Breeding of *Moricandia arvensis* Monosomic Chromosome Addition Lines ($2n=19$) of Alloplasmic (*M. arvensis*) *Raphanus sativus*

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By backcrossing to *Raphanus sativus* cv. 'Pink ball', 55 BC₂ plants were obtained from two sesquidiploid BC₁ plants (MaRR, $2n=32$) between *Moricandia arvensis* (MaMa, $2n=28$) and *R. sativus* (RR, $2n=18$). Their somatic chromosome numbers ranged from $2n=18$ to $2n=23$, except for one hyperploid plant with $2n=44$. In the BC₃ generation, 64 plants ($2n=19$) were generated from 16 BC₂ plants with $2n=19\sim23$. Each plant with $2n=19$ exhibited both morphological and physiological characteristics diagnostic for the presence of an added chromosome of *M. arvensis* genome, and showed predominantly the chromosome pairing type of $9II + 1I$ at metaphase I of PMCs. They were classified into twelve (a-l) types of monosomic chromosome addition lines (MALs) of alloplasmic (*M. arvensis*) *R. sativus* carrying *M. arvensis* cytoplasm by their morphological, physiological and cytogenetical characteristics. The mean of seed setting in the twelve types of MALs ranged from 2.88 grains (f-type) to 0.51 grains (j-type) per pollinated flower when they were backcrossed to *R. sativus* cv. 'Pink ball'. The transmission rates to the next generation through female gametes ranged from 32.5% (f- and j-types) to 5.5% (h-type) when smaller seeds were selectively grown. The specific characteristics of each type of MALs were transmitted from the BC₃ generation to the BC₄ and BC₅ ones without any modification. The MALs of the distinctive twelve types were also identified using RAPD markers in the BC₅ generation. Pollen fertility of the twelve types of MALs ranged from 85.6% (c-type) to 3.4% (l-type), although four types (g-, h-, i- and j-types) exhibited complete male sterility. Furthermore, alloplasmic (*M. arvensis*) *R. sativus* plants ($2n=18$) which were derived from male fertile MALs showed complete male sterility. The twelve types of MALs produced in this study should be useful materials to determine the localization of genes for agronomic traits on the individual chromosome of *M. arvensis* and the alloplasmic (*M. arvensis*) *R. sativus* should also be a useful material for the development of a new cytoplasmic male sterility system in *R. sativus*.

Key Words: *Moricandia arvensis*, *Raphanus sativus*, monosomic chromosome addition lines (MALs), alloplasmic (*M. arvensis*) *R. sativus*, male sterility.

Introduction

Moricandia arvensis (L.) DC. ($2n=28$, MaMa) is one of the C₃-C₄ intermediate species of *Brassicaceae* that are characterized by a high concentration of organelles in the bundle-sheath cells and low CO₂ compensation concentrations. In *Brassicaceae*, other C₃-C₄ intermediate species were found within species of the genera *Moricandia*, *Diplotaxis* and *Brassica* (Apel *et al.* 1996, 1997). Apel *et al.* (1984) reported the photosynthetic properties of the F₁ and BC₁ plants between *B. alboglabra* (C₃ species) and *M. arvensis*, and suggested that the C₃-C₄ intermediate trait, especially low photo-respiration activity, might be valuable for the breeding of *Brassica* crops. In order to analyze the genetic characteristics of the C₃-C₄ intermediate trait and to introduce it into the cultivated species, a number of intergeneric hybrids between *M. arvensis* and some C₃ species in *Brassicaceae* have been obtained through the application of the conventional pollinating method, embryo rescue technique and protoplast fusion (Toriyama *et al.* 1987, Takahata 1990, Takahata and Takeda 1990, Takahata *et al.* 1993, Kirti *et al.* 1992, Razmjoo *et al.* 1996, Bang *et al.* 1996). However, very little is known about the evolution and genetic system of the C₃-C₄ intermediate species in *Brassicaceae*.

