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## **Backcross Compatibility in Hybrids between *Brassicoraphanus* (*Brassica oleracea* X *Raphanus sativus*) and Cruciferous Crops, and Clubroot Resistance in Their BC<sub>1</sub> Plants**

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All the F<sub>1</sub> hybrids between *Brassicoraphanus* 'K-11' and cruciferous crops showed very low pollen and seed fertility. Backcrossings of the F<sub>1</sub> hybrids with *Raphanus sativus* and *Brassica campestris* each were successful, whereas those with *B. oleracea* were almost unsuccessful under natural conditions. BC<sub>1</sub> plants could be obtained through embryo culture when F<sub>1</sub> hybrids were backcrossed with *B. oleracea*. In the backcrossings with *R. sativus* and *B. campestris* each, seed fertility was improved as the backcross generation and selection of the plants advanced. One to three trivalents were observed in the PMCs of BC<sub>1</sub> plants obtained by backcrossing F<sub>1</sub> hybrids with *B. campestris*. Clubroot resistant plants were obtained from BC<sub>1</sub> progenies derived from backcrossing of the BC<sub>1</sub> hybrids with *R. sativus* and *B. campestris* each.

### INTRODUCTION

For breeding clubroot resistant cruciferous crops using clubroot resistant *Brassicoraphanus* 'K-11' (Fukushima, 1945; Xing et al., 1989) as a bridge plant, cross compatibility between *Brassicoraphanus* and susceptible cruciferous crops was investigated and their F<sub>1</sub> hybrids obtained were found to be strongly resistant to clubroot (Xing et al., 1989; Long et al., 1992).

To transfer clubroot resistant gene from *Brassicoraphanus* 'K-11' to Chinese cabbage or Japanese radish, successive backcrossings are required. In the present report, backcross compatibility of the clubroot resistant F<sub>1</sub> hybrids and their BC<sub>1</sub> hybrids were investigated to produce BC<sub>2</sub> progenies. Clubroot resistance in the BC<sub>1</sub> progenies obtained from backcrossings of the BC<sub>1</sub> hybrids with *B. campestris* and with *R. sativus* was also investigated to demonstrate the possibility of breeding clubroot resistant Chinese cabbage- and Japanese radish-like reversional plants.

### MATERIALS AND METHODS

F<sub>1</sub> hybrids obtained from the crossings between *Brassicoraphanus* 'K-11' and selected cruciferous crops (Long et al., 1992), grown in a greenhouse, were backcrossed

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with the cruciferous crops from early April to early May in 1988 and 1989.

BC<sub>1</sub> plants obtained from ('K-11' × *B. campestris*) × *B. campestris* and ('K-11' × *R. sativus*) × *R. sativus* were backcrossed with *B. campestris* and *R. sativus*, respectively in the greenhouse from early April to early May in 1989.

Methods of backcrossing, ovary and embryo cultures and pollen tube observation were described previously (Long et al., 1992).

Stainable pollen of the F<sub>1</sub> and BC<sub>1</sub> plants in 1 % acetocarmine was recorded as fertile. For observing meiotic configuration in BC<sub>1</sub> plants, the floral buds were fixed with acetic alcohol (1:3 v/v) for 24 h. After squashing the anther in a drop of 1 % acetocarmine, the pollen mother cells (PMCs) were examined at metaphase-I and -II. For chromosome observation, the root tips from BC<sub>1</sub> plants were pretreated with a solution of 0.002M 8-hydroxyquinoline for 4 h at 20°C and fixed with acetic alcohol for more than 2 h. Then the root tips were hydrolyzed in a mixed solution of 1 N HCl and 45 % acetic acid (1:1 v/v) for 90 sec at 60°C, stained with lacto-propionic orcein (Dyer, 1963) for more than 12 h, and observed under a light microscope.

Table 1. Pollen fertility in F<sub>1</sub> hybrids between *Brassicoraphanus* and cruciferous crops.

