# Colchicine Induced Variability in Saffron

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**Abstract**

**In an effort to overcome the sterility barrier in autotriploid (2n=3x=24) saffron (*Crocus sativus* L.) the present study was undertaken to induce polyploidy by colchinization. Colchicine was applied to the emerging apical buds of saffron corms at 0.05, 0.1, 0.2 and 0.4 per cent aqueous concentration employing cotton plugs for 48 hrs (12 hrs for 4 consecutive days). Studies on developmental, morphological and leaf anatomical characters of Co, C1 generation showed that the colchiploids exhibited delayed flower and leaf emergence. The leaves were thicker, shorter somewhat flat-ridgeless, coarser in feature and darker green in colour with reduction in number of leaves per plant. Stomatal studies revealed decrease in number with a concomitant increase in size of stomata in $C_1$ generation. There was reduction in the number of flowers per plant, floral variants observed included smaller sized flowers, irregularly shaped/reduced tepal number, lobed and dentate tepals, flowers with deep pigmentation in stigmas extending to stylar regions and orange red pigmented anthers. The hereditary behaviour of the variants will be further studied in C2 generation in the ensuing flowering season.**

## INTRODUCTION

Saffron (*Crocus sativus* L.) is an autumn flowering cormose plant, long cultivated for numerous properties ascribed to the stigmatic lobes (Basker and Negbi, 1983) and used as spice, condiment and for medicinal purposes. The corms reproduce annually, only vegetatively as the plant is sterile (Chichiriccò, 1984) autotriploid (2n=3x=24) and seeds are unknown. Studies have revealed that the sterility is related to meiotic abnormalities producing both, pollen grain which displays low/defective germination, and partially non-functional macrospores (Chichiriccò, 1989). Sterility in saffron limits the application of conventional breeding approaches for its further improvement. In Kashmir saffron cultivation is loosing popularity among farmers because of its low productivity of 2.0 to 2.5 kg dry saffron $ha^{-1}$ and lack of availability of improved high yielding and disease resistant clones is one of the major constraints. With the objective of increasing the spectrum of variability for floral traits and recovering auto-hexaploids (6x=48) in saffron (to break the sterility barrier) the present study was undertaken.

## MATERIALS AND METHODS

Saffron corms weighing 7-8 gm were collected from saffron fields of Pampore, and cotton swabs soaked in 0.05 (A), 0.10 (B), 0.20 (C) and 0.4 (D) percent aqueous concentration of colchicine (Sigma) were placed on the emerging apical floral buds 0.5 to 2.0 mm long for 48 hours (12 hours for four consecutive days), in the second fortnight of July and first fortnight of August respectively during 2001. Fifty corms were taken in each treatment. Treated corms were planted along with control in the field on raised beds in rows 30 cm apart, with corm to corm spacing of 10 cm, during last week ($25^{th}$) of August. Data on flower, leaf emergence, leaf morphological and anatomical characters were recorded in the variants. For stomatal studies an abaxial surface epidermal fresh leaf peel was mounted in a drop of 50% glycerine on a glass slide and observed under microscope. Length and breadth of 25 random stomata ($\mu m^2$) were measured using ocular

and stage micrometer.

The quantitative data on frequency and size of stomatal and epidermal cells were based on an average of 10 random observations. Stomatal index was calculated by using formula:

$$\text{Stomatal index (SI)} = \frac{\text{Number of stomata}}{\text{Number of stomata + Number of epidermal cells}} \times 100$$

## RESULTS AND DISCUSSION

The influence of colchicine on the phenotype of the plant was characterized by its effect on the number, shape, size and texture of organs. There was reduction in the number of leaves 3-9/plant (9-11 in control). The leaves were thicker, broader (+66.6%), shorter (-68.75 %), wrinkled, darker green and coarser in texture than control. The stigmatic rays showed a gradual decrease in length (-43.3 %) (Table.1). The flowers with pigmented anthers and stigmas having pigmentation beyond the stylar portion (normally lacking in triploids) were observed. The tepal colour became intense with a decrease in its number and size.

The variant plants showed slower rate of growth as compared to triploids, which was reflected both in vegetative and flowering stages. The leaf emergence got delayed by about 10 days and flowering was delayed by about 12 days. The slower growth rate is due to the reduced number of cell divisions and increase in nuclear size. Reduced number of cell divisions during development is characteristic of polyploids (Emswellerm, 1949; Stebbins, 1971). Nuclear DNA content and developmental rates are inversely related, i.e. the lower the DNA content the faster the development rate (Bennett, 1972).

