Intergeneric Hybridization between *Raphanus sativus* L. and *Brassica nigra* Koch. and Alloplasmic Radish Derivative

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Cytological and morphological observations were made on the intergeneric new hybrids between *Raphanus sativus* and *Brassica nigra* and the derived progenies. Chromosomes of F_1 hybrids in the meiosis of the pollen mother cells (PMC) were amphihaploid except for an amphidiploid plant (NiR F_1 -3) in *B. nigra* $\times$ *R. sativus*. Amphihaploids showed the pairing type of (0-4) II + (9-17) I at metaphase I (MI) and the chromosome number of most of the PMCs at metaphase II (MII) ranged from 5 to 12 with a modal value of 8 or 9. An amphidiploid plant showed a relatively regular meiosis with 17 II at MI and PMCs with 17 chromosomes at MII. The morphology of the amphihaploids was intermediate between that of the parent cultivars except for the presence of white flowers. Due to the meiotic abnormalities, amphihaploids failed to produce viable pollen and seed. An amphidiploid of NiR F_1 -3 was very large and showed seed fertility. Backcrossed generations with *R. sativus* and *B. nigra* were sesquidiploids of BRR ($2n=26$) and BBR ($2n=25$) showing pairing types of 9 II+8 I and 8 II+9 I at MI, respectively. The BRR plants preserved their seed fertility not only in open pollination but in further backcrossing to two cultivars of *R. sativus*. Derived plants were euploid ($2n=18$) or hyperploid ($2n=19-22$). Prevailing occurrence of univalents and bivalents in both amphihaploids and derived progenies suggested the occurrence of more frequent autosyndetic pairings than allosyndetic ones at MI. Subsequently new alloplasmic radish lines, viz. *R. sativus* with *B. nigra* cytoplasm were bred. These lines produced fertile seeds in the next backcrossing to *R. sativus*.

KEY WORDS: *Raphanus sativus*, *Brassica nigra*, embryo and ovary culture, intergeneric hybrids, alloplasmic radish.

Introduction

Among the cultivated *Brassica* crops, *B. campestris* ($n=10$), *B. nigra* ($n=8$) and *B. oleracea* ($n=9$) are recognized to be the donors of A, B and C genomes, respectively. Intergeneric hybridization between *Raphanus sativus* ($n=9$, R genome) and these *Brassica* species has been carried out by many workers and reviewed by HARBERD and McARTHUR (1980). It was demonstrated that hybridization was more successful between *R. sativus* $\times$ *B. oleracea* and *B. campestris* $\times$ *R. sativus*. These hybrids were named *Raphanobrassica* (KARPECHENKO 1927) and *Brassicoraphanus* (TERASAWA and SHIMOTOMAI 1928, TOKUMASU 1976), respectively. SARASHIMA et al. (1980) produced another *Brassicoraphanus* between *B. oleracea* $\times$ *R. sativus* by means of embryo culture in vitro.

However, as far as we know, reciprocal hybrids between *R. sativus* and *B. nigra* have not yet been obtained. In this report the new hybrid plants and their progenies are described from the view point of breeding alloplasmic lines of radish.

Materials and Methods

Plant materials used in this study are intergeneric F_1 hybrids between *R. sativus* $\times$ *B. nigra* which were obtained previously by means of embryo culture (MATSUZAWA 1983). Other hybrid plants between *B. nigra* $\times$ *R. sativus* were produced by ovary culture according

to the method of INOMATAA (1977); namely young ovaries were cultured on the 0.8% agar medium of WHITE (1963) supplemented with 0.5 g/l casein hydrolysate and 30 g/l sucrose 7 days after pollination. Ovaries were cultured at ca. 25°C for 60 days, the resulting hybrid embryos were excised and immediately cultured on the same medium at 15°C. Hybrid plants thus obtained were carefully potted and nursed at 15°C for 5 weeks to avoid a spindly shape. Bud pollination was predominantly adopted to obtain the following hybrid progenies.

