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Arati D Rathod
P.G. Students, Agril Botany Section, College of Agriculture, Nagpur, India

Shanti R Patil
Professor (CAS), Agril Botany Section, College of Agriculture, Nagpur, India

GW Karad
P.G. Students, Agril Botany Section, College of Agriculture, Nagpur, India

Duppala Manoj Kumar
P.G. Students, Agril Botany Section, College of Agriculture, Nagpur, India

Shubhangi K Maraskole
P.G. Students, Agril Botany Section, College of Agriculture, Nagpur, India

Correspondence
Shanti R Patil
Professor (CAS), Agril Botany Section, College of Agriculture, Nagpur, India

Effect of colchicine on cytological traits in African marigold

Arati D Rathod, Shanti R Patil, GW Karad, Duppala Manoj Kumar and Shubhangi K Maraskole

Abstract
An experiment on effect of colchicine on polyploidy induction in white marigold was conducted in CRD with six treatments and five replications during year 2016-2017. The seeds of 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 germinated in protray. Thirty days old seedlings were transplanted in the field with spacing of 45 cm × 30 cm in ridges and furrow. Observations on cytological traits were recorded on each and every plant in each treatment and the data were subjected to statistically analysed. These effects on seedling growth were most evident at the higher colchicine concentrations (1.5 to 2.5 %). Effect of colchicine treatment on different cytological traits showed significant variation among the different colchicine treatments. Increase in the concentration of colchicine increased guard cell length and width while decrease the pollen grain size and pollen grain frequency per mm^2.
This study lead to the conclusion that seeds treated with 0.5 % colchicine for 12 hrs. was optimum for inducing variation in marigold, and stomatal frequency, length and breadth of stomata and guard cells and pollen grain diameter were the good indicator for identifying variants / polyploid in marigold.

Keywords: Marigold, colchicine, variation

Introduction
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 Sused for colouring food stuffs. Purified extract of marigold petals containing lutein dipalmitate is marketed as an ophthalmologic agent under the name adaptinol.
Marigold is widely cultivated as bedding plant in landscape design. Beside the pristine uses as loose flower and the bedding plants, marigold occupies anthelmintic, analgesic, anti-inflammatory, aromatic, bronchodilatory, digestive, diuretic, emmenagogue, sedative and stomachic properties. It is also widely used as perfumes, herbal, gulal, organic manure, anticarcinogenic agent, antioxidant in retinotherapy and for oil extraction. The floral extract of marigold is used for treating eye diseases and ulcers. Flowers are important for their economic use as well as aesthetic value. Among the flowers grown by farmers, marigold has its own importance. It has gained popularity among flower growers because of its easy cultivation and wide adaptability. The growers are attracted towards marigold flower as it has a habit of free flowering, short duration to produce marketable flowers of attractive colours having good keeping quality.
Total area under marigold crop in India during the year 2014-2015 was 56.04 thousand ha. with the production of 497.59 thousand metric tonnes of loose flowers and 4.28 lakhs number of cut flowers (Anonnymous, 2015). Flower characters of marigold are generally one of the important aspects that acquire a tremendous attention from morphological breeders and growers. Those of undesired flower characters could possibly be improved by polyploidization. According to Azmi*et al*. (2016) perfection of flower in the floriculture plants have probably been achieved by polyploidy. In recent times, polyploidy program had been used to bring about variation in chromosome number, in order to fill up the needs of the industry. Polyploidy can increase genetic variability and improve flower characteristics,

therefore, floriculture is probably the most benefited from this techniques. Those of several previous studies have been showed valuable role of polyploidy in marigold and various crops improvement.

Keeping in mind the above views, this study was planned in white marigold (*Tagetus erecta*, 2n = 2x = 24) using colchicines with the objective of creating more genetic variability,high yield and novel flower characters.

Materials and Methods

The seeds of diploid white marigold were collected from Horticulture section of College of Agriculture, Nagpur. Before sowing the seeds were soaked in water for 12 hr and after that water soaked seeds were treated with different concentration of colchicine for 12 hr. The seeds at the rate of 50 per treatment were soaked in aqueous solution of colchicine with a concentration of 0.00 (control), 0.5, 1.0, 1.5, 2.0 and 2.5 per cent w/v at room temperature (25º C) for 12 hrs. Five replications of 10 seeds for each treatment were planted in the holes of protray filled with potting mixture of coco pit and vermicompost which was then gently covered with the 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 transplanted in the field with 45 × 30 cm^2 spacing in ridges and furrow. The experiment was conducted in CRD design with five replications. The data were subjected to statistical analysis as per the method given by Pansen and Sukhatme 1954. Per cent data were subjected to arcsine transformation before analysing the data.

