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Crossability of *Brassica tournefortii* and *B. rapa*, and morphology and cytology of their F_1 hybrids

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Abstract The crossability between *Brassica tournefortii* (TT, 2n = 20) and *Brassica rapa* (AA, 2n = 20) and the cytomorphology of their F_1 hybrids were studied. Hybrids between these two species were obtained only when *B. tournefortii* was involved as a female parent. The hybrid plants were intermediate for most of the morphological attributes and were found to be free from white rust under field conditions. The F_1 plants showed poor pollen fertility, although occasional seed set was achieved from open pollination. Self-pollination or backcrosses did not yield any seeds in these plants. The occurrence of chromosome association ranging from bivalents (0–7), trivalents (0–2) to a rare quadrivalent (0–1) in the dihaploid hybrids indicates pairing between the T and A genomes. The homoeologous pairing coupled with seed set in the F_1 plants offer an opportunity for interspecific gene transfers from *B. tournefortii* to *B. rapa* and vice-versa through interspecific hybridization.

Keywords *Brassica tournefortii* · *Brassica rapa* · Crossability · Interspecific hybrid · Morphology · Cytology · Homoeologous chromosome pairing

Introduction

In India, Indian mustard (*Brassica juncea* L. Czern and Coss), as well as three ecotypes of *Brassica rapa* L (AA, 2n = 20), i.e. yellow *sarson*, brown *sarson* and *toria*, are grown widely as oilseed crops (Prakash and Chopra 1996). These species are well adapted to a dry environment and mature earlier than other *Brassica* species (Kimber and McGregor 1995). The available cultivars of both species are susceptible to aphids and *Alternaria* blight and do not have plant types suitable for intensive cultivation (Prakash and Raut 1983). On the other hand, *Brassica tournefortii* Gouan (TT, 2n = 20), which grows wild throughout the Indian peninsula (Narain and Prakash 1972), has been reported to be a good source of resistance/tolerance against drought, aphid and several pathogens such as *Leptosphaeria maculans*, *Alternaria* and *Albugo candida* (see Ljungberg et al. 1993 and references therein). However, it has been found to be susceptible to *Peronospora parasitica* (Rajpurohit and Choudhary 1995).

Interspecific hybridization is a valuable tool for transferring economic characters from one species to another and has been applied widely for the improvement of *Brassica* crops (Chiang et al. 1977; Roy 1984; Raney et al. 1995a, b; Brown et al. 1995; Choudhary 1997). The study reported here was undertaken to combine the useful attributes of both *B. tournefortii* and *B. rapa* and to generate variability through interspecific hybridization. This paper deals with the crossability of *B. tournefortii* with *B. rapa* and discusses morphological and cytological details of the F_1 hybrids.

Materials and methods

Three promising diverse genotypes of *Brassica rapa* (AA, 2n = 20) – yellow *sarson* (BRYS), brown *sarson* (BRBS) and *toria* (BRT) – and two of *B. tournefortii* (TT, 2n = 20) – RBT 63 (yellow seeds) and RBT 58 (brown seeds) – were selected from the germplasm collections of the Agricultural Research Station, Mandor, India, and used in the hybridization programme. Reciprocal crosses between the selected genotypes of both species were attempted in the field. Normal crosses produced interspecific hybrids that were used for cytomorphological studies.

For meiotic observations, flower buds of the appropriate size were fixed in Carnoy's fluid (ethanol:chloroform:acetic acid – 6:3:1) with ferric chloride as a mordant for 48 h and then stored in 70% ethanol. Affinity of the chromosome was observed at diakinesis/ metaphase I stages of meiosis in pollen mother cells (PMCs) by squashing anthers in a drop of acetocarmine (1%). Pollen fertility was estimated on the basis of percentage stainable pollen.