Since the monosomic chromosome addition lines (MALs) of *Raphanus sativus*-*B. oleracea* and *B. rapa*-*B. oleracea* were produced by Kaneko *et al.* (1987) and Quiros *et al.* (1987) respectively, several of MAL series have been successfully bred through interspecific and intergeneric hybridization (Quiros *et al.* 1988, Jahier *et al.* 1989, Chen *et al.* 1992, Srinivasan *et al.* 1998, Kaneko *et al.* 2001). These MALs were also examined for the analysis of agronomic traits and gene(s) that were assumed to be located on the added chromosomes from the B and C genomes of *Brassica* species (Zhu *et al.* 1993, Chevre *et al.* 1996, 1997, Kaneko *et al.* 1996, 2000, Chen *et al.* 1997). The advantages of using MALs include the possibility of assigning species-specific gene(s) and/or characteristics to particular chromosomes,

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and the potential to transfer desirable agronomic traits between species (Namai 1987, McGrath and Quiros 1990, Matsuzawa *et al.* 1996).

In our previous paper, it was reported that the intergeneric F_1 hybrids between *M. arvensis* and *R. sativus* (C_3 species) were produced through the application of the embryo rescue technique when *M. arvensis* was used as a pistillate parent, and then BC_2 seeds were obtained by successive backcrosses to *R. sativus* cv. 'Pink ball' (Bang *et al.* 1996). In this study, twelve types of MALs of alloplasmic (*M. arvensis*) *R. sativus* will be described with reference to their morphological, physiological and cytogenetical characteristics in addition to RAPD markers.

Materials and Methods

Plant materials and production of monosomic chromosome addition lines (MALs)

M. arvensis (strain 4) is an accession of Cruciferae genetic stocks from the Laboratory of Plant Breeding, Tohoku University, Japan. *R. sativus* cv. 'Pink ball' for the recurrent parent is an accession from the Laboratory of Plant Breeding, Utsunomiya University, Japan. BC_2 seeds used in this study were obtained from two sesquidiploid BC_1 plants (MaRR, $2n=32$) between *M. arvensis* (MaMa, $2n=28$) and *R. sativus* cv. 'Pink ball' (RR, $2n=18$) by backcrossing to *R. sativus* cv. 'Pink ball' (Bang *et al.* 1996). Out of them, the fully mature 83 seeds were sown in small pots and then 55 BC_2 plants were grown in a greenhouse after being transplanted to ceramic pots 18 cm in diameter. The BC_2 plants with $2n=19\sim23$ were successively backcrossed to *R. sativus* cv. 'Pink ball' to maintain and newly generate the MAL with $2n=19$ in the BC_3 generation. All the plants with $2n=19$ were firstly classified into twelve types of MALs based on the morphological, physiological and cytogenetical characteristics in the BC_3 generation, and were successfully maintained as distinctive MALs to the BC_5 generation through successive backcrosses to *R. sativus* cv. 'Pink ball'.

Morphological, physiological and cytogenetical characteristics of twelve types of MALs

Twelve types of MALs were identified based on the morphological and physiological characteristics of their leaves, roots and flowers and the cytogenetical features in the BC_3 generation. Chlorophyll content was determined using a chlorophyll meter (SPAD-502, Minolta, Japan) and several leaf characters were measured when the leaves were fully extended. Root diameter was measured on the more enlarged part during the period of seed setting. The measurements of petal length, petal width and ovary length were made using ten flowers in full bloom. The number of ovules was counted using 10 ovaries under a fluorescence microscope after staining with 0.1% aniline blue. Bolting was recorded by observing the mean days from sowing to bolting when flower buds protruded from the rosette. Plant height was measured when the individual plants were in full bloom.

