Cytomorphological Studies in a Spontaneous Triploid of *Capsicum annum* L.

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Polyploidy is one of the major factors in the evolution and breeding of plants. Stebbins (1950) is of the opinion that it is one of the best evolutionary processes in producing radically different and well adapted genotypes. Naturally occurring polyploidy is not usually known in *Capsicum*. A single case of naturally occurring triploid in the variety NP 51, highly sterile with varying number of trivalents and multivalents is known from the science reports of IARI New Delhi (1942). However, Pal and Ramanujam (1939) reported a triploid from colchicine treated seed lot, assuming the origin of it as a spontaneous one. Attempts were made by Nishiyama and Karasawa (1954) and Ohta (1962) to produce triploids by crossing diploid and tetraploid reciprocally. These resulted in securing triploids ($2n=36$) and hypotriploids ($2n=35$) with varying chromosome configurations at metaphase (four to eleven trivalents with a mode of nine trivalents and eight trivalents in the triploids and hypotriploids). Kormos (1954) reported a triploid paprika while studying the sterility in paparikas.

The authors while making the cytogenetic studies of different species of *Capsicum*, found a plant with bigger leaves and flowers in collection number 7 of *Capsicum annum*. When this plant was subjected to cytogenetical analysis, it was found to be a triploid with $2n=36$ chromosomes. The present paper deals with the cytology and morphology of this plant.

Observations

Comparative sizes of diploid and triploid plants, their leaves and flowers are shown Figs. 3 and 4. The increase in plant parts, delay in growth, prolonged flowering and deep green foliage compared to the diploid are obvious in the triploid plant. Table 1 reveals that the size of the leaves, flowers, pedicels, corolla, anther and ovary in the triploid are almost double the size of those in the diploid. However, no appreciable difference is seen in respect of branching and pedicel length.

Cytology:

The diploid and the triploid revealed $2n=24$ and $2n=36$ chromosomes respectively (Figs. 1, 2). For describing the karyotype the following categorisation of

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Table 1. Comparative morphological characters of diploid and triploid plants of *Capsicum annum*

Characters	Diploid	Triploid
Plant habit	8-10 months	17-18 months
Plant height	76 cm	76 cm
Branching	erect with few branches	erect with few branches
Leaves	acuminate, small	acuminate, big
Leaf thickness	1-1.5 mm	3-4 mm
Leaf size	small, 41×14 mm	big, 81×47 mm
Stem	succulent	woody, green
Stem size (diameter)	0.4 cm	0.7 cm
Fruit nature	erect	erect
Fruit colour	green-red	green-red-purple
Pungency	pungent	pungent
Flower colour	white	white
Anther colour	blue	blue
Anther size	4 mm	7 mm
Pollen fertility	95%	highly sterile
Pedicle length	17 mm	15 mm
Petal length	10 mm	12 mm
Sepal length	4 mm	6 mm
Petal breadth	3.5 mm	5 mm
Stamen length	4 mm	6 mm
Filament length	1.5 mm	2 mm
Style length	6 mm	10 mm
Ovary (diameter)	2.5 mm	5.0 mm

chromosomes has been used. The satellited ones, mostly the chromosomes with submedian centromere or constrictions having the satellites on short arm and infrequently the satellite being borne on the short arm with subterminal centromere, median chromosomes, and submedian chromosomes and subterminal chromosomes. These for convenience have been designated as S, V, J and I respectively.

The diploid has the karyotype formula $2n=S_4 + V_4 + J_{14} + I_2$ with four types of chromosomes. There are two pairs of satellited chromosomes with median centromeres, the satellites measuring 0.3 and 0.4 μ . The triploid has also four types of chromosomes as in the diploid and has the karyotype formula $2n=S_6 + V_6 + J_{21} + I_3$. Six satellited chromosomes which have submedian centromeres are observed, the satellites measuring 0.3 and 0.4 μ . Three chromosomes are seen with subterminal centromeres as compared to two in the diploid. This reveals the triploid to be an autotriploid with $2n=36$ chromosomes.