Cross combination	No. of pollen grains examined	No. of fertile pollen grains	Pollen fertility (%)
<i>Brassicoraphanus</i> × <i>Brassica campestris</i>			
K-11 × Hiroshimana	519	18	3.47
K-11 × Santo	463	22	4.75
K-11 × Kyoto No.3	373	84	22.52
K-11 × Nagasakiaka	630	18	2.86
K-11 × Hakatasuwari	402	7	1.74
Total or mean	2387	149	6.24
<i>Brassicoraphanus</i> × <i>B. oleracea</i>			
K-11 × Miikechusei	371	5	1.35
K-11 × Hakushin	693	7	1.01
K-11 × kale (W) <sup>2</sup>	652	85	13.04
K-11 × kale (Y) <sup>3</sup>	917	12	1.31
Total or mean	2633	109	4.14
<i>Brassicoraphanus</i> × <i>Raphanus sativus</i>			
K-11 × Minowase	569	87	15.29
<i>Brassicoraphanus</i> × amphidiploid			
K-11 × Shore?	854	597	69.91
K-11 × JS <sup>4</sup>	1067	560	52.48
Total or mean	1921	1157	60.23

<sup>2</sup>kale with white flowers.

<sup>3</sup>kale with yellow flowers.

<sup>4</sup>*B. napus* (*B. oleracea* × *B. campestris*).

<sup>5</sup>*Brassicoraphanus* (*B. japonica* × *R. sativus*).

Clubroot resistance in BC<sub>2</sub> progenies of *Brassicoraphanus* ‘K-11’ backcrossed with Chinese cabbage or Japanese radish was compared with that in their pollen parents. Pathogens were Williams’ races 2 and 4. Culture and inoculation methods of the pathogens and evaluation of clubroot resistance were described previously (Xing et al., 1989). The spore load was 4.1 X 10<sup>5</sup> per gram of dry matter. The seeds were sown on

Table 2. Backcross compatibility in F<sub>1</sub> hybrids between *Brassicoraphanus* ‘K-11’ and cruciferous crops.

Pollen parent and backcross combination	P.G.I.”	No. of flowers pollinated	No. of siliques set (A)	% of silique set	No. of seeds obtained (B)	B/A
<b>B. campestris</b>						
(K-11 x Hiroshimana) x Hiroshimana	4.0	667	574	86	5	0.009
(K-11 x Santo) X Kyoto No.3	4.0	501	451	90	9	0.020
(K-11 x Kyoto No.3) x Kyoto No.3	4.0	1020	377	37	16	0.042
(K-11 x Nagasakiaka) x Nagasakiaka	3.5	174	96	55	11	0.115
(K-11 x Hakatasuwari) x Hakatasuwari	4.0	584	485	83	6	0.012
Total or mean	3.9	2946	1953	66	47	0.024
<b>B. oleracea</b>						
(K-11 x Miikechusei) x Miikechusei	3.9	948	910	96	2	0.002
(K-11 x Miikechusei) x Hakushin	3.8	645	613	95	3	0.005
(K-11 X Miikechusei) x kale(W)	3.7	666	626	94	5	0.008
(K-11 x Miikechusei) X kale(Y)	3.6	760	714	94	0	0
(K-11 x Hakushin) x Hakushin	4.0	518	357	69	9	0.025
(K-11 x Hakushin) x Miikechusei	4.0	316	196	62	6	0.030
(K-11 x kale(W)) x kale(W)	3.8	475	290	61	0	0
(K-11 x kale(W)) X Miikechusei	4.0	327	252	77	0	0
(K-11 x kale(Y) x kale(Y)	3.6	594	77	13	1	0.013
Total or mean	3.8	5249	4035	77	26	0.006
<b>R. sativus</b>						
(K-11 x Minowase) x Minowase	4.0	367	187	51	167	0.893
Amphidiploid						
(K-11 X Shoren) x Shoren	4.0	76	42	55	52	1.238
(K-11 x JS) x JS	3.8	289	211	73	130	0.616
(K-11 x Shoren) open pollinated	-	-	-	-	-	1.580
(K-11 x JS) open pollinated	-	-	-	-	-	0.190

“pollen germination index = (b+2c+3d+4e)/(a+b+c+d+e)(0≤P.G.I.≤4). No. of pistils without pollen grains on the stigma (a), with pollen grains not germinated (b), with pollen grains germinated (c), with pollen tubes reaching to the style (d) and with pollen tubes penetrating into the ovule (e).

