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REGULAR ARTICLE

CYTOGENETIC STUDIES OF F_1 HYBRID *CAPSICUM ANNUUM* L. X *CAPSICUM CHACOENSE* (HUNZ).

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SUMMARY

Interspecific F_1 hybrid between *Capsicum annuum* var. cerasiformis (cultivated) and *Capsicum chacoense* Hunz. (wild) was obtained. The F_1 hybrid resembled *C. chacoense* parent more in gross morphological features such as growth habit, leaf structure and position, shape and size of the fruit. The mean chiasma frequency in the F_1 hybrid was less compared to either of the parents indicating reduced homologies between the parental taxa. The meiotic studies further revealed that the two parents involved in the cross differed from each other by two translocations, an inversion and some minor structural alterations. The hybrid was weak and highly sterile (pollen and seed). Isolating mechanisms such as hybrid weakness and hybrid sterile on operative among the parental taxa.

Keywords: *Capsicum annuum*, *Capsicum chacoense* (Hunz)., Cytogenetics, Interspecific hybrid.

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1. Introduction

The genus *Capsicum* commonly known as chili pepper is a major spice crop and is almost cosmopolitan in distribution. The genus comprises of five domesticated and twenty wild species [1]. The cultivated taxa are widely used as condiment and vegetable. Though cross compatibility relationships among some taxa of this genus have been reported by quite few workers [2-7] these were mostly confined to the breeding behaviour of the F_1 hybrids. Further the interspecific relationships and genome homologies are not well understood even today. Information on cytogenetic analysis of species hybrids of *Capsicum* is meager [8-12]. Similarly not much is known about the interspecific relationships and cytogenetic behaviour of F_1 hybrids between cultivated and wild species. Therefore the present study is taken up to elucidate cytogenetic relationships between *C. annuum* var. cerasiformis (cultivated) and *C. chacoense* (wild) on the basis of meiotic chromosome pairing behaviour and fertility of the F_1 hybrid and the results are documented in the present communication.

2. Materials and Methods

Seeds of *C. chacoense* Hunz., a wild species and *C. annuum* var. cerasiformis cultivated form were obtained from Dr. E. Pochard (France) and from Lam-farm (Guntur) respectively. The parental species were selfed for two generations before employing them in the hybridization programme. Reciprocal crosses have been attempted by controlled pollinations between *C. annuum* var. cerasiformis and *Capsicum chacoense*. Viable F_1 hybrid was obtained when *C. annuum* was the seed parent in the cross. The data on morphological features of the both parents and F_1 hybrid was recorded.

For cytological analysis the young flower buds of the parents and the F_1 hybrid were

fixed in acetic acid and alcohol mixture (1:3) and transferred to 70% alcohol after 24 hours of fixation. Squashes were made in 2% acetocaramine to study meiosis. Pollen fertility was determined by staining the ripe and mature anthers with 2% acetocaramine. The well filled and stained pollen grains were considered as fertile while, half filled or empty and unstained or partly stained grains and of unequal sizes were treated as sterile.

3. Results

Crossabilty

The reciprocal crosses between *C. annuum* var. cerasiformis and *C. chacoense* and *C. chacoense* and *C. annuum* var. cerasiformis yielded fruits and seeds. Nevertheless, seedlings could be raised from the seeds of *C. annuum* var. cerasiformis and *C. chacoense* crosses only. While the seeds from the reciprocal cross of *C. chacoense* X *C. annuum* var. cerasiformis did not germinate (Table 1).

Table 1. Crossability relationships between *C. annuum* var. cerasiformis and *C. chacoense.*

S. No.	Particulars	*C. a* var. cerasiformis X *C. chacoense*	*C. chacoense* X *C. a* var. cerasiformis
1	No. of crosses made	200	250
2	No. of fruit set	28	16
3	Average No. of seeds/fruit	24	9
4	Seed germination (%)	30.8	-
5	No. of plants survived till flower formation	6	-
6	No. of plants survived till fruit set	6	-

Morphology of the parents and F_1 hybrid

The *C. annuum* var. cerasiformis and *C. chacoense* conform to the taxonomic description of IBPGR booklet [1]. The six F_1 plants survived till maturity were weak and resembled more *C. chacoense* parent in gross morphological features such as growth habit, leaf structure and position, size and shape of fruits etc. (Table 2 & Figure 1-3). The F_1 hybrid flowered late than either of the parents.

Cytology of the parents and their hybrid

In the two parents 12 bivalents per PMc regularly formed both at diakinesis and metaphase I and meiosis was normal and regular. However, the chromosome synapsis was relatively poor and meiosis was irregular in the F_1 hybrid. Association of four and three chromosomes or both up to a maximum of two per PMc and variable number of univalents and bivalents were observed in the F_1 hybrid (Figure 4-6). The nucleolus organizing chromosome was not involved in multivalent formation. Significant intra-plant differences were not observed in respect of chromosome pairing and therefore the data was pooled for studying the mean frequencies of chromosome configurations and chiasmata. The mean frequencies of chromosome associations and chiasmata in both parents and F_1 hybrid are listed in Table 3. All the 24 chromosomes were paired as bivalents in 30% of the PMcs in the hybrids and were mostly rods. Higher chromosome associations were mostly chains. The mean chiasma frequency both at diakinesis and metaphase I was low in the F_1 hybrid compared to corresponding parents (Table 3).

