J. Soils and Crops 27 (2) 30-35, Dec. 2017

ISSN 0971-2836
NAAS Rating - 4.46
Scientific Journal Impact Factor - 4.338

EFFICIANCY OF COLCHICINE TREATMENT METHODS FOR INDUCING VARIATION IN AFRICAN MARIGOLD

Shanti R.Patil[1], Arathi Rathod[2] , M.M.Wakode[3], Vandana B.Kalamkar[4] and Priyanka Taksande[5]

ABSTRACT

Colchicine at 0.0%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5% was used for induction of variation through polyploidy in marigold by two methods *viz.,* seed treatment and root treatment. The seedling obtained after treatment by both the methods were transplanted in the field in CRD replicated five times with a spacing of 45 cm x 30 cm in ridges and furrow during 2016 at the experimental farm of Botany Section, College of Agriculture, Nagpur. Observations related to stomata, guard cell and pollen grain along with survival rate and morphological observations were recorded in each and every plant. Significant variation was observed between seed treatment method and root treatment method for all the traits studied. In seed treatment method colchiploid plants survived in all the concentrations, but in root treatment method colchiploid plants survived only upto 1.5% concentrations. The plants were not able to withstand the higher concentration of colchicines when roots were treated. The traits related to stomata, guard cell and pollen grain served as useful indicator for induction of variation through polyploidy. Frequency of stomata and pollen grain decreased with the increase in concentration of colchicines. On the contrary the size of stomata and guard cell in terms of length and breadth and pollen grain size on terms of diameter increased with the increase in concentration. In addition to stomatal, guard cell and pollen grain changes colchiploid plants as expected were having larger and thick leaves with deep green pigmentation as compared to the untreated control. Maximum number of types of variation were recorded in 0.5% colchicine in both seed and root treatment methods. Seed treatment method was found to be more efficient than root treatment method in inducing variation through polyploidy. Seeds soaked in colchicine solution at 0.5% concentration for 12 hrs was found most effective in inducing large number of variants types. The colchicploid treated variant marigold plants scored on the basis of stomatal, guard cell and pollen grain traits were labeled and harvested separately for further confirmation and evaluation.

(Key words : Colchicine, seed treatment, root treatment, variation)

INTRODUCTION

Marigold (*Tagetes* sp.) is one of the most common grown flowers in India and used extensively on religious and social functions in different forms. Because of ease in cultivation, wide adaptability to varying soil and climatic conditions, long duration of flowering and attractively coloured, long duration of flowers endowed with excellent keeping quality, the marigolds are one of the most popular flowers 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 high-lights, floral heads are typically 0.1 to 4-6 cm diameter, generally with both ray and disc florets. Marigold in general tends to be planted as annuals, although the perennial varieties are gaining popularity. The method of inducing polyploids in plants artificially through the application of colchicine was first demonstrated by Derman (1938) in *Rhoeo.* Still it is the frequently used method of increasing the chromosome number of plant. Colchicine is a poisonous chemical ($C_{22}H_{23}O_6N$) isolated from the seeds and bulbs of autumn crocus (*Colchicum autumnale*). Colchicine can also be isolated from different species of *Gloriosa* (Bharthi *et al.,* 2006 and Sarkar *et al.,*2011). It is readily soluble in water, alcohol and chloroform. Polyploidy in most cases is associated with gigantism in different plant organs like leaves, flower, fruits and stomata.

In horticulture, the induction of polyploidy 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 larger reproductive and vegetative organs) enhanced enzymatic activity, prolonged flowering time, no seed (or fewer seeds), as well as increased pest resistance and stress tolerance (Dhooghe *et al.,* 2009). In this study an attempt was to find the efficient method of inducing variation through colchicine treatment.

