CHROMOSOME BEHAVIOUR IN TRIPLOID PETUNIA1

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ABSTRACT

A total of 12 triploid plants of garden Petunia (3n=21) obtained from $4n \times 2n$ crosses were studied cytologically in some detail. Number of trivalants varied from 1 to 7 per cell, with a mean of 4.06, while that of univalents from 0 to 6 with a mean of 2.75. Four III per cell was represented at maximum frequency, followed by those with 5 and 3, occasionally, the number of chromosome associations exceeded the basic number, which may be due to the presence of small, duplicated segments in otherwise non-homologous chromosomes. The co-orientation of trivalents at metaphase-I, and their bearing on first and second division segregations of chromosome was studied which reflects in the nature and frequency of an analoid gametes formed by the triploid. Pollen fertility of 52.9% indicates, that gametes carrying 1, 2, 3 or more additional chromosomes were probably viable and functional in Petunia, as demonstrated by the appearance of various an euploid plants in triploid progenies.

INTRODUCTION

Although there were several reports in literature on the occurrence of euploids and aneuploids in *Petunia*, data on the degree and types of multivalent formation in the former and the cytology and breeding behaviour of the latter are not adequate. The present studies are intended to fill some of the lacunae in our knowledge, by adding to the existing information, particularly on chromosome associations at various levels of aneuploidy. Detailed cytological aspects of induced autotriploid are presented in this paper.

MATERIALS AND METHODS

White flowered variety of P. axillaris (Lam) B. S. P., employed in the present study, was obtained from horticultural seed suppliers, Poona. Tetraploids, used in the reciprocal crosses with diploids were obtained from colchicine

treatments. Smear preparations were made in 1% acetocarmine.

RESULTS AND DISCUSSION

Triploids were obtained only from 4n×2n crosses, but not from the reciprocals. The triploid seedlings remained weak with pale green leaves, for about 40 days of growth. Gibberellic acid of 0.0001 concentration was repeatedly sprayed over the stem tips, in order to promote growth. Leaves on the mature plant, were larger than diploids in size, male parent in breadth. But, they did not possess the prominent epidermal hairs characteristic of tetraploid leaves, and resembled only the diploid parent in texture. First formed flowers were abnormal and were arrested from further development, when they were 0.3 to 0.6 cm in length.

Quantitative data on chromosome associations, co-orientation of multivalents at metaphase I, frequencies of tri-

^{1.} Accepted for publication on December 15, 1982.

valents, bivalents and univalents were gathered from 106 PMCs at diakinesis and metaphase I (Table-I). Number

CHROMOSOME ASSOCIATIONS IN TRIPLOID AT DIAKINESIS

TABLE I

PMC's		Type of association		
%	No.	Univalents	Bivalents	Trivaleats
4.20	3			7
8.50	6	1	1	6
18.50	13	2	2	5
1.42	I	-	3	5
30.00	21	3	3	4
4.28	3	1	4	4
24.28	17	4	4	3
2.85	2	2	5	3
2.85	2	5	5	2
1.42	I	3	6.	2
1.42	1	6	6	1

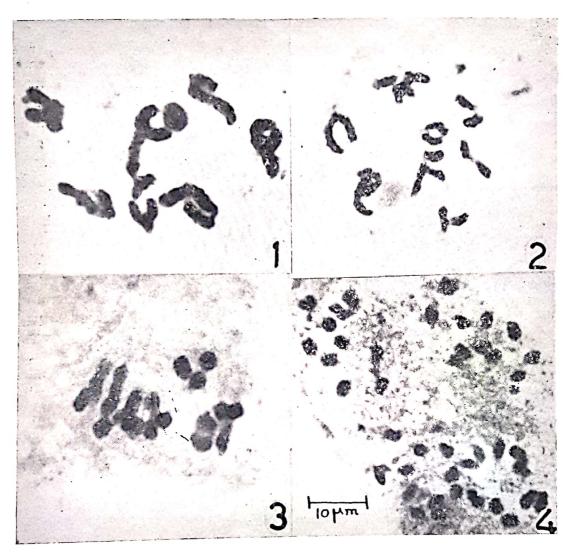
of trivalents varied from 1 to 7 per cell, with a mean of 4.06 while that of univalents from 0.6 with a mean of 2.75 (Figs. 1 and 3). Average number of bivalents was 3.00, 56.68% of which were of rod type.

Trivalent types 7, 8 and 9 were observed, and the chain type was most frequent. Out of a total of 456 tri valent associations analysed at diakinesis and metaphase I, 339 (74.37%) were of chain type, 85 (18.64%) were of type 8, and 32 (7.02%) were of type 9. Altogether, 61.46% of the chromosomes were involved in their formation. In the frying pan type, generally the chiasmata were terminalised, but in some, the third chromosome was joined to the bivalent ring interestitially. Among

the chain type trivalents observed at diakinesis some configurations with 'L' shape were found, where the connection between 2 of the chromosomes was very thin, while the third chromosome showed a prominent, interstitial chiasma. Average number of chiasmata per PMC, was 12.87 at diakinesis and 12.44 at metaphase I, with terminatisation coefficients of 0.621 and 0.635 respectively.

