Increase of Resistance of Alfalfa to Sclerotinia Crown and Stem Rot through Recurrent Selection

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Summary

Breeding of alfalfa (*Medicago sativa* L.) for resistance to Sclerotinia crown and stem rot (SCR; causal agent *Sclerotinia trifoliorum* Eriks.), which is a serious disease in Japan, was started in 1983. Increase of resistance to this disease has been examined following repeated selection of surviving plants from artificially inoculated field plots to the 9th generation. The strains selected for SCR resistance showed a higher resistance than Natsuwakaba and Tachiwakaba which were used as breeding materials and control cultivars. The effectiveness of selection based on the survival rate of progenies increased gradually as generations in which the recurrent selection was performed advanced. Realized heritability was low ($h^2=0.078$) in the 1st and the 2nd generations in which selection was performed but was high ($h^2=0.364$) in the 3rd to 9th generations. It is considered that the selection effect on SCR resistance became higher in the later generations, probably due to the accumulation of genes which have minor or polygenic effects on resistance.

Key Words: Alfalfa crown and stem rot, *Sclerotinia trifoliorum*, *Medicago sativa*, recurrent selection, heritability, disease resistance.

Introduction

Sclerotinia crown and stem rot (SCR) is one of the serious problems leading to failure of stand establishment in alfalfa and in other legumes in the Northern part of Europe, humid area of America and all over Japan, especially in areas with heavy snow. Alfalfa plants of all ages are susceptible to this disease but the damage is most severe at the seedling stage under cool and moist conditions. The damage may be extensive in late winter and early spring following the fall seeding of alfalfa (Elgin *et al.* 1988). Breeding of cultivars resistant to SCR was considered to be difficult because of the non-specific character of the host-pathogen relationship reported in several pathological and breeding studies (Welty and Busbice 1978, Dickinson and Lucas 1982, Nakane and Kaneda 1987). However, differences in the degree of disease severity were observed among alfalfa cultivars under artificial inoculation in the test field of Aichi Agric. Res. Cent. (AARC) which is located in the

warmer region of Japan. It was suggested that the inoculation method which employed hardening under natural conditions enabled to evaluate the resistance to SCR (Kanbe *et al.* 1993). We observed that the growth conditions for the seedling test were optimum at 16 °C under a 9hr day-length to reveal significant differences among the cultivars (Kanbe *et al.* 1996). The nondormant cultivars showed a high susceptibility to the disease, whereas cultivars of the Flamande type were found to be more resistant than the others (Kanbe *et al.* 1993). Several other authors have observed differences among alfalfa and red clover cultivars in the damage caused by the occurrence of SCR (Elgin and Beyer 1968, Raynal 1981). Trials to select resistant strains have been reported recently (Pierson *et al.* 1994, Pratt and Rowe 1996). We have initiated breeding work for resistance of alfalfa to SCR since 1983, and studies based on long-term selection for SCR resistance have been carried out in artificially inoculated field plots. In this paper, we report on the accumulated effects obtained in continued selection, and its important implication in the breeding work. The estimation of heritability of SCR resistance is also presented.

Materials and Methods

The following 10 cultivars and strains were used as the original population for SCR selection: Natsuwakaba, Tachiwakaba, LR43112-7, CRSY541-1, CR488-3, Everest, Derby, Lutece, Furez507 and Delta. These cultivars were higher percentage of survival than nondormant cultivars, and there were no significant difference among the 9 cultivars except for Natsuwakaba (Kanbe 1993). Selection of surviving plants was repeated for 9 generations in artificially inoculated fields. The selection method for each generation consisted basically of mass selection, but in the 3rd and 5th generations, selection based on maternal-line comparison was added. The selection rate was 2.0-7.0 % and 55 to 100 plants were selected depending on the generations (Table 1).

Evaluation for resistance of selected strains was carried out in two evaluation groups, in different years at the National Grassland Research Institute (NGRI) and AARC. Selected strains used in the two experiments are shown in Table 2.

Evaluation 1: The strains from the 1st to 5th generations and 14 populations were sown on August 23 and transplanted to the field on October 2 1989 at NGRI.