Somatic chromosomes were observed using the Feulgen stain squash method followed by 1% acetocarmine staining. At least 20 clear metaphases I (MI) in pollen mother cells (PMCs) per MAL plant were observed using the 1% acetic orcein smear method for the determination of meiotic chromosome pairing. Pollen tetrad was observed in at least 500 sporads after staining with 1% acetic orcein. Pollen fertility was ascertained by observing 1000 pollen grains after staining with 1% acetocarmine. Seed setting was determined by counting the number of mature seeds per pollinated flower when the MAL plants were backcrossed to *R. sativus*. Transmission rate of the added chromosome in each type of MALs through female gametes was calculated by observing about 30 plants per each type in the BC_4 and BC_5 generations that were grown from the selected smaller seeds according to the method of Kaneko *et al.* (1991).

RAPD analysis

RAPD markers were employed to confirm the validity of the twelve types of MALs in the BC_5 generation according to the method of Kaneko *et al.* (2001). Total DNA was extracted from young leaves of MAL plants using the Extraction Kit (Nucleon Phyto Pure, Scotlab Bioscience, Buckingham, UK). Random 10-mers primers used in this study were selected from 40 primers (kit OPA, OPE, OPR and OPT, Operon Tech. Co., Alameda, CA, USA). For DNA amplification, a thermocycler (Program-tempucontrol system PC-700, ASTEC Co., Fukuoka, Japan) was used; the DNA templates that were resolved by Analysis Kit (Ready-To-Go, Pharmacia, NJ., USA) in 0.5 ml tubes were amplified according to the schedule for DNA amplification developed by Kaneko *et al.* (2000). The samples were run in 5% polyacrylamide for 50 min at 40 mA to separate the amplified products, which were visualized by ethidium bromide (0.5 $\mu\text{g/ml}$) staining. These procedures were repeated twice. The reliable bands that were not observed in *R. sativus* cv. 'Pink ball', but obtained consistently in *M. arvensis*, were assigned to RAPD bands specific to MALs.

Results

Production of twelve types of MALs and their morphological, physiological and cytogenetical characteristics

The somatic chromosome number of 55 BC_2 plants ranged from $2n=18$ to $2n=23$, except for one hyperploid plant with $2n=44$ (Table 1). The plants with $2n=20$ were obtained in the highest frequency (25.5%), followed by $2n=18$ (21.8%), $2n=19$ (20.0%), and both $2n=21$ and $2n=22$ (14.6%). When 16 BC_2 plants with $2n=19\sim23$ were successively backcrossed to *R. sativus*, 214 BC_3 plants were obtained. Their somatic chromosome number ranged from $2n=18$ to $2n=21$, except for 2 plants with $2n=27$ (Table 1). Most of them were $2n=18$ (138 plants), followed by $2n=19$ (64), $2n=20$ (8) and $2n=21$ (2). Sixty-four plants with $2n=19$ were consecutively and newly generated from 16 plants of BC_2 , consisting of 2 primary MAL plants with $2n=19$, 7 plants

Table 1. Distribution of somatic chromosome numbers in BC₂ and BC₃ plants derived from two sesquidiploid BC₁ plants (2n=32) after successive backcrosses to *R. sativus* cv. 'Pink ball'

Line	No. of plants Observed	Somatic chromosome number (2n)						
		18	19	20	21	22	23	23<
BC ₂	55	12	11	14	8	8	1	1
	(%)	21.8	20.0	25.5	14.6	14.6	1.8	1.8
BC ₃ ¹⁾	214	138	64	8	2			2
	(%)	64.5	29.9	3.7	0.9			0.9

¹⁾ BC₃ plants were obtained from 16 BC₂ plants with 2n=19~23 by backcrossing to *R. sativus* cv. 'Pink ball'.

with 2n=20, 3 plants with 2n=21, 3 plants with 2n=22 and one plant with 2n=23. Another 26 plants of BC₂ with 2n=19~22 showed a lower seed setting or complete sterility, resulting in the failure to transmit the added chromosome(s) to the BC₃ generation.

