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Induced Autotetraploidy in *Zinnia elegans* Jacq[1]

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The cytogenetic studies in the family Compositae were initiated by senior author in the year 1968 when he was located at Gorakhpur. After having moved to Meerut in 1969, these studies were continued in this laboratory. Part of the results of these studies were earlier published in a series of papers. Two of these earlier reports (Gupta 1969, Gupta *et al.* 1972) included chromosome surveys of some ornamental and wild taxa. The other two reports contained results on meiotic abnormalities in *Carthamus oxyacantha* (Srivastava and Gupta 1970) and interchange heterozygosity in *Chrysanthemum parthenium* (Gupta and Agarwal 1972).

Subsequently some genera including *Zinnia* were selected for intensive study. Tetraploids are being produced in some species of *Zinnia* in this laboratory for two different objectives. Firstly the possibility or producing Zinnias of better ornamental value will be explored. Secondly these tetraploids will be used for the production of trisomics if possible.

Two species of *Zinnia*, namely *Zinnia linearis* and *Z. elegans* are commonly used as ornamentals in tropics during summers. Colchicine treatment in the genus *Zinnia* was given by Srivastava (1965) but no detailed observations on the results are available. Bose and Panigrahi (1969) gave a detailed account on the tetraploids produced in *Zinnia linearis*. The present communication contains the observations made on autotetraploids produced in *Z. elegans*.

Material and methods

The seeds of *Zinnia elegans* were obtained from Sutton and Sons, Calcutta. These were soaked in colchicine solutions of three different concentrations viz., 0.05%, 0.1% and 0.2%. In each case the treatment was given for six hours. The seedlings obtained from soaked seeds were again subjected to the treatment with the colchicine solutions of corresponding concentrations for a duration of eight hours with the help of cotton plugs. The cotton plugs were kept wet by regular application of colchicine solution by a dropper. The seedlings were then washed and transplanted in beds.

Preliminary screening of polyploids was done on the basis of stomata size. The suspected tetraploids were cytologically analysed with the help of mitosis (leaf tips) and meiosis. The material for mitosis was pretreated in a saturated solution of α-bromonaphthalene and fixed for 24 hours in absolute alcohol: acetic acid (3: 1)

[1] The experimental work was done by the junior author (RK).

before staining with 1% acetocarmine solution. The material for meiosis was fixed for 24 hours in absolute alcohol: chloroform: acetic acid (6: 3: 1) and stained in 1% acetocarmine. Photomicrographs were obtained from temporary preparations.

Results

Colchicine treatment

The data on the effect of different concentrations of colchicine solutions are presented in Table 1. It can be noticed that the survival in 0.05% and 0.1% concentrations was comparable with that in the control. In 0.2% solution, however, there was considerable lethality. Only two out of 27 surviving plants in this treatment were tetraploids.

Table 1. Effect of colchicine on survival in *Zinnia elegans*

Colchicine conc.	Seeds treated	Duration of seed treatment	Seedlings obtained	Duration of seedling treatment	Plants survived	Tetra-ploids
Control	100	6 hrs	60	8 hrs	59	—
0.05%	100	6 hrs	58	8 hrs	57	—
0.1%	100	6 hrs	56	8 hrs	50	—
0.2%	100	6 hrs	48	8 hrs	27	2

Cytology of diploids and tetraploids

Zinnia elegans is known to have a somatic chromosome number of $2n=24$ (Torres 1962, 1963). This number has been confirmed in the present investigation through mitotic as well as meiotic studies. At metaphase I, 12 bivalents were observed (Fig. 1).

