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# Variation Induced in $C_2$ Generation of African marigold Using Colchicine Treatment

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**A B S T R A C T**

Variation induced in $C_2$ generation of African marigold using colchicine treatment was conducted in non-replicated trial with six treatments. The seeds of diploid white marigold were treated with 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 % colchicine at room temperature for 12 hrs. and the seeds collected from individual plants of $C_1$ generation were used for the experiment to raise $C_2$ generation and germinated in protray. Thirty days old seedlings were transplanted in the field with spacing of 45 × 30 cm in ridges and furrow. Observations on seventeen different types of variants *viz*. tall, dwarf, broad leaf, narrow leaf, twin flower, orange flower, yellow flower, light yellow flower, multi-layer spreaded flower, single layer spreaded flower, bilayer spreaded flower, button shape flower, flower without central disc, small flower, large flower, plant with single stem and profuse branches were identified and isolated. Variation frequency, treatment efficiency and treatment effectiveness did not follow any specific trend in relation to dose of colchicine. Significant variation among the treatments for all the variants was recorded in $C_2$ generation of African marigold and hence, offers scope for identifying variants.

## Introduction

Marigold is one of the commercially exploited flower crop that belongs to the family *Asteraceae* and genus *tagetus*. Marigold has Indian origin although it appears that its natural origin is Mexico compared to other flowering annuals. Marigold is one of the most commonly grown flower in India and used extensively on religious and social functions in different forms. They have special importance especially on Diwali and Dashera. There is a constant demand for flowers throughout the year for functions, festivals, marriages and floral decorations. Because of ease in cultivation, wide adaptability to varying soil and climatic conditions, long duration of flowering and attractively coloured flowers endowed with excellent keeping quality, marigold has become one of the most popular flower in India. Due to its variable height and colour, marigold is especially used for decoration and included in landscape plants. *Tagetes* species vary in size from 0.01 - 2.2 m tall. Most species have pinnate green leaves. Blooms are naturally in golden,

orange, yellow and white colours, often with maroon highlights. Floral heads are typically with both ray and disc florets. Marigold in general tends to be planted as annuals, although the perennial varieties are gaining popularity.

Marigold is grown for loose flowers, making garlands, decoration during puja and several religious functions, besides its use in landscape gardening. Apart from its significance in ornamental horticulture, it has been valued for other purposes too. The aromatic oil extracted from marigold, is called as "Tagetes oil". It is used in preparation of high grade perfumes and also as an insect fly repellent. Recently dried flower petals of marigold are used as poultry feed in order to improve the colour of egg yolk as well as broiler's skin. Flowers of African marigold can be used for extraction of L-limonene, ocimene, L-linalylacetate, L-linalool. Marigold petals are used for extraction of xanthophylls. Lutein which is the major constituent of xanthophylls is used for colouring food stuffs. Purified extract of marigold petals containing lutein dipalmitate is marketed as an ophthalmologic agent under the name adaptinol.

Total area under marigold crop in India during the year 2016-2017 was 56.04 thousand ha. With the production of 497.59 thousand metric tonnes of loose flowers and 4.28 lakh number of cut flowers (Anonymous, 2017). Major marigold growing states are Karnataka, Maharashtra, Andhra Pradesh and West Bengal. The major centers of flower marketing are metropolitan cities like Mumbai, Kolkatta, Chennai, Bangalore, Delhi in India and Pune, Mumbai, Nasik, Ahmednagar, Sangali, Kolhapur, Thane, Satara and Nagpur in Maharashtra.

In horticulture, the induction of variation is a valuable route to obtain useful and novel characteristics that are not present in the diploid progenitor. These characteristics can include increased, cell size (which leads to large reproductive and vegetative organs), enhanced enzymatic activity, prolonged flowering time, no seed (or few seeds) as well as increased pest resistance and stress tolerance (Dhooghe *et al.,* 2009). It has been claimed that the induction of variability can enhance the production of secondary metabolites with medicinal properties (Dhawan and Lavania, 1996).

Keeping in mind the above views, this study entitled "Effect of cochicine treatments to induce variation in $C_2$ generation of African Marigold." was planned and executed with the objective of creating more genetic variability, high yield and novel flower characters.