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Table 1. Intensity of selection for *S. trifoliorum* in each generation

Gener- ation ¹⁾	Selection method	No. of plants (M. lines) ²⁾	No. of selected plants (M. lines) ²⁾	Selection rate ³⁾	Developed strain
1	Mass select.	2736	90	3.3	SR 58-1
2	Mass select.	3960	90	2.3	SR 58-2
3	Maternal-line select.	2430 (90)	70 (7)	2.9	SR 58-3
4	Mass select.	1960	62	3.2	SR 58-4
5	Maternal-line select.	3952 (52)	80 (8)	2.0	SR 58-5
6	Mass select.	2880	60	2.1	SR 58-6
7	Mass select.	960	55	5.7	SR 58-7
8	Mass select.	1634	60	3.7	SR 58-8
9	Mass select.	1435	100	7.0	SR 58-9

¹⁾ Generation in which selection was performed.²⁾ M. line : maternal lines, within which individual plants were selected.³⁾ Selection rate was calculated by (Number of plants selected after observation of surviving plants/total number of plants in the field) × 100**Table 2.** Selected strains used in the two experiments

Strain*	Objectives	Origin of breeding stock
SR 58 - 1~9	Resistance for <i>Sclerotinia trifoliorum</i>	Natsuwakaba, Tachiwakaba, LR 43112 - 7, CRSY 541 - 1, CR 488 - 3, Delta, Everest, Derby, Lutece, Furez 507
SHR 61 - 1~2	do.	HR 533 - 4, HT 61 - 1, TH 61 - 1, HR 581 - 1,
SLR 61 - 1~2	do.	Tachiwakaba
CR 488 - 3	Resistance for <i>Corticium rolfsii</i>	Natsuwakaba, Sabina, Moapa
HR 533 - 5	Tolerance for humid-soil	Aikei 16, B 13 - AL 4
Aikei 23	do.	Aikei 16

* Number after hyphen (—) indicate number of generations in which selection was performed

They were inoculated with mycelium from cultures of *S. trifoliorum* on April 11 at the base of the plants, and covered with a cheese cloth (85 % shade) for 7 days.

Evaluation 2: The strains from the 5th to 9th generations were sown on September 29 and thinned in early November, 1994 at AARC. They were inoculated with *S. trifoliorum* by the same method on December 16. The experimental plots were arranged according to a randomized block design using 19 entries and 5 blocks in Evaluation 1, 9 entries and 8 blocks in Evaluation 2. The numbers of surviving plants three and four months after the inoculation were counted and their percentages were used as values to examine the effect of selections.

The isolate of *S. trifoliorum* used in this study was obtained from an infected alfalfa plant in the field at NGRI.

Results

Evaluation 1: Almost all the leaves and stems of alfalfa had rotted two weeks after the inoculation with *S. trifoliorum*. Regrowth of the surviving plants was observed at three stages 18, 33 and 86 days after inoculation. The number of regrowing plants increased gradually for three months (Table 3). In the first observation, most of the cultivars did not show any evidence of regrowth, while but 4 selected strains showed a slight regrowth. In the second observation on May 14, all the strains displayed signs of regrowth and the survival rate of 3

Table 3. Differences in resistance among populations based on the percentage of plants which survived in plots inoculated with *S. trifoliorum*
: evaluation 1 — selected strain vs commercial varieties —

Cultivar or strain	Percentage of surviving plants			Multiple range test*
	Apr.29	May.14	Jul. 6	
SR 58 - 5	3.6	24.8	57.6	a
SR 58 - 4	1.6	19.6	35.2	b
SR 58 - 3	2.4	15.6	31.2	b
SHR 61 - 2	2.4	9.6	20.8	c
SR 58 - 2	0.8	8.0	17.6	cd
Natsuwakaba	0.0	4.0	16.0	cde
HR 533 - 5	0.0	8.0	15.2	cdef
SR 58 - 1	0.0	4.4	12.4	cdefg
CR 488 - 3	0.4	7.2	11.8	defg
SLR61 - 1	0.0	7.2	9.6	defg
Williamsburg	0.0	4.8	9.6	defg
Tachiwakaba	0.0	4.0	7.2	efg
Thor	0.0	0.8	6.4	efg
Aikei 23	0.0	2.4	6.4	efg
Vertace	0.0	2.4	5.6	fg
Kitawakaba	0.8	3.2	4.8	g
du Puits	0.0	6.4	4.8	g
Orca	0.0	2.4	4.0	g
CUF 101	0.0	4.0	3.2	g

* Duncan's multiple range test at 5% level.

selected strains was higher than that of Natsuwakaba by 15 %.

