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Effect of varying concentrations of colchicine on polyploid induction in *Jasminum sambac* (L.) Aiton

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Abstract
For breeding ornamental crops, particularly those like jasmine, in which very little successful breeding has been done, biological interventions like polyploidization may accelerate the selection of novel variants. The present investigation was carried out with the objective of developing commercially viable polyploid variants of *Jasminum sambac* through colchicine treatment. Rooted cuttings of *J. sambac* Cv. Ramanathapuram Gundumalli were treated with colchicine at several concentrations, *i.e.,* 0.25%, 0.50%, 0.75%, and 1%, for 6 hours. The survival rates for each treatment were calculated 15 days after treatment (DAT), and the phenotypic and stomatal traits of the treated plants were assessed at 150 DAT. In comparison to the control, colchicine treatments resulted in decreased plant height (42.69 cm at 1%) and internodal length (3.83 cm at 1%), whereas stem girth (7.44 mm at 1%), leaf area (20.72 cm^2 at 0.5%), and number of leaves per plant (54.67 at 0.5%) had increased. Improved floral characters, including flower bud length (2.79 cm) and flower bud girth (2.75 cm), were observed in 0.5% colchicine treated plants. Thus, this study has proven that colchicine can be used to induce polyploidization in *J. sambac.* The induced polyploid plants with improved floral characters can be used to evolve new cultivars or as donors in breeding programs.

Keywords: *Jasminum*, Polyploidization, Colchicine, Variations

INTRODUCTION

J. sambac (L.) Aiton of the family Oleaceae, is one of the most important and popular traditional flowers in India. It is a tropical and subtropical evergreen shrub with erect or scandent growth that can reach 1-3 m with glabrous leaves producing attractive white sweet-scented flowers in clusters of 3-12 together at the end of branches. In addition to being a widely used garden plant and a popular loose flower, it is used to make jasmine concrete, which is used in the cosmetic and fragrance industries (Green and Miller, 2009). In India, Tamil Nadu state is the leading producer of *J. sambac* with an annual production of 128963.05 MT from the cultivated area of 12893.31 ha during the year 2021-2022 (Department of Horticulture and Plantation Crops, Government of Tamil Nadu, 2022). The flowers produced in Tamil Nadu are being exported to neighbouring countries like Sri Lanka, Singapore, Malaysia and to Middle Eastern countries.

Improvement of vegetatively propagated crops like *J. sambac* depends on the presence of genetic variation in the existing germplasm. Being a triploid species (2n = 3x = 39), the *J. sambac* Cv. Ramanathapuram Gundumalli is sterile and does not set seeds. Hence, conventional hybridization attempts with this cultivar have not

succeeded till date. The primary objective of a breeder is to create variation so that the material can be used either as a new variety or as a source for further plant breeding programmes (Ghosh, 2018).

Ploidy modification is one of the useful methods for creating genetic variation in vegetatively propagated crops like jasmine. Polyploidy breeding is an effective method for doubling the chromosome number of a species. Polyploid plants are noticeably different from or sometimes superior to their diploid counterparts in terms of their phenotypic expression as well as other economic traits. Polyploidy induction enhances ornamental characteristics, environmental tolerances, biomass, and heterosis, and restores fertility in wide hybrids. Such new variations or forms with improved plant attributes provide good material for breeding programmes and further development of cultivars (Mata, 2009).

Colchicine ($C_{22}H_{25}NO_6$) is a natural alkaloid and anti-mitotic agent extracted from the seeds and bulbs of the Autumn Crocus (*Colchicum autumnale* L.) and is used to induce polyploidy in plants. Colchicine prevents the formation of microtubules and causes discontinuation in the formation of spindle fibres during the cell division. It stops cell division at the early anaphase stage. In this way, chromosomes are duplicated without mitosis and cell wall formation (Bhattacharyya *et al.,* 2008). This results in polyploidy of the plant cells. The polyploid cells are generally larger than their diploid cells and develop into thicker tissues, resulting in large-sized plant organs. A large number of morphological effects have been obtained from induced polyploidization. The aim of the present study was to investigate the effect of different colchicine concentrations on polyploid induction in *J. sambac* and assess ploidy variations based on morphological, flowering and physiological parameters.

