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Cytogenetic studies of the F_1 hybrids of *Capsicum annuum* with *C. chinense* and *C. baccatum*

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Summary. Partially sterile interspecific hybrids were obtained between *C. annuum* var. 'cerasiformis' and *C. chinense* var. 'mishme' (H_1), and *C. annuum* var. 'cerasiformis' and *C. baccatum* var. 'pendulum' (H_2). Morphologically the F_1 hybrids were intermediate between the corresponding parents. Meiosis was irregular in the two F_1 hybrids. Cytological analysis of the two F_1 hybrids revealed that the genome of *C. annuum* differs from *C. chinense* by two translocations and some minor structural alterations and from *C. baccatum* by two translocations, a single inversion and some minor structural alterations. Isolation barriers such as hybrid inviability, weakness and hybrid breakdown in the H_1 hybrid and, inaddition, desynapsis in the H_2, were operative in these taxa. The differences between the present findings and those reported earlier on the two F_1 hybrids were attributed to differences in the genetic architecture of the taxa employed in hybridization.

Key words: *Capsicum annuum, C. chinense, C. baccatum* – Interspecific hybrids – Chromosome pairing – Genome homologies

Introduction

Chili pepper (*Capsicum* L.), an important condiment, is more or less cosmopolitan in distribution and is cultivated as a cash crop not only in India but in many parts of the world.

In spite of its economic importance, the cytogenetic work necessary for a proper understanding of the interspecific relationships in the genus has not been carried out. Though crossability relationships among some taxa of this genus have been reported (Smith and Heiser 1951, 1957; Hirose et al. 1960; Lippert et al. 1966; Eshbaugh, 1964, 1975; Somanatha Pillai et al. 1977; Yamamoto 1978), these were mostly confined to the breeding behaviour of the F_1 hybrids. Nevertheless, information pertaining to the cytological analysis of a few interspecific hybrids including the F_1 hybrids of *C. annuum* × *C. chinense* and *C. annuum* × *C. baccatum* is available (Ohta 1961; Venkateswara Rao and Yesoda Raj 1974; Pickersgill 1971, 1977; Koompai 1976; Tanksley 1984; Egawaa and Tanaka 1984, 1986; Aniel Kumar 1984; Panda 1985).

In the present paper we describe cytological observations on F_1 hybrids of *C. annuum* × *C. chinense* and *C. annuum* × *C. baccatum,* and relate these to some of the differences previously recorded on these two F_1 hybrids (Pickersgill 1971, 1977; Koompai 1976; Tanksley 1984; Egawa and Tanaka 1984, 1986).

Materials and methods

Seeds of *C. annuum* var. 'cerasiformis' were obtained from N. S. R. Murthy, Agricultural Research Station, Lam Farm, Guntur, India. Seeds of *C. chinense* var. 'mishme' and *C. baccatum* var. 'pendulum' were provided by E. Pochard, Montfavet, Avignon, France. The three species were selfed and maintained for several generations before the hybridization programme was began. Reciprocal crosses were attempted between *C. annuum* and *C. chinense* and *C. annuum* and *C. baccatum* respectively. Two F_1 interspecific hybrids, *C. annuum* × *C. chinense* and *C. annuum* × *C. baccatum,* were obtained and these provided the material for the present study. F_1 hybrids of *C. annuum* var. 'cerasiformis' × *C. chinense* var. 'mishme' and *C. annuum* var. 'cerasiformis' × *C. baccatum* var. 'pendulum' are referred to as H_1 and H_2, respectively, in the text.

For cytological analysis, young flower buds from both the parents and the F_1 hybrids were fixed in a 1 : 3 acetic acid and alcohol mixture. After 24 h, they were transferred to 70% alcohol and stored in a refrigerator. Smears were made in 2% acetocarmine to study meiosis. Pollen fertility – sterility was determined by staining mature and ripe anthers with 2% acetocarmine. Well-filled and stained grains were scored as fertile while shrunken, unstained or half-stained grains were considered to be sterile.

Results

Crossability

Though reciprocal crosses between *C. annuum* and *C. chinense,* and *C. annuum* and *C. baccatum* yielded seeds, viable F_1 hybrids could be raised only from the crosses *C. annuum* × *C. chinense* (H_1) and *C. annuum* × *C. baccatum* (H_2). The seeds from the reciprocal crosses either did not germinate or those few that did germinate died after a few days (Table 1).