Stomatal studies of the abaxial surface of leaf revealed that the average stomatal length was maximum in colchiploids treated with C (0.2 conc.). The average length of the stomata ranged from 44.0 to 54.8 μm and breadth 36.8 to 55.2 μm. Number of stomata ranged from 5 to 17. The average number of epidermal cells ranged between 12 to 17 (21 in control). Treatment B (0.10 conc.) and C (0.20 conc.) showed lesser number of epidermal cells $mm^{-2}$ (12) with increase in size of epidermal cells. The colchiploids from D (0.40 conc.) had smaller, thicker and coarser leaf along with lower stomatal frequency. The range of stomatal frequency in colchiploid was between 3.4 to 11.7 $mm^{-2}$. Decrease in frequency is due to increase in size of stomata. Stomatal index was highest in the control and it decreased in colchiploids, the lowest being 27.7 which was observed in colchiploids with D (0.40 conc.) (Table.2).

## CONCLUSIONS

Slower growth rate could be because of reduced rate of cell division (Eigsti, 1947), lower amount of growth hormone (Larsen and Mintung, 1950) or lower metabolic activities. Increase in size of stomata reduction in its number may be due to higher gene doses as a result of chromosome number increase. Role of colchicine in bringing about chromosome doubling is well known, however sometimes mutation have been also induced (Franzke and Ross, 1952). Colchicine induced partial desynapsis associated with floral characters has been reported in *Lathyrus* and explained due to gene mutations and not due to chromosome structural changes (Harpstead et al, 1954). The plants showing variability for vegetative floral traits have been tagged and will be observed during ensuing season. Evaluation data of $C_3$ generation along with chromosomal (root tip/PMC) studies will further confirm role of mutations/polyploidy in inducing variability in saffron.

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## Tables

Table 1. Comparison of morphological characteristics in triploid and probable colchiploid variants of saffron

| Treatments | Leaf emergence (date) | Number of leaves plant $^{-1}$ | Leaf length (cm) | Leaf breadth (mm) | Flowering Initiation (date) | Number of flowers plant $^{-1}$ | Length of stigmatic rays (cms) |
|---|---|---|---|---|---|---|---|
| Control (Triploid) | 27$^{th}$ October | 9-11 | 32.0 ± 0.35 | 3.0 ± 0.22 | 24$^{th}$ October | 2-3 | 2.5-3.0 |
| A (0.05) | 29$^{th}$ October (2) | 9 | 16.0 ± 0.17 (-50%) | 3.2 ± 0.4 (+0.06) | 28$^{th}$ October (4) | 2 | 2.3-3.0 3.0 |
| B (0.10) | 3$^{rd}$ November (7) | 7 | 14.0 ± 0.16 (-56.25) | 3.5 ± 0.4 (+16.66) | 31st,October (7) | 1 | 1.9-2.5 7.0 |
| C (0.20) | 5$^{th}$ November (9) | 5 | 15.0 ± 0.10 (-53.12) | 4.0 ± 0.35 (+33.33) | 3$^{rd}$ November (10) | 1 | 2.2-2.8 0.8-1.8 |
| D (0.40) | 10$^{th}$ November (14) | 3 | 10.0 ± 0.10 (-68.75) | 5.0 ± 0.30 (+66.66) | 5$^{th}$ November (12) | 1 | 1.5-1.6 43.63 |

Table 2. Comparison of leaf anatomical features in triploid and probable colchiploid variants of saffron

| Treatments | Stomatal length( μm) | Stomatal width ( μm) | Number of epidermal cells (mm$^{-2}$) | Number of stomata (mm$^{-2}$) | Stomatal index | Stomatal frequency mm$^{-2}$ |
|---|---|---|---|---|---|---|
| Control(Triploid) | 44.8 ± 0.25 | 42.4 ± 0.21 | 21 | 22 | 51.4 | 15.17 ± 0.76 |
| A (0.05) | 44.0 ± 0.22 | 36.8 ± 0.34 | 19 | 17 | 47.2 | 11.7 ± 0.66 |
| B (0.10) | 54.4 ± 0.27 | 55.2 ± 0.18 | 12 | 10 | 45.4 | 6.8 ± 0.54 |
| C (0.20) | 54.8 ± 0.26 | 53.2 ± 0.20 | 12 | 9 | 42.8 | 6.2 ± 0.53 |
| D (0.40) | 54.4 ± 0.27 | 40.8 ± 0.23 | 13 | 5 | 27.7 | 3.4 ± 0.48 |