Breeding for Clubroot Resistance of Crucifer Crops in Japan

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Preface

Clubroot disease caused by *Plasmodiophora brassicae* Woronin, is considered one of the most damaging diseases of crucifer crops. In Japan the occurrence of clubroot was first recorded in 1892 on cabbage and turnip, and in 1978 on Chinese cabbage.[7]

For a long time the infested area was small and severity of clubroot was not high except few fields where traditional turnip cultivars were grown successively. Since about 1965, however, clubroot incidence has been reported in many different regions growing crucifer crops, and the severity and percentage of diseased plants have increased very much. At present, clubroot disease can be found almost all over Japan, especially in the central and northern parts, where clubroot is a serious problem. Intensive crucifer cropping was undoubtedly conducive to a population build-up of the organism in the soil. High concentration of pathogen in soil is apt to make the effect of chemical control insufficient,[10] so that crucifer crops are forced to be replaced by other crops. Clubroot is a constant threat to crucifer crops, especially to cabbage, turnip and Chinese cabbage.

Except chemical control, cultivation of resistant cultivars is one of the safest and cheapest methods to combat with clubroot. A breeding work for clubroot resistance was started first on cabbage under a practicable plan at Vegetable and Ornamental Crops Research Station in 1974. At present, breeding works for clubroot resistance are being made by one national, and 7 prefectural research stations as well as by 12 seed companies.[12] Although it is not long since the breeding works were started, an outline of the breeding works for clubroot resistance in Japan will be presented in this paper.

Inoculation methods

In the breeding works, a simple and reliable inoculation method is required for stable and uniform disease development under normal environmental conditions. To test accurately and simultaneously the resistance of a large number of plants, it is important to use the minimum effective quantity of pathogen to avoid its unnecessary spread.

From such standpoints of view, Yoshikawa et al.[16] established a screening method named "insertion method." According to their comparison tests on spore concentrations and inoculation methods, the insertion method showed the highest percentage of diseased plants and highest disease index, with a lower standard error of the disease index under any condition of spore load and soil pH than other dipping, mixing and pouring methods.

Details of the insertion method are as follows:

(1) Insertion method: Preparation of inoculum follows the method of Williams.[13] A mixture of one part clay powder, three part perlite and one part peat constitutes a good medium for plant growth and clubroot development. The mixture was adjusted to pH 5.5 to 6.5 and the spore load to 5×10^6/g of dry mixture. Cylindrical blocks of the mixture (infested soil), 4 cm in length and 3 cm in diameter, were made by using an instrument and inserted either into pots or fields. Seeds were sown on these blocks and

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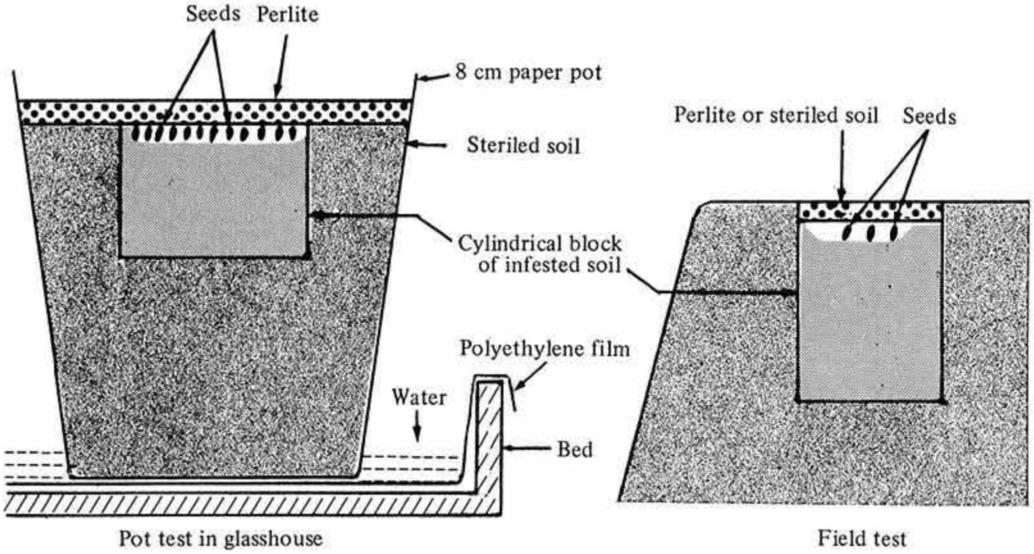

Fig. 1. A sketch of the insertion method for glasshouse- and field-tests

covered with a thin layer of perlite or noninfested soil (Fig. 1).

