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## Clubroot Resistance in *Brassicoraphanus*

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Clubroot-resistance in *Brassicoraphanus* ( $2n=36$ ; *ccrr* genome), which originated from the progeny of *Brassica oleracea* var. *capitata* cv. Miikechusei  $\times$  *Raphanus sativus* var. *longipinnatus* (the cultivar is unknown), was tested using Williams' races 2 and 4 in comparison with some cruciferous crops with an insertion-inoculation method. Three strains of *Brassicoraphanus* were not infected with the two physiological races suggesting this intergeneric plant has a stable and strong resistant gene to clubroot disease. Neither hybrids between *Bmssicoraphunus* and high to moderate resistant cultivars of Japanese radish or cabbage nor hybrids between *Brassicoraphanus* and susceptible cultivars of Chinese cabbage or turnip were infected with the two races. It is concluded from these results that clubroot resistance in *Bmssicoraphunus* is controlled by a dominant gene, and that *Brassicoraphanus* is useful for breeding clubroot resistant cruciferous plants.

### INTRODUCTION

Clubroot is a soil borne disease of cruciferous plants caused by parasitism of *Plasmodiophora brassicae* Woronin. The Mediterranean coast has been supposed to be the center of origin of the disease. At present, the disease has expanded throughout the world and has been considered one of the most damaging disease of cruciferous crops (Watson, 1969).

*P. brassicae* parasitizes only in the roots of cruciferous plants. The infected root enlarges abnormally like a club and becomes fragile. When the main root is infected with the pathogen, the plant wilts and defoliates because of the difficulty in water absorption in the root, and in the worst case it results in death. Since *P. brassicae* lives in soil for several years and effective and safe fungicides have not been improved, cruciferous plants with the disease resistance had been screened by some workers (Walker, 1936, 1939 ; Ashizawa *et al.*, 1978, 1980). Based on these criteria, breeding of clubroot disease-resistant cultivars of cruciferous plants had been conducted (Yoshikawa *et al.*, 1981 ; Yoshikawa, 1983). According to Yoshikawa *et al.* (1988), several clubroot-resistant cultivars of Chinese cabbage have been, to some extent, infected with clubroot in some regions. Hence, the stability of clubroot disease resistance in the cultivars is suspected by them. Thus, further possible measures to estimate the disease resistance will be necessary in cruciferous crops including Chinese cabbage.

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Generally, *Raphanus* plants have high potentials of clubroot disease resistance (Ashizawa et **al.**, 1980). Since it is expected that the intergeneric hybrid *Brassicoraphanus* (Fukushima, 1945) has the potentials of clubroot disease resistance, this is a very important plant for mediating genes to both genera *Brassica* and *Raphanus* through hybridization. The purpose of the present study was to estimate the clubroot resistance in *Brassicoraphanus* and demonstrate its usefulness for breeding the cruciferous plants resistant to clubroot disease.

MATERIALS AND METHODS

Experiment 1. Estimation of clubroot resistance in *Brassicoraphanus*

Three strains of *Brassicoraphanus*, which is symbolized as *K* (2n= 36 ; ccrr genome), and cole crops (Table 2) were used as plant materials. Two physiological races of *Plasmodiophora brassicae* Williams' race 2 and Hagikin were used, Williams' race 2 was provided by the Vegetable and Ornamental Crops Research Station.

The spores of the two races were suspended to count as follows. Fifty grams of clubbed roots stored at -20°C were homogenized in 400 ml water and the homogenate was filtered through the eight layers of cheesecloth. Then, the filtrate was centrifuged at 2,000 g for 5 minutes. The supernatant was discarded and the remaining pellet containing the spores was suspended in water. This centrifugation and suspension steps were repeated five times to remove the impurities. After the final step, the spores were counted to calculate a spore load under a microscope with a hemocytometer.

An inoculation method described by Yoshikawa et **al.** (1981) was applied with some modifications as follows. A mixture of sphagnum moss, perlite and clay powder (1: 3 : 1 by weight) was made and adjusted to pH 5.8 with CaCO<sub>3</sub>. The spores were injected into the mixture of the soils in which a spore load was 5 X 10<sup>5</sup> per gram of the dry matter. Cylindrical medium blocks measuring 4 cm×3 cm were made of the mixture and inserted into plastic pots (11 cm in diameter) stuffed with vermiculite. Seeds of *Brassicoraphanus* and cole crops were sown on the blocks on July 2, 1986 and thinly covered up with a mixture made of perlite and vermiculite (1 : 1 by weight). The young plants were fertilized with a 0.1% solution of a compound fertilizer, OK-F-1 (Otsuka Chemical Company). The extent of clubbing formed in the roots of the plants was examined seven weeks after sowing.