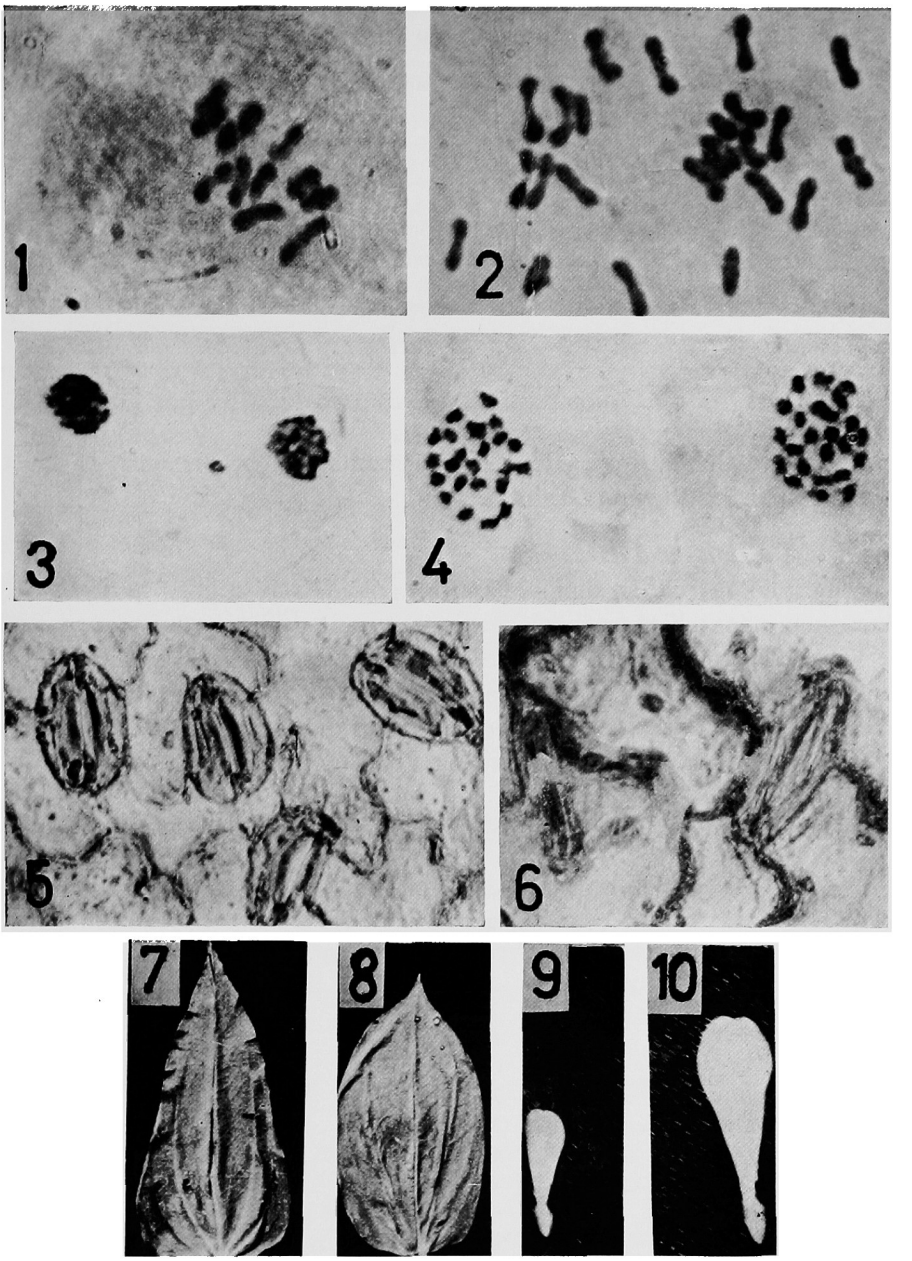
In one of the two tetraploid plants available, the disc florets were completely absent. Since the ray florets were female and did not bear any anthers, meiosis could not be studied in this plant. The tetraploid nature of this plant was confirmed through mitotic metaphase studied from leaf tip squashes. The meiosis, therefore, could be studied only in the other tetraploid plant.

Table 2. Chromosome associations at metaphase I in colchiploid *Zinnia elegans*

Cells examined	Chromosome associations*					Xta per cell	Xta/per chromosome
	I	II			IV		
		Ring	Rod	Total			
35	0–4	0–8	16–24	20–24	0–2	22–36	
	(0.9)	(1.8)	(20.7)	(22.5)	(0.5)	(26.0)	1.1

* Figures in parentheses are per cent values.

