Cytogenetic investigations in colchicine induced tetraploid of *Cosmos sulphureus* (Asteraceae)

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ABSTRACT: Polyploidy or whole genome duplication is an important mechanism for acquiring new genes and creating genetic novelty in plants. In the present study, successful induction of autotetraploidy has been achieved through seedling treatment of colchicine in *Cosmos sulphureus*. Young seedlings were treated with different concentrations of aqueous colchicine (0.15, 0.2%, each for different durations) using the cotton-swab method. Polyploidy was confirmed during meiotic behavior of pollen mother cells. Induced tetraploid was cytogenetically distinguished from diploid by the occurrence of 48 chromosomes at diakinesis/metaphase-I with different combinations of univalent, bivalents, trivalents, and multivalent. In addition, different types of chromosomal anomalies such as laggards, micronuclei etc. were also observed at anaphase/telophase-I. Various cytological features like chromosomal associations (quadrivalents, bivalents and univalents) and chiasmata frequency were recorded at diakinesis/metaphase-I. It is expected that the induced colchiploid, if established, could be used in further cytological and breeding programs.

KEYWORDS: *Cosmos sulphureus*, colchiploid, quadrivalent, capitulum

Polyploidy has been a recurrent process during the evolution of flowering plant that has made a considerable impact on plant species diversity (Wendel and Doyle 2005). In contrast to allopolyploidy which is much prevalent in nature, autopolyploids has several potential advantages since the plants can resort to higher number of genes and higher maximum number of variants (Soltis and Soltis 2000). The application of colchicine in inducing polyploid and their subsequent use in agriculture is well known. Colchicine has been largely used to alter ploidy level frequencies in cell populations to obtain tetraploid plants, starting with diploid material (Ahirwar *et al.* 2015). In fact, polyploid plants usually have larger and thicker leaves, stems and flowers, fruits and seeds, and then, a greater yield.

Cosmos sulphureus, belonging to the family Asteraceae, is an important ornamental as well as medicinal plant. The species is native to Mexico in Central America but has been introduced elsewhere around the world and is now cultivated in India, Cambodia and Indonesia. Besides its antibacterial, antifungal, antiviral and anti-inflammatory properties, the flower heads of this plant are used as an eco-friendly dye in woolen industry (Jansen and Cardon 2005; Kale *et al.* 2005). The present study has been conducted with the primary objective of production of autotetraploids in *Cosmos sulphureus* by using colchicine, and their cyto-morphological characterization which can be exploited for future improvement of the plant.

MATERIALS AND METHODS

Colchicine treatment A total of 20 young potted seedlings were selected for colchicine treatment. In the present study, cotton swab method has been employed for colchicinization. In this method, cotton swabs immersed

either in 0.15 or 0.20% aqueous colchicine were placed on the emerging apical tip between two cotyledonary leaves. To avoid an increase in the concentration, colchicine was added drop by drop at regular intervals on the cotton swabs with the help of sterilized syringe. The treatment was done for two or three days of four, five and six hours each day.

Meiotic analysis For meiotic studies, young flower buds of appropriate size were collected from mature plants between 10 and 11 O'Clock AM and fixed immediately in a freshly prepared 1:3 mixture of glacial acetic acid and absolute alcohol for at least 24 h at room temperature. Pollen mother cells (PMCs) were analyzed from temporary slides for various stages of meiosis like diakinesis, metaphase I, anaphase I, telophase I and II. Chromosome associations, chiasma frequency, distribution of chromosomes and other features were recorded. The preparations were observed under the Olympus microscope and photomicrographs were taken from temporary slides with the help of electronic eyepiece.

RESULTS AND DISCUSSION

The results indicated that colchicine treatment was effective for producing tetraploid plants. Among treated plants, only four plants reached to maturity and one plant showed tetraploid nature. The tetraploid plant has been observed in 0.2% treated population (six hours for two days).

Morphology The tetraploid plant was robust from the initial stages of development and it was maintained till maturity. The plant showed reduction in height due to slow and stunted growth as well as thick stem and hairs over

Figs. Morphology and cytology of diploid and tetraploid *Cosmos sulphureus*.

A: Diploid and tetraploid capitulum, B: Diakinesis of normal diploid ($2n = 24$), C: Anaphase-I (12:12) of normal diploid, D-G: Diakinesis showing quadrivalents, bivalents and univalents of colchitetraploid ($2n = 48$), H: PMC showing Precocious segregation, I: Anaphase-I (24:24) in colchitetraploid, J: PMC showing Leggard at anaphase-I, K: PMC showing Micronuclei, L: PMC showing Bridge, M: Tripolar PMC, N: Pollens of diploid, O: Pollens of colchitetraploid.

them were more prominent as compared to control. The total plant height at maturity was 67.5cm in colchitetraploid as compared to 80cm in their corresponding diploids. The leaves of colchitetraploid plant were darker, thicker and bigger in size as compared to diploid. The general increase in leaf area was a distinguishing feature of colchitetraploids from its diploid. The flower heads or capitulum in colchitetraploids were bigger with the

diameter almost two times more as compared to control and also had deeper color (Fig. A). The longevity of the capitulum in colchitetraploid was also 5-8 days more than the diploids. Individual petals of the capitulum were thicker and fleshier. In general, there was an overall improvement in the important features which are of ornamental value (Table 1).

Table 1. Morphological features of diploid and colchicine-induced tetraploid of *Cosmos sulphureus*.