The normal pairing pattern in which the number of trivalents+bivalents, did not exceed the basic number of 7 was seen in 90.0% of the cells (Table-I), while in the remaining cells, the number of chromosome associations exceeded the basic number, indicating the possible existence of small duplicatd segments in otherwise non-homologous chromosomes (Fig.2). The co-orientation of trivalents at metaphase I, was either linear, convergent or indifferent. In of 163 configurations analysed at metaphase I, 53 (32, 51%) were linear, 104 (63.80%) were convergent and 6 (3.68%) were indifferent. Frequently, the univalents showed delayed migration to the equatorial plate, and they either remained undivided there, or included in one of the poles, or showed division. Out of a total of 181 cells analysed at anaphase I, 11.03% showed division of 1 to 4 univalents. Except for the rare instances of unilateral movement of both chromatids to one pole, generally, the two products of division showed 1 to 1 separation. The division of univalents was not synchronous with the movement of the normal chromosomes to the poles, but was somewhat delayed.

The number of chromosomes at late anphase I groupings ranged from 6 to 15. Segregation of \$10+11chromosome was seen in 43.09% of the cells. Each of the sister nuclei receive haploid set of chromosomes plus one or more in



Figures 1-4.: 1. Diakinesis in triploid (3n=21) Petunia showing 7 III. 2. Diakinesis in the same plant showing non-homologous pairing 1 III+8 II+2 I. 3. Metaphase I showing 4 III+3 II+3 I. 4. Anaphase II showing 10+11+10+11 segregation.

addition. Clearly, the additional chromosomes of one nucleus were not the same, as those of the other, and hence they are qualitatively different. In 14. 36% of the cells, segregation of 10+10 and a laggard was seen. The lagging univalents may either divide at a later stage, or may be ultimately lost in the cytoplasm. In the former cases, the chromatids may also be subjected to the same fate, so that a certain degree of chromosome loss occurs during

meiosis. Segregations of 12+9, 13+8, 14+7 and 15+6 were represented by 17.12%, 18.28%, 4.97% and 1-10% respectively. In spite of such nemerically unbalanced distributions, the percent of sterile pollen was only 47.1, which indicates that gametes carrying 1, 2, 3 or more additional chromosomes were probably viable and functional in *Petunia* as demonstrated by the appearance of various aneuploids plants in triploid progenies (Reddi and Padmaja, 1982).

Complete pairing of 7 sets of chromosomes, n the form of 7 trivalents was observed only in a small fraction of the cells. Variation in the behaviour of individual chromosomes in the triploid may be primarily governed by a similar variation in the length of chromosomes paired at pachytene, and consequently in the number of chiasmata formed, as observed in triploid tulpis (Darlington and Mather, 1932). The average number of chiasmata observed in triploids was 12.87 which is less than one and half times, the frequency of chiasmata obtained in comparable diploid line.

The number of trivalents, far less than the haploid number, has been observed in several plant genera also. In Lycopersicum esculentum with n=12, the number of trivalents varied from 3-7, with an average of 4.9 (cf. Dawson, 1962). Derman (1931) mentioned that chromosomes rarely formed trivalents in triploid hybrids of Petunia. Steere (1932) on the other hand reported that all chromosomes were regularly associated into a complete set of trivalents, in triploid Petunia. Matsud's (1935) report was similar to the present observation of frequent occurrence of 4 III per cell (Fig. 3).

Interchromosomal pairing, observed in a considerable fraction of cells analysed was also reported earlier in Petunia (Kostoff and Kendall, 1931, Matsuda, 1935), in Fragaria (Yarnell, 1929) and in triploid Sorghum (Erichsen and Ross, 1963). In the present study, it may represent cases of chromosome stickiness at times, but the presence of ture chiasmatic connections between the chromosomes appear to exist in others. It is therefore, likely that the apparently non-homologous chromosomes, carry homologous segments, attained through

segmental interchange in the remote ancestor. Sen (1969) and Maizonnier (1974), working with haploid Petunia, also reported non-homologous pairing and expressed the view of the possible occurrence of duplications in the genome. On the other hand, Houl (1964) attributed the multiple associations, observed in Goix aquatica (n=5) to possible association between nonhomologous chromatic segments. Gates (1942) described in grasses, illegitimate pairing in M₁, caused by partial homology, and Grell (1962, 64) as secondary type of pairing in Drosophila. In the present study, the factors responsible for this phenomenon, manifesting only in cytologically unbalanced systems like triploids and some ancuploids (unpublished) may Le due to the tendency of the unpaired chromosomes to pair with non-homologous chromosomes carrying homologous end segment.

The frequency of gametes with 0 to 7 extra chromosomes was variable and agrees in general with earlier reports on triploids of several other plant genera. In the present study, gametes with normal haploid numer were found only in 4.97% of the microspores analysed. Deviating types may be at disadvantage because of selection, as indicated by higher pollen sterility (47.10%). However, relatively high degree of 52.99% fertile pollen in the regular triploid indicate that probably, several of the unbalanced gametic types were viable and functional, to considerable extent, as evidenced by the appearance of various ancuploid plants in progenies (Padmaja, 1979).

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