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Reproductivity of Mixtures of Race 3 and Race 4 of *Heterodera glycines* on Soybean Cultivars

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大豆品種에서의 콩씨스트 線虫 混合 Race의 生殖力

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ABSTRACT

Soybean cultivars were inoculated with mixtures of races 3 and 4 of the soybean cyst nematode(SCN), *Heterodera glycines* Ichinohe. On a susceptible soybean cultivar Lee, the relative female maturation of the race mixture was not significantly different when compared with the maturation potentials for each of the two races alone. However, female maturation was significantly lower for the mixture than for race 4 alone on the soybean cultivas Pickett and Mack which are susceptible to only race 4. Selection of SCN populations consisting of race 3 and race 4 resulted in a decrease in race 4 on soybean cultivars Lee and Bragg which are susceptible to all SCN races and an increase in race 4 on soybean cultivars Pickett and Peking which are susceptible to only race 4. A significant reduction of race 4 was also observed on Lee with most mixtures of race 3 and race 4 combined in several ratios, suggesting that race 3 was more competitive than race 4 on the susceptible soybean.

Key words : mixture of races, reproduction, soybean cyst nematode, soybean cultivars.

要 約

콩씨스트 線虫 Race 3와 Race 4를 混合하여 大豆品種에 접종한 結果, 感受性品種인 'Lee'에서 混合 Race의 암컷의 成熟은 各各의 Race의 암컷 成熟能力과 비교해 볼때 유의차가 없는 것으로 나타났다. 그러나 Race 4에만 感受性인 大豆品種 'Pickett'과 'Mack'에서는 混合 Race 접종시에 Race 4 단독에 의한 것보다 암컷의 成熟比率이 현저히 낮았다. 콩씨스트 線虫의 모든 Race에 感受性인 大豆品種 'Lee'와 'Bragg'에서는 混合 Race의 Race 4 비율이 감소하였으며, Race 4에만 感受性인 'Pickett'과 'Peking'에서는 Race 4의 비율이 증가하였다. 또한 Race 3와 Race 4를 여러비율로 大豆品種 'Lee'에서 증식하여도 Race 4의 비율이 감소되어 Race 3이 Race 4보다 더 큰 경합력을 지닌 것으로 생각된다.

INTRODUCTION

Soybean cyst nematode(SCN), *Heterodera glycines* Ichinohe, is distributed throughout countries where soybeans are grown and is an economically important pest on soybean (*Glycine max* (L.) Merr.)(11). There are 5 described races [3,5] with some variation in distribution. SCN race changes are affected by soybean cultivars upon which SCN reproduces (1, 2, 6, 13, 19, 20). Price et al. (9, 10) demonstrated that races will hybridize and that races in mixtures compete, but did not explore the role of host resistance on these interactions. The objective of this study was to determine SCN female maturation when SCN susceptible and resistant soybean cultivars are inoculated with race mixtures to provide basic information on the nature of the population dynamics.

MATERIALS AND METHODS

Seeds of the SCN susceptible 'Lee' and 'Bragg', the race 4 susceptible 'Pickett' and 'Peking', and the race 3 and 4 resistant 'Bedford' soybean cultivars were germinated in vermiculite. Seedlings in the cotyledon stage were transplanted to sterilized fine river sand in 10−cm−d clay pots and placed in a greenhouse at 24−30°C. Five replications of each cultivar were inoculated with each inoculum except in the host differential test where 3 replicates were used.

Female maturation in race mixtures. Greenhouse stock cultures of SCN that had been race tested by standard procedures were used. SCN second stage juveniles (J2) of race 3 (R3), and race 4 (R4) were hatched from eggs on Baermann funnels and applied to the rhizosphere of Lee, Pickett and Mack soybean seedlings in the unifoliate stage. The races were applied separately or as a 1 : 1 mixture (R3+R4). Thirty days after inoculation mature females from each pot were suspended in water, collected on a sieve with 250 μm pore openings, and counted with the aid of a stereomicroscope. A mixture index was calculated based on the number of females from the mixture divided by the number from either race alone times 100.

Race mixture response to soybean cultivar selection pressure. Lee, Bragg, Pickett, Peking and Bedford soybean seedlings in the unifoliate stage were inoculated with a 1 : 1 mixture of R3 and R4. After 30 days mature females were extracted and counted as in the previous study. The females and egg masses from Lee, Bragg, Pickett and Peking soybeans were homogenized in a blender to free eggs from them. The eggs from each soybean cultivar were used to inoculate all five cultivars again. After 30 days female maturation on each cultivar was determined. The percentage of race 4 in the resulting populations was calculated as a mature female index using the number on Pickett as the base number for each inoculum source.