At metaphase I, usually 24 bivalents (Fig. 2) were quite regularly observed. The bivalents exhibited some degree of secondary association. The detailed analysis of chromosome associations at metaphase I is presented in Table 2. It can be noticed that quadrivalents are formed in a very low frequency (mean, 0.49 per cell). Univalents were also observed. At anaphase I, laggards were observed



Figs. 1–10. 1–4. Meiosis in diploid and tetraploid *Zinnia elegans*. 1, metaphase I in diploid showing 12 bivalents. 2, metaphase I in tetraploid showing 24 bivalents. 3, anaphase I showing a laggard. 4, anaphase I showing 24: 24 distribution. 5–10. Morphological characters in diploid and tetraploid *Zinnia elegans*. 5, 6, leaf epidermis showing stomata size in diploid and tetraploid respectively. 7, 8, leaves of diploid and tetraploid respectively showing leaf shape. 9, 10, ray florets of diploid and tetraploid respectively showing size difference.

Table 3. Frequency of laggards at anaphase I in colchiploid *Zinnia elegans*

Cells examined	PMCs with laggards			Mean laggards per PMC
	0	1	2	
100	64	2	34	0.7

Table 4. Distribution of chromosomes at anaphase I in colchiploid *Zinnia elegans*

Cells examined	PMCs with chromosome distribution			
	24: 24	23: 2: 23	23: 1: 23	23: 25
100	64	12	2	20

(Fig. 3) in a low frequency (Table 3). Distribution of chromosomes to the two poles at anaphase I is shown in Table 4.

Morphology of diploids and tetraploids

The morphology of the diploids and tetraploids was studied in some detail. The tetraploid plant which was male sterile was stunted in growth and was smaller in size when compared with diploids or with the other tetraploid plant available. It is possible that this plant carried some structural alterations in the chromosome complement. Since this plant was male sterile, it did not need any emasculation for hybridization work. This plant was therefore, utilized for crosses with diploids in order to get triploid seeds.

The other tetraploid plant was healthy and data on the morphological features

Table 5. Morphological characters in diploid and autotetraploid *Zinnia elegans*

Character	Diploid*	Tetraploid*
Plant height (in cm)	150.0–225.0	140.0–177.0
	157.5	158.5
Number of branches	6–42	7–19
	34.0	13.0
Length of first internode (in cm)	3.0–7.5	2.0–5.1
	4.2	3.6
Diameter of stem at base (in cm)	4.0–7.5	3.0–5.5
	6.1	4.3
Leaf size (in cm)	6.0–7.5×3.0–5.0	6.0–7.6×3.0–5.5
	7.2×4.4	7.1×4.9
Diameter of head (in cm)	7.0–13.0	6.0–14.0
	9.7	10.3
Stomata size (in μ)	37.0–52.0×15.0–23.0	75.0–90.0×30.0–45.0
	48.0×18.0	82.0×31.0
Pollen diameter (in μ)	13.0–15.0	15.0–18.0
	14.0	16.0
Pollen fertility (per cent)	97.0–99.0	95.0–98.0
	98.0	96.5

* Upper figure range, lower figure mean.

in this plant were collected. The data on the morphology of the diploid plants and the tetraploid plant are summarised in Table 5. The differences in size of stomata, leaf and ray florets between diploid and tetraploid are shown in Figs. 5–10. It should be noticed that the tetraploids have a tendency to show increase in size of cells and the organs.

Discussion

The present investigation indicated that the effective concentration of colchicine will adversely affect the survival of the plants, as a result of which polyploids are available only in classes having low survival values.

The cytological study showed a significant feature of preferential bivalent formation in a raw autotetraploid. One possible reason could be the size of the chromosomes. In diploids as well as tetraploid, there are mainly rod bivalents, and the ring bivalents are only rarely observed (Table 1). This suggests that a single chromosome perhaps can not take part in the formation of two chiasmata. This will obviously eliminate the formation of multivalents. The presence of secondary association between bivalents indicated that the homologous chromosomes were perhaps somatically associated at the premeiotic mitosis, but could not give rise to multivalents. Low frequency of multivalents can be compared with low frequency of laggards at anaphase I. However, unequal distribution of chromosomes (23: 25) could be observed in 20% of pollen mother cells studied. This should lead to aneuploidy, although such gametes (n=23; n=25) may not be functional. One tetraploid plant which was male sterile perhaps carried some structural abnormality as suggested by the nonviable seed obtained due to pollination by the diploid plant. Perhaps the embryo formation in these seeds could not proceed beyond a certain stage of development. Such an abnormality is also suggested by the stunted growth of the plant.

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