Compared with *R. sativus* cv. 'Pink ball' and alloplasmic (*M. arvensis*) *R. sativus* (2n=18), all the plants with 2n=19 exhibited distinctive and diagnostic features based on morphological, physiological and cytogenetical characteristics, resulting from the presence of an added chromosome of *M. arvensis* genome (n=14). Based on their specific characteristics in the BC₃ generation, 64 plants (2n=19) were classified into twelve types of MALs of alloplasmic (*M. arvensis*) *R. sativus*, and tentatively referred to as the a-type to l-type (Table 2). Morphological, physiological and cytogenetical characteristics were also observed for each type of MALs in the BC₃, BC₄ and BC₅ generations (Table 3).

Significant differences between *R. sativus* cv. 'Pink ball' and *M. arvensis* were recognized in the morphological and physiological characteristics of the leaves, roots and flowers. The leaves of *R. sativus* showed numerous lobes, the petals and roots were tinged with pink and the roots were enlarged (Fig. 1A). On the other hand, *M. arvensis* exhibited spatulate leaves, the sepals and petals were tinged with purple, the roots were not enlarged and the plants displayed an early bolting character (Fig. 1B). The a-type plants had twisted leaves with a large number of lobes, and the roots and petal organs were pale-pink. The b-type plants showed crenate lobes, and a conspicuous purple pigmentation on the surface of the roots and both sepal and petal organs. The c-type plants showed whitish roots and wide intervals between lobes, and exhibited the highest pollen fertility (85.6%). On the other hand, the alloplasmic (*M. arvensis*) *R. sativus* (2n=18) and four types of MALs (g-, h-, i- and j-types) showed male sterility but their flowers had well-developed anthers and nectaries. In the d-type plants, the leaves were lighter green and thinner, and the flowers were smaller and

purplish-pink with a low pollen fertility (Fig. 1C). The e-type plants, however, were characterized by green leaves with denticulate lobes and larger petal and ovary organs than those of the other types. The f-type plants showed an erect type and had sleek leaves with wider intervals between the lobes, and exhibited the highest seed setting (2.88 grains) and the highest transmission rate of 32.5% when smaller seeds were selectively grown. The g-type plants had the darkest green leaves and small roots. The h-type plants exhibited the longest leaves with a small number of lobes, and showed the lowest transmission rate (5.5%). The i-type plants had short and narrow leaves with many lobes compared with those of the c-, f- and h-types, and the petals were purplish-pink. In the j-type plants, the leaves were light green and short with a small number of lobes, and the plants exhibited the highest transmission rate of 32.5%. The k-type plants had stiff and wrinkled leaves with a dentate leaf margin (Fig. 1D), and showed a lower pollen fertility (12.0%). The l-type plants closely resembled the alloplasmic (*M. arvensis*) *R. sativus* (2n=18) plants except for the slightly dark green leaves, the long and wide petals and some fertile pollen (3.4%).

The plants of all types of MALs exhibited predominantly the chromosome pairing type of 9II + II at MI of PMCs (Fig. 2, Table 3) and had pollen tetrads that appeared normal as in the case of *R. sativus* cv. 'Pink ball'. However, their pollen fertility showed a significant difference, as mentioned above. No significant correlation was recognized between pollen fertility and seed setting although seed setting in each type of MALs ranged from 2.88 grains (f-type) to 0.51 grains (j-type). Accordingly, both pollen fertility and seed setting could be utilized as a potential genetic marker to identify each type of MALs.

RAPD analysis

RAPD markers in the twelve types of MALs are shown in Table 4. The number of markers for each type of MALs

Table 2. Classification of twelve types of MAL (a~l, 2n=19) derived from BC₂ plants (2n=19~23) by backcrossing to *R. sativus* cv. 'Pink ball'

No. of plants observed	Plant type											
	a	b	c	d	e	f	g	h	i	j	k	l
64	8	4	3	2	1	2	3	2	5	5	8	1

¹⁾ Unidentified type of MALs.

Fig. 1. Comparison of morphological characteristics of parental species and MAL plants. A: *Raphanus sativus* ($2n=18$), B: *Moricandia arvensis* ($2n=28$), C: d-type of MAL ($2n=19$) and D: k-type of MAL ($2n=19$).

ranged from 9 (d-type) to 24 (i-type). Among these bands, RAPD markers specific to each type alone ranged from one for the a- type and b-type to five for the i-type. No marker was observed alone for the d-type, e-type, g-type and h-type of MALs. The g-type and h-type of MALs closely resembled each other in the pattern of bands in spite of their distinctive plant morphology.

