# *Original Research Article*

**Potentiality of colchicine in induction of polyploids in *Jasminum sambac* (L.) Aiton cv. Ramanathapuram Gundumalli**


## ABSTRACT

The study was commenced in a compulsion to accomplish a successful approach in developing a commercially viable polyploidy variety in *Jasminum sambac*. Rooted cuttings of *J. sambac* cv. Ramanathapuram Gundumalli were treated with different concentrations of colchicine (0.05%, 0.10%, 0.15%, 0.20% and 0.25%) for 48 hours. The survival and sprouting rates for the different treatments were determined after 45 days of treatment and the phenotypic and stomatal characteristics of the treated plants were recorded. Colchicine treatments registered reduction in plant height (76.00 cm to 27.33 cm), internodal length (3.88 cm to 2.64 cm) and number of flowering cymes per plant (56.33 to 20.44) in comparison with the control. In comparison to untreated plants, polyploidy-induced plants developed leaves with larger stomata and lower stomatal frequency. ~~Polyploidy induced plants developed leaves with larger stomata with lower stomatal frequency in contrast to the untreated plants~~.



*Keywords: Jasminum sambac, colchicine, polyploidy, flower bud length and stomatal size*


## INTRODUCTION

*Jasminum sambac* (Arabian jasmine, Tuscan jasmine) is a hardy, evergreen, perennial flowering shrub belonging to the family Oleaceae and native to tropical Asia. It is cultivated commercially in large scale for loose flowers and concrete extraction in southern states of India. One of the common generic interests among home gardeners is the cultivation of major Jasminum species~~Growing of major *Jasminum* species is one of the shared generic interests among home gardeners~~ and hence jasmine prevails as a household flowering plant in Tamil Nadu. Jasmine is the leading loose flower crop in area and production in Tamil Nadu's floricultural wealth specifically *J. sambac* is the predominant contributing species of 13,719 ha and 1,40,105.13 MT to the total jasmines value [1].

The cultivars or ecotypes of the jasmine crop under commercial cultivation are the outcomes of sustained period of extensive selections facilitated by the local people of different regions. The evolution of different cultivars or ecotypes in J. sambac could be attributed to autopolyploidization, spontaneous mutation, and natural crossing over time.~~Origin of different cultivars or ecotypes in *J. sambac* naturally might be due to autopolyploidization, spontaneous mutation and natural crossing that transpired in course of time.~~ The basic chromosome number of all the species and varieties of *Jasminum* genus is 13. There is only a released variety in the *J. sambac* cv. Soojimalli named Arka Aradhana from Indian Institute of Horticulture, Bengaluru noted for its double whorled bold buds. Hence to develop a variety featuring improved yield in terms of quality and quantity, artificial induction of changes in the cultivated variety is pressing priority in jasmine crop improvement program.

Polyploidy is a naturally occurring phenomenon in living organisms at a slower pace that involves process like abnormal cell division in mitosis or failing of chromosomes to detach in meiosis. The frequency of polyploidy in flowering plants is about one per 1 lakh (1/1,00,000) [2]. Stable polyploidy is common in plants due to high development rate and the tolerance of plants to polyploidy changes. Polyploid plant progenies obtained through polyploid breeding can be utilized in introgression of germplasm over heterogenous ploidy levels [3].

Colchicine ($C_2H_{25}NO_6$) is one of the mitotic inhibitors employed in development of polyploidy cells through chromosome doubling. It is a plant-based alkaloid derivative, isolated principally from the bulb-like corms of *Colchicum autumnale* [4], seeds and tubers of *Gloriosa superba* [5] and fractionally from corms and seeds of *Iphigenia indica* [6]. The earliest illustration of chromosome doubling by colchicine and their significance in plant breeding was contributed by improved experimental results in different horticultural and agricultural crops [7]. Freshly prepared aqueous solution of colchicine in required concentrations ranging anywhere between 0.01 to 1.00% is generally used for treating different plant parts or stages *i.e*., the actively dividing meristematic regions of either shoot or roots are identified as the most absorptive and responsive plant organ. Due to the toxic nature of colchicine, lower doses with longer exposure periods are bound to provide a higher rate of polyploid development. ~~Lower doses of colchicine with longer exposure period are bound to offer better polyploid development rate owing to its toxic nature~~ [8]. Induced polyploidy or neo-polyploidization has been strived in commercially important *Jasminum* species but none of the polyploids outperformed the existing diploids and natural triploids [9]. Polyploidy breeding of triploids are known to aid in overcoming the barriers that prevented self-fertilization.