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Results

Crossability

From 595 *B. tournefortii* buds pollinated with *B. rapa* pollen, 13 F_1 plants were obtained, whereas in reciprocal crosses, none of the plants grown were found to be true hybrids. Hybrid recovery in the *B. tournefortii* × *B. rapa* cross ranged from 0 to 6.0% and four out of six crosses of this combination yielded hybrids (Table 1). There was a marked difference between the two genotypes of *B. tournefortii* and their interaction with *B. rapa* subspecies. For example, RBT 63 produced 6.0% hybrids with BRYS and 3.7% with BRBS, whereas RBT 58 showed a 3.2% and 0.9% crossability with the corresponding pollen parents. However, both the genotypes failed to yield any hybrid with BRT. Amongst the three ecotypes of *B. rapa* used as pollen parents, yellow *sarson* produced the maximum hybrids (4.5%) followed by brown *sarson* (2.3%); no hybrid was obtained with *toria*.

Morphological features of F_1 hybrids

The hybrid plants were bushy, medium in height, profusely branched and intermediate to their putative parent species for most of the morphological and inflorescence attributes (Fig. 1a). The basal leaves were petiolate and pinnetisect with a sinuate-dentate margin (Fig. 1b). The flower size and colour (pale yellow) were intermediate to these of the parents (Fig. 2c). A few characteristics, like hairy leaves, profuse branching, flower shape and white rust tolerance from *B. tournefortii*, and earliness and longer racemes from *B. rapa*, were expressed in the hybrids. Few seeds were set in the F_1 plants due to open pollination in the surroundings of *B. rapa*. However, no seed set was achieved under self-pollination or in backcrosses. Although no *in vitro* resistance test against white rust was carried out, the hybrid plants were found to be free from white rust under field conditions.

Meiotic characteristics of F_1 hybrids

The chromosome associations observed in PMCs of the TA dihaploid hybrids (2n = 20) are given in Table 2, and some representative cells are shown in Fig. 2. Chromosome pairing varied from cell to cell both within and between the hybrids. Of the 241 PMCs analysed, 51 (21.2%) exhibited all the 20 chromosomes as univalents (Fig. 2a); the remainder of the cells had bivalents and multivalents in addition to compensating number of univalents. Although the occurrence of univalents was frequent, a maximum of seven bivalents were still observed in two PMCs. One (Fig. 2b, c), two (Fig. 2d, e), three (Fig. 2f, g), four (Fig. 2h, i), five (Fig. 2j) and six bivalents (Fig. 2k) were observed in 18.7%, 15.8%, 17.4%, 10.8%, 3.3% and 2.1% of the PMCs, respectively. Conspicuously, some of the chromosome associations were heteromorphic in nature (Fig. 2g, h). Trivalents (Fig. 2n) and quadrivalents (Fig. 2o) were also noted in few cells. The hybrid *B. tournefortii* × *B. rapa* var. yellow *sarson* showed a higher mean chromosome pairing (0.01 IV + 0.13 III + 2.20 II + 15.17 I) than the *B.*

Table 1 Crossability relationship between *Brassica tournefortii* and *B. rapa*

Cross	Number of buds pollinated	Percentage hybrid recovery
B. tournefortii × *B. rapa*		
RBT 63 × BRBS	107	3.7
RBT 63 × BRT	91	0
RBT 63 × BRYS	83	6.0
Total/mean	281	3.2
RBT 58 × BRBS	109	0.9
RBT 58 × BRT	112	0
RBT 58 × BRYS	93	3.2
Total/mean	314	1.3
B. rapa × *B. tournefortii*	489	0

Table 3 Chromosome distribution at Anaphase I and II (A I, II) in *Brassica tournefortii* × *B. rapa* hybrids (TA, 2n = 20)

Chromosome distribution	PMCs observed	
	Number	Percentage
Equal distribution (10:10)	8	8.7
Unequal distribution	13	14.1
Laggards at A I	44	47.8
Laggards at A II	16	17.4
Bridge-fragment configuration	11	11.9
Total	92	

Table 2 Mean (±SE) chromosome pairing at diakinesis/metaphase I in pollen mother cells (PMCs) of *Brassica tournefortii* × *B. rapa* hybrids (TA, 2n = 20)

