B.R. Choudhary · P. Joshi · K. Singh

Synthesis, morphology and cytogenetics of *Raphanofortii* (TTRR, 2n = 38): a new amphidiploid of hybrid *Brassica tournefortii* (TT, 2n = 20) × *Raphanus caudatus* (RR, 2n=18)

Abstract Amphidiploid *Raphanofortii* was synthesized by colchicinization of the F_1 hybrid *Brassica tournefortii* (TT, 2n = 20)×*Raphanus caudatus* (RR, 2n = 18). The crossability between these two species, and the cytomorphology of the F_1 plants and the amphidiploids were investigated. Intergeneric hybrids between the species were obtained only when *B. tournefortii* was involved as female parent. The hybrid plants were intermediate for most of the morphological attributes and showed very low pollen fertility compared to the parents. Although a majority of the pollen mother cells of the dihaploid hybrid (TR, 2n = 19) harboured univalents, a maximum of six bivalents were also observed. Of the 37 colchicine-treated F_1 plants analyzed cytologically, 21 were found to be true amphidiploids (2n = 38), whereas seven were mixoploids. Meiosis in the amphidiploids was characterized by the occurrence of 19 bivalents, though multivalents and univalents were also observed in a few cells. Most of the amphidiploid plants exhibited a fairly high pollen and seed fertility, which was further enhanced with the advancement of generations. Out of 69 plants investigated in the A_2 generation, 64 were euploids while the remaining five were aneuploids (2n = 36, 37, 39, 40 and 42). The newly synthesized *Raphanofortii* has great potential as a new commercial crop, as well as a bridge species for the transfer of economically important attributes of both the species to other *Brassicas*.

Key words *Brassica* · *Raphanus* · Intergeneric hybrids · Amphidiploid · *Raphanofortii* · Morphology · Cytogenetics

B.R. Choudhary (✉) · P. Joshi · K. Singh
Agricultural Research Station, Rajasthan Agricultural University, Mandor-Jodhpur 342 304, India

Introduction

Intergeneric and interspecific hybridization have great potential for the improvement of cruciferous crops (Inomata 1994) and are widely used to investigate crossability among related genera and species (Hinata et al. 1974; Nishiyama et al. 1991; Choudhary and Joshi 1999), and genomic homology with each other (Mizushima 1980; Choudhary et al. 2000). Such crosses generate useful genetic variation (Prakash 1973a; Choudhary 1997) and also help in the transfer of valuable characters (Gundimeda et al. 1992; Lelivelt et al. 1993). Artificial synthesis of otherwise naturally occurring *Brassica* amphidiploids, viz., *Brassica juncea* (Prakash 1973b), *Brassica carinata* (Prakash et al. 1984) and *Brassica napus* (Rashid et al. 1994), has led to the creation of new species such as *Brassica napocampestris* (McNaughton 1973a), *Raphanobrassica* (Honma and Heeckt 1962; McNaughton 1973b) and *Brassicoraphanus* (Tokumasu 1970; Lange et al. 1989) through intra- and inter-generic hybridization.

Various species of *Brassica* are grown in different parts of the world as per their importance and specific adaptability. Wild turnip (*Brassica tournefortii* Gouan) (TT, 2n = 20), grown sporadically as an oilseed crop in few pockets, has been reported as a good source of resistance/ tolerance against aphids (*Liphaphis erismi*), pathogens such as *Alternaria*, *Albugo candida* and *Leptosphaeria maculans*, and drought (Ljungberg et al. 1993). The rat-tail radish (*Raphanus caudatus* L.; RR, 2n = 18) (syn. *R. sativus* var. *caudatus* L. Vilmorin), an annual herbaceous species very similar to the common radish (*Raphanus sativus*) except for a characteristic fleshy root, is grown in Southeast Asia mainly for its long slender unripe pods which are eaten raw as a salad or cooked as a vegetable (Anonymous 1989). Its oil is also used as an ingredient in folk medicine (Kumar 1999).

The present investigation was carried out with the objective of developing an amphidiploid having the long slender pods of *R. caudatus* along with all the economi-

cally important attributes of *B. tournefortii*. In addition, it was aimed to study the crossability between species, the morphological and cytological details of di-haploid F_1 hybrids (TR, $2n = 19$) and the amphidiploids. The newly synthesized amphidiploid (TTRR, $2n = 38$) has been named *Raphanofortii,* suggested by Dr. N. Inomata (personal communication).

Materials and methods

Plant materials

Two promising diverse genotypes of *B. tournefortii,* viz. RBT 1 (yellow seeded) and RBT 48 (brown seeded), and one of *R. caudatus,* i.e., RCM 1, selected from the germplasm collection of the Agricultural Research Station, Mandor, were used for the intergeneric hybridization programme.

Crossing programme

Crosses including reciprocals were attempted among the selected genotypes under field conditions during the winter season of 1995–96. Flower buds of appropriate size were emasculated in the afternoon, and all the other buds and flowers were removed from the selected inflorescences. The emasculated buds were covered by paper bags. The following morning these buds were pollinated with the pollen of desired parents and re-covered with the bags.

Planting of seeds and colchicine treatment

About half of the seeds harvested from the crosses were grown in field, while the remaining seeds were germinated in pots for colchicinization. After seedling emergence, small cotton swabs soaked in 0.3% aqueous colchicine (Sigma) were placed on growing apical buds of young seedlings for 4 h, and the treatment was repeated for 3 successive days (Dijkrstra and Speckmann 1965). The cotton was constantly kept wet by adding the solution at regular intervals. At the end of each day treatment, the apical buds were washed thoroughly with water. Three-week-old seedlings were transferred carefully to the field. All the recommended agronomic practices were followed to raise the crop plants.

Cytological studies

For meiotic observations, flower buds were fixed in Carnoy's fluid (ethanol: chloroform: acetic acid- 6:3:1) containing ferric chloride as a mordant for 48 h and then stored in 70% ethanol. Anthers were squashed in a drop of acetocarmine (1%). Chromosome pairings were examined at diakinesis/ metaphase-I of meiosis. The pollen fertility of the parents, hybrids and amphidiploids was estimated by the percentage pollen stainability using acetocarmine.