## Materials and Methods

The seeds of individual plants harvested from each treatment of $C_1$ generation were used for the experiment to raise $C_2$ generation. Black coloured protray of 52.5 × 26.5 $cm^2$ size were filled with potting mixture of coco pit and vermicompost. Seeds of diploid white marigold were sown in tray which was then gently covered with soil. Trays were watered lightly with the help of hands. After about 3 to 4 days the seeds started germinating and potential germination was completed within ten days.

Thirty days old uniform well developed and healthy seedlings of 10-15 cm length were selected for transplanting. One day before the transplanting light irrigation was given to the plot. Seedlings were transplanted in the field with spacing of 45 × 30 $cm^2$. in ridges and furrow. Light irrigation was given immediately after transplanting. The plants were time to time supplemented with nutrients along with RDF for the proper growth and development of flower bud. Irrigation was

given to the plants at proper interval. Weeding, plant protection were adopted as and when found essential. The variation frequency in percentage for inducing visible variants were calculated for each treatment as suggested by Gaul (1958) and the efficiency and effectiveness of colchicine in different treatments in $C_2$ generation were estimated as per the formula given by Konzak *et al.,* (1965).

## Results and Discussion

In the present investigation 17 different types of morphological and economical variants were identified and isolated from $C_2$ population. Their frequencies are presented in table 1.

### Dwarf variant [Plate 1]

Dwarf variant were induced in the treatment0.5% and 1.0% colchicine. The high frequency for the dwarf varitants was noticed in $T_1$ (1.07%) and low in $T_2$ (0.53%). These variants were short in stature with compact forms having height of 60 to 78cm as compared to control (102cm).

Some variants were short and thick stemmed with reduced internodal length. Similar to this result Rathod (2017) also reported the occurrence of dwarf variants in 1.5% and 2.0% colchicine treatments in white marigold.

### Tall variant [Plate 1]

The tall variants were induced in the treatments0.5% and 1.0% colchicine. The maximum frequencies were obtained in 1.0% colchicine (1.26%) and the low frequencies in 0.5% colchicine (0.97%). The range for the character was from 191 to 219 cm as compared to control $T_4$ (124 cm).In accordance to this result Rathod (2017) also obtained tall variant in 0.5% and 2.0% colchicine treatments in white marigold.

### Broad leaf variant [Plate 2]

Broad leaf variants were found in all the treatments. The highest frequency was found in 2.0% colchicine (2.56%) followed by 2.5% colchicine (1.85%), 0.5% colchicine (1.00%), 1.0% colchicine (0.90%) and 1.5% colchicine (0.43%).

These variants had elliptic shape and broad leaves as compared to control. The broad variants were also earlier reported by Rathod (2017) in white marigold at 0.5%, 1.0% and 1.5% colchicine concentration.

### Narrow leaf variant [Plate 2]

Narrow leaf variants were found in all the treatments. The highest frequency was observed in $T_4$ (1.28%) followed by $T_2$ (0.92%), $T_5$ (0.92%), $T_3$ (0.86%) and $T_1$ (0.85%).

These variants had elliptic shaped and narrow leaves as compared to control. The narrow leaf variants were earlier reported by Rathod (2017) in white marigold at 0.5%, 1.0% and 1.5% colchicine concentration.

### Twin flower variant [Plate 3]

Twin flower type variant were recorded in only 0.5% colchicine. The frequency of variant was 0.07 percent. Some variant of this treatment showed normal flower structure with multilayer spreaded flower type.

### Orange flower colour variant [Plate 5]

The orange flower colour variants were isolated from all the treatments. The highest frequency for the character was observed in 2.5% colchicine (1.85%) and the lowest in 1.0% colchicine (0.87%). Variants showed orange flower colour as compared to control with white flower.

### Yellow flower colour variant [Plate 5]

The yellow flower colour variants were isolated from all the treatments. The highest frequency for the character was observed in 2.5% colchicine (1.85%) and the lowest in 1.5% colchicine (0.86%). Variants showed yellow flower colour as compared to control with white flower colour. The yellow flower colour variants were earlier reported by Rathod (2017) in white marigold at 1.5% and 2.5% colchicine concentration.