Hybrid	PMCs observed	Chromosome pairing per PMCs			
		Univalents	Bivalents	Trivalents	Quadrivalents
B. tournefortii × *B. rapa* var. brown *sarson*	98	16.42 ± 0.29 (10–20)[a]	1.74 ± 0.14 (0–5)	0.03 ± 0.02 (0–1)	–
B. tournefortii × *B. rapa* var. yellow *sarson*	143	15.17 ± 0.30 (6–20)	2.20 ± 0.15 (0–7)	0.13 ± 0.03 (0–2)	0.01 ± 0.01 (0–1)
Total	241				

[a] Range

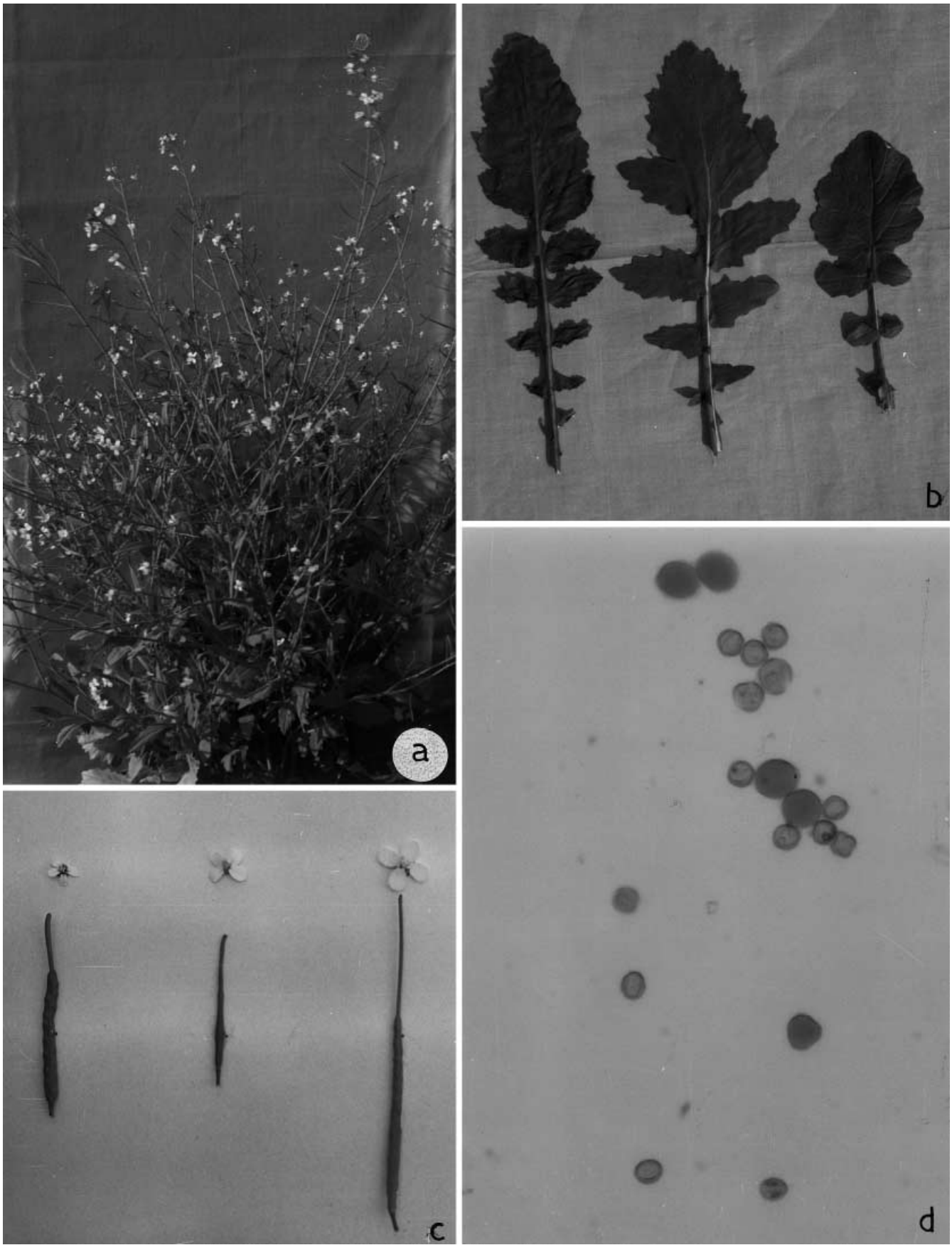
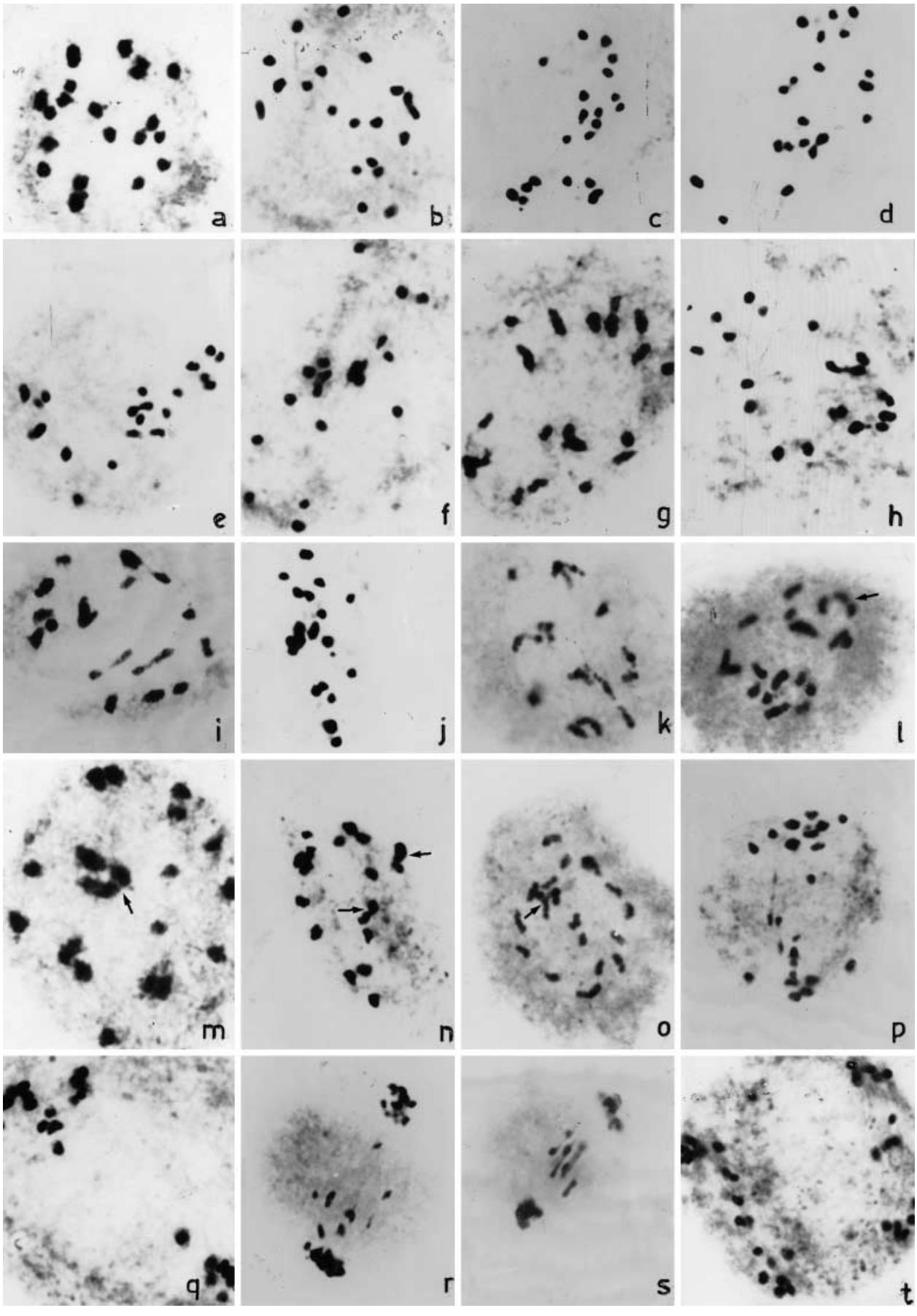