(2) Favorable conditions for infection: A high spore load, usually 5×10^6/g of dry soil, is optimum for disease development. A soil pH of 5.5 is best, but between 5.5 and 6.5 is adequate. The soil should be kept damp throughout the experiment. Soil temperature should be kept between 18° and 30°C, preferably between 18° and 24°C. At high temperatures soil drying becomes a problem and frequent watering is necessary.

(3) Observation and evaluation of resistance: The best time for observation is the time when the susceptible cultivar is completely and severely affected. Observations on clubbing were usually carried out 6 weeks after sowing when conditions favored clubroot development. When conditions were less favorable observations were made one or two weeks after clubs became visible with the naked eye on the root base of the susceptible check.

The degree of infection was rated on a 0 to 3 scale where 0=no infection; 1=few small clubs; 2=considerable clubbing; 3=severe clubbing. The number of plants in category 1 was multiplied by 20, in 2 by 80, in 3 by 100 and the sum of the products was divided by the total number of plants to give the disease index. The trials were usually replicated three times with 10 plants per pot.

Cultivar difference

This field of research has received much attention in recent years.[3,9,14,15] Hitherto it has generally been considered that the *B. campestris* group is more susceptible to clubroot than the *B. oleracea* group. In Chinese cabbage marked cultivar differences have been noticed on soil infested at lower inoculum levels, but this has not been observed on soil infested at high inoculum levels.[1,2,4] From the breeding point of view, it was thought that Chinese cabbage almost completely lacked resistance to clubroot, although some cultivars, including some from northern China, were less severely clubbed. Japanese turnip and mustard green are highly susceptible and lack in resistance source like Chinese cabbage. European turnip showed a wide range of resistance and susceptibility and some cultivars are highly or completely resistant to race 2. In *B. oleracea* group, kale are thought to be the most resistant to clubroot. Cabbage showed a wide range of resistance and susceptibility, and none of the commercial cultivars are highly or completely resistant. Cauliflower, broccoli and kohl-rabi are highly susceptible and lack in resistant source. Brussels sprouts are more resistant than cauliflower or broccoli. Rutabaga exhibited a wide range of susceptibility

Table 1. Breeding materials resistant to the race 2 of *Plasmodiophroa brassicae* in crucifer crops

Chlomosome number	Crop	Resistance[a)]	Cultivars[b)]
n=10	turnip	RR	Novitas, Novitas 4x, Meedjeslander, Leielander, Ponda, Zelder GT, Halflong Purple Top LB, Mosa, Tigra 4x, Marco, Debra, Siloga, Bicolar, Talonda, Klepa 2x, Vollender Zelder MB, Croppa, VIR 1200, VIR 1201, VIR 1153, ECD 02, ECD 04, Orion, Mommersteeg, Polybra, Waalslander, Roskilde, Civasto R, Gelria A, Gelria R
		R	Green Top Market, Manchester Market, Golden Ball, Green Top Scotch, Milan White, Red Globe, Green Top Stone, T-3, Milan Purple Top
n=9	cabbage	R	Bindsachsener 72754, Böhmerwaldkohl 72755, Böhmerwaldkohl 72756, G-6-0, Vologodskaja S. Zimnjaja, Winter White
		M	Ladozhskaja 22, Losinoostrovskaja 8, Zimnjaja Gribovskaja 13, Tajninskaja, Cluseed Early, Aichidaibansei, Badger Shipper
	cauliflower	M	Walcheren Winter Manston
	Brussels sprouts	R	Fillbasket, Winter Harvest, Seven Hills, Roofnerf Seven Hills
	kale	RR	K269, K173, K271, K278, K158, Extra Curled Scotch, Dwarf Green Curled, Sinjaja Kyrgavaja
n=19	rutabaga	RR	Wilhelmsburger, W. F. Neckless
n=9	radish	RR	Sakurajima, Shogoin,Uchikigensuke, Miyashigesou-buto, Shirokubi-miyashigemarujili, Nezumi, Wakayama, Moriguchi, Hooryo, Chubukura, Ookura, Akitsumari, Hakata 40 days, Round Scarlet Giant, Natsumino-wase No. 1 (F_1), Evelest, Hayamaru (F_1)

a) RR: high resistance, R: semi-resistance, M: moderate resistance
b) No resistant cultivars were found in Chinese cabbage, salt greens, broccoli, oil rape and Hakuran.

tibility to resistance, while oil rape, Hakuran and Indian mustard were very susceptible. Radish was more resistant than cabbage or rutabaga, and Japanese radish and *Raphanobrassica* showed the highest resistance of all crucifer crops (Table 1).