### Light yellow flower colour variant [Plate 5]

The light yellow flower colour variants were recorded in the treatments 0.5% colchicine and 1.0% colchicine. The high frequency for the character was observed in 1.0% colchicine (0.34%) and the low in 0.5% colchicine (0.23%). Variants showed light yellow flower as compared to control with white flower colour. The light yellow flower colour variants were earlier reported by Rathod (2017) in white marigold at 1.5% colchicine concentration.

### Multi-layered spreaded flower variant [Plate 4]

The multi-layered spreaded flower variants were isolated from all the treatments. The highest frequency for this variant was observed in 2.0% colchicine (3.19%) and the lowest in 1.0% colchicine (0.74%). Variants showed multi-layer of petals which were spreaded with different colour as orange and yellow and both small and large size. The multi-layered spreaded flower variants were earlier reported by Rathod (2017) in white marigold at 1.5% and 2.5% colchicine concentration.

**Table.1** Frequency of induced variants in different colchicine treatments in $C_2$ generation

| Sr.no. | Type of variant characters | $T_1$ 0.5% | $T_2$ 1.0% | $T_3$ 1.5% | $T_4$ 2.0% | $T_5$ 2.5% | Total |
|---|---|---|---|---|---|---|---|
| **1** | Dwarf | 1.07 | 0.53 | - | - | - | 1.60 |
| **2** | Tall | 0.97 | 1.26 | - | - | - | 2.23 |
| **3** | Broad leaf | 1.00 | 0.90 | 0.43 | 2.56 | 1.85 | 6.74 |
| **4** | Narrow leaf | 0.85 | 0.92 | 0.86 | 1.28 | 0.92 | 4.83 |
| **5** | Twins flower | 0.07 | - | - | - | - | 0.07 |
| **6** | Orange flower | 1.00 | 0.87 | 1.73 | 1.28 | 1.85 | 6.73 |
| **7** | Yellow flower | 0.99 | 1.20 | 0.86 | 1.28 | 1.85 | 6.18 |
| **8** | Light yellow flower | 0.23 | 0.34 | - | - | - | 0.57 |
| **9** | Multi-layer spreaded flower | 1.62 | 0.74 | 1.91 | 3.19 | 1.85 | 9.31 |
| **10** | Bi-layer spreaded flower | 0.07 | 0.17 | 0.43 | 1.28 | - | 1.95 |
| **11** | Single layer spreaded flower | 0.96 | 0.54 | 1.73 | - | 1.77 | 5.00 |
| **12** | Button shape flower | 0.38 | 0.17 | 0.43 | 1.28 | 0.92 | 3.18 |
| **13** | Flower without central disc | 0.15 | - | 0.86 | - | - | 1.01 |
| **14** | Small flower | 1.24 | 1.74 | 2.78 | 3.12 | - | 8.88 |
| **15** | Large flower | 0.51 | 1.00 | 1.73 | 2.56 | - | 5.80 |
| **16** | Profuse branches | 0.82 | 2.10 | 2.21 | 3.41 | 1.77 | 10.31 |
| **17** | Plant with single stem | 0.66 | 1.78 | 1.00 | 3.12 | 2.03 | 8.59 |
| | **Total** | **12.59** | **14.26** | **16.96** | **24.36** | **14.81** | **82.98** |

**Table.2** Effect of colchicine treatments on variation frequency and treatment efficiency and effectiveness in $C_2$ generation

| Treatments | Per cent lethality | Variation frequency | Treatment efficiency | Treatment effectiveness |
|---|---|---|---|---|
| **T1 (0.5%)** | 11.73 | 12.59 | 1.07 | 0.08 |
| **T2 (1.0%)** | 12.35 | 14.26 | 1.15 | 0.05 |
| **T3 (1.5%)** | 16.36 | 16.96 | 1.04 | 0.03 |
| **T4 (2.0%)** | 20.41 | 24.36 | 1.19 | 0.04 |
| **T5 (2.5%)** | 10.00 | 14.81 | 1.48 | 0.02 |


