Cytogenetical Studies on F₁ Hybrid of *Solanum lucani* × *S. surattense* var. violet and white

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Spinous solanums, which are widely distributed throughout the tropical and sub-tropical belts of the world, have great economic importance as vegetable or medicinal plants due to their richness in alkaloid contents (Watt and Brink 1962, Dhar *et al.* 1974, Heble and Bhatt 1976). The earlier cytogenetical work on them by different authors have indicated that there exists a kind of chromosomal compatibility among most members of spinous *Solanum*. Yet sterility emerges despite normal pairing of chromosomes at metaphase I, and without any reduction in chiasma frequency in the reductionally dividing PMCs of the hybrids in respect of their parents (Kirti and Rao 1983, Singh and Roy 1986). This type of meiotic behaviour in hybrid plant is unique and different in nature. It shows major deviation from the normal pattern of behaviour of genomes. In this context some hybridization works have been tried between different members of *Solanum* from India and abroad (Marks 1965, Rao 1981, Kirti and Rao 1981, 1982, Omidiji 1983, Singh and Roy 1986).

In the present investigation genomic relationship was sought for between two members of *Solanum* having different morphology and ecogeographical distribution. Such works can correctly establish or negate the anticipated relationships between the parent forms.

Materials and methods

Seeds of *S. surattense* Burm. f. var. violet and var. white were collected from local populations and those of *S. lucani* F. Muell. obtained from Australia through the courtesy of Dr. David E. Symone of the Waite Agriculture Research Institute, Adelaide, Australia. Pure line plants were raised by single selection method. For successful hybridization work old buds ready to open next morning were emasculated in the evening and bagged to avoid unwanted pollination. Hand pollination was done on the following morning with desirable pollen under aseptic conditions. For facilitating pollen germination, stigmas were smeared with nutrient medium as suggested by Brewbeakers and Kwack (1963). Pollinated flowers were rebagged till fruit setting. Crossability index was recorded after Marks (1965).

For meiotic studies, F₁ buds were fixed in 3:1 ethanol-acetic acid solution for overnight and anthers squashed in 1.5% propionocarmine. Pollen fertility was scored from the percentage of the pollen that stained well with 1% acetocarmine. Seed setting/fruit was determined on the average seeds of ten randomly selected fruits of each plant.

Results

Data on crossability relationships are given in Table 1, which indicates that out of 102 crosses between *S. surattense* var. violet × *lucani*, 28 were successful and 8 of them attained maturity. In reverse direction out of 86 attempts, 22 had set fruits and only 7 of them attained maturity while the remaining fell down. Average number of seeds per berry was 17.50 in forward and 18.00 in reciprocal crosses. However, plant survival was 66.67% in

both ways. Crosses between *S. lucani* × *surattense* var. white showed 7.89% and 6.94% fruit setting out of 76 and 72 attempts in forward and reciprocal direction, respectively. Out of 5 mature fruits of *S. surattense* var. white × *lucani*, two were seedless. The corssability index was 0.23 and 0.21 between *S. surattense* var. violet × *lucani* while 0.10 and 0.16 between *S. surattense* var. white × *lucani* in forward and reciprocal direction, respectively, suggested lower degree of crossability relationships.

Meiosis in parents and hybrids

Meiosis in parental lines showed 12 normal bivalents (Figs. 2–4), mostly ring shaped that disjuncted normally. The mean chiasma frequency were 22.83 ± 0.83 in *S. lucani*, 23.42 ± 0.85 in *S. surattense* var. white and 23.52 ± 0.38 in *S. surattense* var. violet.

