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Potentiality of Colchicine in Induction of Polyploids in *Jasminum sambac* (L.) Aiton cv. Ramanathapuram Gundumalli

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was commenced in a compulsion toaccomplish 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.

Keywords: Jasminum sambac; colchicines; polyploidy; flower bud length and stomatal size.

1. 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.

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One of the common generic interests among home gardeners is the cultivation of major *Jasminum* species 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 auto polyploidization, spontaneous mutation, and natural crossing over 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 phenomenonin 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 ofgermplasm over heterogenousploidy levels [3]. Generally, polyploids possess the advantages of heterosis, gene redundancy, increased heterozygosity, increase in size of certain plant parts and disadvantages of assisting invasiveness of certain plant species, disorganized effects of nuclear and cell enlargement, instability of polyploid mitotic and meiotic processes and deleterious epigenetic mutations.

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 [8]. Induced polyploidy or neo-polyploidization has been strivedin 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 wasto 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.

2. MATERIALS 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% shadein nursery area. 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 analyzed using non-replicated design. All plants were regularly observed after treatments for survival rate and plant growth characteristics. The

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

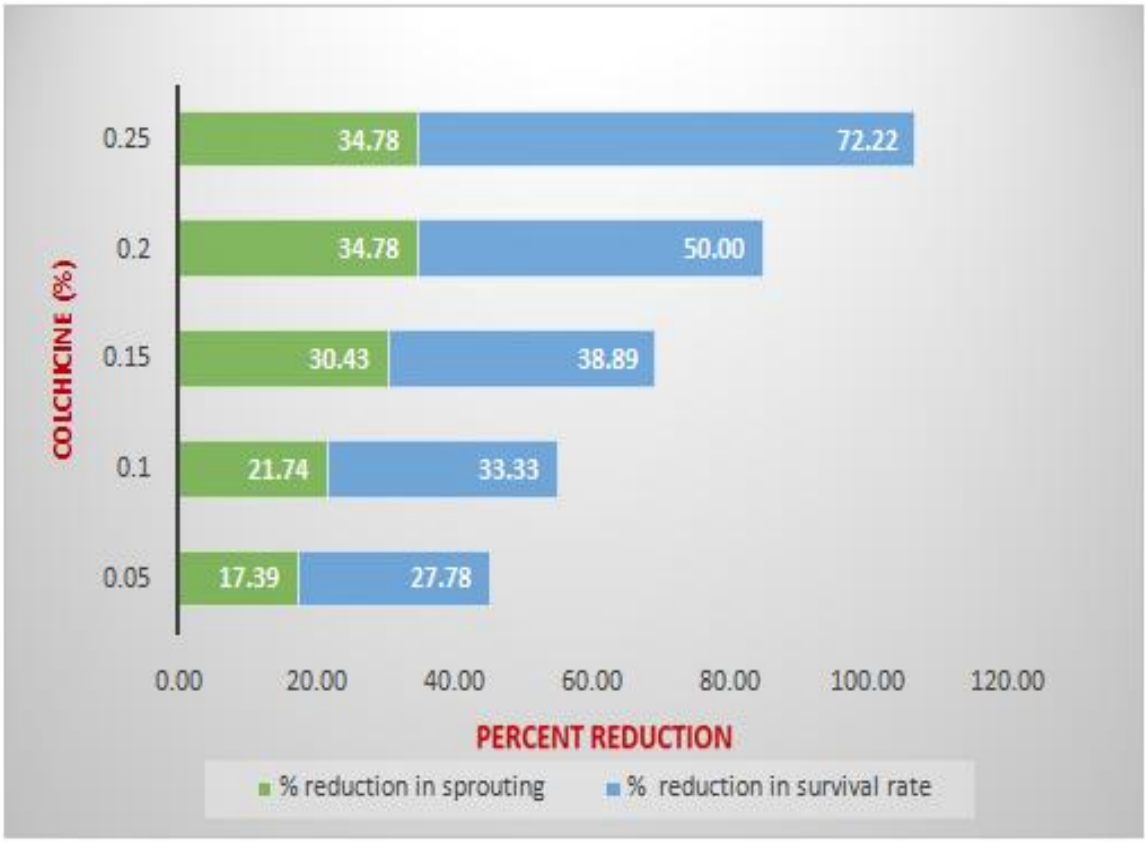

Fig. 1. Influence of colchicine on sprouting and survival rate of treated rooted plants