Chromosome disjunction at anaphase I was irregular, ranging from 11-13 to 10-10 + 4L in 48.6% of the PMcs. Besides, a single persistent bridge was observed in about 15% of the meiocytes. However, no such irregularities were met with in either of the parents. Laggards and polysporic condition were also recorded in some meiocytes at Telophase I and Telophase II respectively (Figure 7-8). Pollen stainabiltiy as a measure of fertility was low in the hybrid compared to the parents (Table 2). The mean seed set per fruit was 2.0 in F_1, 6.0 and 8.0 in *C. chacoense* and *C. annuum* respectively. The

selfed seed of the F_1 hybrid did not germinate.

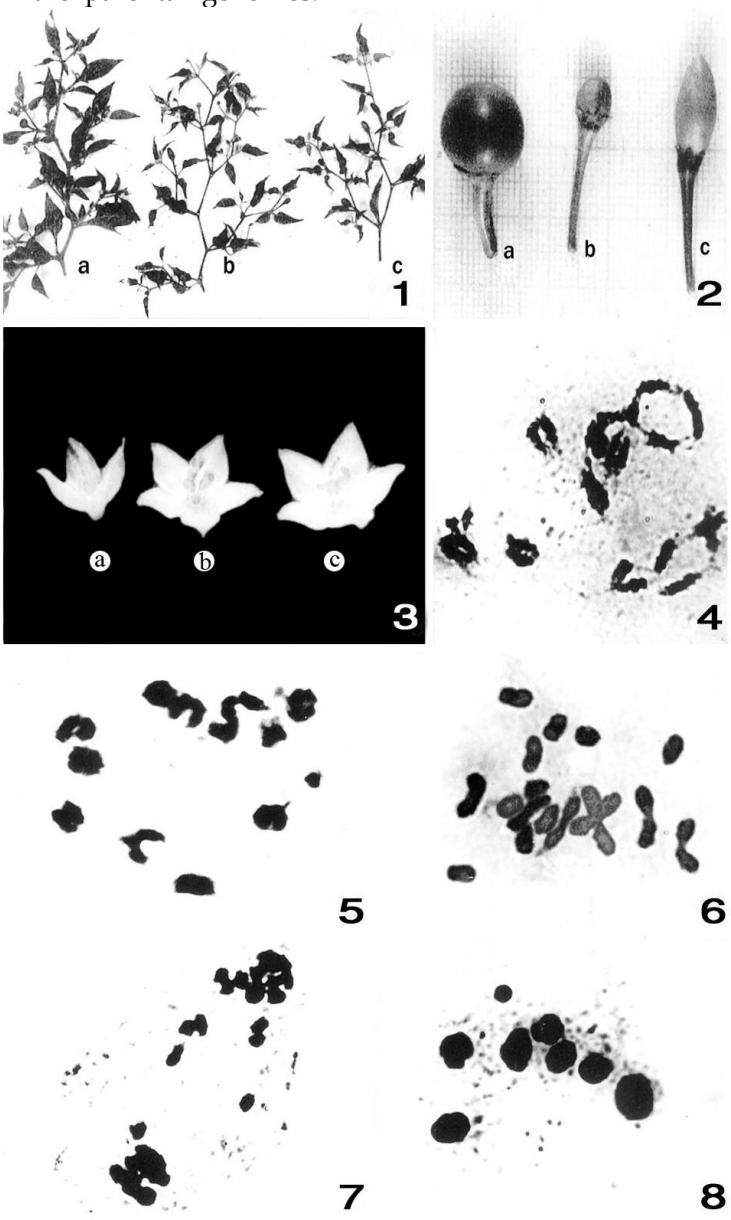
Table 2. Comparison of salient morphological characters of parents *C. annuum* var. cerasiformis, *C. chacoense* and F_1hybrid

S. No.	Characters	*C. annuum* var. cerasiformis	*C. chacoense*	F_1 hybrid
1	Height (cm)	58	54	55
2	Stem	Cylindrical, thick	Angular, narrow	Cylindrical, narrow
3	Leaf			
	Shape	Ovate	Ovate	Ovate
	Size (cm)	8- 10	6-7	5-7
	Colour	Green	Dark green	Dark green
	Texture	Glabrous	Rough	Rough
4	Flower			
	No. per node	1	1	1
	Days to flower	75- 90	120- 150	120- 150
5	Calyx			
	Shape	Saucer shaped	Cup shaped	Cup shaped
	Teeth	Present	Present	Present
6	Corolla			
	Size (cm)	4- 6	3- 4	3-5
	Colour	Milky white	White	White
	Throat spots	Absent	Absent	Absent
7	Stamens			
	Anther colour	Blue	Yellow	Blue
	Stainability (%)	86.0	80.0	27.0
8	Fruit			
	Position	Erect	Erect	Erect
	Shape	Globose	Sub-conical to elliptical	Globose to ovate
	Size (cm)	1-2	1-2	1-2
	Immature colour	Green	Deep- green	Deep- green
	No. per plant	70- 100	80- 120	10
	Seeds per fruit	35- 55	8- 16	0- 3
	Viability (%)	90	55	Inviable