1. Professor (CAS), Agril. Botany Section, College of Agriculture, Nagpur
2. and 5. P.G.Students, Agril. Botany Section, College of Agriculture, Nagpur
3. and 4. Sr.Res. Assistants, Agril. Botany Section, College of Agriculture, Nagpur

MATERIALS AND METHODS

Two procedures were followed for treatment with colchicine such as absorption of colchicine through roots in which the seeds of diploid white marigold were sown in protray filled with potting mixture of coco pit and vermicompost. Five replications of 10 seeds for each treatment were planted in holes of protray. Then trays were kept in shade for 7 to 10 days. Proper care was taken during this period. After 7-10 days the plant grew up to 6-10 cm height and these were tagged and washed well in running water. Colchicine solution of 0.0%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5% concentrations were taken and poured in the test tubes. The plants after washing were kept in the colchicines in tubes for 6 hours. After hardening those plants were transferred to the field. In the second method absorption of colchicines was through seeds. Before sowing the seeds were soaked in water for 12 hrs. and after that water soaked seeds were treated with different concentrations of colchicines (0.0%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5% w/v) for 12hrs. at room temperature (25^0C) . 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 selected for transplanting.

Seedlings were transplanted in the field with a spacing of 45 cm x 30 cm in ridges and furrow with ten plants in each row in the experimental farm of Botany Section, College of Agriculture, Nagpur during the year 2016 in five replications in CRD. Light irrigation was given immediately after transplanting. The plants were time to time supplemented with nutrient along with RDF for the proper growth and development of flower bud. Observations on stomatal frequency, stomatal length, stomatal width, guard cell length, guard cell width, pollen grain frequency and pollen grain size were recorded on each and every plant in each treatment in each replication along with survival rate and morphological variations. The mean value of plants from each treatment were taken to represent the treatment values of each replication. The data were subjected to statistical analysis by following the method described by Panse and Sukhatme (1954).

Method of study of stomata and guard cell: Three leaves were taken from each observational plant one each from top, middle and bottom. The upper side of leaf was smeared with thin coat of adhesive. As it dries, it confirms to the shape of the surface of the leaf. Then the adhesive was peeled away, which now contained a perfect imprint of every stomata on the surface of the leaf. The peel was then placed on different microscope slides and covered with cover slips. The slide was then observed under microscope at 45x X 15x magnification for recording stomatal and guard cell related traits. The ocular meter was calibrated with stage micrometer before recording observations.

Method of study of pollen grain: Fully opened flower was taken to measure the pollen grain frequency. Pollen grains were dehisced with the help of thumbnail and transferred on the slide. Pollen grain was stained with 1% acetocarmine solution and covered with cover slip. The slide was then observed with the magnification of 45x X 15x for recording pollen grain related traits. Occular meter was calibrated with stage micrometer before recording observations.

RESULTS AND DISCUSSION

The conventional method of ploidy determination is done by counting of chromosomes of meristematic tissues (root tip cells), pollen or ovule before meiosis is completed. However, such cytological determination requires trained skills and technique. Chloroplast number in the pair of stomatal guard cell (stomata) is an alternative method of ploidy determination. A positive correlation between the number of chloroplast in the guard cells and ploidy level exists in many plants (Butterfass, 1973). However, chloroplasts in the guard cells can be viewed with or without staining using bright field, phase contrast or fluorescence microscope. Stomatal frequency, stomatal dimension, guard cell dimension, pollen grain frequency and pollen grain size have been commonly used as the alternative method for the determination of ploidy in plants (Borrino and Powel,1988; Setter *et al.,* 1978; Dunstone *et al*., 1973; Chandler and Lyrene,1982; Ho *et al*., 1990; Bamberg and Hanneman, 1991; Sajjid *et al*., 2013 and Sadhukhan *et al*.,2014).

Therefore, in this study the cytological observation on traits related to stomata, guard cell and pollen grain were recorded along with survival rate and morphological observations. The F test was significant for stomatal length and width, guard cell length and width, stomatal frequency, pollen grain frequency and pollen grain size in seed treatment method (Table 1 & 2). The data of root treatment were not statistically analysed as plants were not available in all the treatments. This indicates prevalence of significant variation among the treatments for these traits. Significant variation among the different colchicine treatments for the above mention cytological traits were also reported by Raghunath *et al*. (2014) in African marigold, Liu *et al.* (2007) in *Platanous acerifolia,* Mohammad *et al.* (2011) in *Salvia hains.*

Survival rate (%)

