Crossability of *Brassica carinata* and *B. tournefortii*, and Cytomorphology of Their F₁ Hybrid

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Summary Reciprocal crosses between *Brassica carinata* (BBCC, $2n=34$) and *B. tournefortii* (TT, $2n=20$) were attempted in order to determine crossability between the species, carry out chromosomal associations among the B, C and T genomes, and to study morphology of the interspecific hybrids. A hybrid between these 2 species was obtained only when *B. carinata* was used as a female parent. Morphologically, the hybrid was intermediate between the progenitor species but its leaves resembled *B. carinata*. The F₁ plant was tall but grew very slowly. The hybrid was almost male sterile, showing only 2.3% pollen stainability. Meiotic studies of trigenomic triploid hybrid (BCT, $2n=27$) showed various chromosome configurations including quadrivalents (0–1), trivalents (0–2), bivalents (2–9) and univalents (5–23). Only 5 univalents were recorded to have occurred; otherwise trivalents and bivalents were observed in pollen mother cells of the BCT hybrid. A few chromosomal association could be expected through allosyndesis which suggests the possibility of transferring genes across the species through interspecific hybridization.

Key words *Brassica carinata*, *Brassica tournefortii*, Crossability, Cytomorphology, Interspecific hybrid.

Ethiopian or Abyssinian mustard (*Brassica carinata* A. Braun, $2n=34$) possesses many desirable characteristics that are rare in the other *Brassica* oil crops: heat and drought tolerance, tolerance to various biotic and abiotic stresses and availability of yellow-seeded germplasm (Jiang *et al.* 2007). In spite of these positive attributes, the crop suffers from many agronomic limitations like longer crop duration, poor harvest index, low oil content *etc.*, whereas *B. tournefortii* ($2n=20$), known variously as wild turnip or Sahara mustard or Asian mustard or Tournefort's mustard or Tournefort's Birdrape, grown sporadically in a few pockets of arid and semi-arid areas, has a strategy of early and quick growth because this effectively captures available soil moisture, enables it to build a canopy, reproduce and mature before neighbouring species (Minnich and Sanders 2000). It has also been reported as having a good source of tolerance against aphids (Singh *et al.* 1965) and drought (West and Nabhan 2002). However, lower test weight (2–3 g/1000 seed) and susceptibility to downy mildew (Rajpurohit and Choudhary 1995) are the major constraints in the spread of this species.

Interspecific hybridization has great potential for the improvement of *Brassica*. Besides the transfer of useful traits across the species in genus *Brassica* (Roy 1984, Rahman 2001, Rygulla *et al.* 2007), such crosses were widely used to create genetic variability (Prakash 1973a), genetic diversity (Choudhary and Joshi 2001) and to synthesize artificially new species such as *Brassicoraphanus* (Tokumasu 1970) *Brassica napocampestris* (McNaughton 1973) and *Raphanofortii* (Choudhary *et al.* 2000a). Both *B. carinata* and *B. tournefortii* species have their own advantage and deficiencies. Therefore, the present study was undertaken to examine crossabil-

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ity between *B. carinata* and *B. tournefortii* and to observe the meiotic behaviour of the interspecific F_1 hybrid of these species as it suggests the possibility of genetic introgressions for crop improvements which take place through chromosomal interchange.

Materials and methods

Two genotypes of *B. tournefortii* (TT, $2n=20$) — RBT 58 and RBT 61 — and 1 of *B. carinata* (BBCC, $2n=34$) — BCN selected from the germplasm collection of the Agricultural Research Station, Mandor-Jodhpur, India were used in the hybridization programme.

The reciprocal interspecific crosses were attempted involving the selected genotypes of both the species under field conditions. Emasculation and pollination were conducted as per the given procedure. Immature flower buds of appropriate size were emasculated by removal of anthers in the evening and bagged with butter paper bags. In the following morning, stigma of these buds were pollinated with pollen of the desired parent and the racemes were re-enclosed with bags to prevent cross pollination. Seeds obtained from crosses and parents were grown in the field for evaluation. Morphological comparisons were made, and the meiosis of the interspecific hybrid was studied.

For meiotic analysis, young flower buds were fixed in freshly prepared solution of propionic acid: absolute ethanol (1:3) containing ferric chloride as a mordant. After 24 h the rinsed flower buds were stored in 70% ethanol until slides were made by the acetocarmine (1%) squash technique. Chromosome associations were observed at diakinesis/metaphase I. Pollen fertility of the hybrid and parents was estimated by pollen grain stainability in acetocarmine (1%).