The aim of the current study was to assess the response of the cultivating variety Ramanathapuram Gundumalli of *J. sambac* species to different concentrations of colchicine in induction of practically acceptable growth, floral and yield characters.

## MATERIAL AND METHODS

The ploidy experiment was conducted at the Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore in the time of March 2021 to August 2021. The standardized rooted cuttings of *J. sambac* cv. Ramanathapuram Gundumalli in uniform length and number of axillary sprouts were collected from a jasmine nursery in Ramanathapuram, Tamil Nadu and planted in poly bags filled with growing media for acclimatization for about a month in 50% shade. After a month, immediately after the springing up of new leaf buds in the nodal regions, tips were cut and saturated with different concentrations of colchicine (0.05, 0.1, 0.15, 0.2 and 0.25%) for treatment duration of 48 hours. The afresh colchicine solutions of assorted concentrations were subjected to apical meristematic tissues through the fixed saturated cotton plugs supported by regular moistening of the cotton plugs up until the treatment durations. The experiment was conducted and ~~analysed~~analyzed using non-replicated design. All plants were regularly observed after treatments for survival rate and plant growth characteristics.All plants after the treatments were regularly observed for the survival rate, survived plant growth characters. The stomatal characters were assessed in the paradermic, abaxial surface of three randomly selected leaves of treated plants adhering to the nail varnish technique [10]. The microscopic stomatal observations from the prepared slides were effectuated using under a binocular light microscope (Leica Bio med). Images were obtained by using a camera fitted to a microscope along with a computer at 10X, 40X and 100X magnifications for determination of stomatal size and stomatal density (number of stomata per $mm^2$ of abaxial leaf surface).

## RESULTS AND DISCUSSION

The biological effects of colchicine on the apical tips of sprouts were observed in axillary buds and sprouting branches of J. sambac plants.After the treatment of apical tips of the sprouts with colchicine, the biological effects expressed in axillary buds and sprouting branches of *J. sambac* plants were observed. The growth rate, length of sprouting branches, internodal length, number of flowering cymes per plant are significantly lesser to the control plants whereas the mortality of axillary buds and plants were greater (Table 1 and Fig. 1). With intensifying the colchicine concentration and exposure time, mortality rate of the shoots increases while the number of flower buds per cyme and flower bud length fluctuates among the treatments. Morphological variations of reduced shoot length *i.e*., 18.000 to 48.50 cm, internodal length of 1.90 to 3.10 cm was noted by application of 0.25% colchicine for 48 hours. The number of flowering cymes per plant was found to be reduced in treatments including higher concentrations of colchicine compared to lower concentrations and control plants. The flower bud length of plants in control and polyploid induction treatments showed non-linear changes of 2.2 to 2.9 cm in control; 2.4 to 3.0 cm in 0.05% colchicine; 1.7 to 2.4 cm in 0.10% colchicine; 1.4 to 2.7 cm in 0.15% colchicine; 1.8 to 2.7 cm in 0.20% colchicine and 1.7 to 2.5 cm in 0.25% colchicine. The experimental results are in assent with the colchicine treatment of axillary buds of *J. sambac* in different lengths (0, 3-5 and 8-10 mm) with different concentrations of colchicine (500, 1000 and 2000 ppm) for 24 and 48 hours [11]. Autopolyploidisation of J. sambac with 0.5 percent colchicine was attempted, but there was no economically significant performance in the treated plant materials. Autopolyploidisation has been attempted in *J. sambac* with 0.5% of colchicine and there was no economically significant performance in the treated plant materials [12]. Tetraploids of *J. sambac* showed vigorous growth habit still yielded less number of bolder flower buds than diploids and triploids [13]. *J. auriculatum* seed treatment with colchicine of 1% yielded 11.4 to 23.1 % polyploidy plants [14].