Data regarding the effect of colchicine treatment on survival rate are presented in table 1. Highest survival rate was observed in control (57.5 %) followed by 0.5 % colchicine treatment (35.3 %) and 1 % colchicine treatment (28.8 %). The least survival rate was observed in 2.5 % colchicine (6 %) in seed treatment method. Similarly survival rate was maximum in control (37.83%), followed by 0.5% colchicine (27.27%) and 1.0% colchicine (20.83%) in root

treatment method. The results indicated that increase in the concentration leads to decrease in survival rate in both the methods of treatment. But in root treatment the colchiploid plants were not able to survive in 2.0 and 2.5% colchicines. These findings were in consistent with the finding of Jiranapapan *et al.* (2011) in *Torenia fournieri*, Mohammad *et al*. (2011) in *Salvia hains* and Liu *et al*. (2007) in *Platanus acerifolia,* who reported that the survival rate of colchicine treated shoots were lower, with the extent of the reduction depending on the colchicine concentration. Decrease in survival rate with increase in concentration of colchicine may be due to the cause of tissue necrosis when soaked in different concentrations of colchicine solution. This is because colchicine does not only have an effect on cell division but spreads through the cell, interfering with cellular mechanism and causing toxicity at high concentration as reported by Sasiree *et al.* (2013). Colchicine apparently affect the viscosity of cytoplasm so the cell can not function normally.

Traits related to stomata

Stomatal length and frequency have proved to be reliable indication of ploidy in a number of species and the measurement is simple, largely non-destructive and does not require specialised equipment. Therefore, following colchicine application to seed and root we screened the surviving plants for stomatal size and frequency. Data regarding stomatal frequency per mm^2, stomatal length (µm) and stomatal width (µm) showed significant variation between seed and root treatments and also within seed and root treatments (Table 1). The effect of colchicine on stomatal frequency were higher in colchiploid plants and tends to be low than that of untreated plants both in seed and root treatments. The range of stomatal frequency was 42.22 % (2.5 % colchicine) to 108.63 % (control) in seed treatment method and from 32.66 % (1.5% colchicines) to 66.63 % (Control) in root treatment method. Stomatal frequency was highest in control in both the methods and this was followed by 0.5 % colchicine and 1 % colchicines. It is observed that increase in concentration of colchicine have decreased stomatal frequency. The range of stomatal length was 14.51 µm (control) to 20.05 µm (0.5 % colchicine) in seed treatment method and 14.33 µm (control) to 18.64 µm (0.5 % colchicine) in root treatment method. In both the methods of colchicines treatments 0.5 % colchicine showed maximum stomatal length followed by 1 % colchicine. The range of stomatal width was 7.92 µm (control) to 15.75 µm (0.5 % colchicine) in seed treatment method and from 7.89 µm (control) to 14.98 µm (0.5 % colchicine) in root treatment method. The trend of observations in stomatal length was similar to that observed in stomatal width. Maximum width of stomatal was observed in 0.5 % colchicine followed by 1 % colchicines in both the methods. The data related to stomatal traits revealed that untreated control exhibited maximum stomatal frequency and least stomatal length and in both the methods of cochicine treatment. 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 plant appeared to be greater than the untreated control in African marigold. Liu *et al*. (2007), based on the results in *Platanus acerifolia* 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 $unit^{-1}$ leaf area.

Guard cell length and width (µm)

Like stomatal trait guard cell dimension is also closely connected with the induction of polyploidy. Data regarding guard cell length and width are given in table 2. Guard cell dimensions were recorded in colchiploid plants obtained from both seed and root treatment methods. Significant variation between seed and root treatments and also within seed and root treatment were observed. The range of guard cell length was 17.09 µm (control) to 24.09 µm (0.5 % colchicine) and 17.89 µm (control) to 24.56 µm (0.5 % colchicine) in seed and root treatment methods respectively. In both the methods 0.5 % colchicine showed maximum guard cell length followed by 1 % colchicine. The range of guard cell width was 10.93 µm (control) to 22.64 µm (0.5 % colchicine) and 10.56 µm (control) to 22.79 (0.5 % colchicine) in seed and root treatment methods respectively. The trend of observation in guard cell width was similar to that observed in guard cell length. Maximum width of guard cell was observed in 0.5 % colchicine in both the methods of colchicine treatment and was followed by 1 % colchicine. The results on guard cell length and width measured in two methods of seed treatment 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 *Vaccininium darrowii* 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.*