The interspecific hybrids showed irregular meiosis and chromosomal pairing at first metaphase was reduced (Table 2, Figs. 4–6). The earliest stages of meiosis were not discernible well as the chromosomes were badly entangled then. Some unpaired chromosomes could be made out at pachytene as less stained parts (Fig. 1). *S. lucani* × *surattense* var. violet meiocytes at diakinesis showed varying number of univalents ranging from zero to six

Table 1. Crossability relationships between *S. surattense* (var. violet and var. white) and *S. lucani*

	<i>S. surattense</i> × <i>lucani</i> var. violet	<i>S. lucani</i> × <i>surattense</i> var. violet	<i>S. surattense</i> × <i>lucani</i> var. white	<i>S. lucani</i> × <i>surattense</i> var. white
Number of cross attempted	102	86	72	76
Number of successful cross	28	22	26	20
Number of fruit attained maturity	8	7	5	6
Number of seedless fruit	—	—	2	—
Per cent successful fruit	7.84	8.13	6.94	7.98
Average number of seeds/fruit	17.50	18.00	18.00	14.50
Per cent seed germination	16.00	5.35	10.00	7.40
Per cent survival	66.67	66.67	50.00	100.00
Crossability Index*	0.23	0.21	0.10	0.16

* After marks 1965.

per cell (average 2.18/PMC) and mean number of chiasmata per cell 16.71 ± 1.52 . The average number of bivalents per PMC was 9.45; with most cells showing 3–5 rod and 5–7 ring shaped bivalents. A few PMCs showed 1–2 asymmetrical chain of tri- or hexavalents (Table 2, Figs. 6–8) and occasionally a Y-shaped trivalent and cross shaped quadrivalents (Fig. 6). Average chromosomal configurations in dividing cells of the reciprocal hybrid was 1.60 uni-, 10.13 bi-, and 0.38 chain-shaped trivalent with mean chiasma frequency per cell 17.13 ± 2.37 , which was significantly lower than those of the parents ($P < 0.05$).

Interspecific hybrid between *S. lucani* × *surattense* var. white showed 0–6 (Fig. 5) univalents, 7–10 bivalents, 0–2 tri-, and 0–1 tetravalent. The average configuration per PMC was 3.0 uni-, 9.71 bi-, 0.43 tri-, and 0.14 tetravalent with mean chiasma per PMC 16.57 ± 2.38 ($P < 0.05$). In reciprocal hybrid meiocyte showed 0–4 univalents (1.60), 8–11 bivalents (10.60) and 0–2 chain trivalent (0.40) configuration with mean chiasma per cell 17.80 ± 0.84 , which was significantly lower than those of the parents ($P < 0.05$).

The interspecific F_1 hybrids indicated that both species retain homology to some extent due to occurrence of greater frequency of bivalents in PMCs and atleast seven ring bivalents have full homology to each other and remaining pairs of rod bivalents have partial homo-

Table 2. Mean and range of chromosome pairing in metaphase I, pollen and plant fertility in parental lines of *S. surattense* and *S. lucani* and in their F_1 hybrids

Genotype	No. of PMC observed	Chromosome configurations						X meta (Mean $\pm$ S.D.)	% fertility		
		Uni- valents	Bivalents			Multivalents			Pollen	Seed	
			Ring	Rod	Total	III	IV	VI			
A. parental lines											
<i>S. surattense</i> var. violet	—	11.50 (11-12)	0.48 (0-1)	10.00 (12)	—	—	—	23.49 ± 0.85	98.50	97.20	
<i>S. surattense</i> var. white	—	11.64 (11-12)	0.35 (0-1)	12.00 (12)	—	—	—	23.52 ± 0.38	98.40	89.40	
<i>S. lucani</i>	—	10.83 (10-12)	1.17 (0-2)	12.00 (12)	—	—	—	22.83 ± 0.83	98.80	96.60	
B. Interspecific F ₁ hybrids											
<i>S. surattense</i> var. violet $\times$ <i>lucani</i>	1.49 (0-6)	6.25 (5-7)	3.88 (2-5)	10.13 (8-11)	0.38 (0-2)	—	—	17.13 ± 2.37	10.25	46.40	
<i>S. lucani</i> $\times$ <i>surattense</i> var. violet	2.18 (0-6)	5.27 (3-7)	4.18 (2-5)	9.45 (7-11)	0.20 (0-2)	0.45 (0-1)	0.16 (0-1)	16.71 ± 1.52	8.10	32.80	
<i>S. surattense</i> var. white $\times$ <i>lucani</i>	1.60 (0-4)	6.40 (4-7)	4.20 (3-5)	10.60 (8-11)	0.40 (0-2)	—	—	17.80 ± 0.84	6.66	02.00	
<i>S. lucani</i> $\times$ <i>surattense</i> var. white	3.00 (0-6)	5.57 (4-7)	4.14 (3-5)	9.71 (7-10)	0.43 (0-2)	0.14 (01)	—	16.57 ± 2.38	—	—