Physiological races

So far it has been put forward that seven of the *P. brassicae* physiological races—using the term "race" as defined by Williams[13)]—are present in Japan. Those are named races 1, 2, 3, 4, 5, 8 and 9 (Yoshikawa et al. 1978). This diversity of races has a direct implication on the breeding work and on the types of resistance in the host. Yoshikawa et al. (1978) tested 14 common cabbage, two turnip and 13 Chinese cabbage cultivars to observe their reactions to physiological races 1, 2, 3, 4 and a mixture of all the four. Spore loads were 2×10^6/g dry soil for the individual races and 4×10^6/g for the mixture. Cultivar differences in reactions to the 4 physiological races were relatively small. Highly significant correlation coefficients between percents of diseased plants or disease indexes were observed for each pair of races. Mixing races did not enhance disease incidence. This proves that plants which are strongly resistant to one physiological race are also strongly resistant to others, as described by Nieuwhof and Wiering.[5] It also shows that selection of one of the most virulent races is helpful in screening and breeding works.

Inheritance of resistance to race 2

As Chinese cabbage has no resistant source at all, Yoshikawa et al. (1977) crossed Chinese cabbage with resistant turnip, to transfer the resistant gene to Chinese cabbage (Plate 1).

Plate 1. Chinese cabbage selections with clubroot resistance in B_3 generation of the cross between Nozaki No. 2 (Chinese cabbage) and 77b (turnip)

Resistant turnip 77b; a selection of ECD No. 2, was crossed with susceptible Chinese cabbage; Matsushima New No. 2, to yield F_1, F_2, BC_1 and BC_2 populations. The F_1 was completely resistant. The F_2 segregated in the ratio of three resistant to one susceptible. The BC_1 generation segregated in the ratio of one resistant to one susceptible and the BC_2 generation was all resistant (Table 2). These facts show that the resistance of 77b is governed by one dominant gene. Similar results, produced by the influence of one dominant gene, were observed in 9 F_2 generations of crosses between 5 Chinese cabbage cultivars: Kenshin, Hiratsuka No. 1, Matsushima New No. 2, Nozaki No. 2 and Choosen, and three cultivars of turnip: Siloga, Gelria R and Debra. The resistant gene is considered to be the same as in 77b and is the same as the resistant gene found in the group of European fodder turnips. Leaf character does not correlate with clubroot resistance in any of the 10 F_2 generations described above. Both clubroot resistance and leaf character of Chinese cabbage can be combined in one plant. The dominant resistant gene found in this study should be useful in developing clubroot resistant cultivars of *B. campestris* group and others, especially Chinese cabbage, and the heading character can be improved easily by backcrossing.

Similar genetical analyses were done by Yoshikawa et al. (1978), using the race 2 of the pathogen. In a cross between a resistant cabbage; Böhmerwaldkohl 72755, and a moderately susceptible cabbage: Aichidaibansei, the resistance of

Table 2. Frequency distribution of clubroot resistance in crosses between Matsushima New No. 2 (Chinese cabbage) and 77b (turnip)

Generation and	progeny	Number of plants with disease index 0	1	2	3	Mean disease index	Segregation ratio observed[a] R : S
P_1	Matsushima New No. 2 (M)	0	0	0	22	3.00	
P_2	77b (b)	32	0	0	0	0.0	
F_1	M×b	44	0	0	0	0.0	
F_2	M×b	247	5	6	35	0.42	247(220) : 46(73)
BC_1	(M×b)×M	101	12	9	50	1.05	101(86) : 71(86)
BC_2	(M×b)×b	174	0	0	0	0.0	174(174) : 0(0)

a) R: resistant plants (disease index=0); S: susceptible plants (disease index=1, 2, 3)
Figures in the parenthesis indicate theoretical ratios expected from 3 : 1 segregation in F_2, 1:1 in BC_1, and 1: 0 in BC_2

