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Cytogenetical Studies on Some Auto and Asynaptic Triploids Obtained in an Intervarietal Cross Male Sterile × x206 of *Capsicum annuum* L.

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Cytogenetical studies on one autotriploid and three asynaptic triploids encountered in the hybrid progeny of Ms × x206, revealed trivalents 1-4 and 2-14 univalents in autotriploid and 23-34 in asynaptic triploids. The triploids were partially fertile with 30-47% pollen fertility in contrast to the highly sterile triploids reported earlier. The occurrence of four triploids from the progeny of cross involving the male sterile line and our previous studies indicate that the male sterile line is capable of generating viable unreduced gametes.

Key Words: Male sterile, *Capsicum annuum*. Autotriploid, Asynaptic triploid

Introduction

Polyploidy is one of the best evolutionary processes in producing radically different and well adapted genotypes. Reports of naturally occurring polyploids are fragmentary in *Capsicum*. Earlier, spontaneous triploids were recorded in the genus by Pal and Ramanujam (1939) and Chennaveeraiah and Habib (1973), while Nishiyama and Karasawa (1954) and Ohta (1962) produced triploids by crossing diploids and tetraploids reciprocally. The present paper deals with meiotic and other abnormalities exhibited by four triploid plants, of which one was a normal triploid and the other three sampled were asynaptic triploids in the intraspecific hybrid progeny of *Capsicum annuum*.

Material and Methods

The materials of the present study include four triploids isolated in the hybrid progeny of the intervarietal cross Male sterile (*C. annuum* variety G2) × x206. Usual aceto-carmine technique was followed for meiotic studies. Pollen stainability as an index of pollen fertility was estimated by using 4% iodine potassium iodide solution. Round densely filled pollen grains were considered as fertile, while poorly filled, shrivelled lightly stained as sterile.

Results

All the triploids were morphologically alike. Comparative morphometrics of diploid and triploid plants are set out in table 1. Tripolids in general were very tall, robust with great spread and high number of branches and exhibited an overall increase in size of the leaves (figure 1), stomata and epidermal cells. However, the number of stomata and epidermal cells per unit area were reduced. There was no appreciable difference in the size of the flowers. An alteration in colour of the anthers from bluish green (diploid) to violet (triploids) was noticed. Only few fruits

Table 1 *Comparative morphometrics of the autotriploid: asynaptic triploids and sibling hybrid*

Character	Auto triploid	Asynaptic triploids	Sibling hybrid
Plant height (cm)	131.00	128.00	54.00
Plant spread (cm)	170.00	169.00	122.00
No. of branches	148.00	142.00	56.00
Size of the leaf length/breadth	10/4.1	10.1/3.9	5.98/2.87
Size of the flower length/breadth	1.9/1.2	1.8/1.2	1.3/2.0
No. of fruits/plant	10.00	5.00	130.00
Fruit lenth (cm)	3.60	3.20	9.50
Fruit girth (cm)	3.10	3.10	3.48
No. of seeds/fruit	8.00	4.00	98.00
Pollen fertility	47.06	30.01	94.61
No. of stomata/ unit area	35.00	33.00	44.00
Size of stomata length/breadth	42.56/ 22.96	43.25/ 23.02	36.40/ 18.04

could be secured on open pollination. These were small, pendent, green when immature and red on maturity (figure 2). Seed content was very low (8 per fruit). Though all the triploids were morphologically alike yet further reduction in fruit number and seed content was noticed in the asynaptic triploids.

Detailed meiotic analysis of the four aberrant plants revealed the triploid chromosome number of 2n = 36 (figures 3, 7). In all the plants there was trivalent formation indicating the pairing of three homologues. However, in three asynaptic triploids, univalents were in high frequency.