Discussion

For the successful classification of MALs, it is essential to use specific markers. The morphological characteristics could be suitable markers for classifying the MALs especially when the two species concerned showed significant differences in their morphological characteristics. In this study, *R. sativus* cv. 'Pink ball' and *M. arvensis* showed significant differences in morphological characteristics, as mentioned above. We were able to classify twelve types of MALs of alloplasmic (*M. arvensis*) *R. sativus* ($2n=19$, RR+1ma) based on their morphological, physiological and cytogenetical characteristics, and confirmed that the specific characteristics of each type of MALs were transmitted through female

Fig. 2. Chromosome pairing type of 9II + II at metaphase I of pollen mother cell in a monosomic addition line (MAL).

gametes from BC₃ to BC₄ and BC₅ generations without any modification (Table 3). The twelve types of MALs showed adequate seed setting (0.51 ~ 2.88 grains) and transmission rates (5.5% ~ 32.5%) to permit their successful maintenance when they were backcrossed to *R. sativus*. The MALs of the distinctive twelve types were reconfirmed using RAPD markers in the BC₅ generation (Table 4). These results led to the conclusion that the twelve types of MALs produced in this study were true MALs of alloplasmic (*M. arvensis*) *R. sativus* and the morphological, physiological and cytogenetical characteristics in addition to RAPD-specific markers were essential to classify each type of MALs.

We were unable to obtain fourteen types of MALs corresponding to the *M. arvensis* genome ($n=14$, Ma). In other MALs in *Brassicaceae*, complete series of MALs had not been developed (Kaneko *et al.* 1987, 2001, Quiros *et al.* 1987, 1988, Jahier *et al.* 1989, Chen *et al.* 1992, Srinivasan *et al.* 1998). To produce all the MALs, cross incompatibility within interspecific and intergeneric hybridization and successive backcrosses to background species has to be overcome. In a previous study (Bang *et al.* 1996), we were able to obtain the BC₂ seeds by overcoming the barrier(s) existing in the intergeneric hybridization between *M. arvensis* and *R. sativus* and in the backcrossing of F₁ hybrids to *R. sativus* through the application of the embryo rescue technique followed by chromosome doubling for the F₁ hybrids. In this study, 26 plants out of 42 BC₂ plants with one to five alien chromosomes ($2n=19 \sim 23$) could not generate plants with added chromosomes in BC₃. The missing two types of MALs may be included in these plants. Therefore, it was suggested that a genetic system controlling the cross incompatibility in intergeneric hybridization between *M. arvensis* and *R. sativus* occurred on a few chromosomes of the *M. arvensis* genome, most likely on the missing two chromosomes.

Out of twelve types of MALs (a-type ~ l-type) carrying the *M. arvensis* cytoplasm, four types (g-, h-, i- and j-types) showed a complete male sterility and the other MALs showed a pollen fertility ranging from 3.4% (l-type) to 85.6% (c-type) whereas reverted alloplasmic (*M. arvensis*) *R. sativus* ($2n=18$) predominantly exhibited complete male sterility (Table 3). Therefore, it appears that the restoration

Table 3. Morphological, physiological and cytogenetical characteristics of twelve types (a~l) of MALs in BC₃ to BC₅ generations, *R. sativus* (R), alloplasmic *R. sativus* and *M. arvensis* (Ma)