Table 3. Degeneration of ovule in F<sub>1</sub> hybrid (‘K-11’ x ‘Hakushin’) pollinated with ‘Hakushin’.

Cross combination	No. of viable ovules per silique in indicated days after pollination					
	7	11	15	20	25	27
K-11 x Hakushin	20.1	2.8	0.4	0.3	0.1	0

Table 4. Results of *in vitro* culture of the embryos taken from the F<sub>1</sub> plants 27 days after pollination with *B. oleracea*.

Backcross combination	No. of embryos cultured	No. of embryos with indicated stage of embryo development* at the initiation of culture				Length of embryos cultured (mm)	No. of plantlets obtained
		G	H	T	NM		
(K-11 x Miikechusei) x Hakushin	2	0	0	2	0	1.15	0
(K-11 x Hakushin) x Miikechusei	5	0	0	1	4	1.32	4
(K-11 x Hakushin) x Hakushin	10	0	1	4	5	1.05	5
(K-11 X kale(W)) X kale(W)	1	0	0	0	1	1.70	1

\*G; globular stage, H; heart stage, T; torpedo stage, NM; nearly mature stage.

July 6, 1989 and the degree of clubbing in the roots was examined on August 18, 1989.

## RESULTS

### Backcross compatibility in F<sub>1</sub> hybrids

The F<sub>1</sub> plants grew slowly and resembled the maternal parents in their morphology until they had four to six leaves. Then they rapidly grew into large and vigorous plants of which the morphology was almost intermediate between their parental species. Flowers of all the hybrids were white as *Brassicoraphanus* bears.

Pollen fertilities of the F<sub>1</sub> hybrids of 'K-11' x *B. campestris*, x *B. oleracea* and x *R. sativus* were low (Table 1). Rates of densely stained pollen were only one to 23 % in these F<sub>1</sub> hybrids, while those in the F<sub>1</sub> hybrids between 'K-11' and amphidiploids 'Shoren' and 'JS' were more than 50 %.

Although all the backcrossed F<sub>1</sub> plants showed high pollen germination index (P. G.I.) value, percentage of the siliques set and number of seeds per silique varied depending on the combinations (Table 2). Seed setting was zero or very low in the backcrossings with *B. oleracea* and with *B. campestris* in spite of considerable rate of silique set, while those with *R. sativus* and with the amphidiploids resulted in high backcross compatibilities. Among them, the backcrossing with 'Shoren' produced the greatest number of seeds per silique. The F<sub>1</sub> hybrids from 'K-11' x 'Shoren' and from 'K-11' x 'JS' produced 1.6 and 0.2 seeds per silique by open pollination, respectively. In the backcrossing with 'Hakushin' (*B. oleracea*), number of survived ovules decreased rapidly and no viable ovule was observed 27 days after pollination (DAP) (Table 3).

Most of the hybrid embryos in the backcrossings with *B. oleracea* developed in *vivo* beyond a torpedo or a nearly mature stage' and the lengths of them were approximately 1.2 mm (Table 4). Some of them grew into plantlets after culturing on White's medium supplemented with 300 mg l<sup>-1</sup> casamino acid and 30 g l<sup>-1</sup> sucrose.

Seeds were obtained *in vitro* from the ovaries only from ('K-11' x 'Hakushin') x 'Hakushin' among those from ('K-11' x *B. oleracea*) x *B. oleracea* 4 or 8 DAP (Table 5).

**Backcross compatibility in BC<sub>1</sub> hybrids**

Four types of chromosome number ( $2n=38, 27, 26, 24$ ) were observed in BC<sub>1</sub> plants from ('K-11'  $\times$  *B.campestris*)  $\times$  *B.campestris* (Table 6). Pollen stainability was high in the BC<sub>1</sub> plants with 38 chromosome number, but low in those with 27 chromosome

**Table 5.** Results of ovary culture of F<sub>1</sub> hybrids (*Brassicoraphanus*  $\times$  *B. oleracea*) pollinated with *B. oleracea*.