Results

Crossability

The seed setting was almost double in the cross *B. carinata* × *B. tournefortii* compared to its reciprocal combination. Out of 112 flowers buds of *B. carinata* pollinated with pollen of *B. tournefortii*, 22 seeds were obtained giving rise to only a single hybrid plant. On contrary, in the reciprocal combination, out of 122 buds of *B. tournefortii* pollinated by *B. carinata* pollen, only 11 seeds were obtained which did not yield any true hybrids.

Morphological features of F_1 hybrids

The phenotype of the hybrid was intermediate between parent species. The size and shape of leaves of the hybrid were closer to *B. carinata*. They were obovate-lanceolate, petiolate, pinnatifid to pinnatipartite with entire to slightly dentate margin, obtuse tip, sparsely hairy and dark green in colour. The flowers were medium in size with pale yellow petals but their shape was similar to *B. tournefortii*. The hybrid was tall but grew very slowly and took as many as 95 d to flower and 168 d to mature completely (Table 1). The F_1 plant exhibited enhancement in values of attributes like primary and secondary branches, main raceme length, siliquae on main raceme and siliquae per plant. On the other hand, siliqua length, seeds per siliqua, seed weight and seed yield were recorded much lower in the hybrid compared to either of the parents. Few seeds were set in the F_1 plant due to open pollination. However, no seed set was found under self-pollination or in backcrosses.

Meiotic characteristics of F_1 hybrids

The chromosome associations observed in pollen mother cells (PMCs) of the trihaploid BCT hybrid ($2n=27$) are given in Table 2 and some representative cells are shown in Figs. 1–3. A total of 59 PMCs of the hybrid were analyzed at diakinesis/metaphase I. At least 2 bivalents were observed in all the PMCs, but the maximum of 9 bivalents were observed in 17% of cells (Fig. 2). Many of them exhibited chiasmatic configurations (Fig. 2). Multivalents in the form of trivalents (0–2) and

Table 1. Plant morphological characteristics of *Brassica carinata* and *B. tournefortii* and their F₁ hybrid

Characteristics	<i>Brassica carinata</i>	<i>B. tournefortii</i>	F ₁ hybrid
Days to flowering	63	66	95
Days to maturity	156	137	168
Plant height (cm)	158	131	162
Primary branches per plant	8.3	7.4	11
Secondary branches per plant	23	21	42
Main raceme length (cm)	44	42	56
Siliquae on main raceme	73	27	35
Silique length (cm)	4.9	5.3	2.1
Seeds per silique	12.3	14.8	0.2
1,000-seed weight (g)	3.86	2.92	1.08
Silique per plant	219	256	491
Seed yield per plant (g)	13.4	14.7	0.3
Flower colour	Light yellow	Cream	Pale yellow
Corolla length (cm)	1.55	0.81	1.03
Corolla width (cm)	0.70	0.30	0.41
Leaf hairiness	Sparsely hairy	Densely hairy	Sparsely hairy

Table 2. Chromosome associations at diakinesis/metaphase I in trigenomic triploid BCT hybrid (2n=27) of cross *Brassica carinata* (BBCC, 2n=34)×*B. tournefortii* (TT, 2n=20)

Chromosome association (Number/PMC)				PMCs observed	
IV	III	II	I	Number	Frequency (%)
1	1	6	8	1	1.7
—	2	8	5	1	1.7
—	2	7	7	10	16.9
—	1	9	6	3	5.1
—	1	8	8	5	8.5
—	1	7	10	8	13.6
—	1	4	16	3	5.1
—	1	3	18	4	6.8
—	—	9	9	7	11.9
—	—	8	11	4	6.8
—	—	7	13	5	8.5
—	—	6	15	4	6.8
—	—	3	21	2	3.4
—	—	2	23	2	3.4
Total				59	100
Mean	0.02	0.77	6.69	11.20	
± SE	0.02	0.10	0.25	0.59	
Range	0–1	0–2	2–9	5–23	

quadrivalents (0–1) were also noticed in the majority of PMCs (59.4%). Only 5 univalents were observed in a single cell; the rest were trivalents and bivalents. The mode of chromosome association observed was 2 III+7 II+7 I (16.9%), 1 III+7 II+10 I (13.6%) 9 II+9 I (11.9%), 1 III+8 II+8 I (8.5%) and 7 II+13 I (8.5%). On an average, each PMC had shown an association of 0.02 ± 0.02 IV + 0.77 ± 0.10 III + 6.69 ± 0.25 II + 11.20 ± 0.59 I.

The stickiness and delayed terminalisation of the chiasmata made it difficult to determine the exact nature of the chromosome association observed (Fig. 4). Further, it was not always possible to distinguish between the relatively small bivalents of the B and T genome(s) and the relatively large univalents of the C genome. In the majority of the cases, well-defined metaphase plates could not be recognized as the bivalents did not congress at the equatorial plate but rather were distributed randomly throughout the cell. Bivalent and trivalent associations were observed even at late anaphase I. Distribution of chromosomes at anaphase (I/II) was random due to unequal disjunction.