Results

Crossability

The seed setting was fairly good in the cross *B. tournefortii* × *R. caudatus.* Of the 485 crosses performed using *B. tournefortii* RBT 1 as female parent, as many as 39 hybrid plants were obtained (Table 1). But by involving the other genotype, i.e. RBT 48, only 11 F_1 plants were recovered from 350 pollinations. However, in reciprocal crosses, not a single seed could be obtained, although 595 flower buds of *R. caudatus* were pollinated with *B. tournefortii* pollen.

Morphology and cytology of F_1 hybrids

The F_1 hybrid plants obtained from the crosses, viz. RBT 1 × RCM 1 and RBT 48 × RCM 1, were vigorous, bushy, profusely branched and intermediate for most of the morphological attributes (Fig. 1a). The leaves of these plants were intermediate between the parents in shape, lobbing, hairiness and colour (Fig. 1c). The flowers of hybrids were medium in size and white in colour resembling *R. caudatus* (Fig. 1d). The hybrid plants flowered abundantly and produced few siliquae under open pollination but did not set any seed.

The dihaploid nature of the F_1 plants was reflected in cytological analysis. Of the 349 PMCs analyzed at diakinesis/metaphase-I, 142 (40.7%) were characterized by the presence of 19 univalents (Table 2). Cells having a chromosome pairing of 1 II + 17 I, 2 II + 15 I, 3 II + 13 I, 4 II + 11 I and 5 II + 9 I were found with a frequency of 20.1, 12.9, 10.6, 6.0 and 4.3%, respectively (Fig. 2). A maximum of six bivalents were noticed in seven cells (2.0%), while trivalent associations (0–2) were observed in 12 cells (3.5%). On average the chromosome associations per PMC were 0.04 III + 1.42 II + 16.03 I.

Table 1 Crossability relationship between *B. tournefortii* and *R. caudatus*

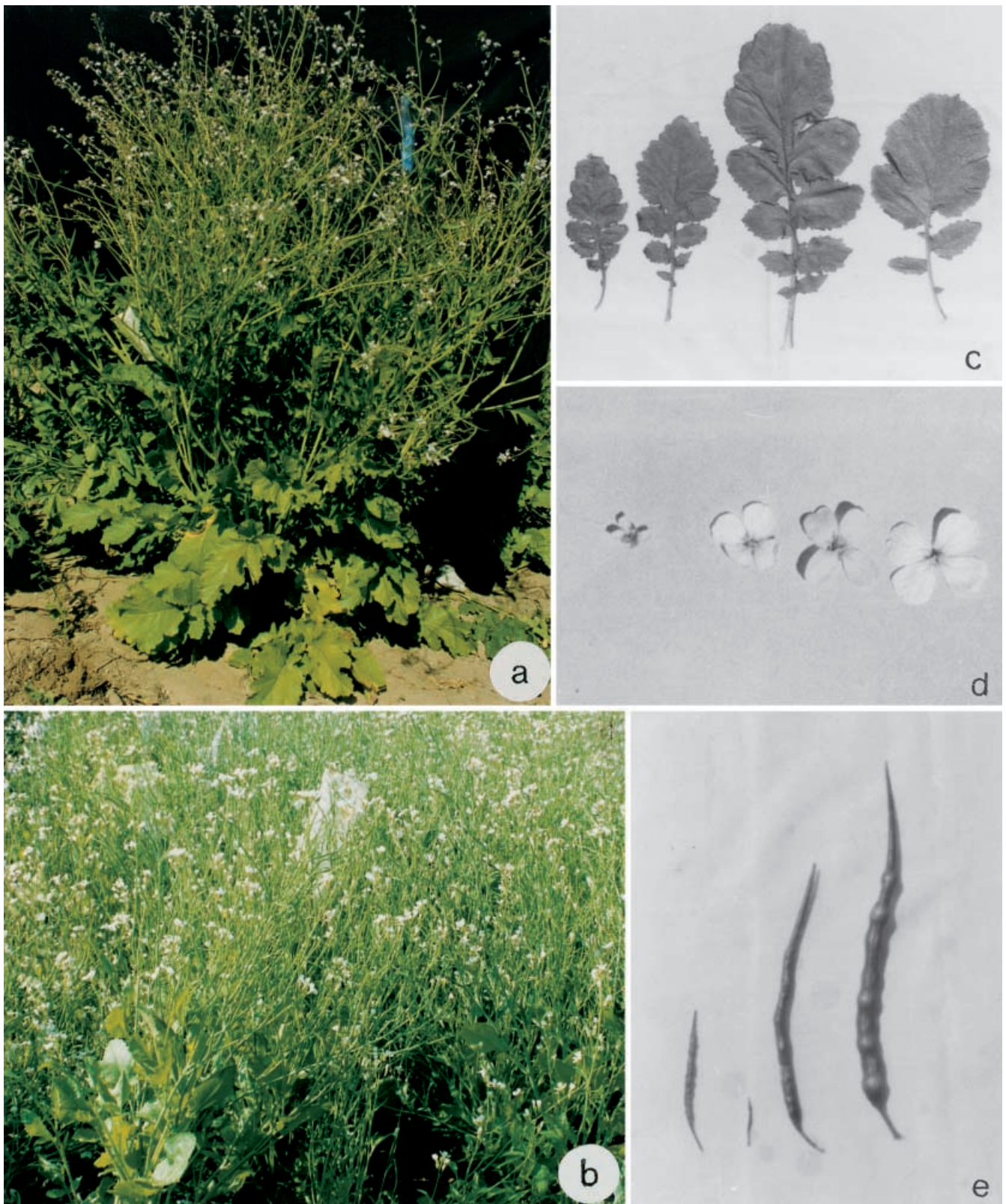| Cross	Number of				Per cent hybrid recovery
	Buds pollinated	Seeds harvested	Hybrids obtained	Hybrids per pollination	
B. tournefortii × *R. caudatus*					
RBT 1 × RCM 1	485	315	39	0.08	8.0
RBT 48 × RCM 1	350	198	11	0.03	3.1
Total/average	835	513	50	0.06	6.0
R. caudatus × *B. tournefortii*					
RCM 1 × RBT 1	325	0	0	–	–
RCM 1 × RBT 48	270	0	0	–	–
Total/average	595	0	0	–	–