The polyploid and vegetatively propagated plants grown under *in vivo* conditions express higher frequency of chimeras and polyploid sequences in their tissues compared to *in vitro* and that encompass the essentiality of determination of ploidy levels [15]. Hence for confirmation of the ploidy levels, reliable direct methods of chromosome counting in mitotic cells of root tips and leaf tissues in flow cytometry [16] and indirect methods of observing morpho-horticultural characters such as length of sprouting branches, internodal length, length and width of the leaf, number of flower cymes and buds per cyme and cytological characters, specifically number of chloroplasts in guard cells, pollen diameter, stomatal length, stomatal width and stomatal density [17] are adopted. Because it is temporally stable, stomatal density assessment is regarded as a reliable parameter.~~Stomatal density assessment is considered to be a reliable parameter as it is temporally stable~~ [10].

The stomatal density, stomatal length and stomatal width of triploid and colchicine treated plants's leaves are presented (Table 2.). Number of stomata per $mm^2$ of hypostomatic leaves of *J. sambac* (stomatal density) was found to be higher in control plants of 24 to 48 stomatas compared to the colchicine treated plants of 11 to 34 stomatas and the other way around in relation to stomatal size (Fig 2.). The plants with stomatal length ranging between 19.17 to 25.37 μm and stomatal breadth between 17.77 and 23.96 μm are categorized as triploids in Ramanathapuram Gundumalli and stomatal length of 20.72 to 43.37 μm and stomatal breadth of 17.08 to 38.55 μm are observed in polyploid induced plants. The polyploidy in plants has a direct impact on the stomatal frequency and stomatal size with lower stomatal frequency per unit leaf area was found in polyploidy plants of *Tagetes erecta* [18], *Celosia argentea* [19] and *Dendranthema indicum* var. Aromaticum [20], *J. nitidum* culture Acc. *Jn*-1 and *J. grandiflorum* cv. White Pitchi [21].

## CONCLUSION

The study on the effect of colchicine for the generation of polyploidy plants helps in understanding the differential response in genetically diversified plant population. The economically viable and stable regenerants can be isolated in future generations and evaluated. The different concentrations and exposure time, method of application of colchicine must be standardized for individual plant species to obtain higher frequency of chromosomal changes.

## REFERENCES


1. Anonymous. Horticulture Crops: Area and Production of Tamil Nadu for 2020-21 (Final Estimates). 2022; 40. Accessed18 June 2022.
   Available: https://www.tnhorticulture.tn.gov.in/upload/pdf/2020-21 FINALAPY.pdf.
2. Ramsey J, Schemske DW. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Syst*. 1998; 29:467–501.
3. Dwivedi SL, Upadhyaya HD, Stalker HT, Blair MW, Bertioli DJ, Nielen S. and Ortiz R. Janick, Jules (ed.): Plant Breeding Reviews. John Wiley & Sons. 2007:179–230.
4. Geiger PhL. "Ueber einige neue giftige organische Alkalien" (On some new poisonous organic alkalis). Annalen der Pharmacie, 1833;**7**(3):274-276.
5. Clewer HWB, Green SJ and Tutin F. XCI.-The constituents of Gloriosa superba. *J. Chem. Soc.*, Transactions. 1915; 107:835-846.
6. Kapadia VH, Dev S, Rao RS and Ansari MY. New source of colchicine in Iphigenia. *Phytochem. Lett*. 1972; 11:1193.
7. Blakeslee, A.F. and Avery, A.G., 1937. Methods of inducing doubling of chromosomes in plants: by treatment with colchicine. *J. Hered*. 28(12): 393-411.