Backcross combination	Days after pollination	Initial ovary length (mm) <sup>1</sup>	Medium <sup>2</sup>	No. of ovaries cultured	No. of seeds obtained
(K-11 $\times$ Miikechusei) $\times$ Miikechusei	8	20.0f2.1	A	<b>30</b>	0
(K-11 $\times$ Miikechusei) $\times$ kale(Y)	8	20.8f1.4	A	<b>30</b>	0
(K-11 $\times$ Miikechusei) $\times$ kale(W)	8	21.6 $\pm$ 2.1	A	<b>30</b>	0
(K-11 $\times$ Miikechusei) $\times$ Hakushin	8	27.6 $\pm$ 1.9	A	<b>30</b>	0
(K-11 $\times$ Hakushin) $\times$ Hakushin	4	15.2f1.9	B	<b>94</b>	<b>6</b>
	4		C	<b>50</b>	<b>2</b>
(K-11 $\times$ Hakushin) $\times$ Miikechusei	4	16.2f1.7	B	<b>50</b>	<b>0</b>
(K-11 $\times$ kale(W)) $\times$ kale(W)	4	15.7f1.6	B	<b>50</b>	<b>0</b>
	4		C	<b>50</b>	<b>0</b>
(K-11 $\times$ kale(W)) $\times$ Miikechusei	4	19.7 $\pm$ 1.5	B	<b>50</b>	<b>0</b>
	4	12.7 $\pm$ 0.8	B	<b>50</b>	<b>0</b>
(K-11 $\times$ kale(Y)) $\times$ kale(Y)	4		C	<b>50</b>	<b>0</b>

<sup>1</sup>mean  $\pm$  S.D.

<sup>2</sup>A; Murashige and Skoog medium (1962), B; White medium (1963) + 500 mg l<sup>-1</sup> casein hydrolysate, C; White medium (1963) + 300 mg l<sup>-1</sup> casamino acid.

**Table 6.** Chromosome number and pollen fertility in BC<sub>1</sub> hybrids derived from *Brassicoraphanus*  $\times$  cruciferous crops.

BC, hybrid with indicated backcross combination	2n	No. of pollen grains examined	No. of fertile pollen grains	Pollen fertility (%)
<i>(Brassicoraphanus</i> $\times$ <i>B. campestris</i> ) $\times$ <i>B. campestris</i>				
KS-K-1 <sup>z</sup>	24	534	63	11.8
KS-K-Z	38	1023	695	67.9
KS-K-3	27	823	10	1.2
KS-K-4	24	968	22	2.3
KS-K-1	27	643	224	34.8
KS-K-2	26	421	1	0.2
KS-K-3	38	615	520	84.6
KS-K-4	27	869	1	0.1
KS-K-5	38	885	727	82.1
KS-K-1	24	1016	38	3.7
KS-K-1	24	844	568	67.3
<i>(Brassicoraphanus</i> $\times$ <i>R. sativus</i> ) $\times$ <i>R. sativus</i>				
KS-M-1	19	910	328	<b>36.0</b>

<sup>z</sup> 'KS-K'; ('K-11'  $\times$  'Santo')  $\times$  'Kyoto No.3', 'KK-K'; ('K-11'  $\times$  'Kyoto No.3')  $\times$  'Kyoto No.3', 'KN-N'; ('K-11'  $\times$  'Nagasakiaka')  $\times$  'Nagasakiaka', 'KH-H'; ('K-11'  $\times$  'Hakatasuwari')  $\times$  'Hakatasuwari', 'KM-M'; ('K-11'  $\times$  'Minowase')  $\times$  'Minowase'.

Italic numbers indicate individual plant number.

number except 'KK-K-I', in those with 24 chromosome number except 'KH-H-I', and in 'KK-K-2' with 26 chromosome number. 'KK-K-I' and 'KH-H-I' showed moderate pollen stainability. Pollen stainability in 'KM-M-I' derived from ('K-11'  $\times$  *R. sativus*)  $\times$  *R. sativus* was also moderate.

All the backcrossed plants showed high P.G.I. values, but the seed set was lower in the BC<sub>1</sub> plants derived from ('K-11'  $\times$  *B. campestris*)  $\times$  *B. campestris* than in those from ('K-11'  $\times$  *R. sativus*)  $\times$  *R. sativus* irrespective of their chromosome number and pollen stainability (Table 7). Only 'KS-K-I' showed a little high seed fertility, while 'KN-N-I'  $\times$  'Nagasakiaka', 'KK-K-I'  $\times$  'Kyoto No.3' and 'KK-K-4'  $\times$  'Kyoto No. 3' gave very low silique and seed settings.