Meiosis in the diploid is normal showing 12 bivalents at diakinesis and metaphase I. Detailed meiotic study of the triploid plant reveals the following. It being an autotriploid, there are three homologous chromosomes which pair to give trivalents. However, one can expect to get in a triploid associations of chromosomes ranging from 12_{III} and 12_I to 12_{III} . It means that we must have cells which

do not show any trivalents. It is really not the case. In about 63 PMCs analysed trivalent formation is met with in every cell and the range is from 6–12 (Figs. 5 to 10, Table 2).

Figs. 1–2. 1, camera lucida drawing of somatic metaphase chromosomes of diploid ($2n=24$) *Capsicum annum* L. $\times 1250$. 2, camera lucida drawing of somatic metaphase chromosomes of triploid ($2n=36$) *Capsicum annum* L. $\times 1250$.

Table 2. Frequency of various chromosome configurations in the triploid

No. of PMC analysed	Configurations			
	I	II	III	IV
19	2	2	10	—
15	1	1	11	—
4	—	—	12	—
1	5	2	9	—
7	3	3	9	—
3	—	1	10	1
1	2	5	8	—
4	5	5	7	—
1	3	4	7	1
1	4	4	8	—
1	6	3	8	—
1	6	6	6	—
1	—	2	8	2
1	1	2	11	—
1	—	6	8	—
1	2	3	8	1
1	1	2	9	1
Total 63				

The extreme case of 12 trivalents is met with in a very few PMCs (Fig. 10). The other extreme case of 12_{II} and 12_I has not been encountered. In most of the

Figs. 3-16. 3, photographs to show comparative plant size in 2x and 3x forms. 4, photograph to show leaf and flower size in 3x and 2x plants. 5-10, diakinesis showing 6, 7, 8, 10, 11 and 12 trivalents respectively. 11-16, metaphase I showing 2, 3, 6, 8 and 11 univalents off the group.

Figs. 5-16 $\times 625$.

Figs. 17-30. 17, telophase I showing 2 univalents. 18, telophase I showing 3 univalents. 19, dyad showing 1 univalent. 20, metaphase II with 5 univalent chromosomes off the groups. 21-23, anaphase II with 2, 7 and 12 laggards respectively. 24, tripolar movement of chromosomes with 10 laggards. 25, 26, telophase II showing 5 and 12 laggards respectively. 27, tetrad with 3 micronuclei. 28, unequal sized nuclei in tetrad. 29, 30, polyspory with 5 and 6 spores respectively.

Figs. 17-30 $\times 625$.

PMCs trivalents are associated with univalents, bivalents and quadrivalents. The range of bivalents and univalents is from 1 to 6. 1 to 2 quadrivalents are seen in a few PMCs. The total of bivalents, trivalents and quadrivalents together in a PMC rarely exceed 13. It may be 13 to 14 in a few cases. The infrequent formation of quadrivalents is probably due to structural alterations in some chromosomes. The range of univalents, bivalents and trivalents is 1 to 11, 1 to 6 and 6 to 12 respectively (Figs. 11 to 16).

Table 3. Fruit and seed setting in open pollinated triploid

Sl no.	Fruit size L×B mm	Seed			
		Setting			Viability
		H	S	T*	
1.	11 × 9	1	1	2	None germinated
2.	9.5 × 6	1	—	1	None germinated
3.	—	2	—	2	Two germinated. One reached maturity and proved to be 2n=26
4.	—	3	2	5	None germinated
5.	14 × 8.5	3	1	4	One germinated and died at seedling stage
6.	10 × 5	—	6	6	All except one shrivelled seed. One apparently looking healthy
7.	8.5 × 7	3	1	4	—
8.	11 × 6	3	—	3	—
9.	6 × 5	2	—	2	—
10.	18 × 13	4	2	6	Many undeveloped small seeds, not considered for seed count
11.	9 × 6	1	1	2	Many black shrivelled seeds
12.	13 × 7	5	1	6	Black healthy seeds
13.	6 × 5.5	2	—	2	Many black seeds, not considered in the count
Total		30	17	47	

* H: healthy, S: sterile, T: total.