There were highly significant differences among the selected strain groups and populations in the percentage of plants which had survived on July 6. All the strains of the SR group (selected strains for SCR resistance) showed a higher percentage of survival than Tachiwakaba, the control cultivar. The highest survival rate of 57.6 % was recorded in the strain SR58-5 which had been selected for five generations for sclerotinia resistance. The second resistant groups (ranked "b" in Duncan's multiple range test) contained SR58-4 and SR58-3 which were selected for four and three generations, respectively. The survival percentage of the commercial cultivars except for Natsuwakaba was low. Strains selected for only one generation: SR58-1 and SLR61-1, did not show regrowth above 13 % even after three months.

The relation between the number of generations in which selection was performed and the percentage of surviving plants of SR58 strains is shown in Fig. 1 and Fig. 2, indicating clearly that the effectiveness of selection was low in the 1st and the 2nd generations but increase from the 3rd to the 5th generations.

Fig. 1. Increase in resistance to SCR in relation to the number of generations in which selection was performed.

Fig. 2. Regrowth vigor of selected strains from 1st to 5th generations.

Evaluation 2: Counts of plants displaying regrowth were made on January 27 and April 30. A significant difference was recognized in both observations (Table 4). Regrowth of the surviving plants was maximum in the investigation on April 30. The strains of advanced generations which were selected from the 5th to 9th generations showed a high percentage of surviving plants, reflecting their superior resistance compared with Tachiwakaba and Natsuwakaba. The strain selected in the 6th generation, SR58-6 showed a 50.4 % survival rate which was 8 % higher than that of SR58-5. Strain SR58-9 showed the highest survival rate of 62.9 %, although for SR58-7 and SR58-8 the gain in resistance was negligible. These results indicated that the increase of the resistance for SCR continued from the 6th generation up to the 9th. On the other hand, SLR61-2, SHR61-2, SR92-1 which were selected in the 1st or 2nd generation showed a low percentage of surviving plants, and the results were similar to those in evaluation 1.

Fresh yield of the first cutting in the SCR-test field was measured on May 30, and there were large differences among the strains and cultivars, as shown in the last column of Table 4. The yield gain of selected strains beyond the 5th generation was much higher than that of the commercial cultivars: among them SR58-9 showed the highest yield of 386 % in relation to Tachiwakaba.

Discussion

Welty and Busbice (1978) reported that the development of cultivars of forage legumes that were resistant to SCR had been difficult because of 1) the non-specific character of the pathogen, 2) the lack of a form of resistance easily identified by plant breeders. On the other hand, Elgin and Bayer (1968) and Raynal (1981) recognized that some alfalfa and clover cultivars sustained less SCR damage than others. Pierson *et al.* (1994) reported the effects of 2 cycles of selection for SCR in Ohio, USA. We confirmed the presence of culti-

Table 4. Differences in resistance among populations

: evaluation 2 — advanced generation vs control cultivars —

Cultivar or strain	Jan. 27	Apr. 30	Multiple range test*	Fresh yield (May 30) ratio to Tachiwakaba, %
SR 58 - 9	41.5	62.9	a	388
SR 58 - 8	34.8	51.3	b	308
SR 58 - 6	35.3	50.4	b	359
SR 58 - 7	28.6	48.2	b	307
SR 58 - 5	21.4	42.4	bc	251
SLR 61 - 2	8.5	34.8	c	135
SHR 61 - 2	13.8	32.6	cd	190
Tachiwakaba	8.0	23.2	d	100
Natsuwakaba	3.1	9.4	e	53

* Duncan's multiple range test at 5% level.