**Plate.1** Variants for profuse branched and height

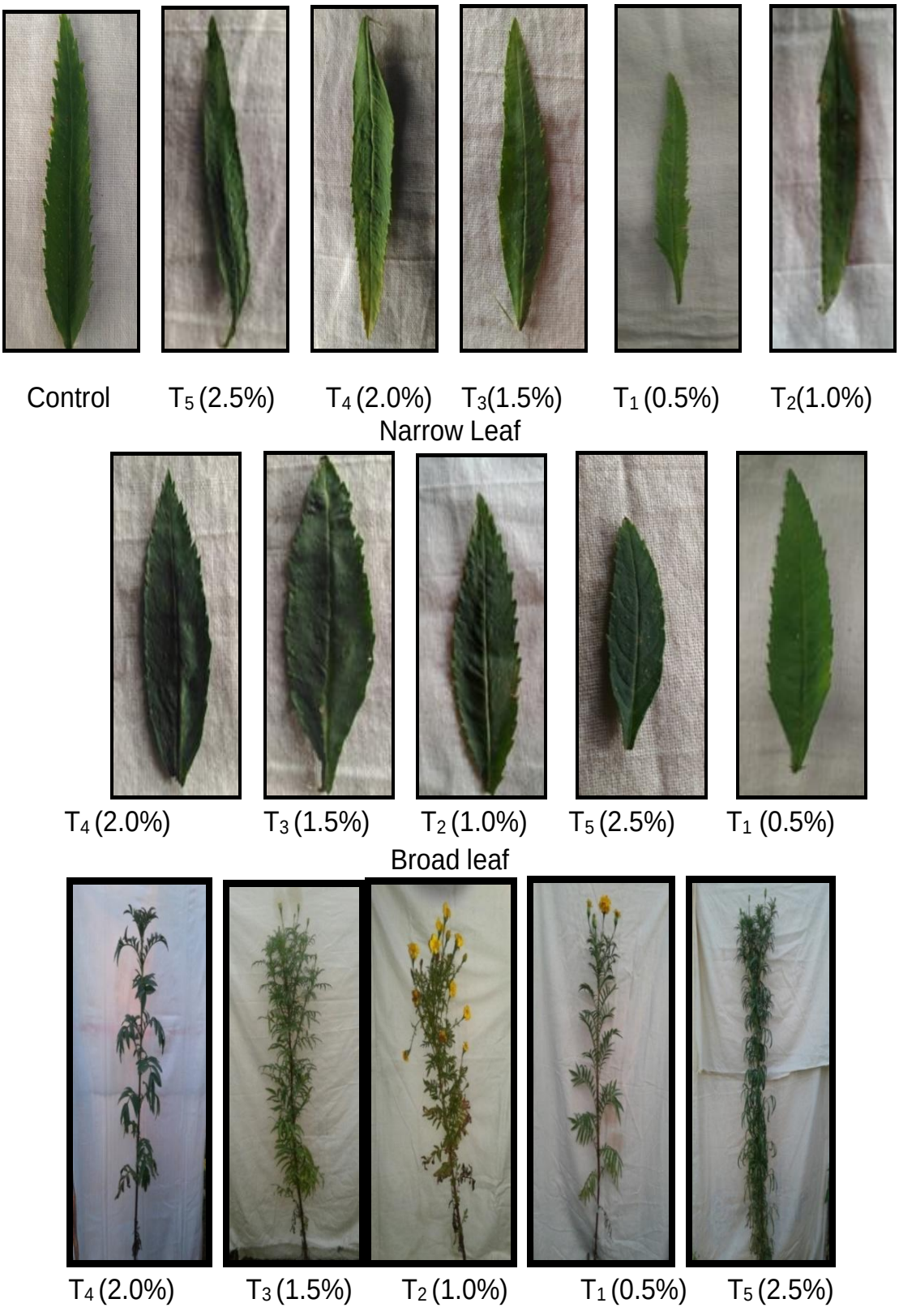



**Plate.2** Variants for leaf size and single stem

$T_1$ (0.5%) $T_1$ (0.5%) $T_1$ (0.5%)

Twin flower

$T_3$(1.5%) $T_2$(1.0%) $T_1$(0.5%) $T_4$(2.0%)