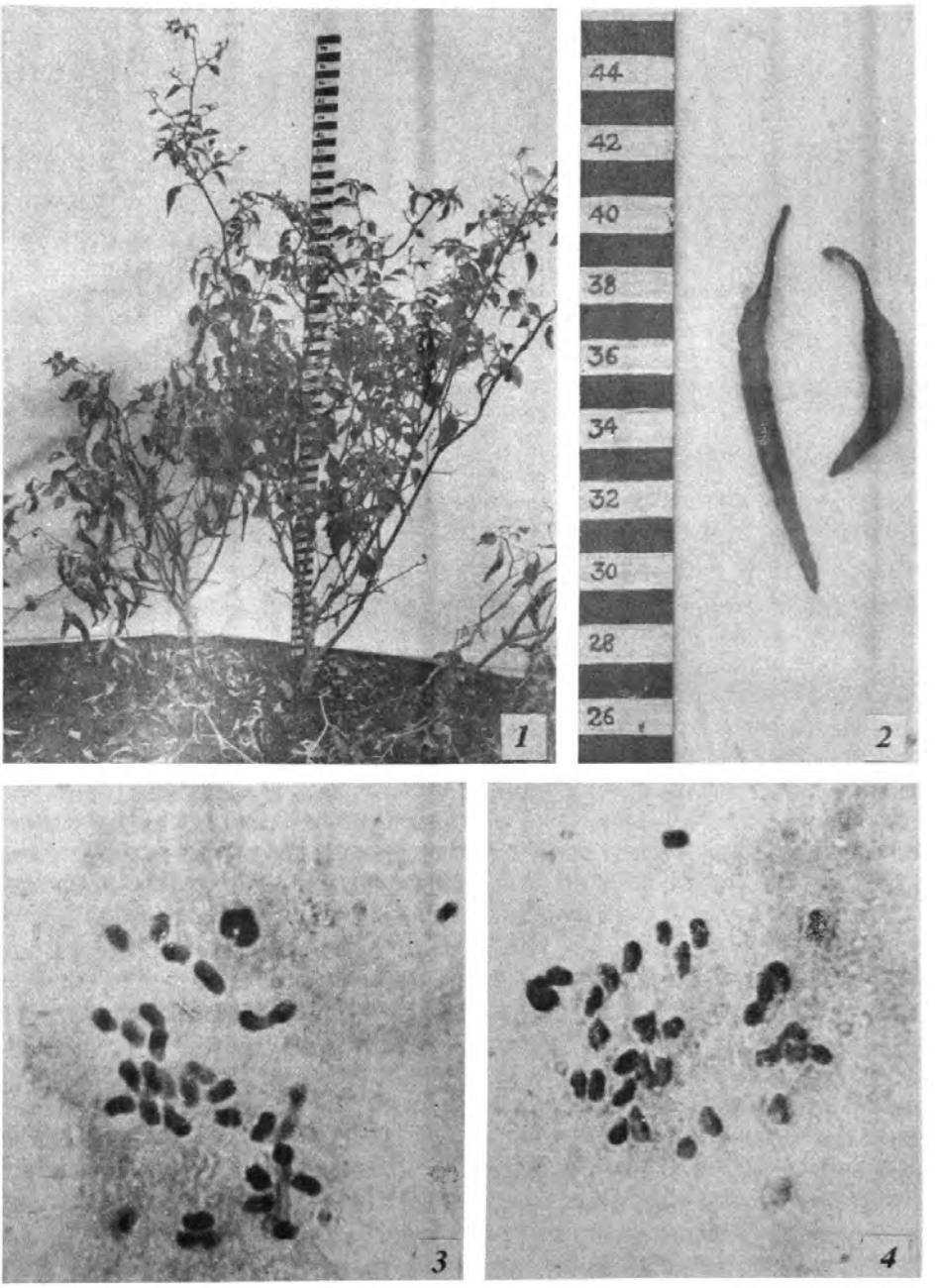
Cytology of Normal Triploid

Of the 6/PMCs analysed at diakinesis, 44 cells displayed trivalent formation ranging in number from 1-4 (figure 4). The extreme cases of the 12 III and 12 II + 12 I have not been met with. The type of chromosome configurations encountered and their frequencies are shown in table 2. In most of the PMCs, trivalents were associated with

Table 2 *Frequency of chromosome associations observed at diakinesis in autotriploid*