**Formatted:** Font: Italic

**Formatted:** Font: Italic

**Formatted:** Font: Italic

**Formatted:** Font: Italic

8. Sajjad, Y., Effect of colchicine on in vitro polyploidy induction in African marigold (*Tagetes erecta*). *Pak J Bot*. 2013;45: 1255-1258.
9. Karale AR and Manjunath SP. Flower Breeding and Genetics. New India Publishing Agency, New Delhi. 2017; Part- I: 579-602.
10. Gitz DC. and Baker JT. Methods for creating stomatal impressions directly onto archivable slides. *J. Agron*. 2009;101(1):232-236.
11. Deng Y, Sun X, Jia X, Liang L, and Su J. Effects of concentration and treatment time of colchicine on growth and development of axillary buds of *Jasminum sambac*. *J. Plant Resour. Environ*. 2016;25(3): 62-71.
12. Krishnaswamy N, and Raman, VS. 1954. South Indian Hort., 1954. 2(4):151-152.
13. Khan WMA, Raman, VS and Raman KR. South Indian Hort., 1969.17:79-85
14. Velusamy P. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.1981.
15. Rêgo MM, Rêgo ER, Bruckner CH, Finger FL. and Otoni WC. *In vitro* induction of autotetraploids from diploid yellow passion fruit mediated by colchicine and oryzalin. Plant Cell Tissue Organ Cult. 2011;107(3):451-459.
16. Dolezêl J, Greilhuber J, Suda J. Flow cytometry with plants: an overview. In: Dolezêl J, Greilhuber J, Suda J, editors. Flow cytometry with plant cells. Wiley, Weinheim. 2007; 41–65.
17. Moghbel N, Borujeni MK, and Bernard F. Colchicine effect on the DNA content and stomata size of *Glycyrrhiza glabra* var. *glandulifera* and *Catharanthus tinctorius* L. cultured *in vitro*. *J Genet Eng and Biotechnol*. 2015;13(1):1-6.
18. Sadhukhan R, Ganguly A, Singh PK, Sarkar HK. Study of Induced polyploidy in African marigold (*Tagetes erecta* L.). *Environ. Ecol*. 2014; 32:1219–1222.
19. Mostafa GG, Alhamd MFA. Detection and evaluation the tetraploid plants of *Celosia argentea* induced by colchicine. *Int. J. Plant Breed. Genet*. 2016; 10:110–115.
20. He M, Gao W, Gao Y, Liu Y, Yang X, Jiao H, Zhou Y. Polyploidy induced by colchicine in *Dendranthema indicum* var. Aromaticum, a scented chrysanthemum. Eur. *J. Hortic. Sci*. 2016; 81:219–226.
21. Ghosh S, Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore. 2018.

**Table 1. Effect of colchicine on growth and flower parameters in *J. sambac* cv. Ramanathapuram Gundumalli**

| Concentration | Shoot length (cm) | | Internodal length (cm) | | Number of flowering cymes per plant | | Flower bud length (cm) | |
|---|---|---|---|---|---|---|---|---|
| | **Mean±SE** | **Range** | **Mean±SE** | **Range** | **Mean±SE** | **Range** | **Mean±SE** | **Range** |
| Control | 79.17±5.48 | 64 -110 | 3.88±0.21 | 3.40 – 5.30 | 56.33±4.60 | 27 - 86 | 2.51±0.065 | 2.2 – 2.9 |
| 0.05 % | 68.36±3.25 | 54 - 81 | 3.74±0.18 | 3.20 – 4.60 | 38.89±3.39 | 12 – 76 | 2.62±0.040 | 2.4 – 3.0 |
| 0.1 % | 49.59±2.94 | 30.50 – 74.50 | 3.78±0.15 | 3.10 – 4.30 | 20.22±1.25 | 10 – 37 | 2.13±0.084 | 1.7 – 2.4 |
| 0.15 % | 43.00±2.01 | 24.00 – 70.00 | 3.17±0.15 | 2.80 – 3.90 | 39.61±3.17 | 12 – 70 | 2.41±0.153 | 1.4 – 2.7 |
| 0.2 % | 35.07±1.87 | 21.00 -49.60 | 2.80±0.14 | 1.90 – 3.70 | 20.44±1.39 | 12 – 34 | 2.28±0.116 | 1.8 – 2.7 |
| 0.25 % | 30.96±1.89 | 18.00 – 48.50 | 2.64±0.15 | 1.90 – 3.10 | 24.11±3.02 | 7 - 48 | 2.19±0.093 | 1.7 – 2.5 |