Figs. 1–12. Pollen mother cells of *S. surattense* var. violet, var. white, *S. lucani* and their F_1 hybrids. All $\times 1600$ except 2–4 $\times 2400$. 1, pachytene bivalents in F_1 hybrid *S. lucani* $\times$ *surattense* var. violet showing paired and unpaired chromosomes (Less staining region with arrow). 2, 3 and 4, diakinesis *S. surattense* var. violet, *S. surattense* var. white and *S. lucani*, respectively, showing normal 12

logy. Significant reduction in chiasma frequency per cell and multivalent association in hybrids indicate that there are some degree of structural heterozygosity between pairing partners. Occurrence of chains of three of six chromosome in hybrid suggested heterozygosity for reciprocal translocation.

Later stages of hybrid meiocytes showed aberrant divisions. There were 7.97% unequal separation, single and double (9.56%) bridges with (3.19%) or without acentric fragments, 0–3 laggards (14.74%) and 0–3 eliminated chromosomes (7.17%) in *S. lucani* $\times$ *surattense* var. violet and in reciprocal direction 7.46, 9.33, 2.61, 16.79 and 4.10% unequal separation, bridge, fragment, laggard and eliminated chromosomes, respectively, were seen.

S. lucani $\times$ *surattense* var. white showed 0–2 single or double bridge with or without acentric fragment (Fig. 9), 0–3 laggards and 0–3 eliminated chromosomes (Figs. 10–11) in meiocytes and their percentage abnormalities are given in Table 3. The frequencies of these

Table 3. Percentage of chromosomal anomalies at anaphase I in F_1 hybrid of *S. surattense* (var. violet and var. white) $\times$ *lucani*

Genotype	No. of PMCs observed	Normal distribution (%)	Bridges (%)	Fragment (%)	Laggards (%)	Eliminated chromosomes (%)	Unequal distribution (%)
<i>S. surattense</i> var. violet $\times$ <i>lucani</i>	268	62.38 (12–12)*	9.33 (0–2)	2.61 (0–2)	16.79 (0–3)	4.10 (0–3)	7.46 (10–14)
<i>S. lucani</i> $\times$ <i>surattense</i> var. violet	251	60.56 (12–12)	9.56 (0–2)	3.19 (0–1)	14.74 (0–3)	7.17 (0–3)	7.97 (10–14)
<i>S. surattense</i> var. white $\times$ <i>lucani</i>	236	57.63 (12–12)	10.59 (0–2)	2.97 (0–1)	18.22 (0–3)	5.51 (0–3)	8.05 (10–14)
<i>S. lucani</i> $\times$ <i>surattense</i> var. white	249	52.21 (12–12)	9.24 (0–2)	2.41 (0–1)	19.67 (0–3)	9.24 (0–3)	9.64 (8–16)

* Range.

reciprocal hybrids were 10.59, 2.97, 18.22 and 5.51% bridges, fragment, laggard and eliminated chromosomes, respectively (Table 3).

These abnormalities, further, indicated genomic differences between *S. lucani* and both the varieties of *S. surattense*. Occurrence of chromatid bridge and acentric fragment proved heterozygosity for a paracentric inversion in the genomes. Acentric fragments were small with less frequency. So it can be assumed that the inversions are small and in subterminal in position.