Fig. 1a–e Comparison of morphological attributes of *B. tournefortii, R. caudatus,* their F$_1$ hybrid and amphidiploid *Raphanofortii*. **a** F$_1$ plant of hybrid *B. tournefortii × R. caudatus*; **b** crop view of *Raphanofortii*; **c–e** (*left to right*) leaf, flower and siliqua of *B. tournefortii*, F$_1$ hybrid, *Raphanofortii* and *R. caudatus*

Table 2 Chromosome pairing at diakinesis/metaphase-I in TR hybrids (2n = 19) of *B. tournefortii* × *R. caudatus*

| Chromosome association	Cells observed					
	RBT 1 × RCM 1		RBT 48 × RCM 1		Total	
	Number	Per cent	Number	Per cent	Number	Per cent
19 I	69	37.3	73	44.5	142	40.7
1 II + 17 I	38	20.5	32	19.5	70	20.1
2 II + 15 I	19	10.3	26	15.9	45	12.9
3 II + 13 I	21	11.4	16	9.8	37	10.6
4 II + 11 I	15	8.1	6	3.7	21	6.0
5 II + 9 I	11	5.9	4	2.4	15	4.3
6 II + 7 I	5	2.7	2	1.2	7	2.0
1 III + 1 II + 14 I	3	1.6	2	1.2	5	1.4
1 III + 2 II + 12 I	2	1.1	1	0.6	3	0.9
1 III + 2 II + 8 I	1	0.5	2	1.2	3	0.9
2 III + 4 II + 5 I	1	0.5	–	–	1	0.3
Cells analyzed	185	100	164	100	349	100
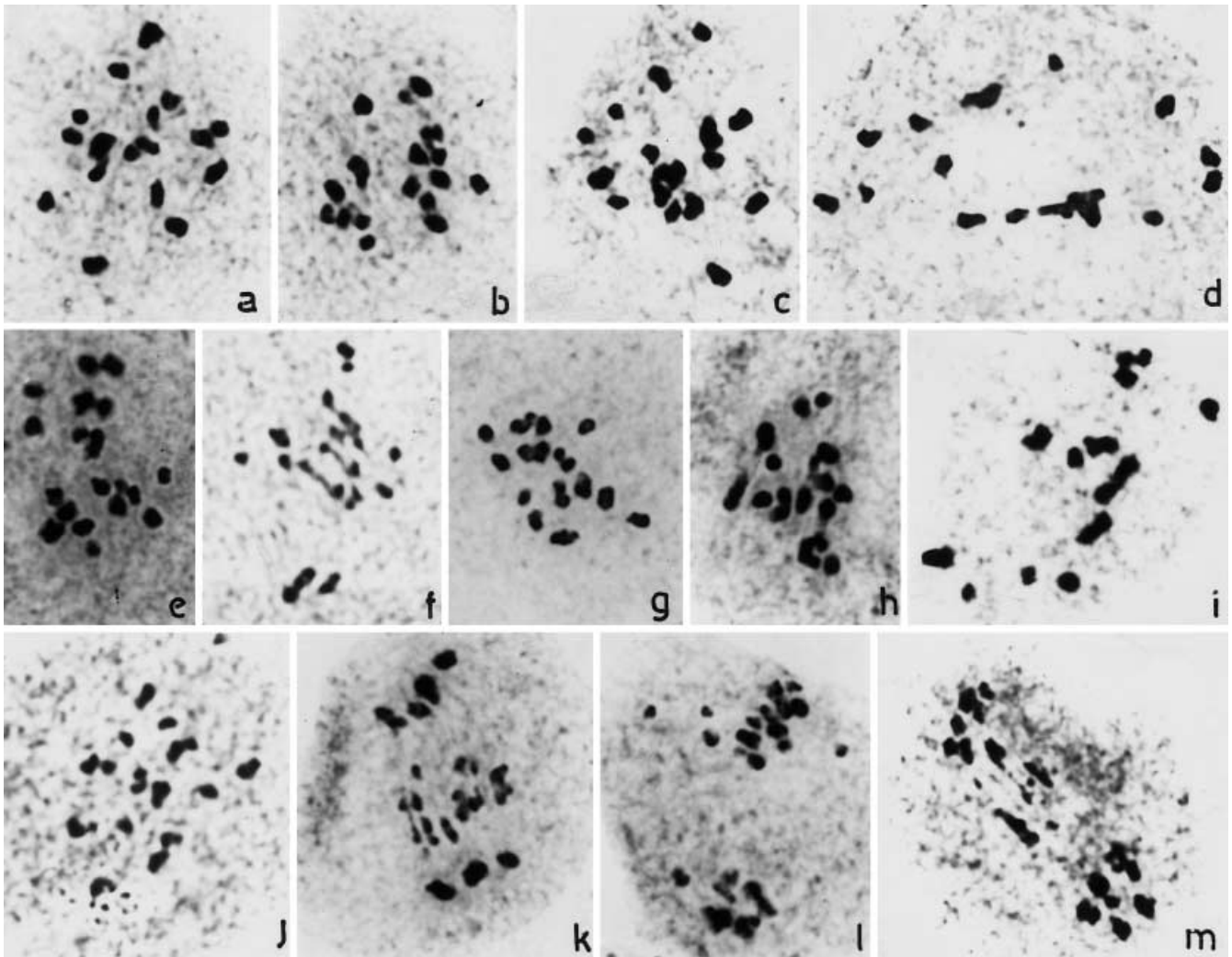
Fig. 2a–m Meiosis in F₁ hybrids of *B. tournefortii* × *R. caudatus*. **a** Metaphase-I (M-I), 2 II + 15 I; **b–c** M-I, 3 II + 13 I; **d** M-I 1 III + 2 II + 12 I; **e** M-I, 4 II + 11 I; **f, g** M-I, 5 II + 9 I; **h** M-I, 1 III + 4 II + 8 I; **i** M-I, 2 III + 4 II + 5 I; **j** M-I, 6 II + 7 I; **k** anaphase-I (A-I), late disjunction of bivalents; **l** A-I, unequal distribution and **m** A-I, bridge-fragment configuration

