

***Review Paper***

**POLYPLOIDY IN ORNAMENTALS**

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**Abstract**

Polyploidy breeding is an effective method for doubling the chromosome number of a species. Genetic variations created can be further used in breeding programme. Polyploidy breeding holds immense prospects in developing desirable varieties in flower crops. With the help of polyploidy, changes in morphology and cytology of plant are observed. Tetraploids are more vigorous and larger in size. Tetraploids produce thick and dark green leaves. Mostly seen consequences of induced polyploidy are increase in size and shape of plants; leaves, branches, flower parts, fruits and seeds. Chemicals like colchicine, oryzalin, triXuralin and amiprophosmethyl (APM) etc. are used in induction of polyploids. Although colchicine remained the most used for induction of polyploidy. Chromosome doubling or polyploidy using various chemicals was observed in ornamentals viz., Crape myrtle, Cyclamens, *Pelargonium*, *Plantanus*, *Petunia*, *Phlox*, Spurflores, Hibiscus, Zinnia, Canna, Dieffenbachia, Colocasia, Iris and Azalea.

Key words: Polyploidy, Ornamentals, Breeding.

**INTRODUCTION**

Polyploidy breeding is an effective method for doubling the chromosome number of a species. Genetic variations created can be further used in breeding programme. Chromosome doubling has been extensively useful in several crops for breeding purpose. These new forms with improved plant architecture provide good material for breeding programme and for further development of cultivars (Mata, 2009). With the help of polyploidy, changes in morphology and cytology of plant are observed. Tetraploids are more vigorous and larger in size. Tetraploids produce thick and dark green leaves (Singh, 1996). Colchicine is extensively used for induction of polyploidy in plants. Concentration and duration of colchicine affects the success percentage in polyploidy induction. The use of colchicine as a means of chromosome doubling has opened a large reservoir of possibilities in plant breeding work. The fact that numerical changes in chromosome number fundamentally entail a mutation which may be expressed in a number of characters of the plant indicates the significance of the above statement (Derman, 1990)

Polyploidy, being defined as chromosome doubling is best determined by chromosome counts. There are a number of characteristics that generally express

polyploidy and are usually associated with it. One major characteristic involves change in size and change in shape of plant species (Chaudhari, 1980).

Mostly seen consequences of induced polyploidy are increase in size and shape of plants; leaves, branches, flower parts, fruits and seeds (Chopra, 2008).

### **Polyploidy in ornamentals**

#### **Crape myrtle**

A protocol for in-vitro induction of crape myrtle (*Lagerstroemia indica* L.) tetraploids using nodes from in vitro-grown shoots ( $2n = 48$ ) was established. Nodal buds were excised from in vitro-grown shoots, maintained on proliferation medium containing Murashige and Skoog medium supplemented with 4.44  $\mu$ M 6-benzyladenine, 0.54  $\mu$ M  $\alpha$ -naphthaleneacetic acid, and treated with a range of concentrations of colchicine under three different conditions. Nodal bud explants treated in liquid proliferation medium supplemented with either 15 or 20 mM colchicine for 24 h turned necrotic and died; whereas, those cultured on solid proliferation medium supplemented with either 125 or 250  $\mu$ M colchicine for 30 days survived, but no tetraploid plants were obtained. However, when explants were cultured in liquid proliferation medium containing 250, 500 or 750  $\mu$ M colchicine for 10 days, tetraploid plants ( $2n = 96$ ) were obtained. Incubation of explants in medium containing 750  $\mu$ M colchicine promoted the highest frequency of survival (40%) of explants and of recovered tetraploids (60%). Morphological and anatomical characteristics of leaves, including leaf index, stomata size and number, stomata index (length/width), and number of chloroplasts in guard cells correlated with ploidy of crape myrtle plants (Zhang, 2010).

The tips of cotyledon-stage seedlings of three crape myrtle cultivars ("Zi Wei", "Hong Wei" and "Yin Wei") were treated with colchicine. Various concentrations of colchicine and different treatment durations were tested. Seedlings of "Zi Wei" treated with 0.5% colchicine for 72 h and seedlings of "Yin Wei" treated with colchicine (0.2% for 96 h, 0.5% for 48 h and 0.8% for 72 h) demonstrated high rates of mutation; "Hong Wei" showed a slightly lower rate. The highest rate of morphological variation was 54.17% and this was achieved when tips were treated with 0.5% colchicine for 72 h. Putative tetraploid plants were identified with morphological and cytological variations, such as larger and thicker leaves, darker green coloration, larger stomata, lower density of stomata across the lower leaf epidermis and increased numbers of chloroplasts per stomata guard cell (Ye, 2009).