Quantitative traits	Diploid	Tetraploid
Plant height (cm)	80	67.5
Stem diameter (cm)	1.8	3
Length of internode of main stem (cm)	8.7	6.6
No. of branches/plant	13	17
No. of capitulum/plant	16	54
Capitulum size (cm)	3.5	6.3
No. of cypsela	16	54
Fertile cypsela	16	24
Sterile cypsela	-	30
Seeds/cypsela	12.35	1.43
Seeds/plant	173	46

Table 2. Chromosomal associations (quadrivalents and bivalents), chiasma frequency and pollen fertility in diploid and colchiploid of *Cosmos sulphureus*.

Ploidy level	Cells Analyzed	Quadrivalents		Bivalents		Chiasma frequency/cell	Pollen fertility (%)
		Mean	Range	Mean	Range		
Diploid (2x=24)	25	-	-	12	0-12	16.6	96.28
Tetraploid (4x=48)	25	1.6	0-4	20.72	0-24	29.28	46.33

Cytology

Meiotic behavior of diploid controls In diploid, clear 12 bivalents were observed at diakinesis/metaphase I in all the pollen mother cells analyzed, of which on an average 2.48 were rings and 9.08 were rod bivalents. Pollen fertility was 96.80% (Table 2).

Meiotic behavior of colchitetraploid Meiotic studies were done from anther of treated population and various chromosomal associations during diakinesis/metaphase-I were recorded (Table 3). The average number of quadrivalents in all colchitetraploids was 1.6 per cell. Similarly, the average number of bivalents in colchiploid was 20.72. At diakinesis/metaphase-I, the most common association was 2IV+20II (56.05%) and 1IV+22II (27.39%). Besides quadrivalents and bivalents, varying frequencies of univalents were frequently present. The average chiasma frequency per cell in colchiploid was 29.28 while in control it was only 16.6. In addition, few abnormal PMCs were also observed showing various types of chromo-

somal abnormalities such as precocious separation and stray bivalents (Fig. H). At anaphase/telophase I, although most of the PMCs showed normal separation, frequent amount of anomalies such as laggards (Fig. J), bridges (Fig. L) and micronuclei were also seen (Fig. K). Table 4 gives an account of major metaphasic and anaphasic abnormalities. Pollen fertility in colchiploid was 46.33 (Table 2).

Induction of polyploidy using colchicine and their cyto-morphological and genetic characterization has been a subject of immense interest among geneticists and breeders for a long time (Verma and Raina 1991a; Ramsey and Schemske 2002). It is estimated that 30 - 70% of the angiosperms are of polyploid origin (Masterson 1994; Hegarty and Hiscock 2007). Polyploidization is known to affect various aspects of morphology, anatomy, physiology and genetics. The most immediate effect is on morphological characters, particularly in the leaf and flower size (Verma and Raina 1991b; Verma *et al.* 2011).

As already mentioned earlier, some morphological

Table 3. Chromosome associations at metaphase I in colchipsoid of *Cosmos sulphureus*.

Chromosome Configuration	No. of cell	Frequency (%)
24II	7	4.46
23II + 2I	6	3.82
1IV + 21II + 2I	6	3.82
1IV + 22II	43	27.39
2IV + 20 II	88	56.05
4IV + 16II	7	4.46
Total	157	

Table 4. The percentage of meiotic abnormalities at metaphase-I and anaphase-I in diploid and colchipsoid of *Cosmos sulphureus*.

Ploidy level	Frequency of abnormal PMCs at metaphase-I			Frequency of abnormal PMCs at anaphase-I			
	Cells Analyzed	Precocious segregation	Stray bivalents	Cells Analyzed	Laggards	Bridges	Micronuclei
Diploid (2x=24)	50	-	-	50	-	-	-
Tetraploid (4x=48)	50	22.0	14.0	75	14.67	9.33	24.0

features showed an increase viz. stem diameter, internode length, number of branches, number of capitulum, capitulum size, cypsela size etc. while few characters showed a decrease thereby exhibiting an unambiguous response of *C. sulphureus* to colchicine treatment. Such observations were previously demonstrated in a large number of plant species (Verma and Raina 1991a; Verma *et al.* 1993; Joshi and Verma 2004; Ahirwar *et al.* 2015).

In autotetraploidy, each chromosome represents itself four times, due to which mostly the quadrivalent associations would be expected. In the present investigations, meiotic analysis of synthesized colchipsoid of *C. sulphureus* showed low quadrivalent frequency and high bivalent average per cell. Several reasons have been put forward to explain the low/high quadrivalent frequency in tetraploids. Sybenga (1975) suggested that the frequency of bivalents and quadrivalents in tetraploids is dependent on the points of pairing initiation. When there is a single point of pairing initiation, bivalents are formed; when there are two points of initiation, both quadrivalents and bivalents are formed in equal frequencies; when the initiation points are more than two, quadrivalents are formed in high frequency as compared to bivalents.

In the present investigation, several abnormalities like

precocious segregation, stray bivalents, laggards, micronuclei and bridges were observed at metaphase and anaphase / telophase I.

According to Darlington (1963), tetraploids always show irregularities and these irregularities are considered to be due to the formation of multivalent associations. It was the common feature in colchitetraploids. The cytological causes, resulting in unviable gametes, include non-disjunction of multivalents, lagging chromosomes, univalents, non-viability and other abnormalities. Gohil and Koul (1981) studied the autotetraploid *Allium tuberosum* and found that anaphase segregation is slightly irregular. They suggested that multivalents were main cause of segregation irregularity at anaphase. In addition, another cause of irregular division of chromosomes at anaphase-I may be due to abnormal behavior of univalents (Verma and Khah 2016).

Thus, the present investigation has shown that colchipsoid produced in *C. sulphureus* has many morphological features which would establish them as better ornamentals.

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