Apart from chiasmatic associations (Fig. 2f), end-to-end, end-to-side and side-to-side sticky chromosome connections appeared commonly (Fig. 2e, g, i). Some of the paired chromosome configurations were found to be heteromorphic in nature (Fig. 2j). At anaphase-I/-II, a majority of the cells (69.6%) had laggards. The frequen-

Table 3 Chromosome distribution at anaphase (I/II) in TR hybrids (2n = 19) of *B. tournefortii × R. caudatus*

Chromosome distribution	Cells observed					
	RBT 1 × RCM 1		RBT 48 × RCM 1		Total	
	Number	Per cent	Number	Per cent	Number	Per cent
Unequal (A–I)	5	12.2	7	14.6	12	13.5
Laggards (A–I)	21	51.2	19	39.6	40	44.9
Laggards (A–II)	9	22.0	13	27.1	22	24.7
Bridge-fragment	6	14.6	9	18.8	15	16.9
Total	41	100	48	100	89	100

Table 4 Chromosome pairing at diakinesis/metaphase-I in amphidiploids *Raphanofortii* (TTRR, 2n = 38)

Chromosome association	Cells observed					
	RBT 1 × RCM 1		RBT 48 × RCM 1		Total	
	Number	Per cent	Number	Per cent	Number	Per cent
19 II	202	76.2	77	59.2	279	70.6
18 II + 2 I	5	1.9	2	1.5	7	1.8
17 II + 1 IV	39	14.7	32	24.6	71	18.0
17 II + 1 III + 1 I	–	–	3	2.3	3	0.8
16 II + 1 IV + 2I	4	1.5	–	–	4	1.0
15 II + 2 IV	12	4.5	14	10.8	26	6.6
13 II + 3 IV	3	1.1	2	1.5	5	1.3
Cells analyzed	265	100	130	100	395	100

Table 5 Chromosome distribution at anaphase-I in amphidiploid *Raphanofortii* (TTRR, 2n = 38)

Chromosome distribution	Cells observed					
	RBT 1×RCM 1		RBT 48×RCM 1		Total	
	Number	Per cent	Number	Per cent	Number	Per cent
19:19	38	66.7	33	55.0	71	60.7
18:20	8	14.0	9	15.0	17	14.5
17:21	4	7.0	5	8.3	9	7.7
16:22	2	3.5	4	6.7	6	5.1
Laggards	5	8.8	7	11.7	12	10.3
Bridge	–	–	2	3.3	2	1.7
Cells analyzed	57	100	60	100	117	100

cies of such cells were 44.9 and 24.7% at anaphase-I and -II, respectively (Table 3). Bridge-fragment configurations were found in 16.9% of the cells (Fig. 2m), while a characteristic phenomenon, delayed terminalization of the chiasmata, was also observed in a few cells (Fig. 2k). On the whole, the meiotic process revealed major irregularities which led to high pollen sterility. Amongst 17 plants analyzed for pollen fertility, five were found completely sterile. The mean pollen fertility was only 2.7% with a variation among the plants from 0 to 9.5%.

Morphology and cytology of amphidiploids

Amphidiploid plants resembled their putative progenitor dihaploid hybrids (F$_1$) for most morphological attributes (Fig. 1). The leaves and flowers of the amphidiploids were larger in size compared to the parents and hybrids (Fig. 1c–d). Siliqua formation in these plants was normal except in shape which was *Raphanus*-like apical and *Brassica*-like basal (Fig. 1e). The siliqua length varied from 4 to 11 cm and had 0–9 seeds per siliqua. The seed weight (1000 seed) was higher in the amphidiploid (4.05 g) than in *B. tournefortii* (2.95 g) but lower than that of *R. caudatus* (11.8 g).

Of the 37 colchicinized F$_1$ plants analyzed cytologically, 21 had 38 chromosomes and were confirmed as true amphidiploids, while seven were mixoploids. Meiosis in the A$_1$ generation of the amphidiploid, *Raphanofortii*, was characterized by the occurrence of quadri-