The degree of infection was divided into five classes, A to E (Table 1) according to Yoshikawa et al. (1981) with some modifications.

Table 1. The degree of clubroot disease in the roots of *K* and cole crops.

Class	Degree of clubing in roots	% of roots infected
B	nonclubbed	0
C	slightly clubbed <sup>a</sup> <sup>b</sup>	1- 10
	moderately clubbed <sup>b</sup>	11- 30
D	severely clubbed <sup>c</sup>	31- 60
E	very severely clubbed <sup>c</sup>	61- 100

a : A few and small clubs formed in roots.  
b : Each club is not jointed in a root.  
c : Each club is jointed even in a fibrous root.

### Experiment 2. Estimation of clubroot resistance in the hybrids between *Brassicoraphanus* and cole crops

Clubroot resistance in 4 hybrids obtained by crossing K-11 with Chinese cabbage cv. Kyoto No. 3, turnip cv. Nagasakiaka, cabbage cv. Miikechusei and radish cv. Minowase was compared with that in their parents. Culture and inoculation methods, pathogens and evaluation of clubroot resistance were the same as those described in Experiment 1. The seeds were sown on July 22, 1987 and the degree of clubbing formed in the roots was examined 45 days after sowing.

## RESULTS AND DISCUSSION

As shown in Table 2, all the plants of Chinese cabbage, Chinese mustard and

Table 2. Response of *K* and cole crops to clubroot disease.

Strain and cultivar	Race of clubroot*	No. of plants examined	Percentage of infected plants	Disease index **
<i>Brassicoraphanus</i>				
K-3	a	24	0	0
	b	19	0	0
K-11	a	24	0	0
	b	21	0	0
K-13	a	22	0	0
	b	23	0	0
Chinese cabbage				
Nozaki No. 2	a	22	100	100
	b	24	100	90
Kashin	a	23	100	76
	b	23	100	83
Chinese mustard				
Komatsuna	a	24	100	90
	b	25	100	95
Green debut	a	23	100	100
	b	25	100	100
Turnip				
Azumakanamachi	a	19	100	87
	b	23	100	87
Hakatasuwari	a	25	100	66
	b	25	100	49
Cabbage				
Miikechusei	a	25	12	1
	b	25	56	32
Natsumine	a	25	52	5
	b	24	88	45
Japanese radish				
Minowase	a	16	31	7
	b	11	100	54
Shijunichi	a	24	0	0
	b	25	0	0

\* a : Williams' race 2 ; b : Hagikin.

\*\* Index =  $\frac{0A+10B+30C+60D+100E}{A+B+C+D+E}$ , A to E : see Table 1

turnip cultivars were infected very severely, whereas none of these strains of *K* plants were infected with either of the two physiological races of *P. brassicae*. In cabbage, disease indices of both cultivars were low to the Williams' race 2(a) but high to the Hagikin(b). The Japanese radish 'Shijunichi' was not infected with any of the two races, but 'Minowase' was. These results suggest that the crops of *K* group are almost stable and strongly clubroot-resistant, that Japanese radish is resistant but not stable depending on the cultivars, and that others are not resistant.

Diversity of physiological race in *P. brassicae* is remarkable, and Williams' races 1, 2, 3, 4, 5, 8 and 9 have also been reported in Japan (Yoshikawa, 1983). However, screening of breeding materials and subsequent resistant breeding have been carried out to use a few special races according to the viewpoint that if plants are strongly resistant to one physiological race they are also strongly resistant to the other races (Nieuwhof and Wiering, 1963 ; Yoshikawa, 1983). A dominant gene, which governs clubroot resistance in European fodder turnip, has been transferred into Chinese cabbage (Yoshikawa, 1983), resulting several clubroot resistant cultivars which have now been growing over wide areas. Recently, these resistant cultivars have been infected with clubroot disease in several regions of Japan. Hence, more permanent stability of clubroot disease resistance in these cultivars is now required (Yoshikawa *et al.*, 1988).

Pathogens used in this experiment were Williams' race 2 and Hagikin. The Hagikin belongs to Williams' race 4 (Tanaka *et al.*, unpublished). Crops of *K* group are not only strongly resistant to Williams' races 2 and 4 but are also strongly resistant to Williams' races 1 and 9 (Tanaka *et al.*, personal communication). Therefore, it may be concluded that *K* crops are stable and resistant to various races of *P. brassicae*.

