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Assessment of genetic purity of F_1 interspecific hybrids of Chilli pepper (*Capsicum* L.)

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ABSTRACT

Two F_1 interspecific hybrids (H1&H2) of chilli pepper (Capsicum) were obtained by reciprocally between C. annuum var. X-235 and C. frutescens L. and were studied for identification and genetic purity via cytomorphological and SDS-PAGE seed protein profiles. Cytogenetic analysis of F_1 hybrids showed that the parental genomes differ from each other by 2 or 1 translocations, 1 inversion and some minor structural alterations. Meiotic irregularities, pollen and seed sterilities were higher in H1 than H2. It was observed that decreased seed protein profiles were encountered in F_1 hybrids

Keywords: *Capsicum*, chiasmata, laggards, PMC, stainability, SDS-PAGE

INTRODUCTION

The genus *Capsicum* commonly known as chilli pepper is a major spice crop and is of cosmopolitan in distribution and the genus comprises of five domesticated and twenty five wild species [1]. The cultivated taxa are widely used as condiment and vegetable. The cross compatibility relationships among some taxa of this genus have been reported by quite few workers [2-7] were mostly confined to the breeding behaviour of F_1 hybrids. Further the interspecific relationships and genome homologies are not well understood even today. However, 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. Hybrid identification in a crop species through molecular finger printing is an effective tool to increase the speed and quality of backcrossing, thus reducing the time to produce crop varities with desirable characteristics. The electrophoretic seed protein banding patterns were useful for identification of cultivars, intra and interspecific crosses in the genus *Capsicum* [13-15]. Therefore the present study is taken up to elucidate cytogenetic relationships between *C. annuum* var. X-235, *C. frutescens* and their two F_1 interspecific hybrids on the basis of meiotic chromosome pairing behaviour, fertility and seed protein profiles.

MATERIALS AND METHODS

Seeds of *C. annuum* var. X-235 and *C. frutescens* were obtained from Sutton seeds, Calcutta, India. The parental species were selfed for two generations before employing them in the hybridization programme. Reciprocal crosses were attempted by controlled pollinations between *C. annuum* var. X-235 and *Capsicum frutescens*. Viable F_1 hybrids were obtained by both directions (reciprocal). The data on morphological features of both parents and F_1 hybrids were recorded.

For cytological analysis the young flower buds of the parents and the F_1 hybrids were fixed in acetic acid and alcohol mixture (1:3) and transferred to 70% alcohol after 24 hours of fixation. Squashes were made with 2% acetocarmine to study meiosis. Pollen fertility was determined by staining the ripe and mature anthers with 2% acetocarmine. 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.

About 200mg seeds from each genotype and F_1 hybrids were homogenized with the help of mortar and pestle using 0.01M Tris-Hcl buffer (pH 7.5). The resulting homogenates were centrifuged at 15000rpm for 10 minutes then the supernatants were filtered with 541 Whatmann filter paper and the obtained residues were boiled at 90^0C for five minutes with 1:1 ratio of 1.0M Tris (pH 6.8), 10% SDS, 2% β-mercaptoethanol, 10% glycerol and 0.002% bromophenol blue.

Extracted soluble proteins were fractionated by one dimensional SDS-PAGE [16] and the data was analyzed by scoring the protein polypeptides on SDS-Polyacrylamide gels as presence (+) or absence (-).

RESULTS

Crossability

The reciprocal crosses between *C. annuum* var. X-235 and *C. frutescens* yielded fruits and seeds (Table-1).

Table 1. Crossability relationships between *C. annuum* var. X-235 and *C. frutescens*

S. No.	Particulars	*C. a.* var. X-235 X *C. frutescens*	*C. frutescens* X *C. a.* var. X-235.
1.	No. of crosses made	200	250
2.	Fruits attained maturity (%)	45	32
3.	Seed set (%)	16	14
4.	Seed germination (%)	43.2	28.6
5.	No. of plants survived till flower formation	4	3
6.	No. of plants survived till fruit set	6	3

Morphology of the parents and F_1 hybrids

The *C. annuum* var. X-235 and *C. frutescens* conform to the taxonomic description of IBPGR booklet [1]. The two F_1's were weak and resembled more to *C. annuum* var. X-235 parent in gross morphological features such as growth habit, leaf structure and position, size and shape of fruits etc. (Table-2 & Figure-1a).