Effect of race ratio on selection of race mixture. Lee soybean plants growing in 15−cm−d clay pots were inoculated with about 5000 eggs of *H. glycines* in the ratios of 1 : 9, 3 : 7, 1 : 1, 7 : 3, and 9 : 1, of R3 and R4, respectively. There were three replications. Two months after inoculation, SCN females and cysts extracted from the pots were homogenized in a blender to free eggs and second−stage juveniles, passed through a sieve with 250 μm openings and the eggs and J2 were used to inoculate the soybean cultivars Lee, Pickett, Peking, and Bedford, and lines PI 88788, and PI 90763. After 35 days, females were collected and counted as previously. A mature female index was calculated based on the number of females from Lee. The percentage of race 4 in the final mixtual was also calculated.

RESULTS

Female maturation in race mixtures. Numerous mature females were found on Lee soybean inoculated with R3, R4 or mixtures of R3 and R4(Table 1). SCN race 4 matured well on Pickett and Mack. The relative number of mature females on Lee inoculated with R3 and R4 mixtures varied with inoculum source but was not significantly

Table 1. Number of females(± standard deviation) produced by separate or mixed race cultures of *Heterodera glycines* on four soybean cultivars

Cultivar	Reaction[a]		No. females/plant			Mixture index(%)[b]
	Race 3	Race 4	Race 3	Race 3+4	Race 4	
Lee	S	S	143±50.6[c]	274±116.4	228±60.6 x[d]	148 N.S.[e]
Pickett	R	S	17±24.6	66± 7.3	287±67.9 x	43 *
Mack	R	S	28± 5.9	90± 16.3	255±17.1 x	63 *
Lee[f]	S	S	114±24.6	111± 37.6	191±58.6 x	73 N.S.

[a]S : susceptible, R : resistant(12).

[b]Mixture index = $\frac{\text{no. of cysts by mixture of the races}}{\text{average no. of cysts by race 3 or race 4}} \times 100$

[c]Average of 5 replicates.

[d]Means followed by the same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

[e]Orthogonal contrast between average no. of cysts formed by two single races and no. of cysts formed by mixture of the races. (N.S. : not significant, * : significant at P=0.05.)

[f]Another inoculum source used to test SCN reproduction.

Table 2. Number of females and mature female index of mixtures of races 3 and 4 of *Heterodera glycines* selected on a soybean cultivar and progeny tested on other soybean cultivars

Inoculum Source	No. females/plant				
	Lee	Bragg	Pickett	Peking	Bedford
1 : 1 mixture	198±72.4[b] (100)[c]	272±72.7 (137)	79±25.2 (40)	31±6.9 (16)	8±3.5 (4)
2nd inoculum source					
Lee	241±4.7 (100)	232±73.1 (96)	19±3.1 (8)	15±1.5 (6)	10±4.0 (4)
Bragg	223±57.1 (100)	209±77.0 (93)	26±8.3 (12)	18±6.4 (8)	10±5.7 (4)
Pickett	95±12.1 (100)	84±34.2 (88)	65±16.9 (68)	46±4.5 (48)	5±5.8 (5)
Peking	41±11.5 (100)	58±7.0 (141)	51±7.1 (124)	29±3.8 (71)	2±0.9 (4)

[a]SCN populations developed after 1 month on each cultvar.

[b]Means±standard deviations of 5 replicates.

[c]Mature female indices in parentheses(number of females as percent of the number on Lee).

different(P=0.05) than the average number of cysts formed by R3 or R4 alone. When inoculated with R3 and R4 mixtures, the number of mature females on Pickett and Mack was 43% and 63% of the average number of females on Lee inoculated with R3 or R4 alone, a significant reduction(P=0.05) in female maturation(Table 1).