Fig. 3a-l Meiosis in *Raphanofortii* (A$_1$) showing chromosome pairing at diakinesis/ metaphase-I. **a** 13 II + 3 IV; **b** 15 II + 2 IV; **c, d** 16 II + 1 IV + 2 I; **e** 19 II; **f** 17 II + 1 IV; **g, h** 15 II + 2 IV; **i** 18 II + 2 I; **j** 15 II + 2 IV; **k** 13 II + 3 IV and **l** 15 II + 2 IV
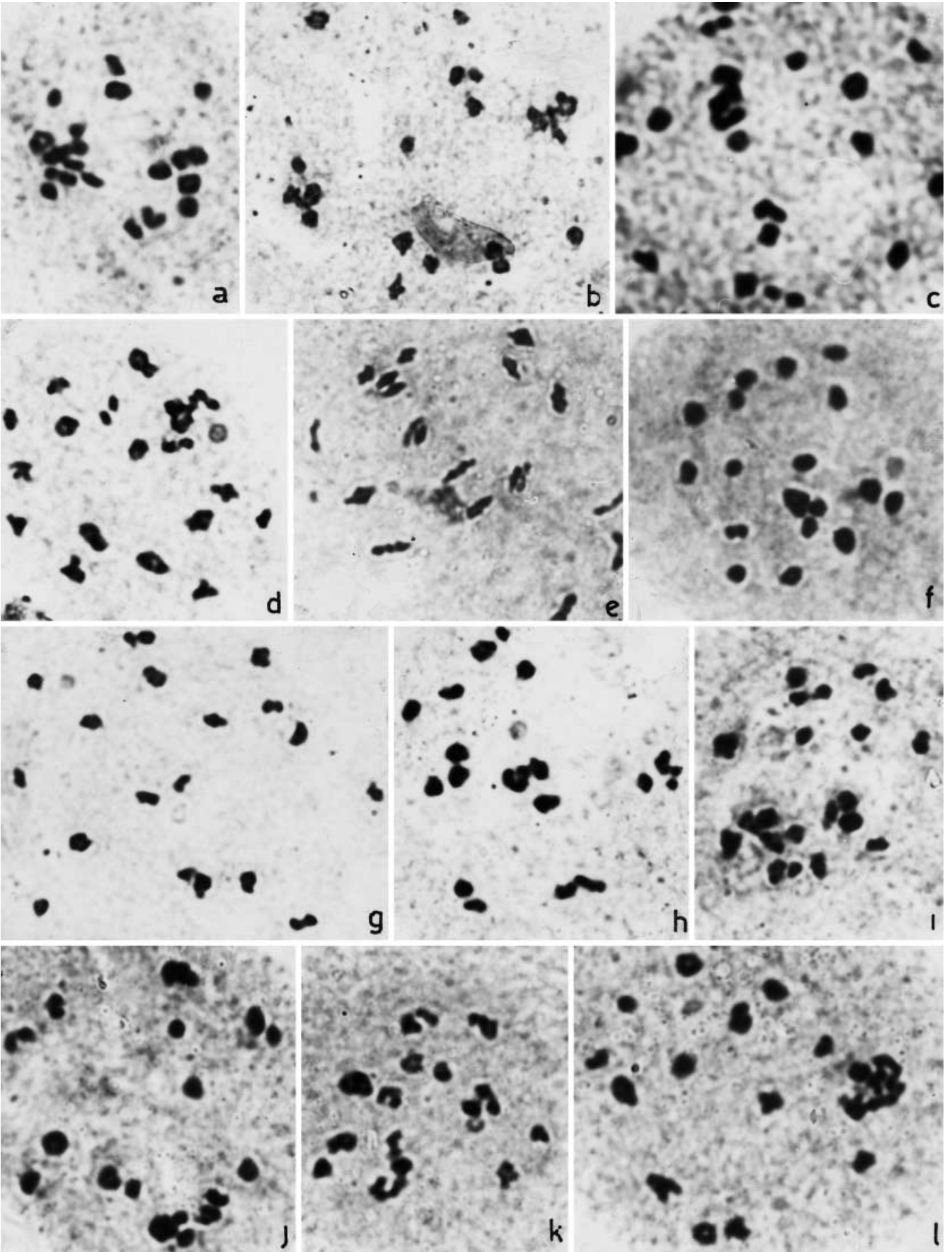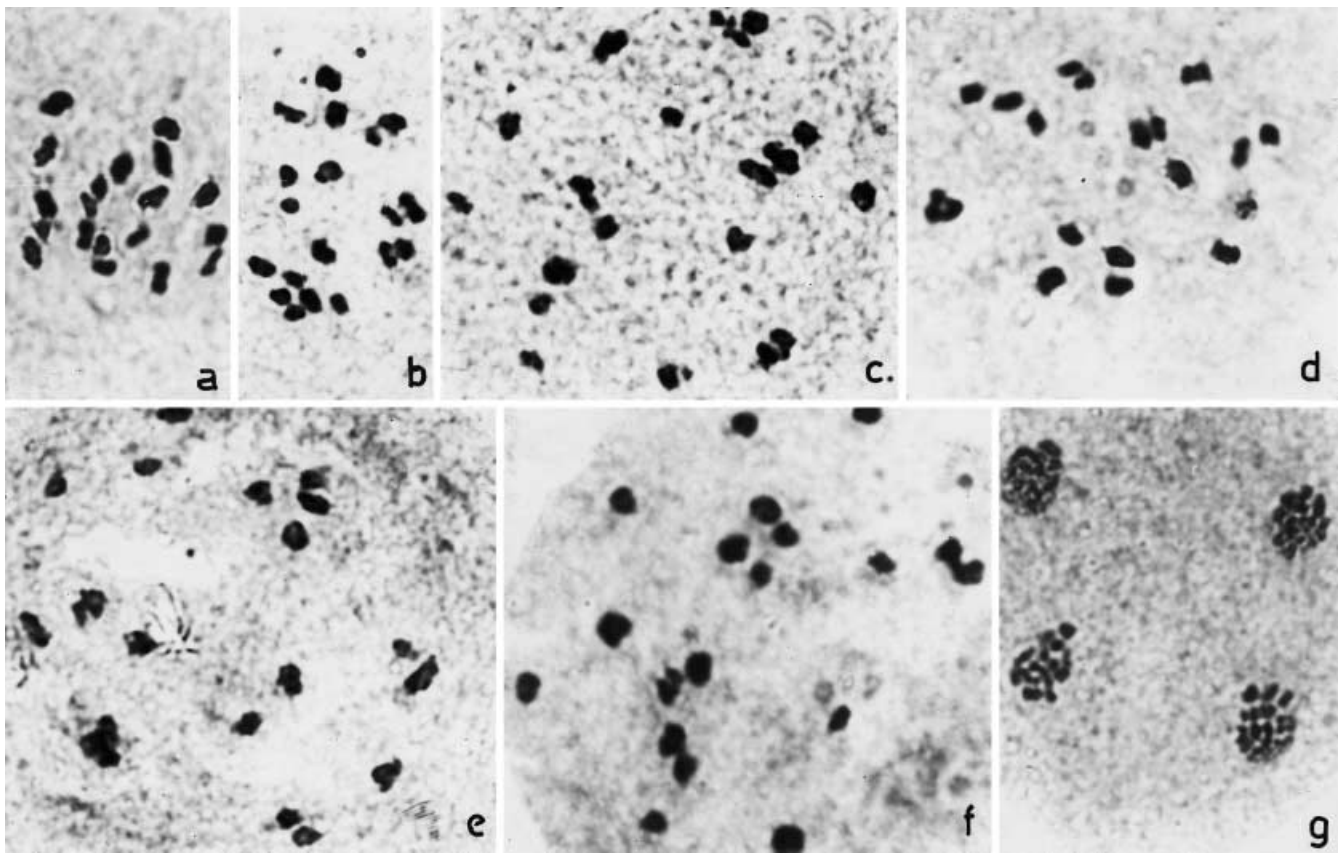
Fig. 4a–g Meiosis in *Raphanofortii* (A$_2$) showing chromosome pairing at diakinesis/ metaphase-I. **a–c** 19 II ; **d, e** 17 II + 1 IV; **f** 15 II + 2 IV and **g** anaphase-II, normal distribution