Observation on cytological traits viz., stomatal length and width, guard cell length and width, stomatal frequency, pollen grain frequency and pollen grain size were recorded on each and every plant in each treatment in each replication. The data recorded were analysed statistically by following CRD design as described by Panse and Sukhatme (1954). Per cent data were subjected to arcsine transformation before analysing the data.

Results and Discussion

Cytological observations

The cytological observations on traits related to stomata, guard cell and pollen grain were recorded. The F test was found to be significant for all the seven traits. Observation from table 1 showed significant treatment mean squares for seven cytological traits studied ie, stomatal length and width, guard cell length and width, stomatal frequency, pollen grain frequency and pollen grain size. This indicates prevalence of significant variation among the treatments for these traits.

The data related to stomatal traits revealed that untreated control exhibited maximum stomatal frequency and least stomatal length and width at all the stages of observation. Colchicine treatment has decreased the stomatal frequency and increased the stomatal length and width. This reveals that variation in the stomatal traits due to colchicine treatments acts as an indicator for the variation induced through colchiploidy. The observed lower frequency of stomata studied from the colchiplants than the untreated control is due to the fact that the length and breadth rate of the treated plants are bigger in size than the untreated control. This indicated that induction of colchiploidy could be done successfully.

Similar to this result Raghunath*et al*. (2014) observed lower frequency of stomata from the leaves of 5 ppm colchicine treated plant than the leaves of untreated control. They also reported that the stomatal dimension (length and breadth) of colchicine treated plants appeared to be greater than the untreated control in African marigold. Liu *et al*. (2007), based on the results in *Platanusacerifolia* reported that an initial screening on the basis of stomata size can be effective in identifying putative polyploids. Mohammad *et al*. (2011) in *Salvia hains* demonstrated from their result that stomatal characteristics were important indicators for determination of ploidy level. They further reported that diploid plants rather than tetraploids had stomata with smaller diameter and increased frequency of stomata per unit leaf area.

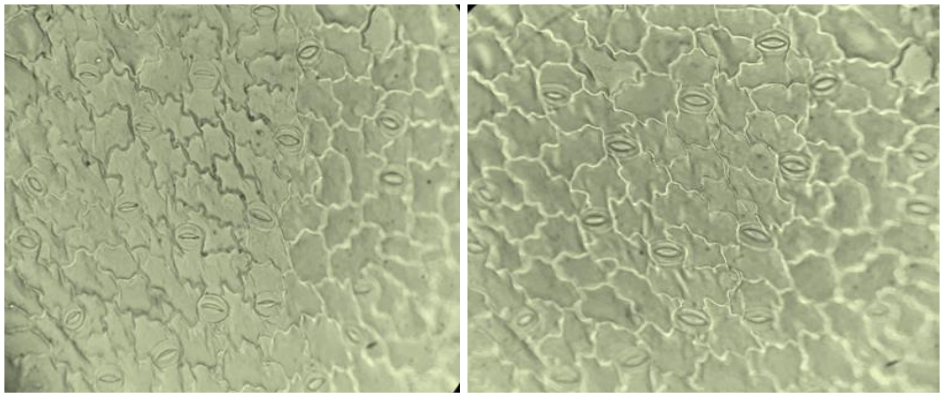
The results on guard cell length and width measured at three stages revealed that colchicine treatment have increased the size of guard cell in terms of both length and width. Increase in size was observed to be more at lower concentration of colchicine as compared to higher concentration.

In accordance to this study Dario and Paul (2009) reported that guard cell length increased and were larger in colchicine treated plants in *Vaccininiumdarrowii* and hence were an efficient way to screen for colchiploid changes after colchicine treatment. Increase in stomatal guard cell length with doubled ploidy level has also been observed in African marigold by Raghunath*et al*. (2014). Mohammad *et al*. (2011) also reported larger guard cells in colchiploid plants than the untreated plants in Salvia hains.