Race mixture response to soybean cultivar selection pressure. The R3 and R4 mixture reproduced well on the susceptible soybean cultivars, Lee and Bragg(Table 2). Female indices for the race mixture were 40 on Pickett, 16 on Peking and 4 on Bedford(Table 2). When the populations selceted on the different soybean cultivars were used to reinoculate, relative female maturation on Pickett and Peking were different. The inoculum selected on Lee or Bragg matured at a lower level on Pickett and Peking compared to the original 1 : 1 race mixture. Mature female indices on Pickett were reduced from 40 to 8-12, and on Peking from 16 to 6-8(Table 2). Mature female indices on Pickett and Peking were higher after

selection of the race mixture on either of these soybean cultivars than when either was inoculated with the original mixture, indicating that the R4 portion of the population had increased. Female maturation on Pickett was not significantly different (P=0.05) from that on Lee.

Mature female indices of the first and second inoculations on soybean cultivars resistant to SCN R3, showed a decrease of R4(fewer females on Pickett and Peking) following susceptible soybean cultivars Lee and Bragg(Fig. 1). R4 ratios increased when mixtures of the reces were cultured on soybean cultivars resistant to R3 but susceptible to R4(Fig. 1).

Effect of race ratio on selection of race mixture. Mixtures of different ratios of SCN R3 and R4 populations cultured on Lee for 2 months reproduced well on Lee with no significant differences in mature females among race levels(Table 3). On Pickett, Peking, and PI 88788, all susceptible to R4(12), cyst formation was significantly(P=0.05) higher in the 90% R4 levels than the other mixture. No significant differences among race levels were observed in cyst formation on PI 90763R and Bedford, both of which are resistant to R3 and

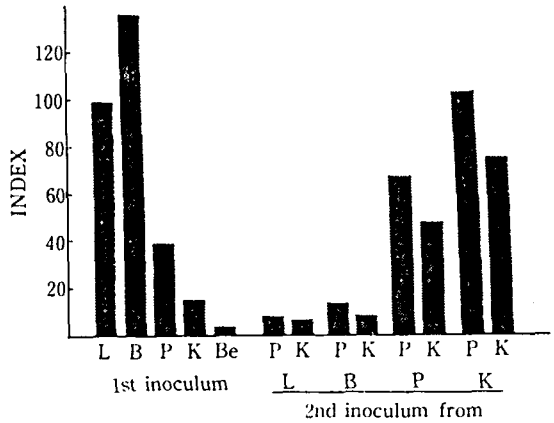

Fig. 1. Female maturation of 1 : 1 mixtures of race 3 and race 4 of *Heterodera glycines* and changes of parasitism on Pickett(P) and Peking(K) as affected by soybean cultivars, Lee(L) Bragg (B), Pickett and Peking Index is the number of females relative to 100 females on Lee. (Be)= Bedford.

R4(12, 17). On Pickett, except 90% R4, cyst formation was significantly less(P=0.05) than expected based on percent R4 in the inoculum. Mature female indices were 85, 4, 5, 7 and 1 in 90%, 70%, 50%, 30% and 10% R4 inoculum levels respectively. The lower indices were related to the decrease of R4 in the populations(Fig. 2).

Table 3. Number of females and mature female indices from mixtures of races 3 and 4 of *Heterodera glycines* selected on Lee soybean and tested on six soybean cultivars and lines

Initial race ratio[a] (% race 4)	No. of females and mature female indices[b]					
	Lee	Pickett	Peking	PI88788	PI90763R	Bedford
90	182 x[c]	154x	17x	15x	5x	15x
	(100)[d]	(85) N. S.[e]	(9)	(8)	(3)	(8)
70	139x	6y	2y	2y	5x	5x
	(100)	(4)*	(1)	(1)	(4)	(4)
50	295x	15y	1y	2y	1x	2x
	(100)	(5)*	(0)	(0)	(0)	(0)
30	83x	6y	1y	4y	2x	6x
	(100)	(7)*	(1)	(5)	(2)	(7)
10	280x	4y	2y	2y	1x	3x
	(100)	(1)*	(1)	(1)	(0)	(1)

[a]Approximately 5,000 eggs of soybean cyst nematode were used for inoculum, which were applied to rhizospheres of 4 plants of each differential in a pot. SCN populations developed on Lee(for 2 months).

[b]Means of 3 replicates(no. of females/plant).

[c]Means folowed by the same letter are not significantly different(P=0.05) according to Duncan's multiple range test.

[d]Mature female index(number of females relative to 100 females on Lee at the same inoculum level).

[e]N.S. : not significnat, * : significant at P=0.05 when tested by comparing cyst formation on Lee and cyst formation on Pickett for each race ratio.