Pollen grain traits

Pollen grain traits have proved to be reliable indication of polyploidy in a number of species and the measurement is simple, largely non-destructive and does not required specialised equipment. Therefore, following *in vivo* colchicine application to seed and root we screened the surviving plants for pollen grain frequency per mm^2 and size (µm). Data regarding pollen grain frequency and size showed significant variation between seed and root treatments and also within seed and root treatments and are presented in table 2. The effect of colchicine on pollen grain frequency were higher in colchiploid plants and tends to be low than that of untreated plants. Frequency was highest in control (74.07 %) followed by 0.5 % colchicine (60.22 %) in seed treatment method and the treatment 2.5 % colchicine (25.18 %) showed minimum pollen grain frequency. In root treatment method pollen frequency was highest in control (57.40 %) followed by 0.5 % colchicine (46.66 %) in root treatment method and the treatment 1.5 % colchicine (20.44 %) showed minimum pollen grain frequency. It is observed that increase in concentration of colchicine have decreased pollen grain frequency in the descending manner. The pollen grain size (µm) ranged from 29.59 µm (control) to 43.17 µm (0.5 % colchicine) in seed treatment method and from 27.43µm (control) to 34.92 µm (0.5 % colchicine) in root treatment method. The treatment 0.5 % and 1 % colchicine showed maximum size of pollen grain in both the methods of treatment. Colchicine treatments were found to increase the size of pollen grain.

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 *Vaccinium darrowii* reported that pollen diameters 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 *Chicoriumin tybus* (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.*

Morphological observation

The aim of this research work was also to induce variation through colchicine treatment and select some useful variant which can be stabilised and used for developing new variety. With this aim two methods (seed and root) of colchicines treatment with 0.0 (control), 0.5, 1.0, 1.5, 2.0 and 2.5 per cent w/v colchicine concentrations were used for this study. The plants were regularly observed from transplanting to harvesting for recording morphological variations. Major morphological and growth habit characteristics observed in seedlings or plants treated with colchicine showed reduced stem elongation, growth and slower node development, relative to control seedlings, and in some cases, the first 1-2 true leaves were morphologically abnormal e.g. wrinkled, subsequent leaves appeared normal. These effects on seedling growth were most evident at the higher colchicine concentrations (1.5 to 2.5 %). In consistent with this results Liu *et al.* (2007) also reported morphological differences of the colchiploid plants which included a more compact growth habit, broader and thicker leaves in marigold.

The data recorded on the type of variants along with their frequency are depicted in table 2. Maximum number of variants of 12 types were observed in the treatment of 0.5 % colchicine followed by 1 % colchicines recording 8 types of variants. Least number of variants were recorded in 2.0 % colchicine (2 variant types) followed by 2.5 % colchicines, (4 variant types) in seed treatment method. But in root treatment method variant types were obtained only in 0.5 and 1.0% colchicine treatments recording 5 and 4 variant types respectively. All the variants identified were labelled and harvested separately. The results on morphological observation revealed that 0.5 % colchicine for 12hrs. was more effective in inducing variation followed by 1 % colchicine for 12 hrs in seed treatment method. In consistent to this result Hanzelka and Kobza (2001) reported 1- 1.5 % colchicine for 5 days to be the best treatment for variant induction in Aster. Kazi (2013) observed maximum variation with 0.2 % and 0.3 % colchicine for 12 hrs in Marigold. In *Torenia fournieri*, the most effective treatments were 5 ppm colchicine for 1 day and 15 ppm for 3 days which yielded maximum variants as reported by Jiranapapan *et al*. (2011).

It is inferred from this study that seed treatment method was more efficient than root treatment method. The optimum concentration of colchicine to induce variation was 0.5%. The colchiploid treated marigold plants scored on the basis of stomatal, guard cell and pollen grain traits requires to be confirmed from further cytological studies.