Somatic chromosomes were observed in root tips which were pretreated with 0.002 M 8-hydroxyquinoline for 3 hrs and fixed in Farmer's fluid overnight. Fixed materials were hydrolyzed in 1 N HCl at 60°C for 6 minutes and then stained in Schiff's reagent for 4 hrs. Root tips were further stained in a drop of acetocarmine on the slides and squashed strongly. For meiotic analysis, pollen mother cells (PMC) were squashed with a coverslip after immersion in acetocarmine and then the slides were warmed gently, followed by sealing with paraffine.

Results

As shown in Table 1, seven new hybrid plants were grown in four cross combinations. They showed the putative sum of gametic chromosomes of their parents, except for NiR F₁-3 which had 34 chromosomes (2n) suggesting the presence of the amphidiploid genome constitution of BBRR. RNi F₁ plants had a compound raceme with white flowers bearing a slight resemblance to those of *R. sativus*. The leaf shape and flower size were intermediate between those of parent cultivars. NiR F₁ hybrids showed a similar morphology to that of reciprocal hybrids except for the relatively tall raceme (Fig.1 A). An amphidiploid hybrid was very large especially with giant flowers more or less yellowish white in color (Fig.1 B).

Meiosis in the PMCs is described in Table 2. Amphihaploid hybrids exhibited the pairing type of (0-4)II + (9-17) I at metaphase I (MI), and more than 80% of the PMCs exhibited no pairing or one at most. Most of the nuclear plates of PMCs showed a number of chromosomes ranging from 5 to 12 at metaphase II (MII), although cells with 8 or 9 chromosomes were more frequent. PMCs with a 2II + 13 I configuration at MI and distribution of 17 chromosomes at MII are shown in Fig.1 C and Fig.1 D, respectively. These amphihaploid hybrids did not produce pollen that could be stained by acetocarmine, nor did they develop pods in backcrossing to the parental cultivars

Table 1. Intergeneric amphiploids between *R. sativus* and *B. nigra*

Hybrids	Cross combination		Somatic chromosomes	Genome constitution
	Female	Male		
RNi F ₁ -1	Shirokubi-nagashiri	Ni 110	2n=17	RB
RNi F ₁ -2	ditto	ditto	2n=17	RB
RNi F ₁ -3	Horyo	ditto	2n=17	RB
NiR F ₁ -1	Ni 110	Aokubi-miyashige	2n=17	BR
NiR F ₁ -2	ditto	ditto	2n=17	BR
NiR F ₁ -3	ditto	ditto	2n=34	BBRR
NiR F ₁ -4	ditto	Shogoin	2n=17	BR

Fig. 1. Intergeneric hybrids between *Raphanus sativus* and *Brassica nigra*.

A : Amphihaploid plant of *B. nigra* $\times$ *R. sativus* (NiR F₁-2, $2n=17$), B : An amphidiploid plant of *B. nigra* $\times$ *R. sativus* (NiR F₁-3, $2n=34$), C : A pollen mother cell at MI in NiR F₁-2, two bivalents and 13 univalents are observed, D : A pollen mother cell at MII in NiR F₁-2, distribution of 17 chromosomes is recognized, E : A pollen mother cell at MI in NiR F₁-3, 17 bivalents are observed, F : A pollen mother cell at MII in NiR F₁-3, pollen mother cell with 17+17 chromosomes is recognized.

Table 2. Chromosome configuration in the meiotic division of PMCs of the F_1 hybrids ($2n=17$) between *R. sativus* and *B. nigra*

Hybrids	pairing in M I					No. of cells observed	Chromosome numbers in M II cells												No. of cells observed
	4II+9I	3II+11I	2II+13I	1II+15I	17I		<5	5	6	7	8	9	10	11	12	12<			
RNi F _I -1		3	7	18	22	50	1	2	6	3	16	18	3	7	2				58
RNi F _I -2		11	10	10	19	50	1	1	0	6	19	19	5	0	1				52
NiR F _I -1		1	4	15	30	50	5	1	6	4	14	13	2	6	1	1			52
NiR F _I -2	1	1	7	9	33	51	2	4	8	5	8	8	5	9	3	1			53
NiR F _I -3			2	8	40	50		2	1	6	17	18	5	1	2				52
	17II	16II+2I	15II+4I	14II+6I				16	16	17	18	18							
NiR F _I -3	30	15	4	1		50		0	2	46	2	0							50