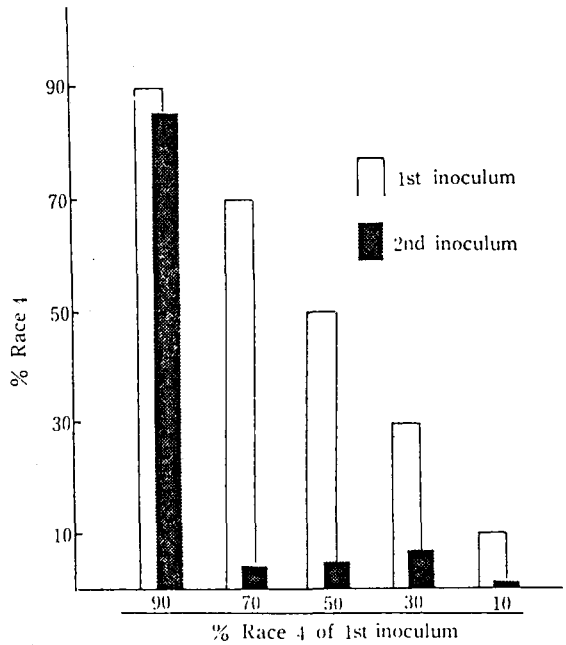

Fig. 2. Changes in race 4 in mixtures of races 3 and 4 of *Heterodera glycines* as affected by Lee.

DISCUSSION

The maturation of fewer females from mixtures of R3 and R4 on Pickett and Mack than on Lee was expected because R3 does not mature on these cultivars(Table 1). The R3 apparently had a few R4 contaminating the population because there was 11.9% as many females on Pickett and 19.6% on Mack as on Lee. The R4 population was apparently better adapted or more efficient in parasitizing Lee than was R3 since the female population for the R3 and R4 mixture on Lee was almost double the R3 population on Lee and R4 produced more than 1.5 times as many females as R3 on Lee. R4 produced 25% more females on Pickett than on Lee but the R3 and R4 mixture produced only 24% as many on Pickett as on Lee. If the mixture was half R4 then we would expect half as many on Pickett as on Lee. The reduction was not quite as great on Mack but the number of females on Mack inoculated with the R3 and R4 mixture also was less than 50% of the number on Lee. The large difference between numbers on Lee and Pickett is difficult to explain considering that R3 and 12% females maturing on Pickett and R4 produced 25% more fefmales on Pickett than on Lee.

O'Brien *et al*. (8)suggested that the penetration of *H. avenae* was inhibited following inoculation of a resistant wheat cultivar with avirulent nematodes. This indicates that the decrease of reproductivity of the mixed population on the resistant soybean cultivars in our experiment may be due to the decreased penetration because R3 penetrated first. Also growth of R4 in soybean roots may be inhibited by physiological or morphological changes of the root tissues induced by the avirulent(R3) nematodes in the population.

Several races of SCN have been identified from field populations(4, 7, 15, 16, 18). In 1970, 4 races were identified in SCN(3), and the fifth race was added in 1979(5). However, Riggs *et al*. (14) demonstrated that the number of races varied depending on the number of differential soybean cultivars used. The complexity of the race situation in fields may result from planting a number of cultivars or lines, because SCN races change according to the cultivars used for SCN reproduction (2, 19).

The R4 portion of the mixed population increased on Pickett or Peking due to inhibition of R3 development on the cultivar. Peking seemed to be a more powerful selector of R4 than Pickett, which agrees with the results of Riggs *et al*. (13). Riggs *et al*. (13) reported that in the inoculation sequences with R3 or R4, all transfers in the concinuous Lee series resulted in similar cyst indices on Pickett and Peking. However, mixtures of R3 and R4(except for 90% race 4) on Lee resulted in a decrease in race 4. Price *et al*. (10) reported that race 3 is more competitive than race 4 and our results support that. One mechanism for greater R3 reproduction may be more intra-race 3 matings than other intraracial matings. That would produce more R3 eggs(9). The R3 males may mature faster and mate with the R4 females before the R4 males mature.

Susceptible soybean cultivas are often recommended to reduce selection pressure and decrease

race selection. However, if the R4 ratio in a SCN population in above a certain level(90% in our experiment), cultivation of a susceptible soybean cultivar may not be effective. Steps must be taken to reduce the total population and equalize the ratios of R4 to other races. Therefore, quantitative as well as qualitative evaluation of the races in fields may be important in order to manipulate SCN populations by manipulating soybean cultivars.

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