Pak. J. Bot., 45(3): 1255-1258, 2013.

EFFECT OF COLCHICINE ON *IN VITRO* POLYPLOIDY INDUCTION IN AFRICAN MARIGOLD (*TAGETES ERECTA*)

YASAR SAJJAD[1*], MUHAMMAD JAFAR JASKANI[1], ASIM MEHMOOD[1], IFTIKHAR AHMAD[2] AND HAIDER ABBAS[3]

[1]*Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan*
[2]*College of Agriculture, D.G. Khan Campus, University of Agriculture, Faisalabad, Pakistan*
[3]*Karachi Institute of Biotechnology and Genetic Engineering, University of Karachi, Pakistan*
[*]*Corresponding author's email: yasr_uaf@yahoo.com*

Abstract

Colchicine was used as chemical mutagen for *In vitro* induction of polyploids. Nodal segments of *In vitro* grown marigold plantlets were cultured on MS medium supplemented with four different concentrations of colchcine (0.0%, 0.001%, 0.01% and 0.05%) for 12h, separately. After treatment, nodal segment were shifted to colchicine free MS medium for normal growth. Highest mortality rate (72.74%) and restricted shoot growth (3.80 cm) was observed in 0.05% colchicine treatment. Stomatal size and number used as cytological parameters to identify polyploids. Stomatal size was increased in 0.01% (811.34 µm) and 864.70µm in 0.05% treatments while 289.30 µm was in control. The stomatal number was reduced to 9 in 0.05% colchicine treatment as compared to control (16). The polyploids showed an increase in stomatal size and decrease in stomatal number per unit area. Thus 0.01% and 0.05% treatments found effective in inducing polyploidy in African marigold.

Introduction

The ornamental flowering plants have great value in the international floral market. In Pakistan the production of flowering plants has been increased and more farmers are attracted to produce different flowers, the most important are rose (Nadeem *et al*., 2011), gladiolus (Memon *et al*., 2009) and tuberose (Ikram *et al*., 2012). Pakistan has great potential to develop its floriculture industry and can earn reasonable foreign exchange by export of flowers to Gulf countries (Nawaz *et al*., 2009).

African marigold (*Tagetes erecta* L.) is seasonal flowering plant, belongs to the family Asteraceae and is native to the South and Central Americas, especially Mexico. It has great importance for landscaping and can be used as bedding and potted plant. Its flower petals serve as a major source of Carotenoids and leaves are effective to control root-knot nematodes (Hussain *et al*., 2011) so it is being grown for flowers in addition to its medicinal value like other ornamental plants, lilies (Ozen *et al*., 2012).

Polyploidy is a prominent process and has been important in the evolutionary history of plants. Recent estimates suggest that 70% of angiosperms have experienced one or more episodes of polyploidization (Masterson, 1994; Jaskani *et al*., 1996, 2004).

Polyploidy in plants has been investigated to understand and perhaps make use of its effects since the 1930s (Stebbins, 1947). In some instances polyploidy has increased flower, seed or fruit size, increased photosynthetic or respiration rates, or increased tolerance of extreme temperatures, drought or flooding (Tal, 1980).

The manipulation of ploidy level is a valuable tool in improving crops. Out of many applicable methods, the use of chemicals to induce changes in chromosome number has been well established (Thao *et al*., 2003; Jaskani *et al*., 2005). Many inhibitors can be used to block cell cycle progression in cycling plant cells, such as the anti-mitotic drug colchicine is used to induce blocking at metaphase of mitosis. Colchicine is an alkaloid extracted from *Colchicum autumnale* L. which acts by binding to the tubulin dimmers preventing the formation of microtubules, and, consequently, spindle fibers during cell division (Petersen *et al*., 2003).). As colchicine is toxic to plants but its prolonged exposure in low dose reduces its toxic effects and enhance the production of polyploids (Vajrabhaya, 1983).

Polyploidization can help to increase the size of flowers, intensify the flower color and modify plant shape. Under *In vitro* conditions, polyploidization has been successfully applied in several ornamental plants such as Rhododendron (Vainola, 2000), Alocasia (Thao *et al*., 2003), Astragalus (Chen & Gao, 2007) and Scoparia (Escandon *et al*., 2005). During the last few decades polyploidization has become increasingly successful and polyploids of several ornamental plants have been induced (Roberts *et al*., 1990). Ploidy manipulation has been found a valuable tool in the genetic improvement of many plants.

Cytological analysis provides information for the identification of polyploids. The rapid, early and precise detection of polyploid individuals from a treated plant population is a fundamental requirement for genetic improvement programs based on chromosome duplication (Vainola, 2000).

The aim of this study was to find a suitable level of colchicine that would effectively induce maximum polyploids in African marigold.

