Effect of colchicine on meiosis of *Chrysanthemum carinatum* Schousb.  
(Asteraceae)

Rakesh Chandra Verma, * Rakesh Purbiya and Rekha Solanki  
School of Studies in Botany, Vikram University, Ujjain-456010, India  
*Author for correspondence (rakeshpurbiya@rediffmail.com) 
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**ABSTRACT:** The effect of colchicine was studied from the cytological point of view to induce mutation and develop polyploidy. The present study was conducted to determine the effect of colchicine on cell division and some morphological traits. Young seedling of *Chrysanthemum carinatum* Schousb. (2n=18) was treated with 0.2% colchicine. During meiotic analysis, reciprocal translocation heterozygote has been isolated among population of the colchicine treated *Chrysanthemum carinatum* Schousb. The translocation heterozygote showed characteristics feature such as multivalent formation (mainly quadrivalents and hexavalent), univalent formation, bivalent associations, chromatin stickiness, chromosomal laggards, fragments and multipolar PMCs. At anaphase I, some pollen mother cells (PMCs) showed abnormal 8:10 chromosomal separation in addition to normal 9:9 separation. The treated populations showed reduction in plant survivability, plant height and delayed flowering. Induction of permanent chromosomal structural changes may also sometime bring out favorable morphological variation. It is expected that the mutant, when established, could be used in further cytological and breeding programs.

**KEYWORDS:** Colchicine, *Chrysanthemum carinatum*, Meiosis, Translocation, APG-IV

The Asteraceae (Compositae) is the largest family of the flowering plants. The most obvious and characteristic feature of Asteraceae is that the florets (small flowers) are grouped into compact heads (capitula or pseudanthia) that often superficially resemble individual flowers (e.g. sunflower or daisy). According to Bentham and Hooker (1883) containing about 1100 genera and probably 30,000 species, the family is cosmopolitan distributed worldwide except for Antarctica. Today a revised and updated modern classification for the families of flowering plants is known as Angiosperm Phylogeny Group system (APG IV) (2016) the family Asteraceae of the angiosperm containing about 434 genera and probably 3,780 species.

The genus *Chrysanthemum* is believed to be native of China, distributed worldwide where it was being cultivated more than 2,000 years ago. It is characterized by the annual or perennial herb, leaves alternate, dissected, long peduncles, flowers arranged in corymbs clusters, ray floret pistillate; disc florets fertile and perfect, involucre scales are imbricated. Amongst the ornamental plants of Asteraceae, the *Chrysanthemum* is one of the widely cultivated and popular composit and ranks third among commercial flowers in the world (Prasad and Kumar 2000).

Interchanges are those structural changes in chromosome, where terminal segments of non-homologous chromosome have exchanged positions. These changes are also called reciprocal translocation. Interchanges in plants are usually associated with semisterility of gametes; such semisterility is observed is only in those plants which have translocation in only one set of chromosomes, the other set being normal, these plants are called interchange heterozygotes (Burnham 1956). On the contrary, there can be plants which have same interchange in both sets of chromosomes. These would, therefore, be called interchange homozygotes.

Mutation is an important tool for breeding new cultivars, and many cultivars have been produced using induced mutations. Gamma rays and colchicine widely used for mutation induction in *Chrysanthemum*. Radiation treatment induces plant damage, by producing chromosomal aberration and other abnormalities, in reduction in chromosome number and other abnormalities as reported in *Chrysanthemum* (Dowrick and El-Bayouni 1966). Furthermore, reduction in chromosome number and induced reciprocal translocation reported in *Pisum sativum* (Verma and Goyal 2012).

**MATERIALS AND METHODS**

Dry and healthy seeds of *Chrysanthemum carinatum* have been used for cytological analysis collected from School of Studies in Botany, Vikram University, Ujjain, India. Material for chromosomal studies were collected during the months of October (2016) in winter season. Seeds were sown in pots, after 5-7 days seedlings came out. When seedling was 4-6 day old they were treated with 0.2 % colchicine solution for 5-6 hrs. per day at least for two to three days; taking care that only the seedling showing cotyledonary leaves and not the third leaf. The method by using for colchicine treatment was "cotton swab method" after treatment short tips was thoroughly washed with water. For meiotic study, young flower buds of appropriate size were collected in the between 9 to 11
AM and fixed in the freshly prepared Carnoy's fluid (Absolute alcohol and acetic acid in 3:1 ratio). Anther were separated, teased in a drop of 2% iron acetocarmine on a clean slide and squashed under a cover glass. Pollen mother cells (PMCs) were analyzed for suitable stages of meiosis. However, in colchicine treated population various cytological abnormalities were recorded in different stages of meiosis.

RESULTS AND DISCUSSION

_Chrysanthemum carinatum_ Schousb. exhibits a somatic chromosome 2n=18 with normal size chromosome. During meiosis, regular nine (n=9) bivalents were observed at diakinesis metaphase-I (Fig. 1A). At anaphase-I, a clear segregation of 9:9 chromosomes (Fig. 1B) was seen in normal cytokinesis.