Table 3. Seed fertility in the backcrossing and selfing of an amphidiploid hybrid ($2n=34$) of *B. nigra* $\times$ *R. sativus*

Cross combinations	Flowers pollinated	Seeds obtained	True hybrid plants
NiR F_1 -3 $\times$ <i>B. nigra</i> (Ni 110)	89	22	18
NiR F_1 -3 $\times$ <i>R. sativus</i> (Horyo)	103	6	3
<i>B. nigra</i> (Ni 110) $\times$ NiR F_1 -3	183	11	0
<i>B. oleracea</i> (Taiwan-wase) $\times$ NiR F_1 -13	155	1	0
<i>B. campestris</i> (Kyoto No. 3) $\times$ NiR F_1 -3	94	*	0
<i>R. sativus</i> (Horyo) $\times$ NiR F_1 -3	84	0	—
NiR F_1 -3 selfed	136	0	—
NiR F_1 -3 open pollination	—	1	0

*: Young ovaries were cultured in vitro.

Table 4. Chromosome configuration in the meiotic division of PMCs of NiR F₁-3 × *B. nigra* and NiR F₁-3 × *R. sativus*

Hybrids	Pairing in M I			Chromosome numbers in M II cells											
	8 II + 9 I	7 II + 11 I	6 II + 13 I	10	11	12	13	14	15	16					
[NiR F ₁ -3 × Ni 110]-1 (2 <i>n</i> =25)	20	5	1	8	7	11	11	5	4	4					
-5	21	4		8	11	16	9	4	5						
-10	22	3		1	3	20	20	5	1						
	10 II + 6 I	9 II + 8 I	8 II + 10 I	7 II + 12 I	6 II + 14 I										
[NiR F ₁ -3 × Horyo]-1 (2 <i>n</i> =26)		19	5	1		3	7	11	7	14	7	2			
-2		23	2			1	9	8	10	13	8	1			
-3	1	22	2			4	4	11	19	8	2	2			

Table 5. Seed fertility in the hybrid of [NiR F₁-3 × *R. sativus* (cv. Horyo)]-1 (2*n*=26) when pollinated with radish

Cross combinations	Flowers pollinated (A)	Seeds obtained (B)	B/A
[NiR F ₁ -3 × Horyo]-1 × Okura	35	73	2.1
[NiR F ₁ -3 × Horyo]-1 × Round Red	16	34	2.1
[NiR F ₁ -3 × Horyo]-1 Open pollination	78	251	3.2

Table 6. Somatic chromosome number of the alloplasmic radish lines derived from successive backcrossing between NiR F₁-3 (2*n*=34) and *R. sativus*

Line	Lineage	Chromosome number (2 <i>n</i>)						No. of plants observed
		18	19	20	21	22		
OK	[NiR F ₁ -3×Horyo]×Okura	12	5	3	0	1	21	
RR	[NiR F ₁ -3×Horyo]×Round Red	11	6	2	1	1	21	
OP	[NiR F ₁ -3×Horyo] open pollination	19	13	2			34	

or in open pollination. As a result the hybrids could not be reared for the following generation. An amphidiploid hybrid of NiR F_1-3 , on the other hand, showed a relatively regular meiotic behavior with 17 bivalents at MI and 17-17 distribution type at MII (Fig. 1E and Fig. 1F) and produced pollen grains that could be stained.

Pollination was carried out in NiR F_1-3 used as pistil and pollen parents. As a result, 18 and 3 backcrossed hybrids were obtained between NiR $F_1-3 \times B. nigra$ and NiR $F_1-3 \times R. sativus$, respectively (Table 3). Table 4 shows the meiosis in three plants of both crosses. All of them were confirmed to be sesquidiploids of BBR ($2n=25$) or BRR ($2n=26$) in their genome constitution. Two plants in the former cross were hyperploids ($2n=26$) which were not listed in Table 4. Hybrid plants between NiR $F_1-3 \times B. nigra$ and NiR $F_1-3 \times R. sativus$ showed the most frequent pairing types of $8\text{II}+9\text{I}$ and $9\text{II}+8\text{I}$, respectively. Nuclear plates at MII had 10 to 16 chromosomes with a mode of 12, 13 or 14 chromosomes. The pollen of the hybrids between NiR $F_1-3 \times B. nigra$ was sterile and they did not produce any seed when crossed to cultivars or lines of *R. sativus* and *B. nigra*, although a few seeds were obtained in open pollination.