Materials and Methods

Seeds were collected from diploid African marigold plants (*Tegetes erecta*) and washed with distilled water to remove dust particles. The seeds were sterilized in 70% alcohol for 3 minutes and after washing with autoclaved double distilled water further sterilized in 5% freshly prepared $NaHClO_3$ solution for 5 minutes followed by several washings with autoclaved double distilled water to remove residues of $NaHClO_3$ solution. These seeds were cultured on MS medium in test tubes to produce plants.

Nodal segments were excised from *In vitro* grown plantlets and inoculated in MS medium containing 4 concentrations of colchicine (0.0, 0.001, 0.01 and 0.05%) for 12 hours in order to induce polyploid variations.

After the treatment, the nodal segments were washed three times with sterilized water and then cultured on MS medium supplemented with 0.25mg/L 6-benzyl amino purine (BAP) for shoot induction. The length of multiple shoots was measured on regular basis at interval of 15 days. Finally, shoot length was measured after 50 days of culture.

The cytological features of treated plants were evaluated to screen ploypoid plants. Stomatal size and density have very important role in identification of polyploids. The stomata were isolated by removing thin layer from lower side of leaves and studied under microscope. Stomatal size of all treated plants was measured by measuring the length and width of stomata. The stomata were counted under Nikon microscope by using 40X objective.

Results and Discussion

Morality rate is very important while applying colchicine to explants for *In vitro* induction of polypoids. Explants showed stress symptoms due to colchicine but symptoms were variable depending upon colchicine treatment. Mortality rate was observed after 20 days of treatment and non-growing, brown buds were considered as dead. The mortality rate was varied from 12 to 72% depending on the dose of colchicine (Table 1). Maximum mortality rate was observed with 0.05% colchicine and only 2% in control which might be due to some other stresses. The results evaluate the effect of colchicine on mortality rate for each treatment. Each treatment showed some lethality depending on treatment concentration. The fatality of colchicine is different for different plants depending upon its concentration high mortality rate can occur with increasing concentrations of colchicine (Sanguthai *et al*., 1973). For marigold 0.05% colchicine was found as lethal treatment in our studies.

Table 1. Influence of colchicine on mortality, regeneration, shoot proliferation and shoot length.

Colchicine	Mortality	Regeneration	Shoot proliferation	Shoot length
(%)	**(%)**	**(%)**	**(%)**	**(cm)**
0.00	2 d	98 a	44 c	9.93 a
0.001	12.17 c	87.83 b	71.67 b	8.26 b
0.01	40.14 b	59.86 c	94 a	7.63 b
0.05	72.74 a	27.26 d	11.67 d	3.80 c

The effect of colchicine on regeneration of explants was assessed after 20 days of treatment and sprouted buds were considered for regeneration percentage. The Table 1 shows that regeneration rate was high in colchicine free treatment while in colchicine treatments, the regeneration rate ranged from 27.26% to 87.83% on 0.05% and 0.001% colchicine treatment, respectively. The results indicate that colchicine had direct effect on regeneration of explants. Thus there was an inverse relation between colchicine concentration and regeneration rate. These results agree with the work of Duren *et al*., (1996) who found the negative effect of high colchicine concentration on regeneration of explants. The inverse relationship between colchicine concentration and explant survival was also found on other plants (Chakarborti *et al.,* 1998).

After regeneration the buds started to grow and produced multiple shoots which were counted from each treatment. The shoot proliferation rate varied from 11.67% to 94.0% depending on colchicine concentration (Table 1). The maximum number of shoots was produced on 0.01% treatment. In comparison with control, other treatments had more multiple shoots except the 0.05% colchicine treatment. Explant tissues undergo stress due to colchicine which leads to the formation of lateral shoots. Thus induction rate of multiple shoot increased with the increase in colchicine concentration but high concentration had negative effect on shoot proliferation as in 0.05% treatment. High colchicine concentration has lethal effect which leads much stress on plant cells, causes death of explant.

In untreated explants, shoot initiation was observed within 4 days after inoculation whereas treated explants started growth within 6-9 days after their transfer to colchicine free medium. It can be seen that maximum shoot length (9.93 cm) was obtained in control and the minimum (3.80 cm) at 0.05% colchicine (Table 1). The results indicated that colchicine had adverse effect on shoot length of plant.

The growth of treated nodes was slow than that of control (Fig. 1). The concentration of colchicine was observed to have a marked influence on the growth of shoots. The shoot length decreased with the increase in colchicine concentration. Hence shoot length was high in case of control. The retarded growth was due to reduced rate of cell division that caused by colchicine. Similar observations in which shoot length decreased due to initial retardation of growth are also reported by Skidar & Jolly (1994).

The 0.01 and 0.05% treatments showed significant results for stomatal number and size than the 0.001% treatment and control (Table 2). The polyploid plants have large size stomata and less number as compared to diploid plant (Fig. 2). So the explants treated with 0.01 and 0.05% treatment showed less stomatal number as compared to control plants.