valents (0–3), trivalents (0–1), bivalents (13–19) and univalents (0–2) (Table 4, Fig. 3). Although a majority of cells (70.6%) showed 19 bivalents (Fig. 3e), few cells with chromosome pairings of 17 II + 1 IV (18.0%) (Fig. 3f), 15 II + 2 IV (6.6%) (Fig. 3g) and 18 II + 2 I (1.8%) (Fig. 3i) were also observed. Three quadrivalents per cell were recorded in five PMCs (Fig. 3k), while a maximum of two univalents were observed in 11 cells. The mean associations per cell recorded was 0.36 IV + 0.01 III + 18.24 II + 0.06 I. Amongst 117 cells analyzed at anaphase-I, 71 (60.7%) showed a regular distribution of chromosomes (19:19), while irregular distributions of 18:20, 17:21 and 16:22 were observed in 14.5, 7.7 and 5.1% of the cells, respectively (Table 5). Laggards at anaphase-I were observed in 10.3% of the cells. The average pollen stainability was 58.3%, and varied among plants (29.6–73.5%).

Of 69 plants from the A$_2$ generation investigated cytologically, 64 (92.8%) having 2n = 38 chromosomes were confirmed as euploids, while the remaining five were aneuploids. Amongst 385 PMCs of euploid plants observed at diakinesis/metaphase-I, 315 cells (81.8%) had the expected 19 II (Fig. 4a–c), while 53 PMCs (13.8%) showed 17 II + 1 IV (Fig. 4d, e) and 17 cells (4.4%) ex-
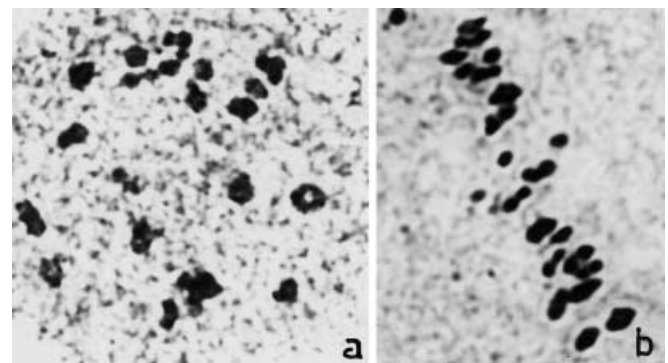

Fig. 5a, b Meiosis in aneuploid *Raphanofortii* (2n = 40) showing chromosome pairing at diakinesis/metaphase-I. **a** 16 II + 2 IV and **b** 18 II + 4 I

hibited 15 II + 2 IV (see Fig. 5f) chromosome associations. The chromosome distribution at anaphase-I and, -II appeared regular. As many as 63 (78.8%) out of 80 cells analyzed, exhibited a normal distribution of chromosomes, whereas an unequal distribution with 18:20 and 17:21 was recorded in eight (10%) and four (5%) cells, respectively. The average pollen stainability and seed fertility of A$_2$ plants was 77.3% and 4.2 seeds per siliqua, respectively. Five plants identified as aneuploids, possessing 2n = 36, 37, 39, 40 (Fig. 5a, b) and 42 chromosomes, were vigorous like the euploids but exhibited low pollen and seed fertility compared with the euploids.

Discussion

Crossability

In view of the excellent possibilities to transfer desirable traits across the genera/species a knowledge of the inter-relationships and crossability among Brassicas is quite important (Lelivelt et al. 1993; Raney et al. 1995a, b). Subramanyam (1954) studied crossability between *R. sativus* and *B. oleracea*, and pointed out that the pollen tube of *R. sativus* did not penetrate the *B. oleracea* style. McNaughton (1973b) correlated his observations with earlier reports on the crossability of *R. sativus* with *B. oleracea* and concluded that hybridization between these two species considerably easier when *R. sativus* involved as the female parent, although few reciprocal hybrids had previously been obtained (Moskov and Makarova 1969). Similarly, the hybridization between *B. campestris* and *R. sativus* was reported to be difficult (Prakash and Chopra 1992), yet a few successful crosses were obtained in either direction (Tokumasu 1970; Ellerstrom and Sjodin 1973).

In the present study, the hybrids from the cross between *B. tournefortii* and *R. caudatus* could only be obtained when *B. tournefortii* used as the female parent. These findings are in good agreement with Hinata et al. (1974), who also suggested that *B. tournefortii* crossed easily with other species when it involved as the female parent. Harberd (1976) also supported the opinion of Hinata et al. (1974) and further pointed out that the pollen grains of *B. tournefortii* were unsuccessful to germinate on the stigmas of other species, resulting in a failure of reciprocal crosses.

The per cent success of hybrids obtained from the cross *B. tournefortii* × *R. caudatus* also differed at the varietal level. The genotype of *B. tournefortii* RBT 1 as female produced as many as 8.0 per cent hybrid plants with *R. caudatus,* while only 3.1 per cent hybrid plants were obtained when RBT 48 was used as the female parent. These results clearly demonstrated that the success of a cross depends not only on the direction of the cross but is also influenced by the genotypes of the species involved. These observations are in close agreement with those of McNaughton (1973b) and Akbar (1989). The success in obtaining hybrids from the cross *B. tournefortii* × *R. caudatus*, and complete failure of the reciprocal combination, confirmed the advantage of using a species with a higher basic chromosome number as the female parent, which lead to a greater recovery of hybrids (Quazi 1988; Ripley and Arnison 1990; Choudhary et al. 2000).

Morphology and cytology of F$_1$ hybrids

The F$_1$ plants were found vigorous and intermediate between the parent species in several morphological traits. These observations are in conformity with those reported in different intergeneric hybrids within crucifers (McNaughton 1973b; Ripley and Arnison 1990; Inomata 1994; Brown et al. 1997; Choudhary and Joshi 2000). The flowers in hybrid plants had a white corolla, as in *R. caudatus*, indicating the dominance of white over the pale-yellow colour of the petals.

