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Chromosomal Associations in B Carrier and Noncarrier Diploid, Tetraploid and Octoploid *Impatiens balsamina* L.

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Polyploidy was induced with 0.2% colchicine in B carrier bicolor variety of *Impatiens balsamina* L., B chromosomes are being retained in induced tetraploids and octoploid. The number of B chromosomes remains unaffected despite the duplication of A chromosomes at tetraploid and octoploid level. Presence of 2 homologous B chromosomes in this species may be an adaptive device for their retention in population. Chiasma frequency decreases from 2x to 8x. A good percentage of chromosomes were involved in bivalent formation. Statistical analysis of plant to plant variation in diploids and tetraploids revealed insignificant difference for chiasma frequency and different meiotic configurations. In octoploid cell to cell variation showed significant difference for univalent frequency. Reasons for low frequency of multivalents have been discussed.

Key Words: Chromosomal associations, B chromosomes, *Impatiens balsamina*, B carrier diploid, Tetraploid and octoploid

Introduction

B chromosomes have been investigated in a large number of species, in various families of higher plants (Battaglia 1964 and Rees 1974) and most of the mare diploids. A few species of grasses are polyploids (Bosemark 1957), however, according to Jones (1975) about 31% of all B-containing angiosperm species are polyploids. These B chromosomes present a wide spectrum of variability in their shape, size and stainability in comparison to A chromosomes. Sharma and Aiyanger (1961) reported a correlation between accessory chromosomes and ecology in *Allium stracheyii* in this case a shift between diploidy and tetraploidy is involved. If diploid plants of *Allium stracheyii* with accessory chromosomes are moved from cold climate to hot climate they are soon changed to tetraploids without accessory chromosomes. Several varieties of *Allium stracheyii* having high chromosome number do not have B chromosomes (Sen 1974). This indicates that tetraploids have high tolerance range and do not require the presence of B chromosomes for adaptation. In *Impatiens balsamina* B chromosomes have been

reported from our laboratory (Raghuvanshi & Joshi 1968), these B chromosomes are euchromatic and indistinguishable during meiosis from other chromosomes of normal complement. To study different chromosomal associations in diploid, tetraploid and octoploid, polyploids were produced in carrier bicolor (mixture of purple and white) variety of *Impatiens balsamina*.

Materials and Methods

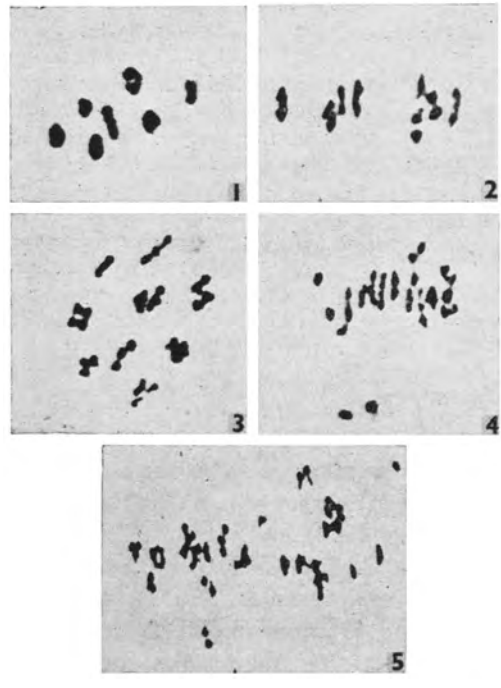
Polyploids were raised in B carrier bicolor (mixture of purple and white) variety by treating shoot tips of 60 young seedlings with 0.2% colchicine for 12 hr. Some of the newly-raised young tetraploid plants were further treated to raise octoploid. For meiotic analysis buds were fixed in 1 : 3 acetic alcohol fortified with iron between 8 to 10 AM. Acetocarmine squashes were made to study the presence of B chromosomes. Photomicrographs were taken from temporary slides and later on the slides were made permanent by ethanol butanol schedule. Data regarding chiasma frequency and different chromosomal associations was statistically analysed to test the significance level.

Results

Chromosome number of this bicolor variety is 2n=14. Twenty diploid plants were analysed. The 11 noncarrier plants have all the PMCs with 7 bivalents while the 9 carrier plants have two types of PMCs, viz. (i) PMCs with 7 bivalents (figure 1), and (ii) PMCs with eight bivalents (figure 2). This eighth bivalent was formed by B chromosomes.

Out of 60 treated diploid seedlings, we were successful in obtaining 18 tetraploids and one octoploid. Out of 18 tetraploids, fifteen were noncarrier having chromosome number 2n=4x=28 (figure 3) and three were carrier, these carrier plants have most of the PMCs with 28 chromosomes while some of the PMCs have 4x=28+2B chromosomes (figure 4). The single octoploid was a carrier having most of the PMCs with 56 chromosomes (figure 5) and some of the PMCs with 56+2B chromosomes.

In this variety diploids had fairly regular meiosis in both noncarrier (OB) and carrier (+2B) plants. However, both carrier and noncarrier tetraploids exhibited meiotic instability to a considerable extent. Although multivalent configuration was observed but multivalent frequency appears to be low. Univalents ranging from 0–6 have been observed in



Figures 1–5 *1*, Diploid (2n=14) $M_1 5(_{II})2_{II}$; *2*, Carrier diploid (2n=14+2B) M_1 $6(_{II})$ 2_{II}; *3*, Tetraploid (4x=28) M_i $1(_{iv})$ $6(_{II})$ 6_{II}; *4*, Carrier tetraploid (4x=28+2B) M_i $5(_{II})$ 8_{II} 4_I; and *5*, Octoploid (8x=56) M_1 $1(_{iv})$ $1(_{iv})$ $1(_{iii})$ $12(_{II})$ 7_{II} 7_I

tetraploids(table 1). Octoploid has greater meiotic instability than tetraploids however, no association higher than quadrivalent was observed.