MATERIALS AND METHODS

Uniform sized rooted cuttings of *J. sambac* Cv. Ramanathapuram Gundumalli were grown under 50% shade net for about a month for acclimatization. Colchicine solutions of different concentrations were prepared with distilled water. For making 0.25, 0.50, 0.75, and 1.00 per cent concentrations, 25 mg, 50 mg, 75 mg, and 100 mg of colchicine were dissolved in separate glass beakers respectively in small quantities of absolute alcohol and transferred to 1000 ml measuring flasks, and distilled water was added to make up the volume. Care was taken to keep the solutions in the dark.

For polyploidy induction, plants were uprooted from polybags and washed with running tap water to remove the adhering soil particles and dust. The plants were subjected to complete submergence in the colchicine solution for 6 hours and then taken out, washed thoroughly with running tap water, and planted back in polybags with growing media. The treated plants were observed for survival rate after 15 days and morphological characters after 150 days. The experiment was conducted in non-replicated design.

The nail varnish technique was used to study the stomatal characteristics of three randomly chosen leaves of treated plants, where the varnish was applied on the abaxial surface of the leaves. A binocular light microscope (Leica Bio med) with camera and computer attachment was used for analyzing the stomatal traits, and images were assessed at 40X magnifications.

RESULTS AND DISCUSSION

In the present investigation, the survival of colchicine treated cuttings of *J. sambac* was reduced gradually with an increase in concentration (**Fig. 1**). Reduced cell division and physico-chemical disturbances in the cells, which are all indicative of the polyploidization process, may be the cause of the decreased survival. A higher level of colchicine appears to affect the cytoplasm's viscosity, disrupting normal cell activity (Boonbongkarn *et al.,* 2013) and impairing the growth of live tissues, which causes mortality (Addink, 2002). A reduction in survival percentage due to colchicine treatment at higher concentrations has been reported in *Buddleja davidii* (Eeckhaut *et al.,* 2004), *Tagetes erecta* (Nandhini *et al.,* 2019) and *Phlox drummondi* (Tiwari and Mishra, 2012).
. Anti-mitotic agents have a well-established relation in inducing early growth retardation. In the present study, plant height gradually decreased from 51.21 cm (0.25%) to 42.69 cm (1%) with an increase in the concentration of colchicine used (**Table 1**). Control recorded the maximum plant height (52.22 cm). Reduced growth could be attributed to severe hormonal imbalances that cause physiological disturbances (Behera, 1975). These findings are in agreement with the observations made in *Agastache foeniculum* L. by Talebi *et al.*(2017).

Increased stem girth ranging from 6.6-8.5 mm was observed in 1% treatment compared to the other treatments (**Table 1**). The usual tendency of polyploid plants to produce thicker stems has been reported earlier (Rose *et al.,* 2000). The internodal distance was significantly shortened in 0.75% (4.31 cm) and 1% (3.83 cm) colchicine treatments, which indicates the scope for selection of dwarf and compact variants. The genotypic characteristic that affects the amount of foliage is internodal distance. Colchicine exhibited a significant effect on leaf production, with significant increase in the mean number of leaves in 0.5% colchicine treated plants (54.67 leaves per plant) as compared to control (45.6 leaves per plant). The highest concentration decreased the production of leaves, as observed in 0.75% and 1% colchicine treated plants (**Table 2**). Colchicine doubling caused an increase in the number of leaves in *Salvia coccinea* cv. Coral Nymph (Kobayashi *et al.,* 2008), *Lilium sp*. (Balode, 2008), orchid *Dendrobium nobile* (Vichiato *et al.,* 2007), ornamental ginger