Table 2. Salient morphological characters of *C. annuum* var. X-235, *C. frutescens* and their F_1hybrids

S. No.	Characters	*C. annuum var.* X-235	*C. frutescens*	F_1 hybrid (H1)	F_1 hybrid (H2)
1.	**Height (cm)**	68	46	56	54
3.	**Leaf**				
	Shape	Round	Quadrangular	Round	Round
	Size(cm)	3.5	5.8	3.2	3.3
	Colour	Dark green	Light green	Green	Green
4.	**Flower**				
	No. per node	1	2	1	1
5.	**Calyx**				
	Shape	Saucer shaped	Cup shaped	Cup shaped	Cup shaped
	Teeth	Present	Present	Present	Present
7.	**Stamens**				
	Anther colour	Yellow	Bluish	Yellowish	Yellowish
	Stainability (%)	94.0	89.0	43.7	42.7
8.	**Fruit**				
	Position	Pendent	Erect	Pendent	Pendent
	Shape	Elongate	Conical	Conical	Conical
	Size(cm)	5.5	2.3	5.3	5.3
	Immature colour	Deep green	Green	Deep green	Deep green
	No. per plant	250	215	220	209
	Seeds per fruit	66	15	43	35
	Viability(%)	85	65	78	70

Cytology of the parents and their hybrids

The two parents exhibited 12 bivalents per pollen mother cell (PMC) regularly formed both at diakinesis and metaphase I and the meiosis was normal and regular (Figure-1b). However, the synapsis was relatively poor and meiosis was irregular in the F_1 hybrids. 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 hybrids (Figure-1c). Significant intra plant differences were not observed with respect to chromosome pairing hence 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 hybrids are listed in Table 3. All the 24 chromosomes were paired as bivalents, 30% of the PMC's in the hybrids were mostly rods on the other hand higher chromosome associations were mostly in chains. The mean chiasma frequency both at diakinesis and metaphase I was low in the F_1 hybrids

compared to corresponding parents and the pollen stainabiltiy was low in the hybrids when compared to their parents.

A total of 21 protein polypeptide bands were scored on 10% SDS-polyacrylamide gel in the parents and F_1 hybrids. Out of which 13 protein bands were polymorphic while the remaining 8 protein bands were monomeric (Table 4 & Figure-1d). A polymorphic protein polypeptide with 40kDa molecular weight recorded in F_1 hybrids. However, band no. 8 with 46.4kDa, 12 with 31.2kDa, 18 with 19.6kda and 20 with 18.8kDa were found only in F_1 hybrids.

Table 3. Chromosome pairing behaviour at metaphase I, chiasma frequency and pollen stainability in the parents and their F_1 hybrids of chilli peppers

Species/hybrid	No. of cells	Stage	Chromosome associations				Chiasma frequency	Pollen stainability (%)
			Is	IIs	IIIs	IVs		
C. annuum var. X-235	200	M	-	12	-	-	19.60±0.07	94
C. frutescens	200	M	-	12	-	-	19.39±0.03	89
H1 : *C. annuum* var. X-235X *C. frutescens*(F_1)	200	M	2.28±0.02	9.26±0.18	0.40±0.36	0.50±0.40	15.36±0.24	43.7
H2 : *C. frutescens* X *C. annuum* var. X-235(F_1)	200	M	2.16±0.16	9.00±0.18	0.36±0.26	0.48±0.42	15.21±0.16	42.7

M: Metaphase-I

DISCUSSION

Assessment of hybrid purity is one of the most important quality control parameters in hybrid seed production. In the present study the degree of crossability varied in both combinations. Viable F_1 hybrids were obtained reciprocally when *C. annuum* var. X-235 and *C. frutescens* are seed parents. However Lippert *et al.* [4], Aniel Kumar *et al.* [10] reported F_1 interspecific hybrids involving *C. chacoense* as the seed parent and *C. annuum* as the male parent but failed to obtain the reciprocal hybrids. The two F_1 hybrids were weak in mean chiasma frequency in F_1 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 PMC's suggests that the parental genomes are partially homologues. Similar findings were reported in F_1 hybrids of chilli peprres (*Capsicum* L.) [4,9,17].

Table 4. Comparison of Rm values, molecular weights and band presence or absence in the parents *C. annuum* var. X-235, *C. frutescens* and their F_1 hybrids

Band No.	Rm value	MW (kD)	Band presence(+) / absence(-)			
			C. a var. X-235	*C. f*	*C. a.* var. X-235 X *C. f* (H1)	*C. f* X *C. a.* var. X-235 (H2)
1.	0.230	72.0	+	+	+	+
2.	0.269	63.2	+	-	-	-
4.	0.323	56.8	+	-	-	-
5.	0.346	54.4	-	+	-	-
7.	0.415	47.2	-	+	-	-
8.	0.423	46.4	-	-	+	+
9.	0.430	46.0	+	-	-	-
10.	0.500	40.0	-	+	+	+
11.	0.576	33.6	+	-	-	-
12.	0.607	31.2	-	-	+	+
13.	0.623	30.0	-	+	-	-
14.	0.676	26.4	+	-	-	-
15.	0.730	23.6	+	+	-	-
16.	0.769	22.0	+	+	-	-
17.	0.823	20.0	-	+	-	-
18.	0.830	19.6	-	-	+	+
19.	0.838	19.4	+	-	-	-
20.	0.869	18.8	-	-	+	+
21.	0.876	18.6	+	+	+	+
Total number of bands			**10**	**9**	**7**	**7**

A single persistent bridge and laggards ranging from 0-4 were present in some PMC's in the F_1 at anaphase I suggestive of inversion heterozygosity. However, Aniel Kumar *et al.* [10] reported two persistent bridges at anaphase I besides fragments and laggards in the F_1hybrid *C. chacoense* and *C. annuum*. Pollen sterility is very high, although considerable bivalent formation was pronounced in the PMC's of F_1. The sterility observed in the F_1's may be attributed mostly to cryptic structural differences which effectively prevent free exchange of genes located within or close to such regions. 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.