Small flower

$T_3$ (1.5%) $T_2$ (1.0%) $T_1$(0.5%) $T_4$(2.0%)

Large flower

**Plate.3** Variants for twin flower and flower size

Control (0.0%) $T_3$ (1.5%) $T_4$ (2.0%) $T_2$ (1.0%) $T_1$ (0.5%) $T_5$ (2.5%)

Multi layerspreaded flower

$T_3$ (1.5%) $T_1$ (0.5%) $T_2$ (1.0%) $T_4$ (2.0%)

Bilayer spreaded flower

$T_1$ (0.5%) $T_2$ (1.0%) $T_3$ (1.5%) $T_5$ (2.5%)

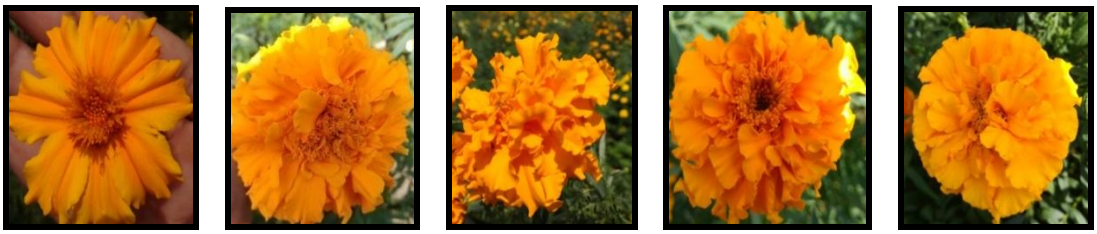
Single layer spreaded flower

$T_5$ (2.5%) $T_3$ (1.5%) $T_4$ (2.0%)

$T_1$ (0.5%) $T_2$ (1.0%) $T_1$ (0.5%) $T_3$ (1.5%)

Button shape flower Flower without central disc

**Plate.4** Variants for flower structure



$T_5$ (2.5%) $T_1$ (0.5%) $T_2$ (1.0%) $T_3$ (1.5%) $T_4$ (2.0%)

Orange flower

$T_3$ (1.5%) $T_4$ (2.0%) $T_5$ (2.5%) $T_1$ (0.5%) $T_2$ (1.0%)

Yellow colour flower

$T_1$ (0.5%) $T_2$ (1.0%)

Light yellow flower

**Plate.5** Variants for flower colour

**Bi-layered spreaded flower variant [Plate 4]**

The bi-layered spreaded flower variants were induced in all the colchicine concentration except 2.5%.The highest frequency for this variant was noticed in 2.0% colchicine (1.28%) and the lowest in 1.0% colchicine (0.07%). The flowers of this variant showed two layer of petals which were spreaded with different colour as orange and yellow and both small and large size. The bi-layered spreaded flower variants were earlier reported by Rathod (2017) in white marigold at 1.0% and 2.5% colchicine concentration.

**Single layered spreaded flower variant [Plate 4]**

The single layered spreaded flower variants were induced in all the colchicine concentration except 2.0% colchicine. The highest frequency for the variant was noticed in 2.5% colchicine (1.77%) and the lowest in 1.0% colchicine (0.54%).

Flowers of the variants showed single layer of petals with different colour as orange and yellow and both small and large size of flower. The single-layered spreaded flower variants were earlier reported by Rathod

(2017) in white marigold at 1.0% and 2.5% colchicine concentration.

**Button shape flower variant [Plate 4]**

The button shape flower variants were isolated from all the treatments. The highest frequency for the variant was observed in 2.0% colchicine (1.28%) and the lowest in 1.0% colchicine (0.17%).

Variants showed button shaped flower with different colour as orange and yellow and of different size. The button shape flower variants were earlier reported by Rathod (2017) in white marigold at 2.0% colchicine concentration.

**Flower without central disc variant [Plate 4]**

The flower without central disc variants were induced in the treatment 0.5% and 1.5% colchicine. The high frequency for the variant was noticed in 1.5% colchicine (0.86%) and the low in 0.5% colchicine (0.15%). Variants resulted in flowers without central disc with different colour as orange and yellow showing both small and large size of flower. The flower without central disc variants was earlier reported by Rathod (2017) in white marigold at 0.5% colchicine concentration.