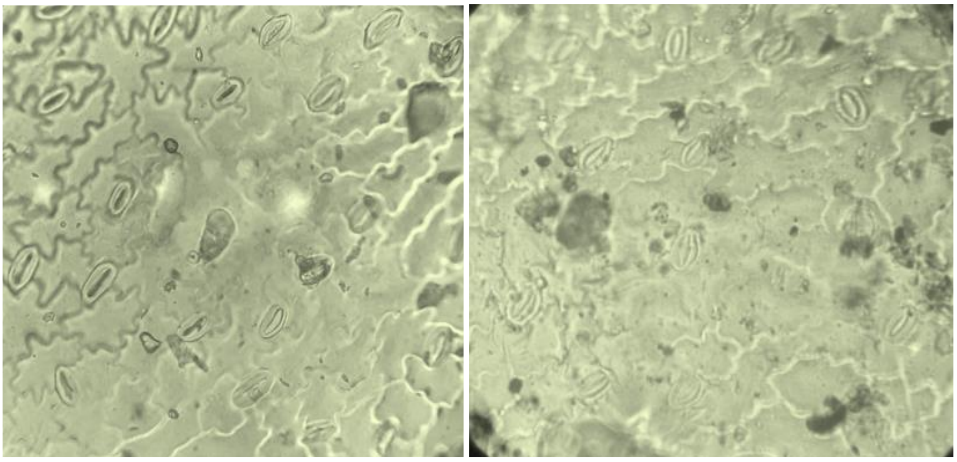
The results on pollen traits revealed that colchicine treatment have decreased the pollen grain frequency and increased the pollen grain size. In consistent to this observation Dario and Paul (2009) in *Vacciniumdarrowii* reported that pollen diameter were larger in some colchicine treated plants and guard cell length along with pollen diameter measurement were an efficient way to screen for polyploidy changes after colchicine treatment, Ravandi*et al*. (2013) also observed that in *Chicoriumintybus* L. diploid plant produced flower with pollen grain smaller in size than those of tetraploids. Mohammad *et al.* (2011) also reported larger pollen size in colchiploid plants in *Salvia hains*.

Table 1: Effect of colchicine treatments on different cytological traits

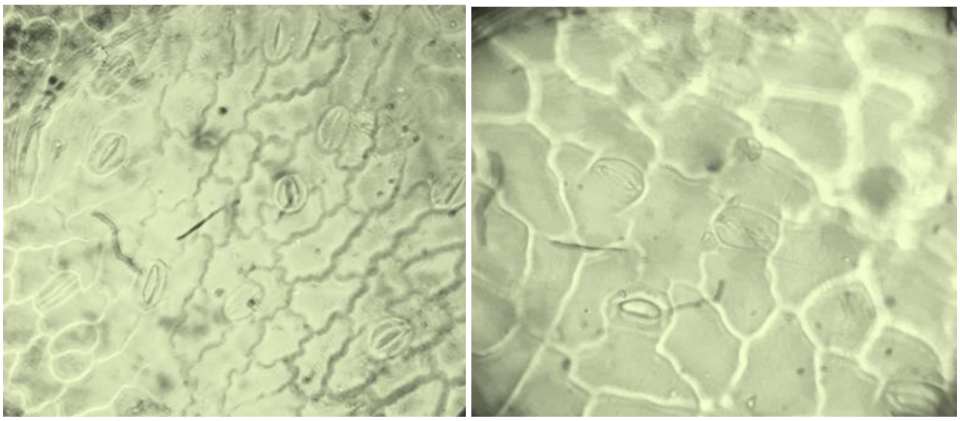
Tr.No	Colchicine concentrations	Stomatalfrquency per mm^2	Stomatal length (µm)	Stomatal width (µm)	Guard cell length (µm)	Guard cell width (µm)	Pollen grain frequency per mm^2	Pollen grain size (µm)
T_0	0.0 %(Control)	109.62	17.35	9.22	17.95	12.55	74.07	29.59
T_1	0.5 %	72.89	22.80	16.21	25.22	27.16	60.22	43.17
T_2	1.0 %	84.44	21.34	15.52	23.28	22.31	43.70	41.71
T_3	1.5 %	65.92	20.37	12.13	20.37	20.37	39.25	34.44
T_4	2.0 %	53.33	18.75	11.16	19.40	13.21	34.81	33.95
T_5	2.5 %	46.66	18.63	10.67	18.92	13.10	25.18	31.41
	SE (m) ±	5.59	0.81	1.18	1.56	1.56	4.40	1.78
	CD (5%)	16.18	2.34	3.41	4.53	4.51	12.72	5.15
	CV (5%)	17.34	9.12	21.14	16.98	19.26	21.29	11.15