Table 1. Effect of colchicine on vegetative parameters of *J. sambac*

Colchicine Treatment	Plant height (cm)		Stem girth (mm)		Internodal length (cm)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
0%	52.22±0.93	44.2-58.7	5.90±0.06	5.4-6.3	4.88±0.24	3.1-6.5
0.25%	51.21±1.50	39.70-63.3	6.01±0.13	5.1-7.2	4.74±0.25	2.4-6.2
0.50%	50.95±2.02	38.2-64.2	6.52±0.17	5.7-7.3	4.68±0.20	3.4-6.3
0.75%	44.97±1.78	31.9-53.4	6.96±0.09	6.1-7.5	4.31±0.12	3.1-4.9
1%	42.69±1.8	28.5-49.6	7.44±0.15	6.6-8.5	3.83±0.11	3.2-4.5

Table 2. Effect of colchicine on leaf parameters of *J. sambac*

Colchicine Treatment	Number of leaves		Leaf area (cm^2)		Chlorophyll (SPAD)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
0%	45.60±2.16	25-52	15.08±0.38	12.48-18.72	40.32±1.42	28.4-48.6
0.25%	52.00±0.85	46-56	18.96±0.39	17.2-22.12	43.35±1.91	32.2-53.9
0.50%	54.67±1.12	47-62	20.72±0.64	17.24-25.46	47.78±2.79	34.5-64.2
0.75%	42.47±1.78	26-49	19.38±0.96	15.26-27.40	44.12±1.79	27.4-54.7
1%	40.47±1.46	25-47	19.26±1.07	12.84-28.14	42.84±1.47	31.4-52.4

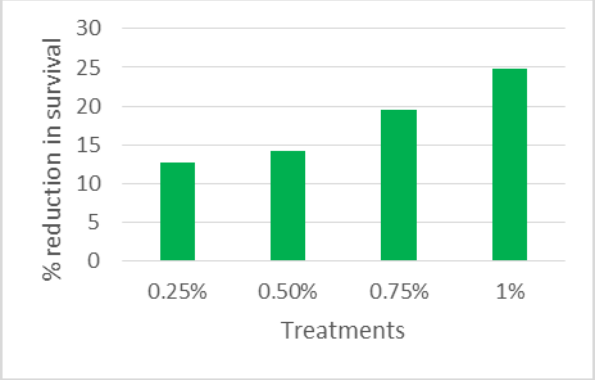


Fig. 1. Effect of colchicine on the percentage reduction in survival of treated plants

(Prabhukumar *et al.,* 2015), (Kazi, 2013) and *Calendula officinalis* (El-Nashar & Ammar, 2016).

The treatment with 0.5% colchicine had a stimulatory effect on the mean leaf area (20.72 cm^2). Polyploidy increases leaf size mainly by increasing the cell elongation rate (Sugiyama, 2005). The increase in leaf area may be attributed to enlargement in palisade and spongy layers, both increasing in length and width. However, increasing the concentration beyond 0.5% resulted in a decrease in the leaf area (**Table 2**), and few leaf abnormalities were observed in treatments 0.75% and 1% (**Fig. 2**). Varying rates of cell division coupled with physiological disruptions in treated plants are the cause of inconsistencies in leaf size and shape. Previous reports also noted increase in leaf area due to induced polyploidy in flower crops like *Impatiens balsamina* (Du *et al.,* 2011), *Torenia fournieri* (Boonbongkarn *et al.,* 2013), *Tagetes erecta* (Sadhukhan *et al.,* 2014), *Vitex agnus castus* (Ari *et al.,* 2015) and *Dendranthema grandiflora* (Lertsutthichawan *et al.,* 2017).

Chlorophyll is the most important parameter used as a physiological index to investigate growth and development. SPAD values reflect the relative content of chlorophyll. Colchicine treated plants have shown higher chlorophyll values than the control (**Table 2**), indicating higher photosynthetic capacity and activity, which in turn leads to enhanced photosynthetic rates.

