commercial lines of Tohoku region.

Estimates of heritability and parent-offspring correlation coefficients of some characters are given in Table 4. Heritabilities for incidence of infection and for disease ratings were 0.68 and 0.69 in T207 × Karikei 114, and 0.53 and 0.60 in T207 × Karikei 123, respectively. As a result of analysis, the two indices of disease showed a similar tendency on heritability in both crosses. In comparison with other agronomic characters, heritabilities for incidence of infection and disease ratings were lower than those of a growing period, number of stem nodes and 100-seed weight, but higher than those of number of pods per plant, total weight and seed yields. This result suggests that heritability for resistance to purple seed stain can be regarded as a medium value in agronomic characters of soybean.

As to parent-offspring correlation coefficients for incidence of infection and for disease ratings, there was no significant correlation between F_2 and F_3 generations. However, significant positive correlation coefficients were obtained between F_3 and F_4 generations in both crosses. The largest cause for the difference in correlation coefficients between generations was due to the variation of the disease incidence between years. Wilcox et al. reported that heritabilities for incidence of infection with purple seed stain were 0.91 in F_2 and 0.51 in F_3 generations[8]. This experimental result also indicates that disease incidence varies widely from year to year and it involves great difficulties in effective selection.

Phenotypic correlation coefficients between incidence of infection with purple seed stain and other characters are shown in Table 5. Most of correlation coefficients varied with generation, but significant positive correlation was obtained with disease ratings in each generation. In F_3 generation, there were significant positive correlations between incidence of infection and days to flowering, growing periods, stem length and number of stem nodes in both crosses. Within the limits of this experiment, such lines as relatively early in maturity or short in height seemed to be relatively resistant to purple seed stain.

Table 4. Estimates of heritability and parent-offspring correlation coefficients of some characters

Crosses	Genetic parameters	Characters									
		Days to flowering	Growing period	Stem length	No. of stem nodes	No. of pods per plant	Total weight	Seed yields	100-seed weight	Incidence of infection[a]	Disease ratings
T 207× Karikei 114	Heritability[b]	0.76	0.79	0.52	0.81	0.20	0.22	0.29	0.79	0.68	0.69
	F_2-F_3 correlation coefficient	—	—	0.756**	—	0.001^{ns}	0.363**	0.240*	0.754**	−0.026^{ns}	−0.063^{ns}
	F_3-F_4 correlation coefficient	0.636**	0.803**	0.781**	0.688**	0.407*	0.395*	0.324^{ns}	0.774**	0.524**	0.527**
T 207× Karikei 123	Heritability[b]	0.77	0.75	0.80	0.74	0.32	0.46	0.30	0.80	0.53	0.60
	F_2-F_3 correlation coefficient	—	—	0.629**	—	0.275*	0.273*	0.301**	0.289**	−0.044^{ns}	0.017^{ns}
	F_3-F_4 correlation coefficient	0.716**	0.578**	0.632**	0.419*	0.106^{ns}	0.419*	0.356^{ns}	0.599**	0.572**	0.629**

*, ** Significant at the 0.05, 0.01 probability level, respectively.
a) : Before analysis, percentage data were transformed to arcsine $\sqrt{\%}$.
b) : Estimated by the analysis of variance in the F_3 generation.

Table 5. Phenotypic correlation coefficients between incidence of infection of purple seed stain and other characters[a]

Crosses	Generation	Characters								
		Days to flowering	Growing period	Stem length	No. of stem nodes	No. of pods per plant	Total weight	Seed yields	100-seed weight	Disease ratings
T 207× Karikei 114	F_2	—	—	0.282*	—	0.283*	0.360*	0.362*	0.336**	0.910**
	F_3	0.433**	0.532**	0.509**	0.580**	−0.217^{ns}	0.187^{ns}	0.030^{ns}	0.521**	0.949**
	F_4	0.158^{ns}	0.344^{ns}	0.182^{ns}	0.455**	−0.065^{ns}	0.174^{ns}	0.112^{ns}	0.473**	0.803**
T 207× Karikei 123	F_2	—	—	−0.036^{ns}	—	0.115^{ns}	0.052^{ns}	0.081^{ns}	0.074^{ns}	0.879**
	F_3	0.399**	0.402**	0.337**	0.308**	0.126^{ns}	0.325**	0.307**	0.165^{ns}	0.879**
	F_4	0.145^{ns}	0.605**	0.155^{ns}	0.221^{ns}	0.064^{ns}	0.499**	0.394^{ns}	0.305^{ns}	0.937**

*, ** Significant at the 0.05, 0.01 probability level, respectively.
a) : Before analysis, percentage data on incidence of infection were transformed to arcsine $\sqrt{\%}$.

Table 6. The relation of some characters between F_3 lines and selected F_4 progenies

Crosses	Classification by selection objectives	F_3 Growing period (days)	F_3 Seed yields (g/plant)	F_3 100-seed weight (g)	F_3 Incidence of infection (%)	F_3 Disease ratings	F_4 Growing period (days)	F_4 Seed yields (g/plant)	F_4 100-seed weight (g)	F_4 Incidence of infection (%)	F_4 Disease ratings
T 207× Karikei 114	Low incidence of infection	133	25.4	24.6	5.9	0.9	130	20.1	18.5	7.7	1.1
	High yield	139	33.4	27.1	23.3	1.9	137	22.3	20.5	12.4	1.4
	High incidence of infection	146	25.5	28.4	48.6	3.1	145	17.2	20.5	17.2	2.0
T 207× Karikei 123	Low incidence of infection	140	24.4	25.5	13.1	1.6	140	20.5	20.0	13.8	1.7
	High yield	145	31.9	26.2	30.5	2.5	144	24.1	20.0	27.1	2.4
	High incidence of infection	145	26.8	26.9	46.5	3.2	147	23.0	21.0	28.3	2.6

Selection method for resistance to purple seed stain

Selections based on three objectives, low incidence of infection, high yields, and high incidence of infection were conducted in F_3 generation. Table 6 shows the relation of some characters between F_3 lines and selected F_4 progenies. The lines selected with the objectives of low incidence of infection in F_3 generation showed low values in incidence of infection and disease ratings in F_4 generation. It suggests the possibility of effective selection for resistance to purple seed stain. The growing period of them was short in both generations, so they gave less seed yields than other classes. On the contrary, the lines with high incidence of infection had longer growing periods than other classes. The lines selected with objectives of high yields showed relatively high values in incidence of infection and disease ratings.

As a result of this experiment, intensive selection in early generation seems to provide progenies with poor productivity. Since incidence of infection is very variable by weather condition and its heritability is medium value, moderate selection for resistance to purple seed stain, noticing other characters may be regarded as appropriate. It is desirable to apply such a way of selection during some generations for development of resistant varieties. As to the simplification for the procedure of selection, disease ratings are convenient and reliable indices for the evaluation of seed infection with purple seed stain.

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