Table 2. Effect of colchicine on stomatal size and number.

Colchicine (%)	Stomatal size (µm)	Stomatal number
0.00	289.30 b	16 a
0.001	322.77 b	15 a
0.01	811.34 a	10 b
0.05	864.70 a	9 b

Fig. 1. Difference in shoot length due to colchicine treatment.

The identification of polyploids can be based on stomatal morphology (Hamill *et al.,* 1992). The size of stomata has been used to differentiate diploid and polyploid regenerants of orchids (Watrous & Wimber, 1988), daylilies (Arisumi, 1972), and rye-grass (Speckmann *et al*., 1965). The differences in stomatal number have also been used as to identify polyploids (Vandehout *et al*., 1995).

The results indicate the effectiveness of colchicine treatments significant differences between treated plants and control. On the basis of stomatal morphology only 2 marigold plants in 0.01% and 1 plant with 0.05% treatment showed higher ploidy level than diploid plants. The lowest colchicine concentration (0.001%) found to be ineffective to induce polyploidy in African marigold plant. The stomatal size inicates the ploidy level of plants as according to Chen & Gao (2007), the polyploids possessed larger stomatal size than diploids.

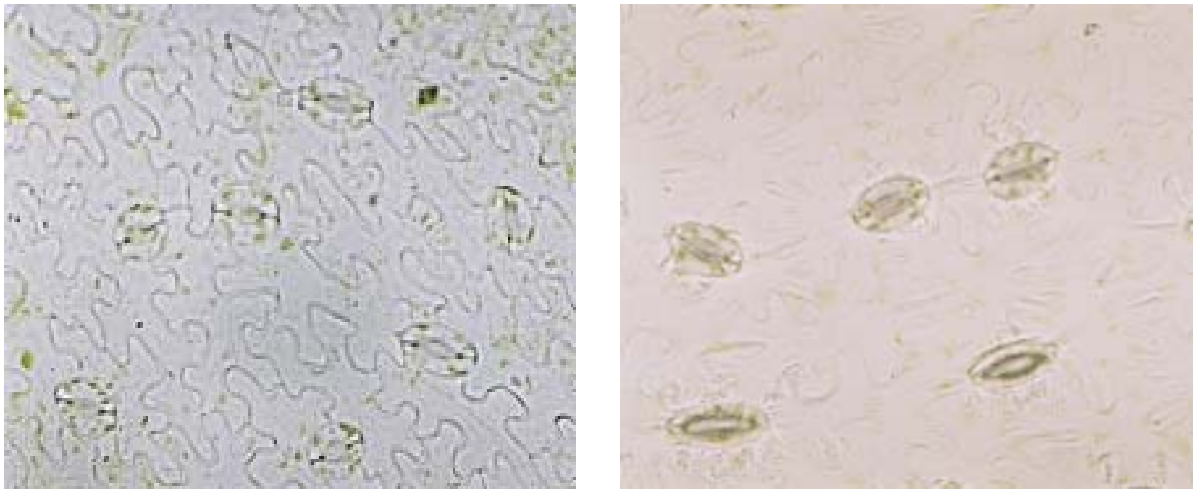

Fig. 2. Difference in stomatal size and number between diploid (a) and polyploid plants (b).

References

Arisumi, T. 1972. Stabilities of colchicine-induced tetraploid and cytochimeral Daylilies. *J. Hered*., 63: 15-18.

Chakraborti, S.P., K. Vijayan, B.N. Roy and S.M.H. Qadri. 1998. *In vitro* induction of tetraploidy in mulberry (*Morus alba* L.). *Plant Cell Rep*., 17: 799-803.

Chen, L.L. and S.L. Gao. 2007. *In vitro* tetraploid induction and generation of tetraploids from mixoploids in *Astragalus membranaceus*. *Sci. Hort*., 112: 339-344.

Duren, M.V., R. Mmorpurgo, J. Dolezel and R. Afza. 1996. Induction and verification of autotetraploids in diploid banana (*Musa acuminata*) by *In vitro* techniques. *Euphytica*, 88: 25-34.

Escandon, A.S., I. Miyajima, L.M. Alderete, J.C. Hagieara, G. Facciuto, D. Mata and S.M. Soto. Wild ornamental germplasm exploration and domestication based on biotechnological approaches. *In vitro* colchicine treatment to obtain a new cultivar of *Scoparia montevidiensis*. *Electron. J. Biotechnol*., [online]. 15 August 2005, vol. 8, no. 2 [cited 5 September 2007]. Available from: http://www.ejbiotechnology.info/content/vol8/issue2/full/2/index.html. ISSN 0717-3458.