**Small flower variant [Plate 3]**

The small flower variant were observed in the treatment 0.5, 1.0, 1.5 and2.0% colchicine. The highest frequency for the variant was noticed in 2.0% colchicine (3.12%) and the lowest in 0.5% colchicine (1.24%).

Variants showed small flower with different colour as orange and yellow. The small flower variants were earlier reported by Rathod (2017) in white marigold at 0.5%, 1.5% and 2.5% colchicine concentration.

**Large flower variant [Plate 3]**

The large flower variants were observed in the treatment 0.5, 1.0, 1.5 and2.0% colchicine. The highest frequency for the variant was noticed in 2.0% colchicine (2.56%) and the lowest in 0.5% colchicine (0.51%). Variants showed large flower with different colours as orange and yellow with different structure. The large flower variants were earlier reported by Rathod (2017) in white marigold at 0.5% and 2.5% colchicine concentration.

**Profuse branched variant [Plate 1]**

The profuse branched variants were isolated from all the treatments. The highest frequency for the character was observed in 2.0% colchicine (3.41%) and the lowest in 0.5% colchicine (0.82%). Variants showed increased height and more number of branches (6 to 9 branches) as compared to their respective control (5 branches).The profuse branched variants were earlier reported by Rathod (2017) in white marigold at 0.5% colchicine concentration.

**Single stem variant [Plate 2]**

The variant plant with single stem was isolated from all the treatments. The highest frequency for the character was observed in 2.0% colchicine (3.12%) and the lowest in 0.5% colchicine (0.66%). Variants showed plant with single stem without any branch but which increased height. The single stem variants were earlier reported by Rathod (2017) in white marigold at 1.0% colchicine concentration.

**Variation frequency in $C_2$ generation**

Variation frequency of each visible variant in $C_2$ generation was calculated as suggested by Gaul (1958) and is represented in table 2. The

table revealed that the treatment 2.0% colchicine induced the highest variation frequency (24.36%) followed by $T_3$-1.5% colchicine (16.96%), $T_5$- 2.5% colchicine (14.81%), $T_2$– 1.0% colchicine (14.26%) and the lowest in $T_1$-1.0% colchicine (12.59%). The frequency of variation was comparable in all the treatments.Similar to this result Thayyil *et al.,* (2016) observed the variation frequency in watermelon and also found that spectrum and frequency increased by colchicine.Mensah *et al.,* (2007) observed high frequencies of variation due to colchicine treatments.

## Treatment efficiency and effectiveness

The efficiency and effectiveness of variants were estimated as suggested by Konzak *et al.,* (1965) and are presented in table 2. It is noticed from the data that 2.5% colchicine exhibited the highest treatment efficiency (1.48), while 1.5% colchicine (1.04) showed the lowest. It was observed that the treatment efficiency increased in high doses and decreased in low doses of colchicine. Among the treatments the highest treatment effectiveness was observed in $T_1$- 0.5% colchicine (0.08) followed by $T_2$- 1.0% colchicine (0.05), $T_4$– 2.0% colchicine (0.04) and $T_3$– 1.5% colchicine (0.03) while the lowest was noticed in $T_5$– 2.5% colchicine (0.02). Further it was noticed that the treatment effectiveness reduced with the increase in the dose of colchicine.

In accordance to the above result Niu *et al.,* (2016) also reported increase ineffectiveness and efficiency in low concentration and decrease in high concentration level in *Jatropha curcas*. Roychowdhury and Tah (2011) also reported that effectiveness and efficiency reduced with the increase in concentration / dose of colchicine in carnation. The results obtained in this study revealed that the variation frequency and treatment effectiveness and treatment efficiency decreased with the increase in doses of colchicine. Among the different treatments studied 0.5% colchicine was found to be more effective as this treatment gave maximum number of variants. From the $C_2$ population of five different treatments 318 desirable and superior variant single plants were identified. All these variant plants were suggested to be forwarded to $C_3$ generation as progeny rows till homozygosity is reached and after attainment of homozygosity superior progeny should be evaluated in yield trails.

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