T0 Control (0.0 %) **T1 (0.5 %)**

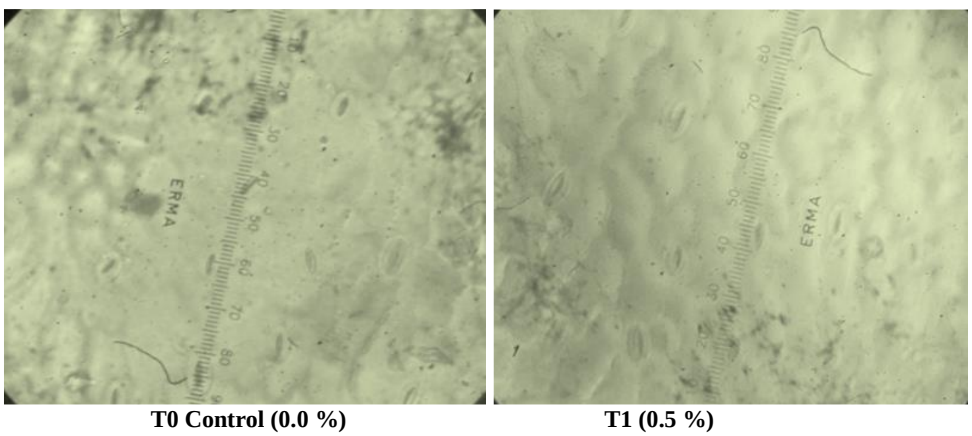


T2 (1.0 %) **T3 (1.5 %)**

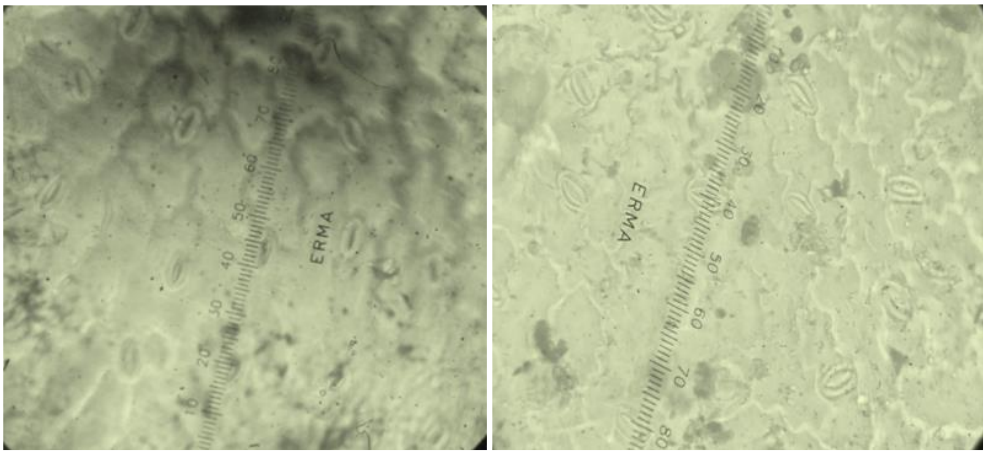


T4 (2.0 %) **T5 (2.5 %)**

Plate 1: Stomatal frequency in different colchicine treatments



T0 Control (0.0 %) **T1 (0.5 %)**



T2 (1.0 %) **T3 (1.5 %)**

Plate 2: Stomatal size in different colchicine treatments

References

1. Anonnymous. Area, production and productivity of flowers. Ministry of Agriculture and farmers welfare, Gov. of India, 2015. http://www.mafw.gov.in
2. Azmi TKK, Sukma D, Aziz SA, Syukur M. Polyploidy induction of Moth Orchid (*Phalaenopsisamabilis* (L.)Blume) by colchicine treatment on pollinated flowers. J Agri. Sci. 2016; 11(2):62-73.
3. Dario JC, Paul ML. Production and identification of colchicine-derived tetraploid*Vacciniumdarrowii* and its use in breeding. J Amer. Soc. Hort. Sci. 2009; 134(3):356-363.
4. Liu G. Colchicine-induced chromosome doubling in *Platanusacerifolia* and its effect on plant morphology. Euphytica. 2007; 157:145-154.
5. Mohammad SH, Meftahizade GH, Lotfi N, Rahimiand V, Baniasadi B. Doubling the chromosome number of *Salvia hains* using colchicine: evaluation of morphological traits of recovered plants. J Med. Plants Res. 2011; 5(19):4892-4898.
6. Panse VG, Sukhatme PV. Statistical methods for Agricultural workers, I.C.A.R. Publication, New Delhi.2nd Edn. 1954, 63-66.
7. Raghunath S, Ganguly A, Singh PK, Sarkar HK. Study of induced polyploidy in African marigold (*Tageteserecta* L.). Environ and Ecol. 2014; 32(4):1219-1222.
8. Ravandi EG, Rezanejad F, Zolala J, Dehghan E. The effect of chromosome-doubling on selected morphological and phytochemical characteristics of *Cichoriumintybus* L. J Hort. Sci. and Biotech. 2013; 88(6):701-709.