Type of association	Number of cells observed	Percentage
1 IV + 2 III + 10 II + 3 I	1	1.64
1 IV + 2 III + 9 II + 8 I	2	3.28
1 IV + 1 III + 11 II + 7 I	1	1.64
4 III + 10 II + 4 I	1	1.64
4 III + 9 II + 6 I	1	1.64
4 III + 8 II + 8 I	1	1.64
4 III + 8 II + 10 I	1	1.64
3 III + 11 II + 5 I	1	1.64
3 III + 10 II + 7 I	2	3.28
3 III + 8 II + 14 I	1	1.64
3 III + 2 II + 23 I	1	1.64
2 III + 14 II + 2 I	2	3.28
2 III + 13 II + 4 I	1	1.64
2 III + 12 II + 6 I	5	8.20
2 III + 11 II + 8 I	6	9.84
2 III + 10 II + 10 I	3	4.92
1 III + 15 II + 3 I	2	3.28
1 III + 14 II + 5 I	1	1.64
1 III + 13 II + 7 I	3	4.92
1 III + 12 II + 9 I	6	9.84
1 III + 11 II + 11 I	2	3.28
17 II + 2 I	2	3.28
16 II + 4 I	3	4.92
15 II + 6 I	5	8.20
14 II + 8 I	3	4.92
13 II + 10 I	2	3.28
12 II + 1 I	1	1.64
11 II + 14 I	1	1.64
Total	61	

bivalents and univalents and their range per cell was 0-4, 7-17 and 2-14 respectively. In an extremely low percentage of cells (4 out of 61) a quadrivalent was present. This infrequent formation of quadrivalents is probably due to structural alterations in some chromosomes. The total of quadrivalents, trivalents and bivalents in a PMC rarely exceeds 13. There was significant reduction in chiasma frequecy per cell (15.23) compared with that of the normal disomic (18.57). The reduced chiasma frequency in the present triploid can be attributed to the high frequency of univalents.



Figures 1-4 Plant, fruit and meiotic stages of the autotriploid (Ms × x206). **1**, Plants of diploid sibling hybrid and autotriploid respectively; **2**, Fruits of diploid hybrid and autotriploid; **3**, Diakinesis with 2n = 36 chromosomes; **4**, Metaphase I with three frying-pan trivalents, two bivalents and 23 univalents

Table 3 *Frequencies of nuclei with different kinds of chromosome associations at diakinesis in asynaptic triploids*

Types of associations	Number of cells	Percentage
3 III + 2 II + 23 I	4	8.00
1 III + 3 II + 27 I	2	4.00
1 III + 2 II + 29 I	2	4.00
1 III + 1 II + 31 I	10	20.00
1 III + − + 33 I	2	4.00
5 II + 26 I	2	4.00
4 II + 28 I	6	12.00
3 II + 30 I	6	12.00
2 II + 32 I	10	20.00
1 II + 34 I	6	12.00
Total	50	

Metaphase I was quite irregular with a number of chromosomes remaining non-oriented on the equatorial plate (figure 4). Fusion of cells was observed in a low percentage of cells. Anaphase I was highly irregular with unequal distribution and a number of lagging chromosomes (2-18) (table 4, figures 5, 6). A maximum of 18 chromosomes were found to be lagging which organize into different groups as a consequence of which telophase II also was highly abnormal with the formation of as many as 3-6 nuclei and 1-13 micronuclei. Pollen fertility was partial (47.06%). The sterility of the triploid can be ascribed to the formation of gametes with unbalanced chromosome numbers. Generally only those PMCs which receive a complete haploid set or two haploid sets of chromosomes are likely to survive. The pollen grains are highly polymorphic ranging in size from 4.5 to 9.5 μm.