Character	Twelve types of MALs												R	aR ¹⁾	Ma
	a	b	c	d	e	f	g	h	i	j	k	l			
No. of plants observed	18	14	10	16	7	21	8	5	10	24	17	13	5	5	5
Leaf															
Chlorophyll content ²⁾	32.2 ^{bc3)}	31.2 ^{bc}	34.7 ^{bcd}	19.9 ^a	34.7 ^{bcd}	28.8 ^b	42.5 ^d	32.4 ^{bc}	32.4 ^{bc}	28.8 ^b	35.8 ^{bcd}	38.8 ^{cd}	54.9 ^e	31.5 ^{bc}	56.5 ^e
Length (cm)	31.4 ^{def}	30.9 ^{cdef}	29.7 ^{bcd}	32.3 ^{ef}	29.7 ^{bcd}	26.8 ^b	27.0 ^b	32.5 ^f	28.5 ^{bcd}	23.8 ^a	30.2 ^{cdef}	30.0 ^{cdef}	27.9 ^{bc}	29.3 ^{bcd}	— ⁴⁾
Width (cm)	10.9 ^d	10.7 ^d	10.8 ^d	10.1 ^{cd}	10.8 ^d	9.2 ^{bc}	8.3 ^b	8.3 ^b	7.0 ^a	10.7 ^d	11.2 ^d	10.8 ^d	10.5 ^d	10.0 ^{cd}	—
Thickness (mm)	0.33 ^{cde}	0.38 ^f	0.30 ^{abc}	0.26 ^a	0.37 ^{ef}	0.32 ^{bcd}	0.30 ^{abc}	0.32 ^{bcd}	0.27 ^{ab}	0.38 ^f	0.37 ^{ef}	0.35 ^{def}	0.37 ^{ef}	0.33 ^{cde}	—
No. of lobe	12.5 ^{cd}	11.5 ^{bcd}	9.0 ^b	10.3 ^{bcd}	13.0 ^{cd}	9.0 ^b	10.3 ^{bcd}	10.0 ^{bc}	13.5 ^d	10.8 ^{bcd}	12.8 ^{cd}	12.0 ^{bcd}	10.3 ^{bcd}	11.0 ^{bcd}	0 ^a
Root															
Diameter (cm)	4.1 ^{ab}	4.4 ^{abc}	4.3 ^{abc}	4.4 ^{abc}	4.9 ^{abc}	3.7 ^a	3.5 ^a	4.5 ^{abc}	5.6 ^c	4.7 ^{abc}	4.0 ^a	4.4 ^{abc}	7.5 ^d	5.5 ^{bc}	—
Color	pale-pink	purple	white	pink	pale-pink	pink	pale-pink	pink	pink	pink	pink	pink	pink	pink	pale-green
Flower															
Petal length (mm)	20.7 ^{def}	19.6 ^{bcd}	20.3 ^{cde}	15.1 ^a	21.6 ^f	21.3 ^{ef}	19.9 ^{cd}	18.6 ^b	19.6 ^{bc}	23.9 ^e	21.4 ^{ef}	21.4 ^{ef}	21.7 ^f	18.5 ^b	23.7 ^e
Petal width (mm)	10.5 ^e	9.0 ^{cde}	9.2 ^{de}	6.0 ^a	10.0 ^{fg}	10.8 ^g	8.4 ^{bc}	6.5 ^a	8.6 ^{bcd}	10.5 ^e	9.2 ^{de}	10.6 ^g	9.5 ^{ef}	8.0 ^b	8.1 ^b
Petal color	pale-pink	purple	pale-pink	purplish-pink	pale-pink	pale-pink	pale-pink	pale-pink	purplish-pink	pale-pink	pale-pink	pale-pink	pink	pale-pink	purple
Ovary length (mm)	10.0 ^f	8.4 ^c	9.1 ^d	7.2 ^a	10.0 ^f	8.0 ^b	9.0 ^d	9.0 ^d	10.2 ^f	10.0 ^f	9.6 ^e	9.9 ^{ef}	11.0 ^g	9.0 ^d	11.9 ^h
Ovule number	4.6 ^a	4.2 ^a	3.9 ^a	4.5 ^a	5.8 ^{ab}	4.0 ^a	6.9 ^b	6.9 ^b	6.7 ^b	5.0 ^{ab}	5.8 ^{ab}	5.5 ^{ab}	4.9 ^{ab}	4.3 ^a	73.2 ^c
Bolting (day)	191.3 ^b	193.5 ^b	185.5 ^b	192.7 ^b	186.7 ^b	182.0 ^b	188.0 ^b	185.0 ^b	184.0 ^b	184.0 ^b	192.2 ^b	184.5 ^b	189.8 ^b	180.3 ^b	50.7 ^a
Plant height (cm)	46.7 ^{ab}	41.7 ^{ab}	51.3 ^{abc}	55.3 ^{abc}	43.0 ^{ab}	63.0 ^c	47.0 ^{ab}	55.8 ^{abc}	47.0 ^{ab}	52.5 ^{abc}	39.7 ^a	48.5 ^{abc}	57.2 ^{bc}	55.0 ^{abc}	49.8 ^{abc}
Chromosome pairing															
at M I of PMCs	9II + II	9II + II	9II + II	9II + II	9II + II	9II + II	9II + II	9II + II	9II + II	9II + II	9II + II	9II + II	9II	9II	14II
Pollen tetrad (%)	99.2 ^{ab}	98.1 ^{ab}	99.3 ^{ab}	98.7 ^{ab}	99.6 ^b	99.4 ^{ab}	99.8 ^b	99.7 ^b	99.5 ^{ab}	98.8 ^{ab}	97.6 ^{ab}	99.6 ^b	100.0 ^b	99.7 ^b	100.0 ^b
Pollen fertility (%)	60.2 ^c	53.9 ^c	85.6 ^d	22.9 ^b	71.0 ^{cd}	53.6 ^c	ms ⁵⁾	ms ^a	ms ^a	ms ^a	12.0 ^{ab}	3.4 ^a	97.9 ^e	ms ^a	98.0 ^c
Seed setting (grain)	1.05 ^{ab}	2.32 ^{bcd}	1.66 ^{ab}	1.03 ^{ab}	0.96 ^{ab}	2.88 ^c	2.59 ^{cde}	1.2 ^{abc}	1.46 ^{abcd}	0.51 ^a	1.63 ^{ab}	2.08 ^{bcd}	3.01 ^{de}	2.20 ^{bcd}	8.10 ^f
Transmission rate (%)	18.0 ^{abc}	17.0 ^{abc}	11.5 ^{ab}	24.0 ^{bc}	10.0 ^{ab}	32.5 ^c	8.0 ^{ab}	5.5 ^a	8.5 ^{ab}	32.5 ^c	15.0 ^{abc}	20.0 ^{abc}	—	—	—