The univalents may or may not reach the pole at anaphase I. If left behind they may form micronuclei in the dyad (Figs. 17 to 19). The disturbance in the first meiotic division has adverse effects on the subsequent stages of the second division. Some chromosomes remain off the groups at metaphase II and they are probably univalents of the previous division (Fig. 20). Lagging of chromosomes ranging from 2–12 is observed, 6–8 laggards being more common (Figs. 21 to 26). The effects of irregular II division are found in the tetrad formation, wherein 5–6 microspores of varying sizes are formed (Figs. 27 to 30). Autotriploids are expected to be highly sterile because of the random segregation of the three chromosomes of each of the trivalent. Only those PMCs receiving a complete haploid set or two haploid sets of chromosomes are likely to survive, the remaining with unbalanced

numbers aborting. However, as in the case of *Datura*, we can expect primary $2n + 1$ types or trisomics because triploid will have three chromosomes in each set and which at reduction division will regularly give many gametes with extra chromosomes. Hence if large number of flowers are selfed in the triploid or open pollinated, the progeny is likely to give rise to all the 12 primary trisomic types that may be expected and also $2n + 1 + 1$ and $2n + 1 + 1 + 1$ types.

Fertility and fruit characters in the triploid

The triploid was observed for fruit setting and was found to be highly sterile. Selfing of 26 flowers did result in the formation of some fruits. Two healthy looking seeds were secured which however did not germinate. The plant was later left for open pollination to observe the fruit setting. It resulted in getting 13 fruits. All the fruits showed erect nature, green when young and turned to red at maturity. There is variation in fruit size which is clear from the Table 3. Smallest fruit was of 6.0×6.0 mm and the biggest 18×13 mm. Seed color was white and turned yellow subsequently. The number of healthy and shrivelled is in 2:1 proportion (Table 3). Healthy seeds were kept for germination. Only three germinated. Two of them died early in the seedling stage, whereas the third reached the stage of maturity. This was with $2n=26$ chromosomes. Shrivelled seeds when kept for sometime turned black. Such seeds were also found in the freshly harvested matured fruits. Seeds from the above plant ($2n=26$) have been collected and the cytology of the progeny is awaited.

An attempt was made to secure $2n + 1$ types or trisomics and others by crossing the triploid with different diploid collections of *Capsicum annuum*. Crosses were not successful. Efforts to secure hexaploid from the triploid plant by treating buds with colchicine did not yield positive results.

Discussion

Polyploidy has played an important role in the success of angiosperms as the dominant flora of the world (Stebbins 1950). It bears a degree of correlation with plant form prevalent in herbaceous perennial plants and least among woody forms realised from annual species as a result of polyploidy (Müntzing 1936). Diploids and the triploid forms are of interest in this connection. Diploids grow for a period of 8–10 months and dry up whereas the triploid continued to grow and flower even when the diploids dried. They survived for 17–18 months duration from the date of germination. Hence we can say that triploidy has tended towards perennial nature and this finding is in agreement with that of Müntzing (1936).

The occurrence of triploids among large number of diploids may be either due to crossing between tetraploid and diploid cultivar, or the product of fertilization of a haploid egg by two male gametes. They may also originate as an offspring from diploid parents by the union of unreduced or diploid gametes and the reduced gametes. Triploids were thought to be of rare occurrence in *Capsicum*. In the present case the autotriploid from the diploid population of collection number 7 of *Capsicum annuum* has probably originated from the union of an unreduced

(diploid) gamete and the reduced. This however, is a spontaneous one.