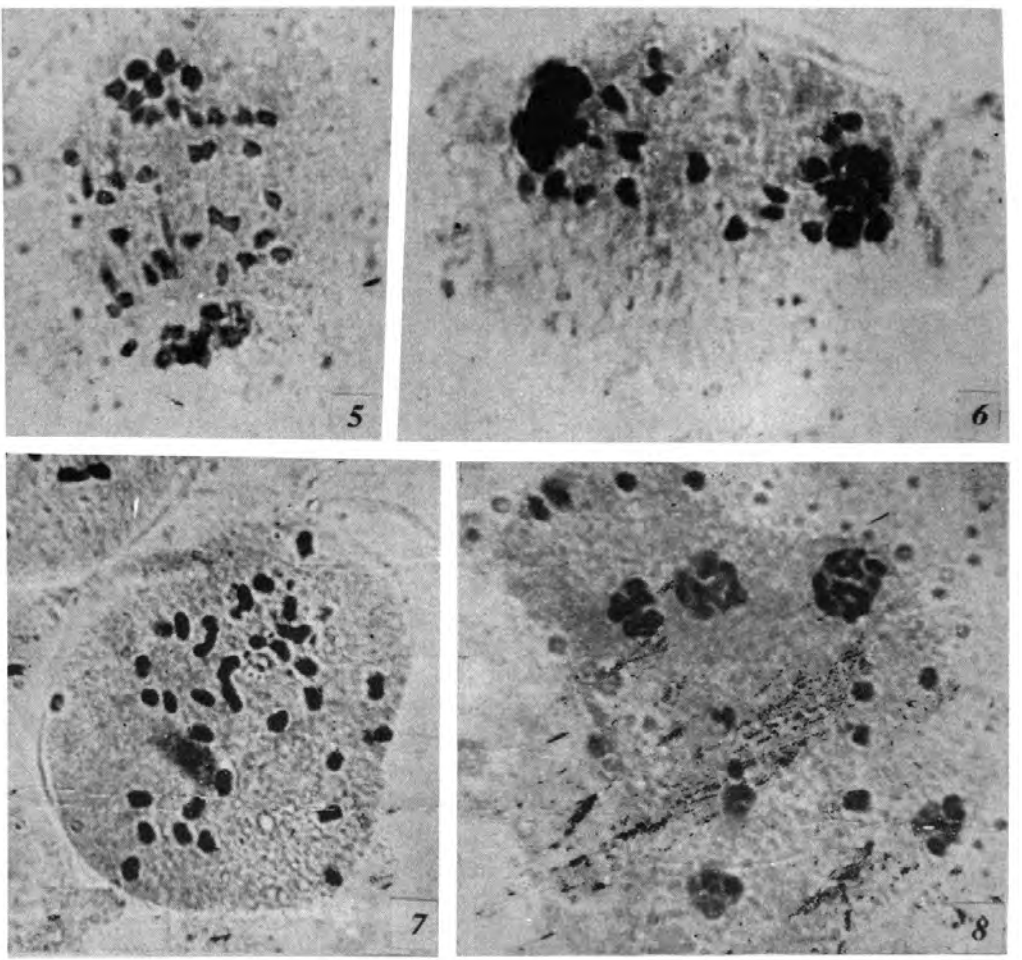
Cytology of Asynaptic Triploids

A critical observation of pachytene stage revealed complete lack of pairing for some chromosomes of the complement, hence, these plants were considered as partial asynaptics. About 50 PMCs were analysed at diakinesis for each of the three asynaptic triploids. In all, the univalent frequency was very high (23-34 per cell) (figure 7). Trivalents were observed in only 40% of the cells analysed with a range of 1-3 per cell (table 3). A feature of interest to note was that, all the bivalents were of rod type and ring bivalents were totally absent. There was significant reduction in chiasma frequency (3.2 per cell) when compared to that of the normal (15.23) which can be attributed to the predominance of univalents and rod biva-

Table 4 *Anaphase I disjunction in autotriploid and asynaptic triploids*

Chromosomal distribution	Number of cells (autotriploid)	Number of cells (asynaptic triploid)
21 : 14 + 1L	4	4
21 : 13 + 2L	1	−
21 : 10 + 5L	4	2
19 : 8 + 9L. 2 B	3	−
19 : 10 + 7L. 2 B	−	2
18 : 18	3	6
18 : 15 + 3L	2	2
18 : 14 + 4L	2	2
17 : 10 + 9L. 1 B	−	8
16 : 14 + 6L	6	2
16 : 9 + 11L	1	2
15 : 13 + 8L	6	7
15 : 12 + 9L	2	3
13 : 20 + 2L. 2 B	2	3
14 : 10 + 12L	2	4
14 : 9 + 13L	2	−
13 : 9 + 14L	3	5
10 : 9 + 17L	1	2
10 : 8 + 18L. 1 B	−	2
10 : 6 + 20L. 1 B	3	−
10 : 5 + 21L. 1 F	4	−
8 : 7 + 21L	5	3
8 : 6 + 22L. 2 F	4	1
Total	60	60