Fig. 1a–d Comparison of morphological attributes of *Brassica tournefortii*, *B. rapa* and their F_1 hybrid. **a** F_1 plant, **b**, **c** leaf (**b**), and flower and siliqua (**c**) of *B. tournefortii*, F_1 hybrid and *B. rapa* var. brown *sarson* (*left*, *middle*, *right*), **d** pollen stainability of F_1 hybrid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tournefortii × *B. rapa* var. brown *sarson* hybrid (0.03 III + 1.74 II + 16.42 I).

Numerous disjunctional abnormalities including late disjunction of bivalents (Fig. 2p), bridges (Fig. 2s) and laggards (Fig. 2r, t) were observed at Anaphase I and II (Table 3). However, a few cells were observed with a normal distribution (Fig. 2q), resulting in some fertile pollen grains in the hybrids (Fig. 1d). Pollen stainability recorded in *B. tournefortii* × brown *sarson* and *B. tournefortii* × yellow *sarson* hybrids was only 5.5% and 7.4%, respectively.

Discussion

Since Sikka (1940) successfully crossed *B. tournefortii* with *B. rapa*, only a few other investigators have also reported hybrids between these two species (Fukushima and Iwasa 1966; Mizushima 1968; Narain and Prakash 1972; Banga et al. 1987; Choudhary 1997). In the present study, successful hybrids were achieved only when *B. tournefortii* was used as a female parent. This is in good agreement with the results of Hinata et al. (1974), Ljungberg et al. (1993) and Choudhary et al. (2000b), who suggested that *B. tournefortii* crossed successfully with other species when it was involved as a female parent. Harberd (1976) pointed out that pollen grains of *B. tournefortii* did not germinate on the stigma of other species resulting in the failure of reciprocal crosses. However, Narain and Prakash (1972) succeeded in obtaining hybrids of *B. campestris* (♀) × *B. tournefortii* (♂), which contradicts the opinion of Harberd (1976).

Although only two genotypes of *B. tournefortii* were used in the present investigation, a marked difference between them was observed in their crossability with *B. rapa*. Similar variations amongst the three ecotypes of *B. rapa* were also recorded. These observations clearly demonstrate that the success of interspecific crosses depends not only on the species and direction of the cross but also on the genotypes of species involved in the hybridization. Seed setting in interspecific and intergeneric crosses is a product of the interaction between genotypes used in the hybridization (Bozorgipour and Snape 1990). This explains the wide range of variation amongst the different *Brassica* genotypes with respect to their ability to set seeds in the interspecific crosses; similar observations have also been reported by Akbar (1989) and Choudhary and Joshi (1999).

Hybrid plants were found to be vigorous and intermediate between the parent species with respect to many morphological characters. These observations are in conformity with those reported by Narain and Prakash (1972) and Banga et al. (1987). The occurrence of characteristics from both progenitor species in the hybrids indicates that the F_1 plants had genes of both parents combined. The meiotic data of the present study and successful introgression in other interspecific crosses (Chiang et al. 1977; Roy 1984) give hope to a successful transfer of useful attributes across the species.