Although the 2n chromosome numbers were both 24 in 'KS-K-I' and 'KS-K-4', the chromosome pairings at metaphase-I were different between them (Table 8). Frequency of trivalent chromosome was higher in 'KS-K-I' than in 'KS-K-4'. The chromosome distributions at metaphase-II were almost the same in the two hybrids, and both of them showed a relatively high frequency of lagging chromosome (Table 9).

### Clubroot resistance in BC<sub>2</sub> progenies

Clubroot resistance was none or low in Chinese cabbage, while it was high in Japanese radish and *Brassicoraphanus* (Table 10). The BC<sub>2</sub> progenies from 'KM-M-I'  $\times$  'Minowase' resembled 'Minowase' in their morphology, and showed almost the same degree of strong resistance to two races of *P. brassicae* as 'Minowase' showed, although the percentage of infected plants was lower in the BC<sub>2</sub> progenies than in 'Minowase'. More than a half of the BC<sub>1</sub> progenies from 'KS-K-I'  $\times$  'Kyoto No.3' were severely infected, but the remainings were slightly or not. Chromosome numbers were 23 in two, 24 in two and 25 in three plants of the BC<sub>2</sub> progenies derived from 'KS-K-I'  $\times$  'Minowase'.

## DISCUSSION

### Backcross compatibility in F<sub>1</sub> hybrids

Low fertility in the F<sub>1</sub> hybrids is in agreement with the previous reports (Honma and Heeckt, 1962; McNaughton, 1973; McCollum, 1979). The loss of seed fertility in the backcrosses also agrees with the findings that successive backcrosses of *Raphanobrasica* with *B. oleracea* are unsuccessful because of low seed fertility in successively backcrossed generations and complete seed sterility in BC<sub>6</sub> hybrids (McCollum, 1979).

Although the seed fertility of 'K-11'  $\times$  amphidiploids, 'Shoren' and 'JS', was very low as compared with that of 'K-11'  $\times$  diploid cruciferous crops (Long *et al.*, 1992), the F<sub>1</sub> hybrids backcrossed with 'Shoren' and 'JS' showed high seed fertility, and they also produced many seeds by open pollination. The reasons for high seed fertility in our results are not clear, but one possibility is that autotetraploidy in the F<sub>1</sub> hybrids being allotetraploid may play an important role.

### Rescuing BC<sub>1</sub> plants through embryo and ovary cultures

The incompatibility occurred in the backcrossings of F<sub>1</sub> ('K-11'  $\times$  *B. oleracea*) with *B. oleracea* was able to be overcome by the culture of embryos which were beyond a torpedo stage. It is, however, extremely difficult to obtain them, and the hybrid seeds

Table 7. Backcross compatibility in BC<sub>1</sub> hybrids derived from (*Brassicoraphanus* X *B. campestris*) X *B. campestris* and (*Brassicoraphanus* X *R. sativus*) X *R. sativus*.

Backcross combination	P.G.I.”	No. of flowers pollinated	No. of siliques set (A)	% of silique set	No. of seeds obtained (B)	B/A
<i>(Brassicoraphanus</i> X <i>B. campestris</i> ) X <i>B. campestris</i>						
KS-K-I’ X Kyoto No.3	4.0	1080	832	77	697	0.84
KS-K-2 X Kyoto No.3	4.0	323	313	97	9	0.03
KS-K-3 X Kyoto No.3	3.9	546	530	97	74	0.14
KS-K-4 X Kyoto No.3	4.0	611	244	40	22	0.09
KS-K-I X Kyoto No.3	4.0	436	4	1	1	0.25
KS-K-Z X Kyoto No.3	3.7	342	277	81	10	0.04
KS-K-3 X Kyoto No.3	3.8	538	253	47	75	0.30
KS-K-4 X Kyoto No.3	4.0	68	0	0	0	0
KS-K-5 X Kyoto No.3	4.0	57	42	74	8	0.19
KS-K-I X Nagasakiaka	3.9	749	37	5	0	0
KS-K-I X Hakatasuwari	3.9	599	377	63	21	0.06
<i>(Brassicoraphanus</i> X <i>R. sativus</i> ) X <i>R. sativus</i>						
KS-M-I X Minowase	4.0	339	149	44	286	1.92