¹⁾ Alloplasmic *R. sativus* carrying *M. arvensis* cytoplasm (2n=18).

²⁾ Chlorophyll content was determined by the Soil Plant Air Determination system (SPAD) 502 value.

³⁾ Numbers in the same column followed by different letters are significantly different at p=0.05 according to Duncan's multiple range test.

⁴⁾ Not investigated.

⁵⁾ Male sterility.

of pollen fertility for the alloplasmic (*M. arvensis*) *R. sativus* may be closely related to the presence of the added chromosome of *M. arvensis*. Alloplasmic *B. juncea* cv. 'Pusa Bold' carrying *M. arvensis* cytoplasm showed also the male sterility, whereas in alloplasmic *B. juncea* with one chromosome of *M. arvensis*, pollen fertility was 53% and the fertility restoring gene(s) were successfully introgressed into the alloplasmic *B. juncea* genome, resulting in the successful development of fertile plants with 93% pollen fertility (Prakash *et al.* 1998). The intergeneric hybrids of either diploid and amphidiploid species of *Brassica* (A, B, C, AC and AB genomes) or *R. sativus* (RR) with *M. arvensis* were obtained through the embryo rescue technique and cytogenetic observations of their hybrids suggested the existence of a partial homology between the *M. arvensis* genome and that of other *Brassicaceae* species (Takahata 1990, Takahata and Takeda 1990, Takahata *et al.* 1993, Bang *et al.* 1996). These results lead to the conclusion that *M. arvensis* cytoplasm has a higher potential for inducing the CMS line in *Brassicaceae* when the fertility restoring gene(s) are transferred from the *M. arvensis* genome to the crop species through meiotic recombination.