Table 1. Effect of colchicine treatment on survival rate and stomatal related traits

Treatment No.	Colchicine concentration	Survival rate (%)		Stomatal frequency mm^2		Stomatal length (µm)		Stomatal width (µm)	
		Seed treatment	Root treatment	Seed treatment	Root treatment	Seed treatment	Root treatment	Seed treatment	Root treatment
T0	0.0 % (control)	57.50	37.83	108.63	66.63	14.51	14.33	7.92	7.89
T1	0.5 %	35.30	27.27	79.75	45.56	20.05	18.64	15.75	14.98
T2	1.0 %	28.80	20.83	68.98	33.90	18.75	17.88	13.58	13.64
T3	1.5 %	11.80	6.00	56.29	32.66	17.78	16.75	10.35	10.42
T4	2.0 %	8.20	-	47.16	-	16.41	-	9.22	-
T5	2.5%	6.00	-	42.22	-	15.59	-	9.00	-
	SE±	**2.04**	**-**	**5.24**	**-**	**0.90**	**-**	**1.22**	**-**
	CD (5%)	**5.90**	**-**	**15.76**	**-**	**2.62**	**-**	**3.55**	**-**

Table 2. Effect of colchicine treatment on guard cell and pollen grain related traits and number of variant types

Treatment No.	Colchicine concentration	Guard cell length (µm)		Guard cell width (µm)		Pollen frequency		Pollen grain size (µm)		No. of variant types	
		Seed treatment	Root treatment	Seed treatment	Root treatment	Seed treatment	Root treatment	Seed treatment	Root treatment	Seed treatment	Root treatment
T0	0.0% (control	17.09	17.89	10.93	10.56	74.07	57.40	29.59	27.43	-	-
T1	0.5 %	24.09	24.56	22.64	22.79	60.22	46.66	43.17	34.92	12	5
T2	1.0 %	22.15	23.22	19.19	20.24	43.70	27.15	41.71	30.55	8	4
T3	1.5 %	20.24	20.49	16.93	16.66	39.25	20.44	34.44	30.31	7	-
T4	2.0 %	19.48	-	12.49	-	34.81	-	33.95	-	2	-
T5	2.5%	18.08	-	11.56	-	25.18	-	31.41	-	4	-
	SE±	**1.56**	**-**	**1.63**	**-**	**4.40**	**-**	**1.78**	**-**	**-**	**-**
	CD(5%)	**4.53**	**-**	**4.73**	**-**	**12.72**	**-**	**5.15**	**-**	**-**	**-**