#### **Cyclamens**

The possibility of obtaining tetraploids from diploid yellow-flowered cyclamen 'Kage Yellow' through colchicine treatment and the effects of polyploidization on the characteristics of yellowflowered cyclamen were investigated. A 4-day treatment of tuber segments with 100 mg 1-l colchicine in vitro yielded two tetraploid plants. The petals of the tetraploids were larger and had a greater ability to accumulate chalcone than those of their diploid relatives. Polyploidization may therefore represent a useful method for the commercial breeding of deeper yellow-flowered cyclamen (Takamura, 1996).

#### **Pelargonium**

Polyploids were effectively pre-selected in colchicine-treated plants of the desirable brown-leaved cultivar Black Velvet Scarlet F1 of the species *Pelargonium × hortorum* L.H. Bailey to obtain the basic breeding material for creating new brown-leaved tetraploid cultivars. The green-leaved cultivar Gizela F1 was used for comparison of quantity and quality of response to colchicine treatments. Water solutions of colchicine in the range from 0.1% to 2.5% induced polyploidy in seedlings with

treatments repeated each day for 2, 3, 5 or 7 days. Polyploid plants were pre-selected according to their morphological changes and stomata length and density and verified using flow cytometry. Some morphological changes (leaf coloration, flower shapes) in colchipooids differed between the genotypes, others were the same in both cultivars (loss of coloration in mixoploids, failure of blooming) (Jadrna, 2010).

#### **PlantanusAcerifolia**

The production of tetraploid plants of *Platanusacerifolia*, with the ultimate aim of improving the ornamental qualities of this important urban landscaping tree. Chromosome doubling was achieved by the application of colchicine to either pre-soaked seed or to the apical meristems of young seedlings. Treatment of the ungerminated seed was the more efficient method in terms of numbers of tetraploid seedlings (up to 40%, as determined by chromosome counting of the root-tip nuclei) but this method produced no mature tetraploid plants due to the deleterious effect of colchicine on subsequent root growth. When colchicine was applied directly to the apical growing tip of cotyledon-stage seedlings, leaf and stem growth was temporarily affected but the plants eventually recovered. We conducted a preliminary screen for putative tetraploids based on the observation in other plant species of a correlation of stomatal size and distribution with ploidy. Plants containing significantly larger stomata and at a lower density across the lower leaf epidermis, were selected for further analysis by flow cytometry and chromosome counting. These techniques confirmed that, of the 12 putative polyploids, four were tetraploid, five were mixoploid and three were, in fact, diploid. Morphological differences of the tetraploids included a more compact growth habit and broader, thicker leaves. These plants are being grown to full maturity in order to test their potential for use in a breeding programme aimed at producing sterile triploid lines (Liu, 2007).

#### **Petunia**

A set of *Petunia hybrida* plants encompassing a range of ploidy levels was developed through colchicine-mediated induction of chromosome doubling. 95% of the shoot tips treated with colchicine for 48 h resulted in polyploid mutant plants, and no difference in this efficiency was observed using concentrations of colchicine between 0.2 and 2.0 mg·mL<sup>-1</sup>. Of the polyploid plants, 10% were found to be tetraploid and 85% were mixoploid (chimeric). Compared with their diploid counterparts, polyploid plants underwent reduced elongation growth during the first 2 weeks and had thicker stems and shorter internodes resulting in dwarfing of the whole plant. In extreme cases, very slow growth rates produced stunted plantlets. Polyploid plants also had larger, thicker leaves and, in some cases, the leaves that developed after 1 month of growth appeared seriously malformed. Octoploid plants were also obtained and these tended to have more extreme phenotypes (Ning, 2009).

#### **Phlox**

Work was undertaken to observe the response of *Phlox drummondito* colchicine treatment. The survival rate and germination percentage is severely affected by various treatments of colchicine. In this investigation, doses related effects of the ploidy treatments on quantitative traits were noticed. Result indicates reduction in plant height, number of leaves per branch, but increase in number of branches. Stomatal size was negatively correlated with stomatal frequency. Stomatal size and stomatal frequency can be used as indirect methods for identification of ploidy level of *Phlox* (Tiwari, 2012).

#### **SPURFLOWERS**

Allotetraploids were created in Spurflowers (WILD SOUTH AFRICAN *PLECTRANTHUS*) on growing shoots of infertile diploid ( $2n=28$ ) hybrid selections, using colchicine. Tetraploids had enlarged leaves and flowers and showed loss of vigour but seed fertility was restored (Brits, 2006).