4. Discussion

For assessing relationship between species from a cytogenetic stand point three parameters viz. a) the direction and ease with which two species can be crossed, b) the nature and fate of the hybrids and c) chromosome behaviour at meiosis are to be considered. The former two parameters indicate genetic incompatibility while, the third reflects the synaptic affinities between the parents. In the present study the degree of crossability varied in both combinations. Viable F_1 hybrid was obtained only when *C. annuum* var. cerasiformis was the seed parent. However a few seeds were obtained in the reciprocal cross but these did not germinate. However Lippert et al [4] Aniel Kumar et al [10] reported F_1interspecific hybrids

involving *C. chacoense* as the seed parent and *C. annuum* as the male parent but failed to obtain the reciprocal hybrids. The six F_1 hybrids were weak the mean chiasma frequency in the F_1 was less than that in either of the parents indicating reduced homologies between the parental genomes. The occurrence of 12 bivalents per PMc in certain proportion of the PMcs suggests that the parental genomes are partially homologues. Similar findings were reported by Lippert et al [4] Aniel Kumar et al [10] in their F_1hybrids.



Figs. 1-8: Cytomorphology of parents and interspecific hybrid of *C. annuum* var. cerasiformis X *C. chacoense* (Hunz.). Figs. 1-3: Morphology, Fig. 1: Twigs of a) *C. annuum* var. cerasiformis b) F_1hybrid c) *C. chacoense*, Fig. 2: Fruits of a) *C. annuum* var. cerasiformis b) F_1hybrid c) *C. chacoense*, Fig. 3: Flowers of a) *C. annuum* var. cerasiformis b) F_1hybrid c) *C. chacoense*, Figs. 4-8: Cytology of F_1hybrid (x1200), Fig. 4: Diakinesis -1 IV + 10 II, Fig. 5: Diakinesis -1 III + 10 II + 1 I, Fig. 6: Metaphase I- 7 II + 10 I, Fig. 7: Telophase I showing laggards, Fig. 8: Telophase II showing polysporic condition.

Table 3. Mean chromosome pairing behaviour both at diakinesis and metaphase I, chiasma frequency and pollen stainability in the parents and F_1hybrid.

Species/hybrid	No. of cells	Stage	Chromosome associations				Chiasma frequency	Pollen stainability (%)
			Is	IIs	IIIs	IVs		
C. annuum var. cerasiformis	200	D	-	12	-	-	22.52±0.18	90.70
	200	M	-	12	-	-	21.36±0.19	
C. chacoense	200	D	-	12	-	-	21.95±0.18	82.70
	200	M	-	12	-	-	18.04±0.27	
C. annuum var. cerasiformis X *C. chacoense* (F_1)	200	D	0.74±0.07	10.79±0.07	0.12±0.02	0.33±0.04	13.27±0.06	27.00
	200	M	2.12±0.16	10.19±0.08	0.11±0.02	0.18±0.03	11.10±0.06	

D: Diakinesis, M: Metaphase I

A single persistent bridge and laggards ranging from 0- 4 were present in some PMcs in the F_1 at anaphase I suggestive of inversion heterozygosity. However, Aniel Kumar at al [10] reported two persistent bridges at anaphase I besides fragments and laggards in the F_1hybrid *C. chacoense* and *C. annuum*. This may suggest that though *C. chacoense* parent used in the cross now and earlier by Aniel Kumar et al [10] is the same but the accessions of *C. annuum* were different. The genomic differences among the accessions of *C. annuum* coupled with structural differences discernable now and earlier probably were the reasons for not obtaining the reciprocal hybrids. The present study suggests that the two parental genomes differ from each other by two translocations, a single inversion and minor structural alterations and the two species are largely differentiated.

Pollen sterility is very high, through considerable bivalent formation was pronounced in the PMcs of F_1. The sterility observed in the F_1 may be attributed mostly to cryptic structural differences which effectively prevent free exchange of genes located within or close to such regions. It is also possible that genetic separation between wild and domesticated species is maintained by a strong tendency towards self-pollination in the domesticated as well as geographical and agriculture isolations and the heterozygosity for differences in chromosome structure and independent assortment of non- homologues satellite chromosomes. It is likely that during the course of evolutionary divergence, gene mutations and small chromosomal structural rearrangements might have occurred in the parental taxa resulting in such barriers. Isolating mechanisms viz. hybrid inviability in the reciprocal cross and hybrid sterility in the F_1hybrid are in operation.

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