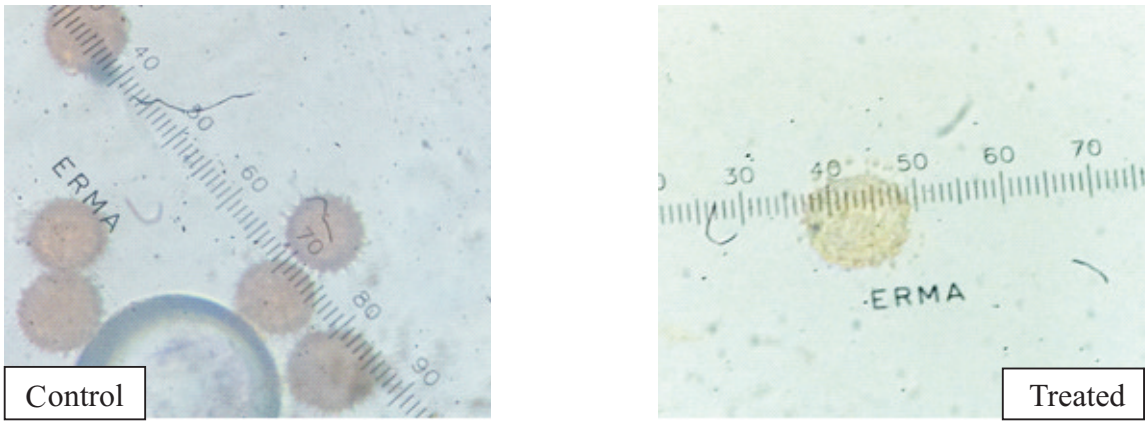

Fig. 1 Pollen frequency and size of control and

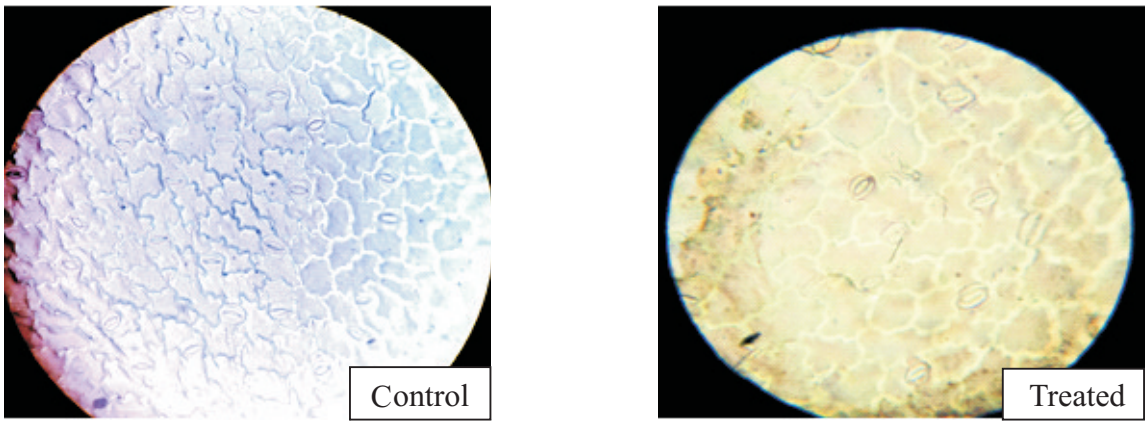

Fig. 2 Stomatal Frequency of control and treated plant

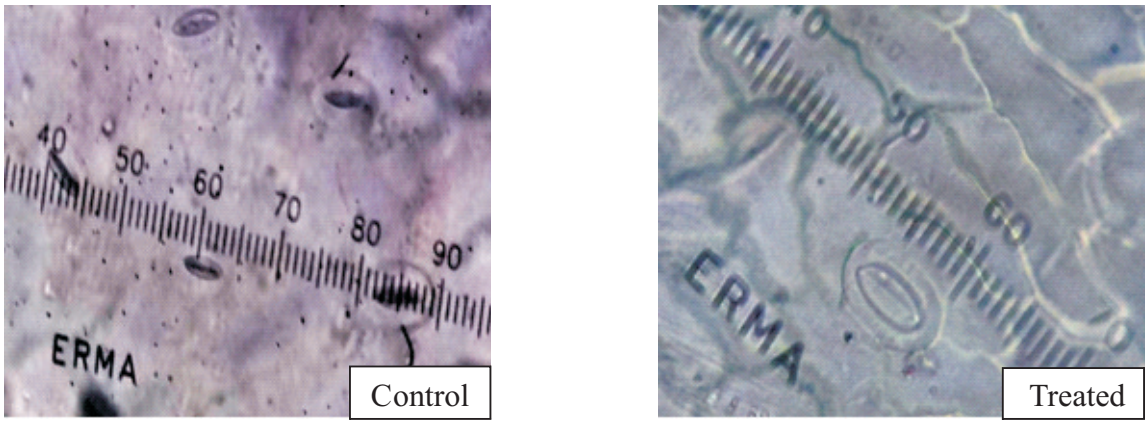

Fig. 3 Stomata size of control and treated plant

REFERENCES

Bamberg, J.B. and Jr.R.E. Hanneman, 1991.Rapid ploidy screening of tuber bearing *Solanum* (potato) species through pollen diameter measurement. Am. Potato J. **68**:279-285.

Bharthi, P., D. Philomina and Chakravarthi, 2006. Estimation of colchicine in six different species of *Gloriosa* grown *in vivo*. Ind. J.Farm.Sci. **68**: 806-809.

Borrino, E.M. and W.Powel, 1988. Stomatal guard cell length as an indicator of ploidy in 105:micro spore derived plants of barley, Genome, **30**:158-160.

Butterfass, T. 1973. Control of plastid division by means of nuclear DNA amount. Protoplasm, **76**: 167-195.

Chandler, C.K. and P.M.Lyrene, 1982. Relationship between guard cell length and ploidy in Vaccinium. Hort. Sci. **17**:53-54.