NiR $F_1-3 \times R. sativus$, on the other hand, was relatively fertile, namely ca. 50% of the pollen grains were stained with acetocarmine and they seemed to be viable. Seed fertility of [NiR $F_1-3 \times R. sativus$ (cv. Horyo)]-1 plant is shown in Table 5 when the plant was pollinated by two cultivars of *R. sativus*. In this plant, seeds were obtained more successfully in open pollination than in backcrossing to *R. sativus*. In Table 6, these three progenies are described as OK, RR and OP lines of radish whose cytoplasm is substituted by that of *B. nigra*. In each line, more than half of the individual plants were euploid with $2n=18$ showing regular meiosis. Twenty four plants were hyperploid with $2n=19$ and they exhibited a consistent pairing type of $9\text{II}+1\text{I}$ at MI. Other aneuploids were $2n=20$, 21 and 22 with less pollen and lower seed fertility. Euploid plants reproduced predominantly euploids with stable chromosome configuration while the $2n=19$ plants produced both euploids and hyperploids so far. Hypoploids were not obtained. Although amphiploid and sesquidiploid plants did not appear to be chlorophyll deficient, the three derived lines showed this unfavorable trait to some extent at seedling stage but chlorophyll deficiency in later growth was no longer present and the lines appeared to be normal. Via these lines, alloplasmic radishes have been bred in some representative cultivars of radish.

Discussion

Intergeneric hybridization between plants of the genus *Raphanus* and *Brassica* has been of great interest to cytogeneticists and plant breeders on account of the undefined genome relationship and its potential to increase the genetic diversity of the two genera. As for crosses of *R. sativus* (R genome) and three monogenomic *Brassica* species, viz. *B. campestris* (A genome), *B. nigra* (B genome) and *B. oleracea* (C genome), up to date, hybrid progenies have been bred in three cross combinations, i. e., *R. sativus* $\times$ *B. oleracea*, *B. campestris* $\times$ *R. sativus* and *B. oleracea* $\times$ *R. sativus*. However, to the authors' knowledge, the breeding of hybrid progenies between *R. sativus* and *B. nigra* had remained unsuccessful. The senior author confirmed the existence of strong barriers

in both pre- and post-zygotic phases for fertilization and the hybrid embryos were so poorly developed as to require in vitro cultivation (MATSUZAWA 1983). In the rearing of the hybrid F_1 plants, embryo culture technique was effective in $R. sativus \times B. nigra$ and three hybrids obtained previously were analyzed cytologically in the present study. In $B. nigra \times R. sativus$, ovaryculture was more effective than embryo culture and 4 hybrids were obtained. The difference in effectiveness between these two techniques may be attributed to the specific cross-incompatibility between the two species and will require further studies.

FUKUSHIMA (1945) postulated that the allosyndetic pairings at MI between chromosome complements of R and B genomes may range from 3 to 5 since the maximum number of bivalents in "R BC" hybrids is 9, on the assumption that "R C" hybrids exhibited 4 to 6 bivalent pairings and that there is no autosyndesis in C genome itself. Moreover by studying the meiotic chromosome behavior and observing the maximum 17 bivalents including 9 complete syndesis in two C genomes, it was estimated that the number of allosyndetic pairings between R and B genomes may range between 0 to 8. In fact, the hybrid plants analyzed in the present study showed some kinds of pairings at MI irrespective to their autosyndetic or allosyndetic nature but trivalents and/or quadrivalents were not detected in either amphiploid or sesquidiploid plants. KANEKO (1980) reported that 35% of the PMCs exhibited one to 3 autosyndetic pairings in haploid $R. sativus$. In haploid $B. nigra$, PRAKASH (1976) observed one to 2 bivalents in autosyndetic pairings in 33% of the PMCs at MI. Considering these facts, it is likely that bivalent formation in the present ampioid plants may be attributed to autosyndesis. Consequently the allosyndesis between R and B genomes may be less frequent than has been considered hitherto and the genetic recombination in these two genomes may rarely take place so as to increase the genetic diversity of *Brassica* crops.