These abnormalities of anaphase I also appeared in anaphase II (Table 4). However, a good percentage of PMCs of the hybrids showed normal telophase in forward and reciprocal direction. The presence of laggards or eliminated chromosomes disturbed the equational segregation of chromosomes resulting into imbalanced telophase nuclei. Eliminated chromosomes during MI and MII witnessed their unequal distribution and some chromosomes

bivalents. 5, metaphase I, in *S. surattense* var. white $\times$ *lucani* showing 9 bivalents and 6 univalents. 6, diakinesis in *S. surattense* var. violet $\times$ *lucani* showing 9 bivalents and chain of 6 chromosomes. 7, diakinesis in *S. lucani* $\times$ *surattense* var. violet showing 7 bivalents, 2 trivalents (chain and Y-shaped) and 1 tetravalent (arrow). 8, metaphase I in *S. lucani* $\times$ *surattense* var. violet showing 9 bivalents, 1 trivalent and 3 univalents (in which one chromosome non oriented at equator, indicated by arrow). 9, anaphase I in *S. lucani* $\times$ *surattense* var. white showing chromatid bridge with acentric fragment (arrow) and breakage of heteromorphic bivalent. 10–11, anaphase I in *S. lucani* $\times$ *surattense* var. white showing eliminated chromosome(s). 12, formation of microunclei in *S. lucani* $\times$ *surattense* var. violet at anaphase I.

Table 4. Frequency of chromosomal anomalies at anaphase/telophase II and abnormal sporad in F_1 hybrids of *S. surattense* $\times$ *lucani*

Genotype	Anaphase/Telophase II					Type of sporad				
	No. of PMCs showing normal distribution	No. of bridge	No. of laggard	No. of eliminated chromosome	No. of PMCs observed	Triad	Tetrad	Pentad	Polyad	Total sporads observed
<i>S. surattense</i> $\times$ <i>lucani</i> var. violet	92 (73.02) 12-12*	4 (3.17) 0-3	18 (14.29) 0-4	12 (9.52) 0-4	126	4 (4.35)	77 (83.70)	9 (9.78)	2 (2.17)	92
<i>S. lucani</i> $\times$ <i>surattense</i> var. violet	79 (70.54) 12-12	3 (2.68) 0-2	18 (16.07) 0-6	12 (10.71) 0-6	112	9 (8.18)	93 (84.55)	7 (6.36)	1 (0.90)	110
<i>S. surattense</i> $\times$ <i>lucani</i> var. violet	96 (68.57) 12-12	4 (2.86) 0-2	22 (15.71) 0-4	18 (12.86) 0-4	140	8 (7.40)	88 (81.48)	10 (9.26)	2 (1.86)	108
<i>S. lucani</i> $\times$ <i>surattense</i> var. violet	90 (68.18) 12-12	5 (3.79) 0-2	20 (15.15) 0-2	17 (12.88) 0-4	132	9 (10.34)	69 (79.38)	6 (6.90)	3 (3.45)	87

* Range; Bracket number showing percent value.

accumulated without order to form micronuclei (Table 4, Fig. 12).

Pollen and plant fertility in parents and hybrids

Parental lines were fully fertile. Interspecific hybrids showed poor pollen and plant fertility, e.g. in *S. lucani* $\times$ *surattense* var. violet pollen fertility in forward and reciprocal directions was 8.40% and 10.25%, respectively, and in both ways such hybrids were partially fertile only. *S. lucani* $\times$ *surattense* var. white showed 6.58% fertile pollen in forward direction while the reverse hybrid was completely sterile.

Discussion

Data on crossability relationships have revealed low crossability index between *S. lucani* and *surattense* (both varieties) as very few seeds were produced by the hybrids between them even after a large number of cross pollinations. This incompatibility of genomes can be ascribed to the isolating mechanism, the wide seas and the spatial difference between India and Australia have aparted the populations since ages to lose homology to the extend now observable in the artificial hybrid. The capacity of hybridization between the two species is reduced because of self pollination. Stimulation of pollen germination after application of pollen germinating media on pistillate stigma indicates nutritional barrier for pollen germination. Likewise, production of parthenocarpic fruits in *S. surattense* var. white $\times$ *lucani* hybrid is due to stimulus of pollination alone as reported by Omidiji (1976) in African *Solanum* hybrids.