Translocation heterozygotes The translocation heterozygotes were isolated in colchicine treated population, the translocation heterozygotes was characterized by the presence of hexavalent, quadrivalent and bivalents in almost all the observed in pollen mother cells. Besides this, some PMCs showed other configurations such as hexavalent along with variable number of univalents. The frequency of various chromosomal associations is shown in Table 1. At diakinesis, out of 92 cells analysed, CVI+RIV+4II configuration was observed in most of the PMCs.

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Fig. 1. A, B) Meiosis in control plant. A) Diakinesis with 9II. B) Normal anaphase with 9:9 segregation. C) Meiosis in translocation heterozygote. C) Chain IV+2I+6II. D) Ring IV+ChainVI+4II. E) RingIV+RingVI+4II. F) Ring IV+5II. G) Ring, Chain IV+5II. H) Ring VI+Ring IV(eight shaped)+3II+2I. I) Chain IV+ChainVI+4II+2I. J) PMC showing stickiness with 4I. K) PMC at A-I with unequal (8:10) distribution. L) Telophase-II with a laggard.
Translocation heterozygotes show much different interchromosomal association such as chain or ring in present study, most of ring and chain associations were observed in different pollen mother cells (PMCs) at different meiotic stages. In the present study, the translocation heterozygotes in *Chrysanthemum carinatum* was observed from, using 0.2% colchicine (6 Hrs. for 3 days) treated seed progeny. There are numerous reports on translocation heterozygotes in various plants such as *Vicia faba* (Verma and Rao 1994), *Pisum sativum* (Verma and Goyal 2012) and *Pennisetum glaucum* (Khah and Verma 2017). Interchanges are those structural changes in chromosome, where terminal segments of non-homologous chromosome have exchanged positions. These changes are also called reciprocal translocation.

Interchanges in plants are usually associated with semisterility of gametes; such semisterility is observed is only in those plants which have translocation in only one set of chromosomes, the other set being normal. These plants are called interchange heterozygotes on the contrary; there can be plants which have same interchange in both sets of chromosomes. These would, therefore, be called interchange homozygotes (Burnham 1962). The orientation of interchange complex at metaphase I have a great bearing on the fertility interchange heterozygotes (Verma and Raina 1990). In addition hexavalent and quadrivalents mostly appeared in diakinesis accompanied by univalent. The associations of hexavalent and quadrivalents along with univalent which indicated that they appeared as a result of weak pairing of one of the translocated terminal bivalent in a hexavalent interchange complex (Khah and Verma 2017).

**Chromatin stickiness** It is characterized by intense clustering of chromosomes during any phase of the cell cycle (Rao *et al.* 1990). Beadle (1932) reported chromosome stickiness in maize for the first time and attributed the irregularity to a recessive mutagen gene called sticky (st). The phenomenon has been reported in present studies (Fig. 1J). As per Evans (1962) it occurs due to partial dissociation of nucleoproteins and changes in the chromosomal organization. With suggestions that chromosome stickiness may be under genetic control or it might also be caused by environmental factors such as X-rays, temperature and soil elements. Clumping of bivalents or chromosomes results in the loss of individuality of these structures and it is presently seen in at metaphase I. In present study such type of behavior observed in metaphase stage with clumping of

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**Table 1.** Chromosomal associations at diakinesis of the translocation heterozygote.

| S. No. | Associations          | No. of PMCs | Frequency (%) |
|-------|-----------------------|-------------|---------------|
| 01    | 9II                   | 20          | 21.73         |
| 02    | 6II+1IV+2I            | 15          | 16.30         |
| 03    | 5II+2IV               | 13          | 14.13         |
| 04    | 4II+2IV+2I            | 14          | 15.21         |
| 05    | 4II+1VI+1IV           | 19          | 20.65         |
| 06    | 3II+1VI+1IV+2I        | 11          | 11.95         |
|       | **Total**             | **92**      |               |

**Table 2.** Types of meiotic separation in colchicine treated population.

| Separations | No. of PMCs | Frequency (%) |
|-------------|-------------|---------------|
| 9:9         | 33          | 39.28         |
| 8:10        | 11          | 13.09         |
| Stickiness  | 24          | 28.57         |
| Laggards    | 16          | 19.04         |
| **Total**   | **84**      |               |
chromosome with four univalents (Fig. 1J). The frequency of metaphasic abnormalities (28.57%) is given in Table 2.

**Unequal distribution** Anaphase I showed varies abnormalities as well, although in a large percentage of PMCs, chromosome showed normal segregation (39.28%). Moreover this, 13.09% of cells had unequal distribution of chromosomes (other than 9:9) in which 8:10 distribution (Fig. 1K). The frequency of anaphasic abnormalities is given in Table 2. In comparison of control some bivalents can either separate earlier (early disjunction) or later (late disjunction) before entering anaphase-I stage. This non-synchronous separation may lead to gametes with extra or deficient number of chromosomes. As per, Kumar and Singh (2003) sometimes adjacent orientations also seems to be responsible for the formation of laggards where there is always a possibility of unequal and delay separation.

**Lagging chromosomes/laggards** Chromosomes (chromatin) that fail to reach respective poles at anaphase or telophase stages form laggard. Failure of one or more chromosomes to move towards respective poles at anaphase or telophase is observed in present study (Fig. 1L) of the meiotically anomalous species. The frequency of laggards (19.04%) which is telophasic abnormalities is given in Table 2. According to Nicklas & Ward (1994) unoriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers and may consequently into laggards.

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