Dario, J. C. and M. L. Paul, 2009. Production and identification of colchicine-derived tetraploid *Vaccinium darrowii* and its use in breeding. J. Amer. Soc. Hort. Sci. **134**(3): 356-363.

Derman, H. 1938. A cytological analysis of polyploidy induced by colchicines and by extreames of temperature. J. Hered. 29:211.f barley. Genome, **30**: 158-160.

Dhooghe, E., W. Grunewald, L. Leus. and M. C. Van Labeke, 2009. *In Vitro* polyploidisation of *Helleborus* species. Euphytica, **165** : 89-95.

Dunstone, R.L., R.M.Gifford and L.T.Evans, 1973. Photoxynthetic characteristics of modern and primitive wheat species in relation to ontogeny and adaptation to light. Aust. J. Biol.Sci. **26**:295-307.

Hanzelka, P. and F. Kobza, 2001. Genome induced mutation in *Callistephus chinensis* and effect of colchicine application on the early plant development. Zahardnictvi Hort. Sci. **28**(1):15-20.

Ho, L. J.M.Wan, M. Widhoi and A.L.Rayburn, 1990. The use of stomatal chloroplast number of rapid determination of ploidy level in maize. Pl. Breeding , **103**:203-210.

Jiranapapan, J., S.Kikuchi, B. Manochai, T. Taychasinpitak, H. Tanaka and H. Tsujimoto, 2011. A simple method chromosome doubling using colchicines in *Torenia* (Linderniaceae), and the behavior of meiotic chromosome in amphidiploids. Chromosome Sci. **14**: 29-32.

Kazi, N.A. 2013. Effect of colchicine on growth and flowering of China aster (*Callistephus chinensis* Nees.). Eco. Env. & Cons. **19** (1): 83-86.

Liu, G. 2007. Colchicine-induced chromosome doubling in *platanus acerifolia* and its effect on plant morphology. Euphytica. **157:** 145-154

Mohammad, S. H., G. H. Meftahizade, N. Lotfi, V. Rahimi and B. Baniasadi, 2011. Doubling the chromosome number of *Salvia hains* using colchicine: evaluation of morphological traits of recovered plants. J. Medi. Plants Res. **5** (19): 4892-4898.

Panse, V. G. and P. V. Sukhatme. 1954. Statistical method for agriculture worker. I.C.A.R. publications, New Delhi. 2nd edn. pp. 63-66.

Raghunath, S. A.Ganguly, P.K.Singh and H.K.Sarkar, 2014. Study of induced polyploidy in African Marigold (*Tagetes erecta* L.). Environ. & Ecol. **32**(4): 1219-1222.

Ravandi, E.G., F.Rezanehjad, J.Zolala and E.Dehghan,2013. The effects of chromosome-doubling on selected morphological and phytochemical characterisitics of *Cichorium intybus* L. J. Hort. Sci. & Biotec, **88**(6): 701-709.

Sadhukhan, R., A. Ganguly, P. K. Singh and H. K. Sarkar, 2014. Study of induced polyploidy in African marigold (*Tagetes erecta* L.). Environ. and Ecol. **3**(4): 1219-1222.

Sasiree, B., T. Taychasinpitak, S.Wongchaochant and S.Kikuchi, 2013. Effect of colchicines tablets on morphology of *Torenia fournieri*, Int. Transaction J. of Engg. Management & Applied Sci. & Tech. **4**:299-309.

Sajjad, Y., M.J.Jaokani, A.Mohmood, I.Ahmed, and H.Abbas, 2013. Effect of colchicines on *in vitro* polyploidy induction in African marigold *(Tagetes erecta)*. Pak J. Bot. **45**: 1255-1258.

Sarkar, H.K., T. Lahri, T.K.Muity and S.Bhattacharya, 2011. Evaluation of glory lily (*Glorioso superba*) using morphological, anatomical and molecular parameters for genetic resource conservation. Environ. & Ecol. **29**: 389-392.

Setter, T.L., L.E. Schrader and E.T. Bingham, 1978. Carbon dioxide exchange rates, transpiration and leaf characteristics in genetically equivalent ploidy levels of alfalfa. Crop Sci. **18:**327-332.

Rec. on 2.05.2017 & Acc. on 20.05.2017