Spontaneous occurrence of amphidiploids is not uncommon in interspecific and intergeneric hybridization of *Brassica* species (U 1935, OLSSON 1960, NAMAI 1971) even when diploid male and female species are used in crosses. This phenomenon is ascribed to the union of unreduced gametes. An amphidiploid obtained in this study was reared presumably through this phenomenon. This amphiploid which yielded several seeds when pollinated by $B. nigra$ and $R. sativus$ failed to do so when used as a pollen parent. Assuming that the pollen of this amphiploid was fertile, it is likely that the cross-compatibility of pistil parents was associated with the insufficient development of hybrid seeds in crosses. Based on the same assumption, the fact that this amphiploid produced few seeds either in selfing or in open pollination may suggest the intrinsic manifestation of a distinct self-incompatibility which is prevailing in some members of *Brassica*.

The derived sesquidiploids also maintained their seed fertility to the extent of enabling the breeding of further generations when they were backcrossed to $R. sativus$. Resulting plants were euploid ($2n=18$) or hyperploid ($2n=18+1-4$) and did not form any multivalents at MI except for bivalents. The absence of hypoploid and the cytological studies performed in this generation made it possible to assume that the chromosome complement

of the female gametes consisted of 9 chromosomes of the R genome supplemented with zero to 4 chromosomes of the B genome. In this regard, $2n=19$ plants may be valuable materials to analyze the genetic potential of each chromosome of the B genome.

KATO and TOKUMASU (1980) reported that Japanese radishes with *B. japonica* (A genome) cytoplasm suffered from chlorophyll deficiency. SARAHIMA (1984) also reported the presence of severe chlorophyll deficiency in *R. sativus* with *B. oleracea* cytoplasm. The alloplasmic new lines bred in this study also showed moderate chlorophyll deficiency at the seedling stage but this defect was no longer detected in later growth. Some lines with only slight or no sign of this deficiency were bred in the next generation. Qualitative and quantitative evaluation of some agronomic characteristics is now in progress for further breeding work of *Brassica* crops in comparison with other kinds of alloplasmic radishes.

Literature cited

- FUKUSHIMA, E. 1945. Cytogenetic studies on *Brassica* and *Raphanus* I. Studies on the intergeneric F_1 hybrids between *Brassica* and *Raphanus*. J. Dept. Agric. Kyushu Imp. Univ. 7 : 281~400.
- HARBERD, D. J. and E. D. MCARTHUR 1980. Meiotic analysis of some species and genus hybrids in the *Brassicaceae*. In "Brassica crops and wild allies" TSUNODA, S., K. HINATA and C. GOMEZ-CAMPO (eds.), Japan. Scientific Societies Press, Tokyo. 65~87.
- INOMATA, N. 1977. Production of interspecific hybrids between *Brassica campestris* and *B. oleracea* by culture in vitro of excised ovaries I. Development of ovaries in various culture media with yeast extract and casein hydrolysate. Japan. J. Breed. 27 : 295~304.
- KANEKO, Y. 1980. Haploid plants of *Raphanus sativus*. Chromosome Information Service 28 : 21~23.
- KARPECHENKO, G. D. 1927. Polyploid hybrids of *Raphanus sativus* $\times$ *Brassica oleracea* L. Bull. Appl. Bot., Genet. P1. Breed. 17 : 305~410.
- KATO, M. and S. TOKUMASU 1980. Nucleus substitution of *Brassica japonica* SIEB. with *Raphanus sativus* L. and its resultant chlorophyll deficiency. Euphytica 29 : 97~106.
- MATSUZAWA, Y. 1983. Studies on the interspecific and intergeneric crossability in *Brassica* and *Raphanus*. Special Bull. Coll. Agr. Utsunomiya Univ. 39 : 1~86.
- NAMAI, H. 1971. Studies on the breeding of oil rape (*Brassica napus* var. *oleifera*) by means of interspecific crosses with the application of grafting method or the treatment of sugar solution. Japan. J. Breed. 21 : 40~48.
- OLSSON, G. 1960. Species crosses within the genus *Brassica* II. Artificial *napus* L. Hereditas 46 : 351~386.
- PRAKASH, S. 1973. Haploidy in *Brassica nigra* Koch. Euphytica 22 : 613~614.
- SARASHIMA, M., Y. MATSUZAWA and T. KIMURA 1980. Intergeneric hybridization between *Brassica oleracea* and *Raphanus sativus* by embryo culture. EUCARPIA Cruciferae Newsletter 5 : 25.
- 1984. On the artificially induced plants in *Brassica oleracea* $\times$ *Raphanus sativus* V. B_2F_2 plants. Japan. J. Breed. 34(Suppl.) : 66~67.
- TERASAWA, Y. and N. SHIMOTOMAI 1928. Bastardierungsversuche bei *Brassica* und *Raphanus*. Sci. Rep. Tohoku Imp. Univ., Ser. N. No. 4 : 827~841.
- TOKUMASU, S. 1976. The increase of seed fertility of *Brassicoraphanus* through cytological irregularity. Euphytica 25 : 463~470.
- U, N. 1935. Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Japan. J. Bot. 7 : 389~452.
- WHITE, P. R. 1963. The cultivation of animal and plant cells. Ronald Press Co., New York.