The cytological study revealed a higher order of chromosome pairing including bivalents (0–7), trivalents (0–2) and a quadrivalent (0–1) in the dihaploid TA hybrids when compared to those reported by other workers (Sikka 1940; Fukushima and Iwasa 1966; Mizushima 1968; Narain and Prakash 1972; Banga et al. 1987). Chromosome pairing in the hybrids could be interpreted to be due to auto- as well as allo-syndesis, respectively within and between the T and A genomes (Prakash 1974; Mizushima 1980; Armstrong and Keller 1981). Prakash (1974) reported chromosome pairing of 1 III + 2 II as a result of autosyndesis in the haploid of *B. tournefortii* (T genome). Similarly, in the *B. rapa* haploid, Armstrong and Keller (1981) noted a maximum association of 1 III + 2 II resulting from autosyndesis within the A genome. These studies suggest that a total sum of 2 III + 4 II or 6 II might theoretically be possible due to autosyndesis within both the T and A genomes. Therefore, out of seven bivalents observed in the hybrids, at least one could safely be explained by allosyndesis between the T and A genomes.

Sikka (1940) observed a maximum chromosome pairing of 1 IV + 3 II + 10 I in the hybrids *B. tournefortii* × *B. trilocularis* (*B. rapa*) and suggested the existence of a weak homology between T and A genomes. Fukushima and Iwasa (1966) noted up to five bivalents and, occasionally, one trivalent as well, they considered that at least three bivalents were allosyndetic in nature. However, Mizushima (1968) was of the opinion that at least two bivalents out of the four observed in the TA hybrids arose allosyndetically. The number of autosyndetic bivalents observed within the T (Prakash 1974) and A genomes (Armstrong and Keller 1981) was much higher than inferred by Mizushima (1968, 1980), indicating an overestimation of allosyndesis between these two genomes. Contrary to earlier observations, Nagpal et al. (1996) observed only some end-to-end and side-to-side associations without any clear discernible chiasmata in allodiploid TA hybrids and therefore concluded that homology between the T and A genomes was non-existend. However, the occurrence of chiasmatic and heteromorphic pairing, multivalent associations and, more importantly, the formation of up to seven bivalents exceeded the amount of pairing that could be accounted for by autosyndesis within the T and A genomes, clearly indicating homoeologous pairing between chromosomes of the T and A genomes.

The bridge-fragment configurations at Anaphase I observed in the present material, as also reported by Sikka (1940), probably resulted from chiasma formation within

◀ **Fig. 2a–t** Meiosis in hybrids of *Brassica tournefortii* × *B. rapa* showing chromosome pairing at diakinesis/metaphase I (**a–o**) and anaphase distribution (**p–t**). **a** 20 I, **b**, **c** 1 II + 18 I, **d**, **e** 2 II + 16 I, **f**, **g** 3 II + 14 I, **h**, **i** 4 II + 12 I, **j** 5 II + 10 I, **k** 6 II + 8 I, **l** 1 III + 3 II + 11 I (trivalent marked by *arrow*), **m** 1 III + 17 I (trivalent marked by *arrow*), **n** 2 III + 2 II + 10 I (trivalents marked by *arrows*), **o** 1 IV + 16 I (quadrivalent marked by *arrow*), **p** late disjunction of bivalents at anaphase I, **q** anaphase I devoid of laggards, **r** anaphase I with laggards, **s** anaphase I with bridges, **t** anaphase II with laggards

a heterozygous inversion. Such chiasmata demonstrate true homology and pairing between the genomes involved (Attia and Robbelen 1986). A notable phenomenon – delayed terminalization of the chiasmata – observed at Anaphase I in the hybrids, was probably caused by non-homologous segments at terminal or subterminal regions of the paired chromosomes (Olsson and Hagberg 1955). Although such configurations had not been reported earlier in TA hybrids, they have been noted in the ABC hybrid (Choudhary et al. 2000a). The occurrence of such a feature supports the allosyndetic nature of pairing between the genomes under consideration.

The low pollen fertility recorded in the present hybrids might be due to meiotic irregularities and segregational anomalies (Stebbins 1966). Narain and Prakash (1972) observed the formation of completely abortive pollen, while Banga et al. (1987) reported higher pollen fertility in TA hybrids. Such differences could be attributed to differences in the genotypes involved.

The homoeologous pairing between *B. tournefortii* and *B. rapa* chromosomes and seed set by interspecific hybrids, when appropriate genotypes of the species are crossed, offers an opportunity for the transfer of useful genes across the species.

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