Bridge-fragment configurations were observed in 12.5% of cells (Fig. 5). The majority of cells had laggards at both anaphases I (Figs. 5, 6) and II. The complete process of meiosis was found to be irregular. Most of the cells did not divide synchronously; monads and dyads were observed in high frequency. These meiotic irregularities might be the reason for pollen being mostly sterile. The percentage of stainable pollen recorded in the hybrid was only 2.3%.

Figs. 1–6. Showing PMCs of *Brassica carinata*×*B. tournefortii* hybrid (BCT, $2n=27$). 1–3: PMCs at metaphase I showing different chromosome associations (a few chromosomes are out of focus and a few are overlapping each other), (1) 3 II+21 I, (2) 1 III+9 II+6 I, and (3) 1 III+4 II+16 I; 4–6: Showing anaphase I distribution: (4) early anaphase I, (5) anaphase I with bridge-fragment configuration, and (6) anaphase I with laggards.

Discussion

The hybrid from cross *B. carinata*×*B. tournefortii* was obtained only when *B. carinata* was used as a female parent. This was not anticipated as in the majority of interspecific crosses reported in *Brassica*, there was greater success when the species with the higher basic chromosome number was used as a female (Quazi 1988, Choudhary and Joshi 1999). Nishiyama *et al.* (1991) reported that crosses between tetraploid and diploid species were partly successful, while the reciprocal, *i.e.* diploid×tetraploid, usually failed. However, the occurrence of the hybrid in the direction of *B. carinata*×*B. tournefortii* in the present study is in contradiction to Harberd's theory that interspecific crosses with *B. tournefortii* were only possible when it was used as a female parent (Harberd 1976).

The sexual hybrid between *B. carinata* and *B. tournefortii* was reported for the first time by us (Joshi and Choudhary 1999). The hybrid plant was observed to be an intermediate in terms of many phenotypic features of both the parents. This is an advantage, since it would allow better selection for specific attributes in segregating progenies.

Meiotic studies of trigenomic triploid hybrid BCT ($2n=27$) revealed various chromosome configurations including quadrivalents (0–1), trivalents (0–2) and bivalents (2–9). Based on earlier studies on haploid genomes of B (Prakash 1973b), C (Armstrong and Keller 1982) and T (Prakash 1974), a total of 7 bivalents were theoretically possible due to autosyndesis in these 3 genomes. Hence, out of the maximum of 9 bivalents observed in the present hybrid, 2 could be expected to be due to allosyndesis among the B, C and T genomes. Mizushima (1968) noticed a maximum of 3 bivalents in a TC hybrid and opined that one of them might be due to allosyndesis between these 2 genomes, while Ljungberg *et al.* (1993) observed a maximum of 1 III+3 II+10 I in a similar hybrid

and suggested the possibility of allosyndetic pairing in T and C genomes. Narain and Prakash (1972) reported a maximum of 3 bivalents in a BT hybrid but did not say anything regarding the nature of origin of such bivalents. Sarla and Raut (1991) found a maximum of 1 III+5 II+4 I in BC hybrid and demonstrated homology between the B and C genomes. Pairing in the present triploid hybrid has been assumed to occur mostly between the B and C genomes. However, less than 10 univalents (possibly of T genome) were counted in 45.8% of all PMCs, suggesting that some T chromosomes of *B. tournefortii* might have been involved in the pairing with B and C chromosomes.

The high level of pairing observed in the hybrid might be due to auto/allosyndesis pairing within and among the genomes. This might be attributed to structural similarities and duplications of some of the chromosomes (Röbbelen 1960). However, the heteromorphic appearance of bivalents in some of the PMCs observed and the presence of only 5 univalents, instead of the 27 expected, indicated the extent of homology among B, C and T genomes. Bridges at anaphase I observed in the present material might have resulted from pairing between partially chromosomes of the parent genomes. Such inter-genomic pairing in *Brassica* hybrid was also observed by Howard (1938). The occurrence of multivalent association at a high frequency in the hybrid studied indicated that parts of the chromosomes of these genomes possess sufficient affinity to allow multivalent association (Newell *et al.* 1984). This showed that some of chromosomes of these 3 genomes had duplicate archetypes and confirmed the hypothesis of the secondary polyploidy origin of the B, C and T genomes. Secondly, the lower average chromosome association observed in the present BCT hybrid, compared to that reported in an ABC hybrid by Choudhary *et al.* (2000b), suggested that the T genome was more distantly related than A to both the B and C genomes. Inter-genomic chromosome associations noticed in the hybrid indicated the possibility of transferring genes across the species through interspecific hybridization.

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