ダイコンとクロガラシとの属間交雑と異質細胞質ダイコンの作出

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ダイコン (*Raphanus sativus* L., $n=9$) $\times$ クロガラシ (*Brassica nigra* Koch., $n=8$) とその逆交雑で, それぞれ胚及び子房培養法によって得られた雑種植物とその後代の細胞学的, 形態的な調査を行った. 複半数性 F_1 植物 ($2n=17$) は, PMC の MI で $(0\sim4) II + (9\sim17) I$ の染色体対合型を示し, 17 I 型のものが 57% で最も多く, ついで $1II + 15I$ 型が約 20% であった. また MII では, 8 ないし 9 個の染色体をもつ核が多かった. これらの植物の花色はダイコンと同じ白色であったが, 他の形態は両親種の間であった. また花粉稔性は極めて低く, 結莢も認められず, 後代植物を得ることができなかった.

クロガラシ $\times$ ダイコンで得られた 1 個体の複二倍体 F_1 植物 ($2n=34$) では, MI で 17II の形成がみられ (60%), また MII で 17 個の染色体をもつ核が多かった (92%). この植物は, 複半数性のものに比べてより巨大型であり, 花色はやや黄白色を呈し, 花粉稔性は高かった. この雑種個体を母親としてダイコンおよびクロガラシの花粉を交配して戻し交雑をしたところ, それぞれ BRR ($2n=26$) 及び BBR ($2n=25$) のゲノム組成をもつ 2 基三倍体 that 得られた. これらの植物は, 多くの PMC の MI でそれぞれ $9II + 8I$ 及び $8II + 9I$ の対合型を示し, また MII では 12, 13 あるいは 14 個の染色体をもつ核が多く観察された. それらの植物は, 雑種 F_1 植物と同様に, 多価染色体を形成することがなく, したがってこれら雑種植物の染色体対合は同親接合によるものと考えられた.

BRR 植物では, ダイコンの 2 品種との戻し交雑と放任受粉とで種子が得られたが, BBR 植物では得られなかった. BRR 植物の後代は, Table 6 に示すように OK, RR および OP 系統とした. 3 系統の植物は, $2n=18$ の正倍数体が最も多く (55%), それらの PMC の MI 及び MII は正常であった. $2n=19$ 植物は, MI で $9II + 1I$ の対合型を示し, また種子が得られた. 正倍数体植物にダイコンの数品種を戻し交雑し, クロガラシの細胞質をもったダイコン系統を育成した. 雑種 F_1 および 2 基三倍体植物は葉緑素欠乏症状を示さなかったが, 異質細胞質系統は生育初期に軽度ながらこの症状を示した. しかし生育後期には回復するものが多く, ほぼ正常と思われる植物も得られた.

今後, 得られた異質細胞質ダイコンの農業的形質を調査することにより, クロガラシの細胞質の比較評価が可能になると考えられる. また, 育成過程で得られた $2n=19$ 植物は, ダイコンの RR ($2n=18$) ゲノムにクロガラシの染色体が添加した植物と思われ, B ゲノムのそれぞれの染色体の遺伝的解析の一助となると期待される.