var differences in the resistance of alfalfa to SCR by the established artificial inoculation method in the field, and found that cultivars of the Flamande type such as Lutece, Euver, and Everest were somewhat more resistant than the others (Kanbe *et al.* 1993). Further more, the selected strain obtained in our field at AARC displayed highest resistance (Kanbe *et al.* 1996). By repeated selection of surviving plants, the increase in resistance to SCR was clearly demonstrated. The strain SR58-5 selected for five generations was the most resistant among all the selected strains and commercial cultivars in the evaluation 1 in the 1989-90 season, and the strain SR58-9 selected for nine generations was more resistant than SR58-5 in evaluation 2 in the 1994-95 season. The effectiveness of selection on the percentage of surviving plants increased gradually with the advance of generations. In order to combine the results of the evaluation in two different years, the heritability estimates of SCR resistance were calculated by Falconer's method (1960). Model of frequency in selection of two populations consisting of parent individuals and progeny individuals in the next generation in which selection was performed using the threshold of the percentage of surviving plants is shown Fig. 3. The difference in population means between the parents and the selected progeny strain indicates the selection response (R). The calculation was performed based on the standard deviations from the threshold ($R = x_0 - x_1$), and the cumulative selection differential was calculated by the same threshold method as that used in each selection. Through the relation between the cumulative selection differential (S) and selection response (R), the expected value obtained as the ratio R/S was considered to be an

estimate of the heritability. Cumulative selection differential and response of each strain are presented in Table 5. SR58-5 was used as common strain from the 1st to 9th generations in the two evaluations. The heritability was estimated separately for the first two generations and later seven generations by fitting two regression coefficients. The response to selection was very low in the 1st and 2nd generations, and distinctly high from the 3rd to the 9th generations. The regression coefficient of the selection response on the cumulative selection was designated as "realized heritability" by Falconer (1960). In our results, the heritability of SCR resistance was low ($h^2=0.078$) in the early genera-

Table 5. Cumulative Selection differential and selection response indicated by the deviation of the threshold from the mean

Strain	Cumulative selection differential ¹⁾	Selection response ²⁾	
		X_i	$X_0 - X_i$
Evaluation 1			
parents (X_0) ³⁾	0	1.19	0
SR 58 – 1	1.84	1.15	0.04
SR 58 – 2	3.18	0.93	0.26
SR 58 – 3	4.09	0.49	0.70
SR 58 – 4	4.83	0.38	0.81
SR 58 – 5	5.57	– 0.19	1.38
Evaluation 2			
(SR 58 – 5) ⁴⁾	5.57	0.19	1.38)
SR 58 – 6	6.20	– 0.01	1.58
SR 58 – 7	6.64	0.05	1.52
SR 58 – 8	7.10	– 0.03	1.60
SR 58 – 9	7.48	– 0.33	1.90

1) The cumulative selection differential was calculated by the product of the selection rate of preceding generations.

2) X_i : Deviation from the population mean of the threshold value of the percentage of surviving plants of each generation in which selection was performed.

$X_0 - X_i$: Selection response from the population mean of parents.

3) Parent data were substituted by average data of 3 cultivars: Natsuwakaba, Tachiwakaba, CR 488 - 3.

4) SR 58 - 5 was used as common strain from 1st to 9th generations.

Fig. 3. Frequency distribution of population with different degree of SCR resistance, and threshold for percentage of surviving plants inoculated with *S. trifoliorum*. (Falconer 1960, revised figure)

Fig. 4. Realised heritability by regression of response to selection on the cumulative selection differential.

tions but relatively high ($h^2=0.368$) in the later generations (Fig. 4).

The theoretical studies on directional selection in autotetraploids (Hill 1971) indicate that the rate of response is low in the early generations and increases as selection proceeds when a low frequency of desirable genotypes was assumed in the initial generation in which selection was performed, as the rate of changes of the gene frequency is dependent on the gene frequency in the population. Response to the selection increases with the repetition of the selection, which results in the increase of the frequency of useful genes. When these theoretical studies were applied to the change of heritability of SCR resistance in our results as the generation in which selection was performed advanced, it appeared that the frequency of occurrence of useful genes responsible for SCR resistance was low in the original population. We were able to demonstrate that recurrent selection is effective for the accumulation of resistance genes with minor polygenic action for resistance. A low response in the early generations but high in the later generations was reported in the increase of verticillium resistance in alfalfa (Sjodin 1990), humid soil tolerance in alfalfa (Kanbe *et al.* 1988), low temperature germinability in colored guineagrass (Kanbe *et al.* 1981) and sugar yield in sugar beet (Fujimoto 1971). These reports indicate the results of selection when a new crop was exposed to new environmental conditions. In the case of plant breeding for the introduction of plant populations from foreign countries where very different climatic conditions prevail, this pattern of response to selection may be important. Change from the low frequency of useful genes to the accumulation of these genes, and reconstitution of useful genotypes may result in higher responses.

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