<sup>z</sup>pollen germination index = (b+2c+3d+4e)/(a+b+c+d+e) (0≤P.G.I.≤4). No. of pistils without pollen grains on the stigma (a), with pollen grains not germinated (b), with pollen grains germinated (c), with pollen tubes reaching to the style (d) and with pollen tubes penetrating into the ovule (e).  
<sup>y</sup>‘KS-K’; (‘K-11’ X ‘Santo’) X ‘Kyoto No.3’, ‘KK-K’; (‘K-11’ X ‘Kyoto No.3’) X ‘Kyoto No.3’, ‘KN-N’; (‘K-11’ X ‘Nagasakiaka’) X ‘Nagasakiaka’, ‘KH-H’; (‘K-11’ X ‘Kyoto No.3’) X ‘Kyoto No.3’, ‘KN-N’; (‘K-11’ X ‘Minowase’) X ‘Minowase’.

Table 8. Chromosome associations at metaphase-I in hybrids derived from (*Brassicoraphanus* X *B. campestris*) X *B. campestris*.

Hybrid	2n	No. of PMCs analyzed	Mean number of associations per cell		
			I”	II	III
KS-K-I’	24	64	1.94	1.66	0.25
KS-K-4	24	27	2.78	1.22	0.04

<sup>z</sup>Univalents(I) through to trivalents (III).  
<sup>y</sup>‘KS-K’; (‘K-11’ X ‘Santo’) X ‘Kyoto No.3’

Table 9. Chromosome distributions at metaphase-II in hybrids derived from (*Brassicoraphanus* X *B. campestris*) X *B. campestris*.

Hybrid	2n	No. of PMCs analyzed	% of PMCs with indicated chromosome distribution at M <sub>II</sub>					
			21-12	11-13	10-14	9-15	8-16	Lagging
KS-K-I’	24	66	30.30	21.21	24.24	4.55	0.00	19.70
KS-K-4	24	84	42.86	13.10	10.71	5.95	1.19	26.19

“‘KS-K’; (‘K-11’ X ‘Santo’) X ‘Kyoto No.3’.



Table 10. Response of *Brassicoraphanus*, cruciferous crops and their BC<sub>2</sub> hybrids to clubroot disease.

Strain, cultivar and hybrid	Race <sup>2</sup>	No. of plants examined	No. of infected plants in 5 classes of infection <sup>3</sup>					% of infected plants	Disease index <sup>4</sup>
			A	B	C	D	E		
Chinese cabbage									
Nozaki No.2	2	15	0	0	0	114		100	97
	4	15	0	0	0	0	15	100	100
Kyoto No.3	2	15	0	0	0	0	15	100	100
	4	15	0	0	0	0	15	100	100
Japanese radish									
Minowase	2	15	13	2	0	0	0	13	1
	4	15	3	12	0	0	0	80	8
Shijunichi	2	15	15	0	0	0	0	0	0
	4	15	15	0	0	0	0	0	0
<i>Brassicoraphanus</i>									
K-11	2	15	15	0	0	0	0	0	0
	4	15	15	0	0	0	0	0	0
BC, hybrids <sup>5</sup>									
KS-K-1 x Kyoto No.3	2	13	5	2	0	3	3	62	38
	4	7	2	0	1	0	4	71	61
KS-K-1 x Minowase	2	15	14	1	0	0	0	7	1
	4	15	10	5	0	0	0	33	3

<sup>2</sup>“2 and 4; Williams’ races 2 and 4, respectively.

<sup>3</sup>Degree of clubbing in roots. A; nonclubbed, B; slightly clubbed, C; moderately clubbed, D; severely clubbed, E; very severely clubbed.

<sup>4</sup>Disease index =  $(0A + 10B + 30C + 40D + 50E) / (A + B + C + D + E)$ , where A to E are the number of infected plants in each degree of clubbing.

