Spraying Colchicine on Flower Buds of *Exacum Ritigalensis* (Binara/Ginihiriya)

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Abstract
Exacum ritigalensis has a high potential to be introduced as a floricultural plant to the floriculture industry of Sri Lanka. In this regard, improvements of its morphological characters through genetic modifications are important. Spraying colchicine is one of the methods which can be applied to plant to alter plant morphology. This study was conducted to find the effect of spraying different colchicine concentrations on flower buds of *E. ritigalensis*. The flower buds at the same reproductive stage were treated with a drop (1ml) of the colchicine solutions (0, 30, 60, 90 and 120mgL^{-1}). Treated plants kept under the green house without wetting stem and bud from other liquids. Water was applied regularly to soil. Experiment was arranged according to the Completely Randomized Design (CRD) with five replicates per each treatment. Observations on flower diameter (cm), color of the flower (deviation from natural colour; light, normal and dark), days to flowering and seeds weight per pod (g) of plants were recorded. Statistical analysis was performed with Duncan's multiple range test using SAS software (version 9.1.3) and Wilcoxon sign rank test using SPSS statistics package (version 20). Results revealed that the spraying of colchicines on flower buds did not have effect on flower diameter and days to flowering. But colchicine concentration 120mgL^{-1} resulted in lighter colour of flowers compared to control but produced no seeds in pods.

Keywords: Bud, Colchicine, *Exacum ritigalensis*, Morphology
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Introduction

Polyploidisation is an important process in nature and in plant breeding that can result in larger and darker leaves, larger inflorescences, a prolongation of the flowering period, apomixis, larger fruits and greater secondary metabolite production and yield (Roy *et al.*, 2001). Colchicine, a poisonous medicinal chemical, has been used since 1937 in plant breeding work to produce changes in plants by doubling the number of chromosomes in cells, a condition referred to as polyploidy. The increased number of chromosomes usually brings about an increase in size of the affected cells and various degrees of changes in their functions. Chromosome losses or rearrangements and gene mutations caused by colchicines are found in flax, sunflower, barley and cotton (Luckett, 1989).In contrast with the normal plants, those developed by colchicine treatment often show changes in height and width; in thickness of branches; in size, shape, and texture of leaves, flowers, fruits, and seeds; in fertility of flowers; and in physiological responses, The degree of changes produced when the chromosome number is doubled cannot be predicted (Dermen and Emsweller, 1961; Katarzyna *et al.*, 2010). *Exacum ritigalensis* has a high potential to be introduced as a floricultural plant to the floriculture industry of Sri Lanka, probably targeting export markets. In this regard, improvements for its morphological characters through genetic modifications are important. Colchicine solution could be sprayed on the growing buds (Mahabal, 2010; Dermen and Emsweller, 1961). This study was conducted to find the effect of spraying different colchicine concentrations on *E. ritigalensis* flower buds.

Materials and methods

E. ritigalensis plantlets were collected from Pannala, Kurunegala, Sri Lanka and maintained under favourable conditions. Healthy *E. ritigalensis* plants with same growth stage were selected for the study. When flower buds were developed, tips of rapidly growing buds were treated with a drop (1 ml) of the colchicine solutions of 0, 30, 60, 90 and 120 mgL^{-1}. It was applied over the tip at once and kept under the greenhouse condition. Watering was done by every day to soil taking care not to wet stem and buds. Experiment was arranged according to the Completely Randomized Design (CRD) with five replicates for three plants per each treatment. Flower diameters (cm), colour of the flower, days to flowering and seed production (seeds weight per pod (g)) of plants were recorded. Colour of the flower was ranked (1, 2 and 3) according to deviation from natural colour (light, normal and dark respectively). Statistical analysis was performed with Duncan's Multiple Range Test (DMRT)using SAS software (version 9.1.3) and Wilcoxon sign rank test using SPSS statics package (Version 20).

Results and discussion

David (1950) believed that the most preferable method to produce polyploidy seed is to spray

colchicines to flower buds of African marigolds. The treatment of seeds which normally produce sterile "mule" plants might be particularly rewarding if an adequate number of seeds were available with colchicines sprayed plants. However, in this study pods with seeds were not observed for all colchicine concentrations which were used (Table 1). Mahabal (2010) observed that whichever method is used, if doubled chromosomes are induced in two compatible fertile flowers, the resulting seeds might produce startling results. According to the Mahabal (2010) the visual changes were induced in flowers but the flowers were infertile.

There were no significant differences in flower length and days to flowering (Table 1). Dermen and Emsweller, (1961) mentioned that all colchicine treated methods gave satisfactory results, particularly with herbaceous plants but, woody and semi-woody plants (buds or young shoot tips) require a somewhat different treatment.

Previous investigations into polyploids of *M. sinensis* induced *ex vitro* by colchicine showed very low effectiveness (0–0.31% of tetraploids and 4–4.2% of mixoploids) (Petersen *et al.*, 2003). The colchicine concentration of 120 mgL^{-1} changed colour of the flower of *E. ritigalensis* natural colour into light (Figure 1). Morphologically, in colchicine treated plants showed leave become large, darker in colour, leathery in texture, leaf lobes overlapped and twisted stomata, bracts, pollen grains, bolls and seeds become bigger than control and maturity is delayed (Mahabal, 2010; Katarzyna *et al.*, 2010). David (1950) believed that the treatment of seeds with colchicine is preferable to spraying the buds. This research observed similar effects as expected.

Conclusions

The spraying of colchicine to flower buds at the initial stage did not change the flower diameter and days to flowering. However, colchicine concentration of 120 mgL^{-1} changed the flower colour into light purple. It was dark purple in control. No seeds were observed in colchicine sprayed mature plants.

Table 1: Effects of colchicine on morphological characters of *E. ritigalensis*

Concentration of colchicines (mgL^{-1})	Flower diameter (cm)	Days to flowering	Colour of the flower	Seedsweight per pod (g)
0	5.96	21	2^a	1.6^a
30	5.91	21	2^a	0.0^b
60	5.94	21	2^a	0.0^b
90	5.92	21	2^a	0.0^b
120	5.90	21	1^b	0.0^b

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test ($P<0.05$).

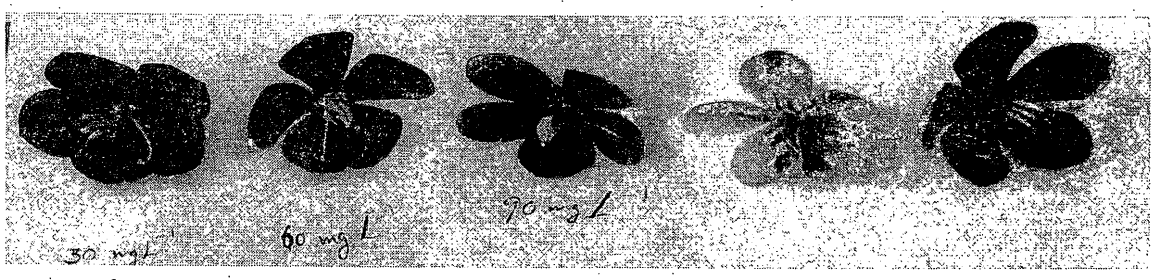

Figure 1: Effect of different colchicine concentrations on flowers of *E. ritigalensis*; A: 30mgL^{-1}; B: 60mgL^{-1}, C: 90mgL^{-1} and D: 120 mgL^{-1} with colchicines solutions and E, control (without colchicines treatment).

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