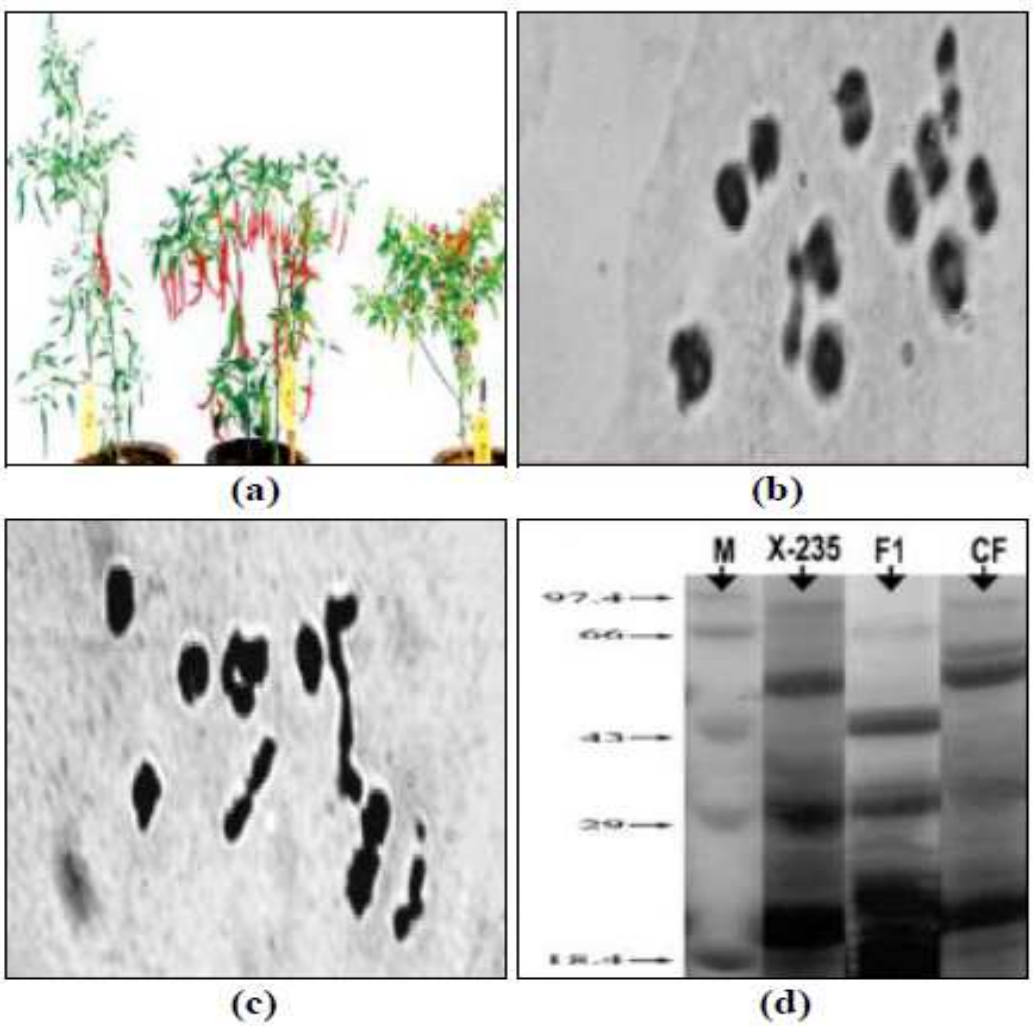

Figure 1. Cytomorphological and SDS-PAGE seed protein profiles of F_1interspecific hybrid: a) Morphology of parents and F_1hybrid; b) cytology of parents shows diakinesis with 12 bivalents chromosomes; c) Cytology of interspecific F_1hybrid shows metaphase-I with 2 IV+8II chromosomes; d) seed protein profiles of parents and F_1hybrid

SDS-PAGE seed protein profiles of parents i.e., (*Capsicum annuum* var. X-235 and *Capsicum frutescens*) and their F_1 interspecific hybrids sheds considerable light on species differentiation, crossability relationships and phenetic relationships in the genus *Capsicum*. This is probably being due to substantial differences in amino acid composition and genetic differences among the taxa. Ahmad and Slinkard [18] also encountered such differences in amino acid composition among the wild and cultivated taxa of the genus *Cicer*.

In the present study, chaisma frequency and seed protein banding patterns strongly support the hybridity.

CONCLUSION

The present study indicates that morphological, cytological and seed protein profiles of the two interspecific hybrids compared with their parents is able to clearly recognize the hybridity and its seed protein profile likely to be promising for identification and genetic testing of commercial chilli seeds and to be a more reliable tool for seed certification.

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