Hamill, S.D., M.K. Smith and W.A. Dodd. 1992. *In vitro* induction of banana autotetraploidy by colchicine treatment of micropropagated diploids. *Aust. J. Bot*., 40: 887-896.

Hussain, M.A., T. Mukhtar and M.Z. Kayani. 2011. Efficacy evaluation of *Azadirachta indica, Calotropis procera, Datura stramonium* and *Tagetes erecta* against root-knot Nematodes *Meloidogyne incognita. Pak. J. Bot.,* 43: 197-204.

Ikram, S., U. Habib and N. Khalid. 2012. Effect of different potting media combinations on growth and vase life of tuberose (*Polianthes tuberosa* Linn.). *Pak. J. Agri. Sci*., 49: 121-125.

Jaskani, M.J., I.A. Khan and S. Husnain. 1996. Morphological description of citrus colchiploids. *Proc. Int. Soc. Citriculture*, 1: 130-132.

Jaskani, M.J., S.W. Kwon, G.C. Koh, Y.C. Huh and B.R. Ko. 2004. Induction and characterization of tetraploid watermelon. *J. Korean Soc. Hort. Sci*., 45: 60-65.

Jaskani, M.J., S.W. Kwon and D.H. Kim. 2005. Comparative study on vegetative, reproductive and qualitative traits of seven diploid and tetraploid watermelon lines. *Euphytica*, 145: 259-268.

Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science*, 264: 421-424.

Memon, N.N., M. Qasim, M.J. Jaskani, R. Ahmad and R. Anwar. 2009. Effect of various corm sizes on the vegetative, floral and corm yield attributes of gladiolus. *Pak. J. Agri. Sci.,* 46: 13-19.

Nadeem, M., M.A. Khan, A. Riaz and R. Ahmad. 2011. Evaluation of growth and flowering potential of rosa hybrida cultivars under Faisalabad climatic conditions. *Pak. J. Agri. Sci*., 48: 283-288.

Nawaz, A., S. Gul, M.A. Anjum and F. Naveed. 2009. Effect of various sucker sizes and planting times on growth and flower yield of chrysanthemum. *Pak. J. Agri. Sci.,* 46: 7-12.

Özen, F., H. Temeltaş and Ö. Aksoy. 2012. The anatomy and morphology of the medicinal plant, *Lilium candidum L*. (*Liliaceae*), distributed in Marmara region of Turkey. *Pak. J. Bot.,* 44: 1185-1192.

Petersen, K.K., P. Hagberg and K. Kristiansen. 2003. Colchicine and oryzalin mediated chromosome doubling in different genotypes of Miscanthus sinensis. *Plant Cell Tiss. Org. Cult*., 73: 137-146.

Roberts, A.V., D. Lloyd and K.C. Short. 1990. *In vitro* procedures for the induction of tetraploidy in a diploid rose. *Euphytica*, 49: 33-38.

Sanguthai, O., S. Sanguthai and H. Kamemoto. 1973. Chromosome doubling of a *Dendrobium* hybrid with colchicine in meristem culture. *Na Pua Okika O Hawaii Nei*, 12: 12-16.

Skidar, A.K. and M.S. Jolly. 1994. Induced polyploidy in mulberry (*Morus* spp.): induction of tetraploids. *Sericologia*, 34: 105-116.

Speckmann, G.J., J.J. Post and H. Dijkstra. 1965. The length of stomata as an indicator for polyploidy in rye-grasses. *Euphytica*, 14: 225-230.

Stebbins, G.L. 1947. Types of polyploids: Their classification and significance. *Advanced Genetics*, 1: 403-429.

Tal, M. 1980. Physiology of polyploids. In: *Polyploidy: Biological Relevance*. (Ed.): W.H. Lewis. Plenum Press, New York, pp. 61-75.

Thao, N.T.P., K. Ureshino, L. Miyajima, Y. Ozaki and H. Okubo. 2003. Induction of tetraploids in ornamental Alocasia through colchicine and oryzalin treatments. *Plant Cell Tiss. Org. Cult*., 72: 19-25.

Vainola, A. 2000. Polyploidization and early screening of Rhododendron hybrids. *Euphytica*, 112: 239-244.

Vajrabhaya, T. 1983. Variations in clonal propagation. In: *Orchid Biology: reviews and perspectives*. (Ed.): J. Arditti, Ithaca, New York: Comstock Publ. Assoc., pp. 176-201.

Vandehout, H., R. Ortiz, D. Vuylsteke, R. Swennen and K.V. Bai. 1995. Effect of polyploidy on stomatal and other quantitative traits in plantain and banana hybrids. *Euphytica*, 83: 117-122.

Watrous, S.B. and D.E. Wimber. 1988. Artificial induction of polyploidy in Paphiopedilum. *Lindleyana*, 3: 177-183.

(Received for publication 20 August 2011)