A convincing method for detecting cytogenetical and phylogenetic relationship between different taxa is study of pairing behaviour of chromosomes during meiosis of F_1 hybrids. This suggests the degree of chromosomal homology and structural differences between pairing partners. Chromosome pairing would induce chiasmatic chromosome associations also as chiasma investigated cytological phenomena in general. Reduction in chromosome pairing is influenced by chromosomal differences and the presence of pairing suppression gene (Sears 1976).

Solanum hybrids have been characterized in showing less reduction in chromosome pairing and chiasma frequency, and yet, sterile (Marks 1965, Singh and Roy 1986). Recently few workers have reported structural hybridity in *Solanum* (Veerabhadra Rao and Rao 1977, Choudhuri 1981, Kirti and Rao 1981). In the present study F_1 of *S. lucani* $\times$ *surattense* var. violet and with var. white have shown irregular pairing that supported some degree of chromosomal differences between both the parents. Further, significant reduction in chiasma frequency corroborated the above contention that there is incomplete homozygosity between the genomes of *S. lucani* and *S. surattense*. Of the 10 bivalents that usually appeared in the hybrid 5-7 were of ring type indicating complete homology between 7 chromosomes of *S. lucani* and 7 chromosomes of *S. surattense* and incomplete homology between the pairing partners of the remaining rod shaped bivalents. Sometimes rod shaped bivalents showed outstretched chiasmata or could even appear as univalents due to perhaps early terminalization. The other three pairs of chromosomes indicated heterozygosity for reciprocal translocation. Asymmetrical chain of 3 or 6 chromosomes suggested unequal translocated segments. These chromosomes reduced chiasmata or could even show total failure of pairing. Special configurations such as Y-shaped trivalents have suggested that for some interchanges there were interstitial break points. This type of higher chromosomal associations in *Solanum* hybrids were also reported by Kirti and Rao (1981) and Ramanna and Hermesen (1979). Moreover occurrence of chromatid bridge and acentric fragment of eliminated chromosomes at anaphase were the result of heterozygosity for a paracentric inversion. Lagging or unequal

segregation of chromosomes produced defective pollen, which influenced pollen fertility of hybrids. This has given rise to a good number of abnormal sporads, besides the normal tetrads. The principal cause of hybrid sterility is meiosis followed by unequal segregation of chromosomes. Incomplete pairing, recombination of partially differentiated chromosomes, paracentric inversion etc. lead to the formation of chromosomally and genetically unbalanced gametes with many duplications and deficiencies.

Complete sterility in hybrid such as *S. surattense* var. white $\times$ *lucani* indicates that besides chromosomal differences this can be brought about by interaction between incompatible nuclear and cytoplasmic factors (Buck 1960, Omidiji 1974, Kirti and Rao 1983).

Summary

Interspecific hybrids between *S. lucani* and *S. surattense* (violet and white varieties both) were successful in forward and reciprocal directions. The crossability index between them was low, which suggests low degree of crossability relationships between hybridizing parents. Meiotic studies on parental genomes showed normal bivalents, most of which were ring shaped and chiasma frequency was high.

Interspecific hybrids showed abnormal synapsis and unpaired chromosomes/chromosome parts with less staining ability. Metaphase I was characterized by the appearance of univalents, chains of three or six chromosomes, Y-shaped trivalent and rarely tetravalent association. Most PMCs contained 9 bivalents of which 5–7 were ring bivalents suggesting full homology between 7 chromosomes of *S. surattense* and 7 chromosomes of *S. lucani*, and the remaining 2 pairing partners exhibited partial homology by showing rod bivalents. Failure of synapsis in the rod bivalents could produce occasionally univalents only. Asymmetrical chain made of three or six chromosomes indicated interchange of unequal segments due to heterozygosity for reciprocal translocation, in which 3 chromosomes from each parent were involved. Formation of Y-shaped trivalents was due to joint of interstitial break points. Further, occurrence of chromatid bridge with or without acentric fragments, presence of univalents, lagging and eliminated chromosomes indicated heterozygosity between parental genomes which contributed to formation of sterile pollen. However, all hybrids showed partial fertility except *S. lucani* $\times$ *surattense* var. white which was completely sterile.

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