<sup>5</sup>“KS-K”; (‘K-11’ x ‘Santo’) x ‘Kyoto No.3’, ‘KM-M’; (‘K-11’ x ‘Minowase’) x ‘Minowase’.

are hardly obtainable through ovary culture. It seems that the degeneration of ovules, endosperm and embryos in the backcrosses occurs earlier than in the initial crossings. Further effective embryo and ovary culture techniques including assertion of appropriate embryonal stage for culture and improvement of culture medium to rescue very immature embryos will be required for improving the hybrid plant production.

### Backcross compatibility in BC<sub>1</sub> plants

Our results that there was no relation between pollen fertility in BC<sub>1</sub> plants derived from (‘K-11’ x *B. campestris*) x *B. campestris* are in agreement with Iwasa’s (1964) report. It is noticeable that the seed fertility increases as the backcross generation and its selection advance, and that the backcrosses of BC<sub>1</sub> plants x *B. campestris* and x *R. sativus* are more successful than the initial backcrosses under natural conditions. Generally, it is thought that seed sterility in hybrids is a manifestation of incongruity between different genetic backgrounds of parental species. Hybrids are the complex of various unbalanced combinations of different genes. If the unbalance is recovered, a certain degree of fertility will be restored. It is considered that balance of gene combinations in the present BC<sub>1</sub> plants improved to some extent with the progress of generation.

Chromosome pairings at metaphase-I in the PMCs and seed fertility of ‘KS-K-I’

and 'KS-K-4' pollinated by 'Kyoto No.3' were different in spite of their same chromosome number ( $2n=24$ ). Tokumasu and Kato (1988) suggested that the effect of meiotic difference in plants with the same chromosome number is not large enough to dominate the effect of their genetic differences on the seed fertility. Although it is difficult to ascertain their genetic difference from the present results, the origin of the chromosomes in 'KS-K-I' and 'KS-K-4' may be different. Iwasa and Ellerstrom (1981) reported that there was no correlation between various meiosis disturbances and pollen fertility in *Raphanobrassica*. In our study on chromosome associations, 'KS-K-4' showed almost the same frequency of lagging chromosomes as 'KS-K-I', but the pollen stainability and seed fertility were lower in 'KS-K-4' than in 'KS-K-I'. Our results also indicate that a negative correlation exists between the degree of meiosis disturbances and pollen and seed fertility.

Haga (1938), Fukushima (1945) and Mizushima (1952) indicated that "r" genome in *Raphanus* is partially homologous to "a","b" and "c" genomes in *Brassica*, and Haga (1938) pointed out that in *Brassica* "a", "b" and "c" genomes are partially homologous. There are one to two autosyndetic pairs in "a" genome (Fukushima, 1945; Mizushima, 1972) and two autosyndetic pairs in "c" genome (Thompson, 1956; Armstrong and Keller, 1982). Tokumasu (1970) reported that some homologous parts exist between heterologous chromosomes in *Raphanus*. In the present study, one to three trivalents appeared in the PMCs of 'KS-K-I' which may have "a" genome and a part of "c" and "r" genomes. The facts that two possible autosyndetic pairs are known in "a" and "c" genomes, that "r" genome is partially homologous to "a" and "c" genomes, and that "a" and "c" genomes are partially homologous may lead to the conclusion that autosyndesis and allosyndesis occurred simultaneously and formed a trivalent in the PMCs, although it is not obvious whether autosyndesis occurred within "r", "a" or "c" genome.

### Clubroot resistance in BC<sub>2</sub> progenies

The results that clubroot resistant BC<sub>2</sub> plants ('KS-K-I'  $\times$  'Kyoto No.3') with 23 chromosome number were obtained from the segregation of BC<sub>2</sub> progenies indicate that allosyndesis may occur among "r", "a" and "c" genomes and that chromosome number decreased as the backcrossing generation advanced. Our results suggest the possibility of transferring the clubroot resistance from *Brassicoraphanus* to *B. campestris* and to *R. sativus* and that of obtaining strong resistant Chinese cabbage and Japanese radish reversional plants through selections from the resistant BC<sub>2</sub> plants and further backcrossings of the BC<sub>2</sub> plants with Chinese cabbage and Japanese radish, respectively. However, seed fertility and quality of the reversional resistant plants should be estimated for breeding resistant strains or cultivars of Chinese cabbage or Japanese radish during the backcrossing and selection process.

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