Morphology of parents and hybrids

C. annuum, C. chinense, and *C. baccatum* conform to the taxonomic description of Eshbaugh (1980) and Anonymous (1983). The two F_1 hybrids (H_1 and H_2) were rather weak and morphologically they were intermediate between the corresponding parents. In a few characters they resembled either one of the other of the parents.

Cytology of parents and hybrids

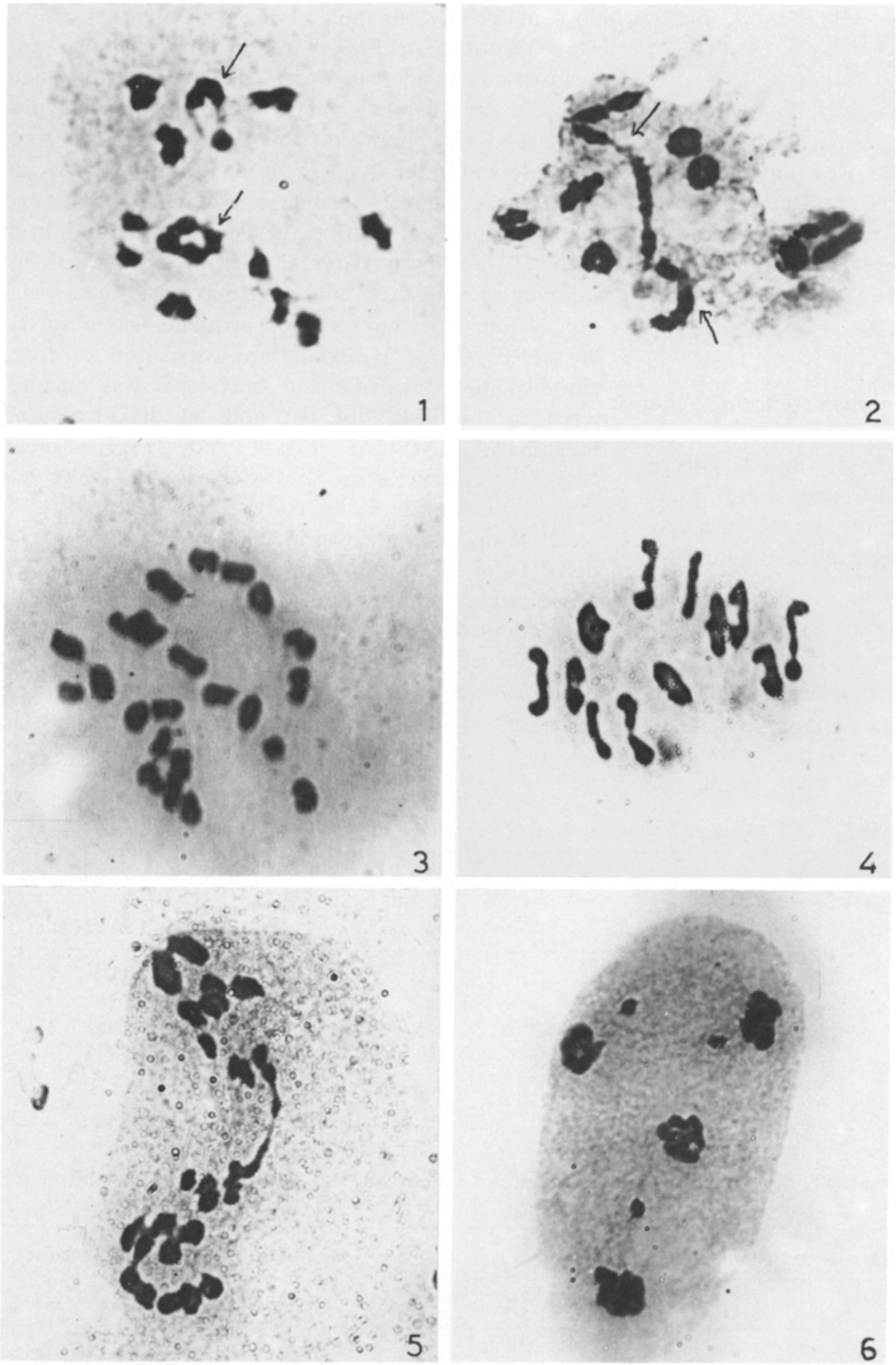
Chromosome pairing and behaviour was studied in 300 PMCs, at both diakinesis and metaphase I, in all taxa under investigation. Twelve bivalents (2n = 24) were recorded in PMCs in all three parents, and meiosis was regular in them. However, chromosome synapsis was relatively poor and meiosis was irregular in H_1 and H_2. Significant interplant differences were not observed in the two F_1 hybrids (H_1 and H_2), non in the parents. Hence, the data for three H_1 plants and five H_2 plants, and for 10 plants chosen at random from each of the three parental taxa, were pooled for studying chromosome pairing behaviour. An association of four chromosomes and bivalents was observed in the PMCs of H_1 while an association of three chromosomes, bivalents and univalents was encountered in the PMCs of H_2, both at diakinesis and metaphase I (Table 2, Figs. 1 and 2). The mean chiasma frequencies in the two F_1 hybrids were less than those found in the corresponding parents (Table 2). A maximum of two higher chromosome associations (IV or III) per PMC was observed in H_1 and H_2. These higher chromosome associations were mostly chains and the nucleolus organising chromosome was not involved in the interchange. The occurrence of twelve bivalents in a PMC was higher in H_1 (37%) and lower in H_2 (21%) (Fig. 4). Univalents ranging in