L, Laggards; B, Bridges; F, Fragments



Figures 5-8 Meiotic stages of the autotriploid and asynaptic triploids. **5**, Anaphase I showing laggards and bridges; **6**, Telophase I with 12 laggards; **7**, Diakinesis showing 2 III + 3 II + 24 I (asynaptic triploid); **8**, Telophase II exhibiting 6 nuclei and 10 micronuclei (asynaptic triploid)

lents. The overwhelming number of univalents that characterized most meiocytes of the asynaptic triploids were dispersed without any order during division phase. At metaphase I, 90.63% of cells were abnormal showing 1-12 non-oriented chromosomes. Fragments were also observed in 6.3% of cells analysed. A variable number of laggards (1-24).were observed at anaphases I and II (table 4). Ultimately they either moved to the poles or remain suspended in the cytoplasm to contribute to variable number of nuclei (3-6) and micronuclei (1-9) of different sizes (figure 8). In 38.46 percentage of cells 4 groups along with 2-9 micronuclei occurred most frequently. Besides laggards, fragments were also noticed in 13.3% of cells. Pollen fertility was still further reduced in these triploids (23.39%) when compared to the normal triploid (47.06%) which was the direct result of meiotic anomalies encountered.

Discussion

The occurrence of triploids in the inter-

varietal hybrid progeny was hitherto not recorded in the genus *Capsicum*. However, in the present investigation, four triploids were secured for the first time in the progeny of the cross Ms × x206. A feature of particular interest to note is that all the triploids were obtained in the hybrid progeny where the male sterile is the pistillate parent. Besides these four, two more triploids were screened by Venkateswara Rao (1985) in our laboratory in the progeny of varietal crosses with the same male sterile line. These observations in conjunction with those of Venkateswara Rao are suggestive of the fact that the Ms line employed is capable of producing viable unreduced gametes.

Triploids generally arise by crossing tetraploids with diploids or due to fertilization of a haploid egg by two male gametes. They may also originate as an offspring from diploid parents by the union of an unreduced diploid gamete with a normal gamete. In the present case, the unreduced gamete might have been contributed by the male sterile parent involved in the cross and its subsequent fusion with a normal male gamete could have led to the production of these triploids.

Chennaveeraiah and Habib (1973) observed double the increase in size of leaves, flowers, pedicels, corolla, anther and ovary, delay in growth, prolonged flowering, deep green foliage with no appreciable difference in plant height, number of branches and pedicel length in the spontaneous triploid reported by them. However, the present triploids exhibited significant increase in plant height, spread, number of branches, size of the leaf, flower, and stomata along with profuse and prolonged flowering and an appreciable reduction in the number of fruits per plant and seeds per fruit. In contrast to the highly sterile triploids reported earlier (Pal & Ramanujam 1939, Kormos 1954, Chennaveeraiah & Habit 1973)., the present triploids were partially fertile with 30-47% pollen fertility.

In the present cases all the environmental factors could be ruled out since the normal and asynaptic triploids were grown under uniform environmental conditions. Hence, it can be inferred that the hybrid gene combinations or the physiological disturbances caused by disturbed hybrid genetic make up led to the formation of asynaptic triploids. However, the genetics of these asynaptic polyploids is still under investigation.

Triploids will serve as a good source of aneuploids which are of immense value in genetic experiments. The triploid seed from 22 fruits was sown and in the progeny eight primary trisomics, two tetrasomics, one multiple trisomic with 28 chromosomes and one asynaptic trisomic were recovered the details of which will be furnished elsewhere after completing the analysis.

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