In the genus *Capsicum* there have been some reports of induced triploids (Pal and Ramanujam 1939, Nishiyama and Karasawa 1954 and Ohta 1962) as having low fertility producing high proportion of aborted pollen, small fruits when selfed or open pollinated. In the present triploid both selfing and open pollination has caused fruit setting. However, it is interesting to note that only such of the seeds obtained from open pollination did germinate and gave rise to aneuploids. In *Capsicum* again there are practically no reports except that of Pal and Ramanujam (1939) who recorded aneuploids for two different chromosomes. Aneuploid secured in the present case is probably a tetrasomic with $2n=26$ chromosomes.

A thorough survey of the literature on polyploidy in the genus *Capsicum* indicates that it is quite rare. As an exception to this Datta (1968 a, b) has concluded that polyploidy and structural alterations of chromosomes have played an important role in the evolution of its varieties. This view however differs from that of authors in that the extensive survey by them is quite contrary to the view expressed by Datta (1968 a, b) regarding polyploidy. However, some spontaneous polyploids in *Capsicum annuum* encountered will be dealt elsewhere. Chromosomal mutation has also played significant role in both natural and artificial evolution of plants and has been widely used by the breeder in plant improvement. Numerical chromosomal changes interfere with the meiotic orientation and has the general effect of reducing gametic efficiency. Raw polyploid is something of a 'hopeful monster' and is a starting point for selection. Autopolyploids obtained from highly homozygous individuals will be poor in vigour and weaker than original diploid type. However, in the present case the autotriploid has excelled the diploid in many respects and is vigorous but sterile. In cultivated forms with asexual propagation more gigas effects are frequently found at the triploid level according to Lewis (1967). Woody branches of the triploid plant were used to ascertain the maintenance of the plant, which was a failure. Polyploids may act as a selective sieve in favouring the unreduced gametes which are occasionally produced by their diploid progenitor and which may have been responsible for their origin.

Summary

Cytomorphology of the spontaneous triploid in *Capsicum annuum* has been studied. Comparative karyomorphology of diploid and triploid revealed marked differences in respect of growth habit, pollen fertility and floral characters as seen from the increased plant parts, delayed growth and prolonged flowering in the triploid. Colour of the fruit and its nature do not show much difference, but marked difference is seen in fruit size and in the ratio of healthy and sterile seeds.

Cytology of the triploid reveals that it is an autotriploid. Meiosis in triploid has various chromosome associations like univalents, bivalents and trivalents 1 to 11, 1 to 6 and 6 to 12 respectively. Quadrivalents were observed in a few cells. Fertility is poor in the triploid and an aneuploid with $2n=26$ chromosomes was obtained from the triploid progeny.

Literature cited

- Datta, P. C. 1968a. Cytogenetics of *Capsicum annuum* L. var. *grossum* Sendt. Proc. Indian Sci. Congr. Part III Abst. 283.
- 1968b. Karyology of Indian varieties of *Capsicum annuum* L. (Solanaceae). *Caryologia* **21**: 121–126.
- Kormos, J. 1954. Investigations on sterility in red pepper. *Ann. Biol. Tihany* **22**: 235–252.
- Lewis, H. 1967. The taxonomic significance of autopolyploidy. *Taxon* **16**: 267–271.
- Müntzing, A. 1936. The evolutionary significance of autopolyploidy. *Hereditas* **21**: 263–378.
- Nishiyama, I. and Karasawa, H. 1954. Somatoplastic sterility in reciprocal crosses between $2x \times 4x$ plants in *Capsicum*. *Jap. J. Breed.* **3**: 36–40.
- Ohta, Y. 1962. Karyotype analysis of *Capsicum* species. *Seiken Zihō Rep. Kihara Inst. Biol. Res.* **13**: 93–99.
- Pal, B. P. and Ramanujam, S. 1939. Induction of polyploidy in chilli (*Capsicum annuum* L.) by colchicine. *Nature* **143**: 245–246.
- Stebbins, G. L. Jr. 1950. *Variation and Evolution in Plants*. Oxford Book Company 1964 Edition pp. 298–369.
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