### **Hibiscus**

*Hibiscus acetosella* Welw. ex Hiern. 'Panama Red' PP20,121 (Malvaceae) has generated public and grower interest due to its attractive red foliage and vigorous growth, however, a horticultural goal is to develop more compact forms. Even though organs of induced polyploids are often larger than the wild type, whole plants are often shorter in stature. Three studies were conducted to induce polyploidy and to evaluate the growth and reproductive potential of the resulting polyploids. In study 1, seeds were soaked for 24 hours in aqueous solutions of 0%, 0.2%, 0.4%, or 0.5% colchicine (w/v) plus 0.5% dimethyl sulfoxide. In studies 2 and 3, apical meristems of seedlings at the cotyledon stage were treated for 1 or 3 days with 0, 50, 100, or 150  $\mu$ moryzalin solidified with 0.8% agar. No induced polyploidy was observed following seed treatment with colchicine at the rates and duration used in this study. In mixoploids plant height was reduced, leaves were smaller, internodes were shorter, and canopy volume was reduced in the octoploid (8x) form compared with the tetraploid (4x) form. This represents the first time oryzalin has been reported to induce polyploidy in *Hibiscus* (Contreras, 2006)

### **Zinnia**

Two species of *Zinnia*, namely *Zinnia linearis* and *Z. elegans* are commonly used as ornamentals in tropics during summers. The seeds of *Zinnia elegans* ( $2n=24$ ) 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 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. In 0.2% solution, there was considerable lethality. In one of the two tetraploid plants available, the disc florets were completely absent. 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 (Gupta, 1976).

### **Canna**

Colchicine at 0.5 to 0.7 per cent was used to treat *Canna indica* and two cultivars of *Canna generalis* for 5-7 hours. More than sixty per cent seedlings of *Canna indica* rendered tetraploids with stunted growth habit, besides broader, thicker and deeper green leaves in comparison to diploid. In colchicine-induced tetraploid, flower size was bigger, but number of flowers was reduced to 9-11 per inflorescence as compared to 58-60 in the original diploids (Khushoo, 1970). Next to hybridisation, triploidy (14 per cent) has been an important mechanism in the origin of cultivars with thicker, more durable and longer flower parts. Flower diameter in triploids was in range of 5.6 cm to 6.3 cm. Number of leaves per plant was reduced to 9-11 (Khushoo, 1975).

### **Dieffenbachia**

Autotetraploid (4n) plants of *Dieffenbachia maculata* 'Perfection' flowered poorly, compared with diploid (2n) plants, following treatment with a 250 or 500 mg foliar spray of GA3/litre. GA3-treated 4n plants produced bracts that normally precede flowering but in this case remained vegetative and produced additional distal shoots instead of flowers (Henny, 1989).

## Colocasia

Studies of several 2x and 3x cultivars of *Colocasia esculenta* Schott and one colchicine-induced tetraploid revealed differences between them in such characters as duration of different phases of flower development, morphology of the flowers and size distribution of the pollen grains (Miyazaki, 1987).

## Iris

Floral anthocyanins of colchicine-induced amphidiploids of *I. laevigata* X *I. ensata* were analysed. The anthocyanin contents of the amphidiploids and the F1 hybrids were 2.81 and 2.45 times higher, respectively, than those of the mid-parent (Yabuya, 1987).

## Azalea

In the course of such studies of azalea (*Rhododendron simsii*, *R. mucronatum*, *R. scabrum* and *R. indicum*), we observed a correlation between flower colour patterns, flower morphology and somatic polyploidy. Using high-resolution flow cytometry of nuclear DNA, the ploidy level was determined in flowers of different azalea sport [mutant] families. Sports exhibiting variegated flowers with broad (>7 mm), differently coloured, petal edges (picotee type) proved to be tetraploid in the petal edge but diploid in the rest of the flower tissue. Neither flower colour pattern nor ploidy differences are periclinalchimaeric in origin, but seem to be correlated with the topographic location of the cells within the flower tissue, i.e. the margin of the petals (Schepper, 2001).

## CONCLUSION

Polyploidy breeding holds immense prospects in developing desirable varieties in flower crops. Scientist should go ahead with polyploidy breeding and mutation breeding when a desirable trait is not available in existing genotypes. Chemicals like colchicine, oryzalin, triXuralin and amiprofosmethyl (APM) etc. are used in induction of polyploids. Colchicine was found to be used in most of the experiments. Colchicine at various concentrations and durations has induced polyploidy in various crops. Polyploidy was found to be successfully induced in in-vitro and other uncontrolled experiments. Chromosome doubling using various chemicals was observed in ornamentals viz., Crape myrtle, Cyclamens, *Pelargonium*, *Plantanus*, *Petunia*, *Phlox*, Spurflowers, Hibiscus, Zinnia, Canna, Dieffenbachia, Colocasia, Iris and Azalea.

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