Mean number, percentage and range of chromosomes involved in quadrivalent, trivalent, bivalent and univalent formation, at diploid, tetraploid and octoploid level was calculated. It was observed that a large number of chromosomes were involved in bivalent formation while a small number were involved in quadrivalent, trivalent and univalent formation (table 1).

Insignificant differences were observed for plant to plant variation in diploids and tetraploids for chiasma frequency and different meiotic features (table 2).

Table 1 *Number, percentage and range of chromosomes involved in different pairing configurations at different ploidy levels in Impatiens balsamina* L.

Ploidy level	Number, percentage and range of chromosome involved				Total number of chromosomes per pollen mother cell
	Quadrivalent	Trivalent	Bivalent	Univalent	
Diploid noncarrier	—	—	13.87 (99.21%) [5–7]	0.11 (0.79%) [0–4]	13.98
Diploid carrier	—	—	13.96 (98.66%) [5–8]	0.19 (1.34%) [0–4]	14.15
Tetraploid noncarrier	0.56 (2.00%) [0–2]	0.02 (0.07%) [0–1]	26.55 (94.96%) [9–14]	0.83 (2.97%) [0–6]	27.96
Tetraploid carrier	0.33 (1.17%) [0–2]	0.03 (0.11%) [0–1]	26.54 (94.35%) [8–14]	1.23 (4.37%) [0–6]	22.13
Octoploid carrier	0.54 (0.96%) [0–2]	0.14 (0.25%) [0–1]	51.90 (92.40%) [19 — 28]	3.59 (6.39%) [0–8]	56.17

Table 2 t-*values for plant to plant variation for chiasma frequency and different meiotic features in non-carrier and carrier diploids and tetraploids of* Impatiens balsamina

Different meiotic features	Diploids		Tetraploids	
	Noncarrier Degree of freedom 10	Carrier Degree of freedom 8	Noncarrier Degree of freedom 14	Carrier Degree of freedom 2
Chiasma frequency	0.4935	0.3829	0.0253	0.0593
Quadrivalent frequency	—	—	0.3443	0.5491
Trivalent frequency	—	—	1.4664	1.0000
Bivalent frequency	0.7241	0.4939	0.2439	0.8351
Univalent	1.3305	0.8862	1.1512	1.0493

Cell to cell variation for octoploid was statistically calculated. Univalent frequency showed significant difference, while chiasma, quadrivalent, trivalent and bivalent frequency was insignificant (table 3).

Discussion

B chromosomes of *Impatiens balsamina* are euchromatic and may be of recent origin. However, B chromosomes of about 60% of the species listed by Battaglia (1964) are described as heterochromatic, the extent of heterochromatization varies.

A gradual decrease in chiasma frequency was noted from 2x to 8x. Chiasma distribution pattern may affect the pairing configuration at two levels, firstly at the level of homologous sets of a complement, depending on whether each set of homologous shares an equal or unequal number of chiasmata (Jones 1967) and secondly within a homologous set, depending on the presence or absence of pairing restriction of homologous chromosomes (Timmis & Rees 1971) when there is no restriction of zygotene, pachytene association of four homologous, one would expect a strong tendency for multivalent association with increasing chiasma frequency. On the other hand, if chromosome association is restricted 'between 'pairs', an increase in chiasma frequency 'will be associated with increase in bivalent frequency as reported in rye (Timmis & Rees 1971).

The frequency of quadrivalent in general was considerably lower in comparison to bivalent frequency in 4x and 8x, Generally 4x with smaller chromosomes have less multivalent frequency and with larger chromosomes have higher frequencies. In the present case chromosome size is not very small, the low frequency of quadrivalent association observed in autotetraploids may suggest that certain other factors may restrict the association of four homologous chromosomes into quadrivalents. On the other hand, according to Rees (1961) chromosome-pairing may be under genetic control in which genes with both major and minor effects participate. Low frequency of multivalents was also observed in artificially induced higher polyploids of *Catharanthus roseus* (Raghuvanshi & Chauhan 1974).

Interestingly enough a good percentage of chromosomes were involved in the formation of bivalents. However, the percentage of bivalents per PMC decreased from 2x to 4x to 8x plants with a simultaneous increase in multivalent and univalent frequency. This may indicate that the disomic association is of predominant type and comparatively less number of chromosomes show tetrasomic association giving rise to quadrivalent formation. Two distinct causes have been recognised for a disomic pairing pattern, one is based on the evidence of genetic control of bivalents pairing (Harberd 1972 and Jauhar 1975) and the

Table 3 *t-test for cell to cell variation for chiasma frequency and different meiotic features in octoploid* Impatiens balsamina L.

Different meiotic features	Degree of freedom	*t*-valve
Chiasma frequency	21	0.2625*
Quadrivalent frequency	21	1.3679*
Trivalent frequency	21	1.0000*
Bivalent frequency	21	0.1087*
Univalent frequency	21	2.9616**

Note: Difference was either insignificant (*) or significant (**)

other is based on structural differences between the 'two pairs' of homologous chromosomes (Reinbergs et al. 1970).

The frequency of univalents was higher in 8x than 4x indicating greater meiotic instability in 8x. In 8x despite eight homologous chromosomes of every type no association higher than quadrivalent was observed, similar findings have also been reported in *Tropaeolum majus* (Raghuvanshi & Pathak 1974).

The most striking aspect of statistical analysis of chromosome-pairing in autotetraploids is the variability that occurs from plant to plant and from cell to cell, regarding the number of bivalent and quadrivalent formation. The variability of chromosome association may be due to variability in the number of chiasmata.

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