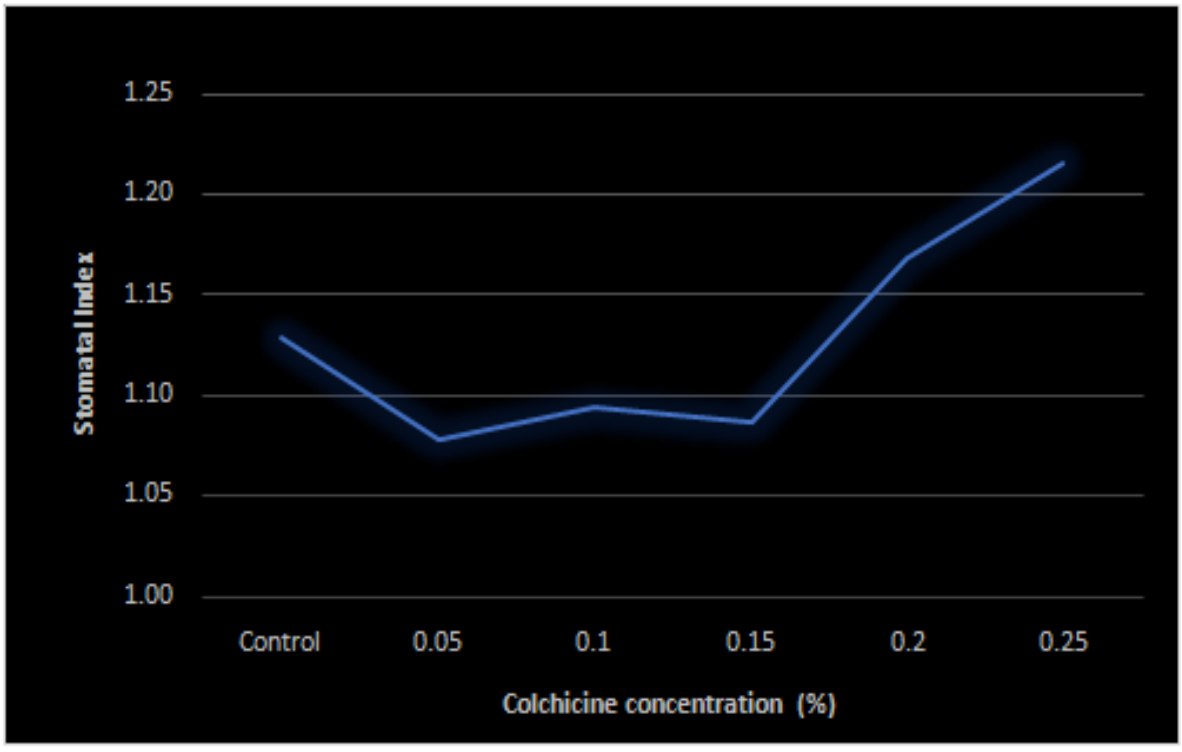

Fig. 2. Influence of colchicine on stomatal index of treated rooted plants

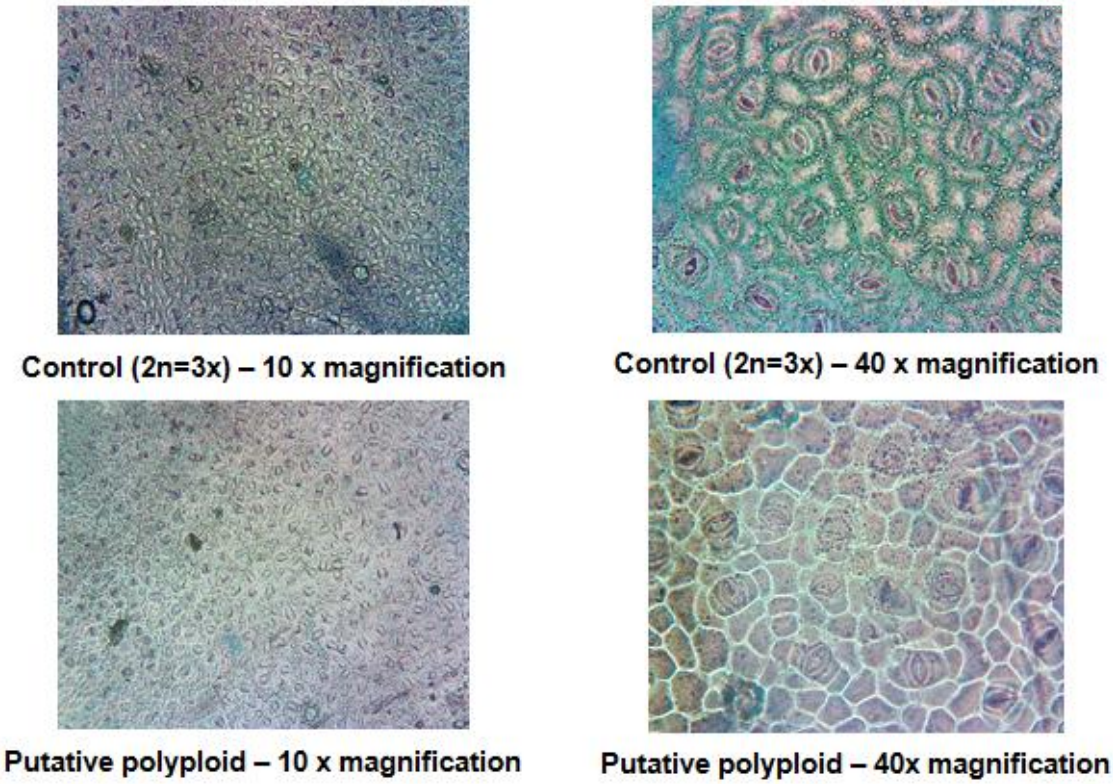

Fig. 3. Variation in stomatal density and size in putative polyploids of jasmine cultivar

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 computerat 10X, 40X and 100Xmagnifications for determination of stomatal size and stomatal density (number of stomata per mm^2 of abaxial leaf surface).

3. 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. 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]. Auto polyploidisation of *J. sambac* with 0.5 percent colchicine was attempted, but 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 inmitotic cells of root tips and leaf tissues in flow cytometry [16] and indirect methods of observingmorpho-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. Stomatal density is temporally stable and hence it's assessment is regarded as a reliable parameter [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 stomatascompared 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].

4. CONCLUSION

The study on the effect of colchicines 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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