When each chromosome of the *M. arvensis* genome

was introduced into the *R. sativus* genome, the characteristics under the control of the single chromosome were dominantly expressed in some organs of *R. sativus*. Accordingly, their characteristics were used as specific markers to identify each type of MALs. *M. arvensis* exhibited the distinctive bolting character (50.7 days) compared with *R. sativus* cv. 'Pink ball' (189.8 days). However, the bolting character of the twelve types of MALs did not show significant differences. These facts suggest that the genome of *R. sativus* cv. 'Pink ball' may be epistatic against each chromosome of the *M. arvensis* genome at least for the bolting character. The late bolting character in Japanese radish was assumed to be controlled by multiple genes and expressed recessively against the early bolting one (Shinohara 1959, Kagawa 1971, Yuasa 1988). Kaneko *et al.* (1987, 2000) reported that the early bolting character expressed by the e-chromosome of the *B. oleracea* genome was dominant to the late bolting character of Japanese radish cv. 'Tokinashi', but recessive to the late bolting one under the g-chromosome of the same *B. oleracea* genome. In this study, the early bolting character of *M. arvensis* was recessively expressed against the bolting character of the *R. sativus* genome when each chromosome of the *M. arvensis* genome was introduced into the *R. sativus*

Table 4. Distribution pattern of RAPD markers derived from *M. arvensis* in twelve types of MALs (bp)

Primer	Type of MALs											
	a	b	c	d	e	f	g	h	i	j	k	l
OPA01	298,310	220,250,298,310	270,298,310		298,310		250,270	250,270	270,298	250	220,250	220,250
OPA07	344	240,344			344	220*,240	240,344	240	344	344		
OPA18						320	320		170*,320	320	270*,320	320
OPE05		298	201,298	201,298	201,298		201	201	201	320*,396*	298	201,298
OPE06	190,517	517	517		517		190	190	517	190,250*	190,517	
OPE13			344*									506*
OPE17	154		154	154	154,420		154	154	154,420	154,420	154	154
OPR05	100	100	85,100		100	85,100	100	100	85,100	100		85
OPR06		344	344	344			344	220,344	220,450*	220	220	220
OPR07	270	270	270	270	270				270,344*	270		270
OPR10			506								130	130,298*,506
OPR11		520*	250,344		250	250	250,344	250,344	250,344	344		
OPR16	1018	120	1100*	170			170	1018	120	170,1018	700	170,700,1018
OPR17	201* ¹⁾	134,850	850	134,850	134	134,850	134		134		506*	
OPT04	344	344	344		344	154*,170*			190*,344		210*	
OPT09		900							506*,900			
OPT12	700	700	190,700	700	700	190			190		190	298*,344*,700
OPT14						270	270	270				
No. of bands	12	18	20	9	14	12	15	13	24	15	14	19

¹⁾ *; The number indicates the RAPD- specific marker for each type of MAL alone.

genome as an added chromosome. Therefore, the early bolting gene(s) in *Brassicaceae* seem to be diverse and to be expressed either dominantly or recessively in interspecific and intraspecific combinations.

Very little is known about the evolution and genetic analysis of the C₃-C₄ intermediate species in *Brassicaceae* including five species of *Moricandia*, *Diplotaxis tenuifolia* (L.) DC. and *B. gravinae* Ten.. In this study, the twelve types of MALs revealed the possibility of transferring useful agronomic traits from *M. arvensis* to *R. sativus* and provided suitable materials for analyzing the characteristics of the C₃-C₄ intermediate species. We are currently attempting to produce MALs between the other C₃-C₄ intermediate species of *D. tenuifolia* and *R. sativus*. Comparative studies of two MAL series of *R. sativus* - *M. arvensis* and *R. sativus* - *D. tenuifolia* may provide more important information to understand the genetic system and evolution of the C₃-C₄ intermediate species in *Brassicaceae*.

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