Results of Experiment 2 are shown in Table 3. In this experiment, the trend of the results was similar to that in Experiment 1 in Chinese cabbage, turnip, cabbage and Japanese radish cultivars. All plants of the cultivars of Chinese cabbage and turnip were very severely infected with the two races of *P. brassicae*. In cabbage, 'Toyomine No. 2' was severely infected with Hagikin whereas the degree of infection with Williams' race 2 ranged from A to E. A Japanese radish cultivar 'Minowase' was not infected with Williams' race 2. About a half of the plants of 'Minowase' was severely infected with Hagikin but the remaining plants were not infected. This clear segregation of clubroot disease-resistant plants in commercial varieties of cabbage and Japanese radish is especially noticeable, because when genes for resistance occur in existing commercial varieties, selection with these varieties will almost always provide the earliest and most satisfactory method of developing resistant strains (Allard, 1960).

Three strains of *K* and four  $F_1$  hybrids between *K* and cole crops were not infected with the two physiological races. Considering that all hybrids between *K* and susceptible cultivars of Chinese cabbage and turnip were resistant, it may be concluded that clubroot resistance in *K* is governed by a dominant gene.

*K* is an amphidiploid bred from the progeny of *Brassica oleracea* var. *capitata* cv. Miikechusei  $\times$  *Raphanus sativus* var. *longipinnatus* (the cultivar is unknown ; Fukushima, 1945). Because of the extinction of the parental lines, it is difficult to prove whether the clubroot-resistant gene of *K* was a parental origin, mutual complement of its parental resistant genes or a mutation during the preservation and selection of the line. However, there are two evidences that *K* may have inherited the resistance from

Table 3. Response of *K*, cole crops and their hybrids to clubroot disease.

Strain, cultivar and hybrid	Race of clubroot *	No. of plants examined	No. of infected plants in 5 classes of infection					Percentage of infected plants	Disease index**
			A	B	C	D	E		
Chinese cabbage									
Kyoto No. 3	a	18	0	0	0	0	18	100	100
	b	24	0	0	0	0	24	100	100
Turnip									
Nagasakiaka	a	24	0	0	0	0	24	100	100
	b	20	0	0	0	0	20	100	100
Cabbage									
Toyomine No. 2	a	23	8	1	2	1	11	65	54
	b	23	0	0	0	0	23	100	100
Japanese radish									
Minowase	a	26	26	0	0	0	0	0	0
	b	19	10	0	0	0	9	47	47
<i>Brassicoraphanus</i>									
K-3	a	17	17	0	0	0	0	0	0
	b	20	20	0	0	0	0	0	0
K-11	a	24	24	0	0	0	0	0	0
	b	22	22	0	0	0	0	0	0
K-13	a	25	25	0	0	0	0	0	0
	b	24	24	0	0	0	0	0	0
F <sub>1</sub> hybrids									
K-11 × Kyoto No. 3	a	11	11	0	0	0	0	0	0
	b	12	12	0	0	0	0	0	0
K-11 × Nagasakiaka	a	4	4	0	0	0	0	0	0
	b	3	3	0	0	0	0	0	0
K-11 × Minowase	a	13	13	0	0	0	0	0	0
	b	13	13	0	0	0	0	0	0
K-11 × Miikechusei	b	2	2	0	0	0	0	0	0

\*See Table 2 ; \*\*See Tables 1 and 2.

the pollen parent Japanese radish. One evidence is that clubroot resistant *Raphano-brassica* has been bred by using the combination of fodder kale and European fodder radish, and the latter is stably resistant to the various races of *P. brassicae* (McNaughton, 1973). Another is that many resistant cultivars existed among Japanese radish to Williams' race 2 (Ashizawa *et al.*, 1980).

Forty years have passed since *K* was bred. Its seed fertility has been obtained through selections during this period. *K* is a self-fertilizing plant and the inbreeding depression has not occurred until now. Because of this breeding potential, *K* is considered to be a bridge plant for breeding clubroot resistant cultivars in cole vegetables. Although hybrids between *K* and radish were easily obtained in our experiment, it was difficult to obtain hybrids by crossing *K* with cabbage, Chinese cabbage and turnip because of seed abortion and cross incompatibility. Development of efficient breeding methods to overcome these problems is necessary for the establishment of breeding of clubroot resistant cole crop varieties.

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