Table 1. Results on crossability relationships of *C. annuum, C. chinense* and *C. baccatum*

Particulars	*C. annuum* × *C. chinense*	*C. chinense* × *C. annuum*	*C. annuum* × *C. baccatum*	*C. baccatum* × *C. annuum*
No. of pollinations made	200	150	200	250
No. of fruits obtained	20	10	20	16
Average no. of seeds/fruit	20	16	13	18
No. of seeds germinated	35	10	40	12
Percentage of seed germination	8.75	6.25	15.38	4.17
No. of hybrid plants surviving	3	–	5	–

Table 2. Mean chromosome pairing, chiasma frequency at metaphase I, pollen stainability and seed set in parents and their F_1 hybrids

Parents and hybrids	Chromosome associations				Chiasmata	% of pollen stainability	Seed set
	IV	III	II	I			
C. annuum var. 'cerasiformis'	–	–	12.00	–	17.36±0.19 (15–19)	83.70±0.42 (76–86)	46±0.15 (26–52)
C. chinense var. 'mishme'	–	–	12.00	–	19.64±0.20 (17–24)	92.70±0.18 (86–94)	35±0.14 (20–45)
C. baccatum var. 'pendulum'	–	–	12.00	–	19.32±0.24 (17–22)	89.80±0.28 (79–91)	40±0.20 (18–45)
C. annuum × *C. chinense*	0.63±0.03 (0–2)	–	10.73±0.06 (8–12)	–	16.99±0.02 (9–24)	42.00±2.05 (30–49)	20±0.10 (6–28)
C. annuum var. 'cerasiformis' × *C. baccatum* var. 'pendulum'	–	0.42±0.05 (0–2)	7.88±0.20 (5–12)	6.98±0.43 (0–16)	11.57±0.54 (6–20)	42.60±0.62 (26–49)	13±0.25 (4–15)



Figs. 1–6. Meiosis in interspecific hybrids of *Capsicum*. **1** Diakinesis showing 2 IV (↑)+8 II (H_1) (× 1,600); **2** Diakinesis showing 2 III (↑)+9 II (H_2) (× 1,600); **3** Metaphase I showing 2 II+20 I (H_2) (× 1,600); **4** Metaphase I showing 12 II (H_1) (× 1,600); **5** Anaphase I showing bridge (H_1) (× 1,600); **6** Telophase II showing laggards (H_2) (× 1,600)

numbers from 0–24 were recorded in H_2 and these were scattered throughout the cytoplasm (Fig. 3).

At anaphase I 200 PMCs were scored for chromosome disjunction for both F_1 hybrids. Irregular chromosome disjunction and laggards were observed in 60% of the H_1 PMCs and a single persistent bridge (Fig. 5) and fragments (0–2) in addition to irregular chromosome segregation and laggards were recorded in 75% of the H_2 cells. The irregularities persisted until the end of the second meiotic division though their frequencies were lower than those found in anaphase I (Fig. 6). Micronuclei varying in numbers from 0–4 were also observed in some of the PMCs of the two F_1 hybrids.

Pollen fertility and seed set was very low in the two F_1 hybrids compared to the corresponding parents

(Table 2). The selfed seeds of the two hybrids were shrivelled with a depression at the centre, consequently these did not germinate. A few seeds were obtained when the H_2 was backcrossed to the *C. annuum* parent and though some of them did germinate these did not survive until maturity. On the other hand, the *C. annuum* × *C. chinense* hybrid (H_1) when backcrossed to either of the parents, and H_2 when backcrossed to the *C. baccatum* parent, did not set seed.