Table 3. Frequency distribution of clubroot resistance in crosses of cabbage between Aichidaibansei and Böhmerwaldkohl 72755

Generation and progeny		Number of plants with disease index				Mean disease index (expected)	Note[a]
		0	1	2	3		
P_1	Aichidaibansei (A)	0	10	48	28	2.21	r=3.69
P_2	Böhmerwaldkohl 72755 (B)	58	17	1	0	0.25	
F_1	A×B	14	31	34	1	1.28 (1.23)	
F_2	A×B	28	81	90	22	1.48 (1.26)	
BC_1	(A×B)×A	0	14	133	53	2.20 (1.75)	
BC_2	(A×B)×B	106	72	29	0	0.63 (1.23)	

a) $r=(\bar{p}_1-\bar{p}_2)^2/8(V_{F_2}-V_{F_1})$

Table 4. Frequency distribution of clubroot resistance in crosses between Masagosanki (cabbage) and K269 (kale)

Generation and progeny		Number of plants with disease index				Mean disease index	Segregation ratio observed[a] R : S
		0	1	2	3		
P_1	Masagosanki (M)	0	0	1	96	2.99	
P_2	K269 (K)	99	0	0	0	0.0	
F_1	M×K	0	12	87	0	1.88	
F_2	M×K	157	95	214	130	1.53	439(447) : 157(149)
BC_1	(M×K)×M	3	11	142	192	2.50	345(348) : 3(0)
BC_2	(M×K)×K	168	87	99	10	0.87	196(182) : 168(182)

a) Same as in Table 2.

Böhmerwaldkohl 72755 was inherited by polygene of four pairs (Table 3). Similar results were shown in the crosses of resistant cabbage; Bindsachsener 72754 or Böhmerwaldkohl 72756, with moderately susceptible cabbage; Aichidaibansei.

In the cross of a resistant curled kale; K269, with a susceptible cabbage; Masagosanki, the resistance of K269 was inherited by one recessive gene and inheritance of leaf character was independent of that of clubroot resistance (Table 4).

Present status and problems of the breeding

(1) Chinese cabbage and other *B. campestris* crops: The breeding work of Chinese cabbage has been carried out by crossing Chinese cabbage with resistant European fodder turnips since 1976 under a practicable plan. Fortunately the resistance inherited by one dominant gene, independently of the leaf character. These facts made the breeding work very successfull. By 4 or 5 times of backcrossing Chinese cabbage to the hybrids between Chinese cabbage and turnip, a good heading quality can be obtained. In the crosses between Chinese cabbage and turnip, lines of various types, such as heading, semi-heading, non-heading Chinese cabbage types, turnip type, chinensis type, nabana type (with buds and young clusters for eating) were segregated. Bolting character was also widely segregated in the F_2 generation.

Today, national and prefectural experiment stations as well as many (12 at present) seed companies are devoting their efforts to the breedings for many types of Chinese cabbage, "Kanamachi, Nozawana, Sugukina and Oonobeni" types of turnip and "Nabana, Meikena and Oosakina" types of salt greens. Some breeding lines have reached the stage of testing their practical use,[6,8,11] and for some of them hybrid seeds production systems were established by using self-incompatibility. On the other hand there still remain some problems, that some lines have undesirable characters such as decreased virus disease resistance, liability to calcium deficiency, poor quality, etc.

(2) Cabbage and other *B. oleracea* crops: The breeding work of cabbage was started earlier than that of Chinese cabbage, using mainly Böhmerwaldkohl 72755 as a resistant source. But the resistance of Böhmerwaldkohl 72755 is not complete especially when the temperature is very high, and was inherited by polygene. Today, heading character is much improved but the resistance is not yet enough for the commercial use. The breeding work is at a standstill.

On the other hand, the resistance of kale "K269" is much higher than that of "Böhmerwaldkohl 72755" and inherited by one recessive gene. The breeding work using "K269" is still at an early stage, and needs many years as compared to the above cross, but lines with complete resistance can be obtained as a genotype of "aa". In this cross, plant characters segregated widely and various types of plant, such as heading cabbage type, broccoli type, cauliflower type, Brussels sprout type, etc., were obtained in F_2 generation. So that it is possible to breed those various types of line with complete resistance.

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