**Table 2. Effect of colchicine on stomatal parameters in *J. sambac* cv. Ramanathapuram Gundumalli**

| Concentration | Stomatal density | | Stomatal length (µm) | | Stomatal breadth (µm) | |
|---|---|---|---|---|---|---|
| | Mean±SE | Range | Mean±SE | Range | Mean±SE | Range |
| Control | 32.67±1.71 | 24 – 48 | 22.36±0.32 | 19.17 – 25.37 | 19.82±0.98 | 17.77 – 23.96 |
| 0.05 % | 27.11±0.22 | 20 - 34 | 24.40±0.24 | 20.72 – 27.20 | 22.64±1.54 | 17.08 – 26.30 |
| 0.1 % | 24.67±0.88 | 17- 32 | 28.67±0.79 | 22.02 – 31.78 | 26.24±1.46 | 20.22 – 30.65 |
| 0.15 % | 19.44±1.09 | 15 – 26 | 31.93±1.20 | 25.19 – 35.75 | 29.40±1.50 | 20.98 – 34.33 |
| 0.2 % | 18.44±1.31 | 14 – 24 | 34.45±1.53 | 26.30 – 39.72 | 29.50±1.65 | 23.84 – 37.10 |
| 0.25 % | 15.11±0.62 | 11 – 19 | 38.24±0.29 | 33.97 – 43.37 | 31.48±2.60 | 23.17 – 38.55 |

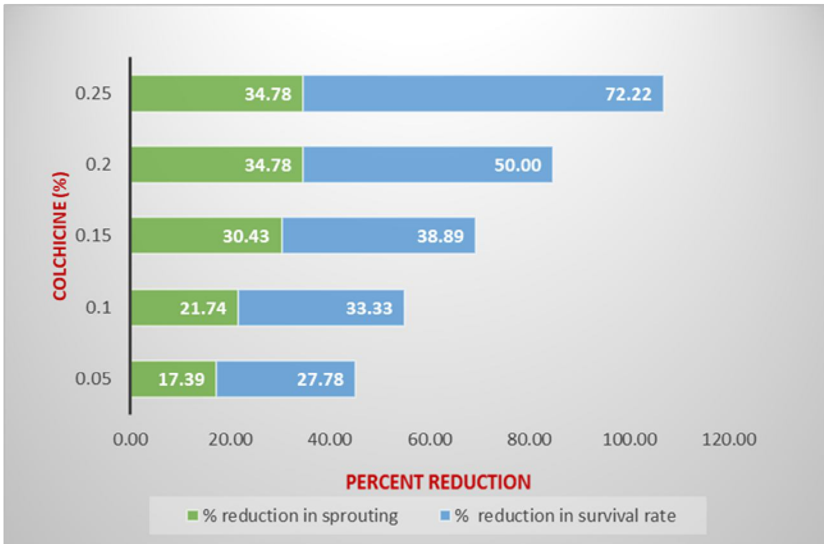


**Fig. 1. Influence of colchicine on sprouting and survival rate of treated rooted plants**

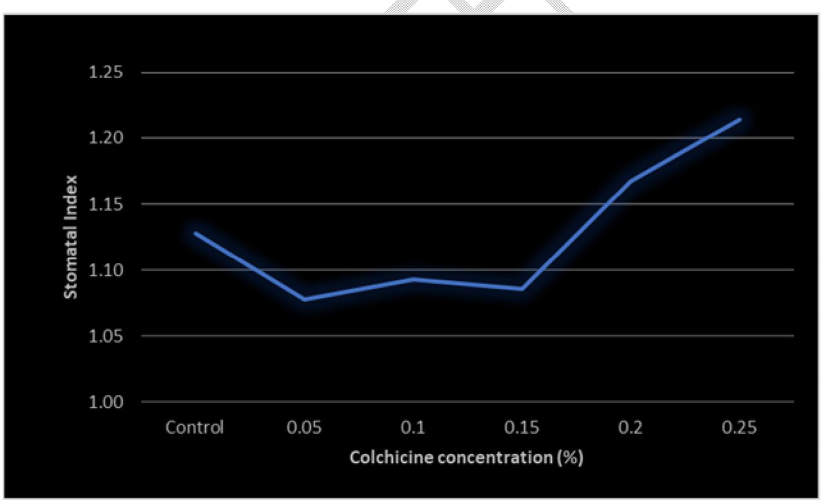


**Fig. 2. Influence of colchicine on stomatal index of treated rooted plants**