Discussion

For assessing interspecific relationships reciprocal crosses were made between both *C. annuum* and *C. chinense*, and *C. annuum* and *C. baccatum*. Viable F_1 hybrids were obtained only when *C. annuum* was the seed parent in each case. However, a few seeds were obtained in the reciprocal crosses for either of the two combinations and some of them did germinate. Nevertheless, these died after a few days. F_2 progeny could not be raised from either of the two hybrids and the few backcross seedlings obtained from H_2 did not survive until maturity. All this suggests the manifestation of hybrid inviability and weakness in the prezygotic stage preventing hybrid formation in the reciprocal direction and the operation of hybrid sterility and hybrid breakdown in the post-zygotic stage of hybrid development resulting in the failure to obtain F_2 and backcross progeny in the two F_1 hybrids.

All three species used in the present study are normal homozygotes while the two F_1 hybrids (H_1 and H_2) are structural heterozygotes as evidenced by the presence of the association of four or three chromosomes in these PMCs. The reduced chiasma frequencies in the two hybrids indicates reduced homologies between the corresponding parental taxa.

The occasional occurrence of twelve bivalents in a PMC in the two F_1 hybrids suggests that despite morphological divergence, the species have retained sufficient ancestral homologies. The low frequency of the higher chromosome associations, and inversions, when present, suggests that the altered segments are not large. Univalent formation in H_2 may be due to the failure of pairing between the chromosomes of the two corresponding parents, which are structurally different. Alternately, these may have resulted from desynapsis of some or all of the chromosomes forming multivalents and bivalents at pachytene and diakinesis. Desynapsis leading to univalent formation may be one of the isolating mechanisms between the *C. annuum* and *C. baccatum* of the present study.

Desynapsis as a isolating mechanism has been reported earlier in the interspecific hybrids of *Oryza* (Shastry et al. 1964). A single persistent bridge with or without fragments was observed in H_2, which is suggestive of inversion heterozygosity.

A variation in pollen fertility in F_1 hybrids (H_1 and H_2) reflects the differences in the cytogenetic architecture of the concerned parents. It is possible that the parents involved in hybridization differed with respect to several genes, a disharmonious assemblage of which might have largely influenced pollen fertility.

Pickersgill (1977) obtained a partially sterile F_1 hybrid between cultivars of *C. annuum* and *C. chinense*. Bivalents and univalents alone were recorded in the F_1 hybrid. She attributed the sterility of the F_1 hybrid to small scale rearrangements of the chromosomes of the two species and also to the cultivated forms. Koompai (1976) and Tanksley (1984) also reported partially sterile F_1 hybrids between *C. annuum* and *C. chinense*. They believed that the two species differ from each other by a single translocation and some minor structural alterations. Egawa and Tanaka (1986) reported a sterile F_1 hybrid between *C. annuum* var. 'minimum' and *C. baccatum* var. 'baccatum'. They concluded that genomes of the two species differed from each other by at least three translocations.

The present cytogenetic analysis of the two F_1 hybrids (H_1 and H_2) indicates that *C. annuum* differs from *C. chinense* by two translocations and minor structural alterations and from *C. baccatum* var. 'pendulum' by two translocations, one inversion and some minor structural alterations.

The differences recorded in the two F_1 hybrids between the present and earlier studies of Pickersgill (1977), Koompai (1976), Tanksley (1984) and Egawa and Tanaka (1986) can be atributed to the inherent genomic differences in the respective taxa employed in the hybridization programme. The results suggest that chromosomal differentiation has occurred between varieties of one or both of the respective species employed in the cross. The inability to obtain reciprocal hybrids in the present investigation can be attributed to reasons other than incompatibility. It is possible that the cytoplasm of the ovules of *C. chinense* or *C. baccatum* may react with gene/genes of *C. annuum* pollen, hindering a viable hybrid.

Meiotic irregularities and partial sterility encountered in the two F_1 hybrids (H_1 and H_2) may be attributed to structural differences found in the respective parents. Such structural changes can inhibit the production of fertile hybrids, as in the present case, and may provide the necessary isolation for the development of diverse species by a further accumulation of genetic differences.

Finally, these structural alterations found in the H_1 and the desynapsis found in the *C. annuum* × *C. baccatum* hybrid acted as effective isolation barriers, in addition to the other isolation mechanisms mentioned earlier, in preventing natural hybridization among the species of the present study. This suggestion gains support from the fact that no naturally occurring hybrids have been reported when these species were grown sympatrically (Pickersgill 1971).

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