In meiotic analysis of the hybrids, a maximum of six bivalents and occasionally trivalents were encountered, whereas Mizushima (1968) noted a maximum of seven bivalents in the hybrid *B. tournefortii* × *R. sativus*. Such differences could be attributed to differences in the species/genotypes used. Chromosome pairing observed in intergeneric dihaploid hybrids might be auto- and/or allo-syndetic in nature. Fukushima (1945) suggested the possibility of one autosyndetic bivalent in the R genome (*R. sativus*) since a trivalent was observed by him in the hybrid of *B. oleracea* (CC) × *R. sativus* (RR), which was further supported by Mizushima (1980). Whereas, in case of the T genome (*B. tournefortii*), Prakash (1974) reported a maximum chromosome pairing of 1 III + 2 II due to autosyndesis. Thus, a sum total of 1 III + 3 II or 4 II may theoretically be possible due to autosyndesis within the T and R genomes. Therefore, out of six bivalents observed in the present hybrids, two could be explained by allosyndesis between the T and R genomes. The occurrence of a notable feature, the delayed terminalization of chiasmata in bivalents, which led to formation of bridges between the separating chromosomes at anaphase-I, might be caused due to non-homologous segments in the terminal or subterminal parts of bivalents (Olsson and Hagberg 1955), and further confirms the allosyndetic nature of chromosome pairing between the genomes under consideration.

The extremely low pollen fertility observed in the present hybrid might be due to meiotic irregularities and segregational anomalies (Stebbins 1966). These hybrid plants were found completely seed-sterile suggesting that sterility was the most effective isolating mechanism active at the the reproductive stage (Biswas and Dana 1975). A much higher degree of sterility, similar to the present observations, was also noticed earlier in the intergeneric hybrids of *R. sativus* × *B. oleracea* (Honma and Heeckt 1962; McNaughton 1973b), *Sinapis alba* × *B. napus* (Ripley and Arnison 1990) and *B. napus* × *S. pubescens* (Inomata 1994).

Morphology and cytology of amphidiploids

Amphidiploids of the genera *Brassica* and *Raphanus*, designated as *Raphanobrassica* or *Brassicoraphanus*, have been studied morphologically and cytologically by several workers (Honma and Heeckt 1962; Tokumasu 1970, 1976; McNaughton 1973b; Iwasa and Ellerstrom 1981; Lange et al. 1989). However, with regard to *Raphanofortii*, this is the first attempt to synthesize and report details on its morphology, cytogenetics and fertility aspects.

The amphidiploid plants obtained were intermediate between the parent species for most of the morphological

characters and had vigorous growth, giving a bushy appearance. The increase in various morphological attributes in the amphidiploids over F_1 hybrids was due to the higher ploidy level. The typical siliqua shape observed in *Raphanofortii* was in close resemblance to that reported in *Raphanobrassica* by Honma and Heeckt (1962) and McNaughton (1973b).

The meiotic analysis of *Raphanofortii* was characterized by the presence of multivalents (quadrivalents and trivalents), bivalents and univalents. These observations are in agreement with those reported in *Raphanobrassica* (Howard 1938) and *Brassicoraphanus* (Tokumasu 1976) but are in contrast to the results of McNaughton (1973b) who noticed only bivalents and occasional univalents in *Raphanobrassica*. This synaptic behaviour of the amphidiploids can be explained by the fact that a high frequency of bivalent associations besides multivalents and univalents could be expected due to: (1) preferential pairing, (2) auto- and/or allo-syndetic associations, (3) low chiasma frequency and (4) disturbed zygomere activity etc. Therefore, the amphidiploids synthesized in the present investigation were presumed to be segmental allotetraploids (Stebbins 1950) since structural differences between the chromosomes of species often result in preferential pairing between duplicated chromosomes producing predominantly bivalent associations. This was followed by a normal distribution of chromosomes at anaphase-I, resulting in higher pollen and seed fertility. However, the low pollen and seed fertility recorded in few plants might be the result of multivalent associations at diakinesis/metaphase-I which led to an unequal distribution and other segregational abnormalities at anaphase-I and -II (Biswas and Dana 1975).

The probable existence of parental genomic homology between the T and R genomes of the species involved in the present intergeneric hybrids might be a reason for the formation of quadrivalents and trivalents at diakinesis/metaphase-I in the amphidiploids. Further, the occurrence of occasional univalents at metaphase-I could be attributed to a minor reduction in chiasma frequency. The occurrence of 7.2% aneuploid plants in the A_2 generation might be the result of the multivalent associations recorded in the A_1 generation, which might have increased the chances of aneuploids in the progeny (McNaughton 1973b).

In general, the higher pollen and seed fertility in *Raphanofortii* compared to that reported in *Raphanobrassica* (Honma and Heeckt 1962; McNaughton 1973b) and *Brassicoraphanus* (Ellerstrom and Sjodin 1973; Tokumasu 1976), and the further increase in the fertility with advancement of generations, indicate the great significance of *Raphanofortii* as a bridge species for the transfer of useful genes of both *B. tournefortii* and *Raphanus* to other *Brassica* species. The agronomical potential of *Raphanofortii* and its aneuploids are under currently evaluation, for their advancement and improvement and their exploitation as a new commercial crop.

References

Akbar MA (1989) Resynthesis of *Brassica napus* aiming for improved earliness and carried out by different approaches. Hereditas 111:239–246

Anonymous (1989) The wealth of India. Publication and Information Directorate, New Delhi, vol VIII, pp 366–367

Biswas MR, Dana S (1975) Black gram x rice bean cross. Cytologia 40:787–795

Brown J, Brown AP, Davis JB, Erickson D (1997) Intergeneric hybridization between *Sinapis alba* and *Brassica napus*. Euphytica 93:163–168

Choudhary BR (1997) Interspecific hybridization in genus *Brassica*. PhD thesis, Rajasthan Agricultural University, Bikaner, India

Choudhary BR, Joshi P (2000) Chytomorphology of intergeneric hybrid *Sinapis alba × Brassica nigra*. J Genet Breed 54: (in press)

Choudhary BR, Joshi P (1999) Interspecific hybridization in *Brassica*. Abstr 10th Int Rapeseed Cong, Canberra, Australia, p 314

Choudhary BR, Joshi P, Ramarao S (2000) Interspecific hybridization between *Brassica carinata* and *B. rapa*. Plant Breed 119 (in press)