In a crop like jasmine, the physical quality of flowers, *viz.* bud length, bud girth, and bud weight, are of much importance. In the present investigation, colchicine treatments substantially impacted the floral characters. The mean flower bud length (2.79 cm) and bud girth (2.75 cm) were higher in 0.5% colchicine treatment

Fig. 2. Leaf abnormalities in colchicine treated plants

than the others (**Table 3 and Fig.3.**). This, in turn, might have increased the single flower bud weight. Similarly, colchicine treatment improved floral characters in various ornamental crops, including *Gerbera jamesonii* (Gantait *et al.,* 2011), *Chrysanthemum carinatum* (Kushwah *et al.,* 2018), *Gladiolus grandiflorus* (Manzoor *et al.,* 2018), *Tagetes erecta* (Rathod *et al.,* 2018), *Torenia fournieri* (Boonbongkarn *et al.,* 2013), and *Vitex agnus castus* (Ari *et al.,* 2015).

Stomata size and density are indicative of ploidy status. In the present study, data pertaining to stomatal characters (**Table 4**) revealed that with an increase in concentration of colchicine, there was a decline in stomatal density and an increase in the size of stomata, compared to control. The lowest stomatal density (17.80 stomata mm^{-2}) compared to control (34.20 stomata mm^{-2}) was observed with 1% colchicine treatment, which might be an indication of polyploidization (**Fig. 4**). The colchicine treatment at 1% level recorded the highest mean stomatal length (30.22 µm) and breadth (23.56 µm). The lowest stomatal length (17.59 µm) and breadth (14.14 µm) were recorded in control. An increase in stomata size and reduction in stomata density might be indications of a change in ploidy. The results pertaining to stomata size obtained in this study are in agreement with those observed in *Tagetes erecta* (Sadhukhan *et al.,* 2014), *Celosia argentea* (Mostafa & Alhamd, 2016) and *Dendranthema indicum* var. *Aromaticum* (He *et al.,* 2016).

Further cytological investigations of the putative polyploids will be taken up in future to confirm the ploidy status. The confirmed polyploids with desirable traits can be utilised as improved varieties or as breeding material for crop improvement programmes.

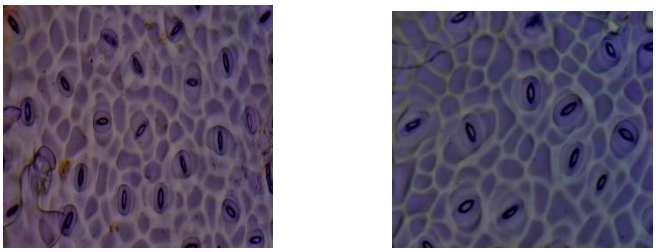
Table 3. Effect of colchicine on flower parameters of *J. sambac*

Colchicine Treatment	Flower bud length (cm)		Flower girth (cm)		Single bud weight (g)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
0%	2.47±0.06	1.9-2.8	2.27±0.07	1.9-2.7	0.16±0.01	0.12-0.22
0.25%	2.55±0.08	1.9-2.9	2.51±0.09	1.6-3.0	0.21±0.01	0.14-0.31
0.50%	2.79±0.06	2.1-3.1	2.75±0.10	1.9-3.4	0.24±0.01	0.17-0.35
0.75%	2.70±0.06	2-2.9	2.48±0.08	1.8-2.8	0.20±0.012	0.16-0.29
1%	2.45±0.07	1.8-2.9	2.29±0.05	1.9-2.6	0.18±0.01	0.14-0.28

Table 4. Effect of colchicine on stomatal parameters of *J. sambac*

Colchicine Treatment	Stomatal density (mm^{-2})		Stomatal length (µm)		Stomatal breadth (µm)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
0%	34.20±1.21	26-45	17.59±0.53	14.28-20.84	14.14±0.18	13.21-15.21
0.25%	23.80±1.22	16-31	24.07±1.24	19.18-32.34	18.54±0.31	15.64-20.32
0.50%	19.93±1.37	13-28	25.63±0.54	23.12-29.15	20.22±0.31	18.33-22.40
0.75%	18.40±1.34	12-26	28.34±0.95	21.47-34.56	22.89±0.83	16.58-27.80
1%	17.80±1.02	13-25	30.22±1.60	20.33-38.64	23.56±1.21	16.46-30.56

Fig. 3. Effect of colchicine on flower bud length observed at 150 DAT



(a) Control (b) Putative polyploid (1% colchicine)

Fig. 4. Lower epidermal peel showing stomatal density at 40 X magnification

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