Dijkrstra J, Speckmann GJ (1965) Germinal pore grains as a selection basis in the production of tetraploid clover. Euphytica 14:244–248

Ellerstrom S, Sjodin J (1973) Species crosses in the family *Brassica* aiming at creation of new fodder crops. In: New ways in fodder crop breeding. Proc Meet Fodder Crop Sec, Eucarpia, pp 26–28

Fukushima E (1945) Cytogenetic studies on *Brassica* and *Raphanus*. I. Studies on inter-generic F_1 hybrids between *Brassica* and *Raphanus*. J Dept Agric Kyushu Imp Univ 7:281–400

Gundimeda HR, Prakash S, Shivanna KR (1992) Intergeneric hybrids between *Enarthrocarpus lyratus*, a wild species, and crop *Brassicas*. Theor Appl Genet 83:655–662

Harberd DJ (1976) Cytotaxonomic studies of *Brassica* and related genera. In: Vaughan JG, MacLeod AJ, Jones BM (eds) The biology and chemistry of the Cruciferae, Academic Press, London, pp 47–68

Hinata K, Konno N, Mizushima U (1974) Interspecific crossability in the tribe Brassiceae with special reference to the self-incompatibility. Tohoku J Agric Res 25:58–66

Honma S, Heeckt O (1962) Investigations on F_1 and F_2 hybrids between *Brassica oleracea* var. *acephala* and *Raphanus sativus*. Euphytica 11:177–180

Howard HW (1938) The fertility of amphidiploids from the cross *Raphanus sativus* x *Brassica oleracea*. J Genet 36:239–273

Inomata N (1994) Intergeneric hybridization between *Brassica napus* and *Sinapis pubescens,* and the cytology and crossability of their progenies. Theor Appl Genet 89:540–544

Iwasa S, Ellerstrom S (1981) Meiosis disturbances, aneuploidy and seed fertility in *Raphanobrassica*. Hereditas 95:1–9

Kumar N (1999) Cytogenetics of cultivated Crucifers. In: Kachroo P (ed) Progress in cytogenetics, Bishen Singh Mahendra Pal Singh, Dehra Dun, pp 133–162

Lange W, Toxopeus H, Lubberts JH, Dolstra O, Harrewign JL (1989) The development of raparadish (×*Brassicoraphanus*, 2n = 38), a new crop in agriculture. Euphytica 40:1–14

Lelivelt CLC, Lange W, Dolstra O (1993) Intergeneric crosses for the transfer of resistance to the beet cyst nematode from *Raphanus sativus* to *Brassica napus*. Euphytica 68:111–120

Ljungberg A, Cheng B, Heneen WK (1993) Investigation of hybrids between *Brassica tournefortii* Gouan and *B. alboglabra* Bailey. Sveriges Utsadesforenings Tidskrift 103: 191–197

McNaughton IH (1973a) *Brassica napocampestris* L. (2n = 58). I. Synthesis, cytology, fertility and general considerations. Euphytica 22:301–309

McNaughton IH (1973b) Synthesis and sterility of *Raphanobrassica*. Euphytica 22:70–88

Mizushima U (1968) Phylogenetic studies on some wild *Brassica* species. Tohoku J Agric Res 19:83–99

Mizushima U (1980) Genome analysis in *Brassica* and allied genera. In: Tsunoda S, Hinata K, Gomez-Campo C (eds) *Brassica* crops and wild allies. Japan Sci Soc Press, Tokyo, pp 89–106

Moskov BS, Makarova GA (1969) High-yielding intergeneric hybrids in the Cruciferae. Bull Appl Bot Genet Pl Breed Ser 2(8):92–102

Nishiyama I, Sarashima M, Matsuzawa Y (1991) Critical discussion on abortive interspecific crosses in *Brassica*. Plant Breed 107:288–303

Olsson G, Hagberg A (1955) Investigation on haploid rape. Hereditase 41:227–237

Prakash S (1973a) Non-homologous meiotic pairing in the A and B genomes of *Brassica*: its breeding significance in the production of variable amphidiploids. Genet Res 121:133–137

Prakash S (1973b) Artificial synthesis of *Brassica juncea* Coss. Genetica 44:249–263

Prakash S (1974) Haploid meiosis and origin of *Brassica tournefortii* Gouan. Euphytica 23:591–595

Prakash S, Chopra VL (1992) Genome manipulation. In: Labana KS, Banga SS, Banga SK (eds) Breeding oilseed Brassicas, Narosa Publishing House, New Delhi, pp 108–133

Prakash S, Gupta S, Raut RN, Kalra A (1984) Synthesis of *Brassica carinata*. Cruciferae Newslett 9:36

Quazi MH (1988) Interspecific hybrids between *Brassica napus* L and *B. oleracea* L developed by embryo culture. Theor Appl Genet 75:309–318

Raney P, Rakow G, Olson T (1995a) Modification of *Brassica* seed oil fatty acid composition utilizing interspecific crossing. Proc 9th Int Rapeseed Cong, Cambridge, vol 2, pp 410–412

Raney P, Rakow G, Olson T (1995b) Development of zero erucic, low linolenic *Brassica juncea* utilizing interspecific crossing. Proc 9th Int Rapeseed Cong, Cambridge, vol 2, pp 413–415

Rashid A, Rakow G, Downey RK (1994) Development of yellow seeded *Brassica napus* through interspecific crosses. Plant Breed 112:127–134

Ripley VL, Arnison PG (1990) Hybridization of *Sinapis alba* L and *Brassica napus* L via embryo rescue. Plant Breed 104: 26–33

Stebbins GL (1950) Variation and evolution of plants, Oxford University Press, London

Stebbins GL (1966) Chromosomal variation and evolution. Science 152:1463–1469

Subramanyam KN (1954) Inter-generic hybridization between *Brassica* and *Raphanus*. Curr Sci 23:60

Tokumasu S (1970) Intergeneric hybrids between *Brassica japonica* and *Raphanus sativus*. Mem Coll Agr Ehime Univ 14:285–298

Tokumasu S (1976) The increase of seed fertility of *Brassicoraphanus* through cytological irregularity. Euphytica 25:463–470