

Effects of Freezing Time on Observation of Chromosomes in *Exacum ritigalensis* (Binara/Ginihiriya)

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Abstract

In this study, freezing time at 0.01% colchicine was practiced to observe chromosome from *Exacum ritigalensis*. Fifty, two weeks old seedlings of *E. ritigalensis* were randomly selected and all actively growing root tips about 10mm in length were excised from each plantlets. These root tips were washed with tap water to remove the residues of the medium. Root tips treated with 0.01% colchicine solution were kept at room temperature for either two hours (control) or at 4°C for either 1, 1.5, 2 and 2.5hrs. The experiment followed a completely randomized design with ten replicates were used for each treatment. Treated root tips were placed on glass slide and stained with one drop of carbofuchsin solution and were observed for chromosomes under a microscope (Axio Lab A1), and photos were taken with the associated apparatus. Then they were ranked using a scale 0, 1, 2, 3, and 4 from 0 to 4 (very poor, poor, satisfactory, good and very good), according to the visibility and for clearness of chromosomes in cells; Statistical analysis was performed by Wilcoxon sign rank test using SPSS statistics package (version 20). It was observed that *Exacum ritigalensis* diploid plants with 28 chromosomes and also results revealed that two hours freezing at 4°C gave the best picture of chromosome than other treatments.

Keywords: Carbofuchsin solution, chromosome, colchicine, diploid

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Introduction

In "higher" organisms (diploids), members of same species typically have identical numbers of chromosomes in each somatic cell (diploid chromosome number = $2n$). Nearly all chromosomes exist in pairs except the sex chromosomes. It means that member of pair is homologous chromosomes. Before polyploidy was induced, the chromosome number of the primary materials was determined in root tips of germinated seeds to confirm the number of chromosome in diploid materials ($2n=2x=48$).

Exacum ritigalensis is up to 85 cm high, thickened at base, 4-angular throughout, narrowly winged at angles. Corolla lobes broadly obovate, 18-28 x 16-18 mm, obtuse to round at apex, pale to dark blue. Leaves are spreading, broadly oval-elliptic, 5.2-16 (-20) x 2.0-5.0 (-6.5) cm, cuneate at base, acuminate to long acuminate. Plant grows in gravelly soil of rocky places and in patanas in the dry intermediate zone towards the central part of the country and in the dry uplands (Andrew *et al.*, 2006; Anderson, 2006; Dahanayake and Perera, 2015). It is difficult to find clear pictures of diploid chromosome in *Exacum ritigalensis* and this study was conducted to identify a simple and efficient metaphase chromosome preparation method on *Exacum ritigalensis* which can also be applied for other plants as well.

Methodology

Two weeks old seedlings of *E. ritigalensis* were randomly selected and from each plant all

actively growing root tips about 10 mm in length were excised. These root tips were washed with tap water for removing the residues of the medium. Root tips treated with 0.01% colchicine solution were kept at room temperature for either two hours (control) or at 4°C for either 1, 1.5, 2 and 2.5hrs. Then they were washed with tap water and transferred to fixing in Carnoy's solution containing acetic acid: ethanol (1: 3, v/v) for at least 18 hours. The fixed root tips were then hydrolyzed in 1 N HCl for 03 min at 65°C. After hydrolysis, root tips were rinsed with distilled water for 10 min and root tips were cut about 1.5 mm. These prepared root tips were then placed on slid glass and stained with one drop of carbofuchsin solution for one minute. After it was squashed under cover slip glass and cell samples of the root tips were observed for chromosomes under a microscope (Axio Lab A1), and photos were taken with the associated apparatus. Photos were ranked using a scale 0, 1, 2, 3, and 4 according to visibility and clearness of chromosomes in cells. Ten replicates were used in every treatment. Statistical analysis was performed by Wilcoxon sign rank test using SPSS statics package (version 20).

Results and Discussion

A plant, *E. ritigalensis* with all the root tip cells showing 28 chromosomes was determined as diploid. The diploid chromosome number ($2n = 28$) shows in Figure 1 (d). Due to the low frequency of metaphase cells in root tips of *E. ritigalensis* selecting of root tips are difficult and time-consuming. The same results were

observed by Omidbaigia et al (2010) for different plants.

Root tips pre-treated with 0.01% colchicine solution for two hours at 4°C were the best compare to other treatments followed by the root tips pre-treated with 0.01% colchicine solution for two hours at 4°C without freezing (Table 1). Figure 1 shows chromosomes which were taken at different time after freezing and without freezing. Many chromosome observation methodologies have done by without freezing (Dhahanayake, 2008; Ghader, 2010; Li and Zhang, 1991). Results of this experiment revealed that pre-treated with 0.01% colchicine solution for two hours at 4°C with visible and clear image of chromosomes in *E. ritigalensis*.

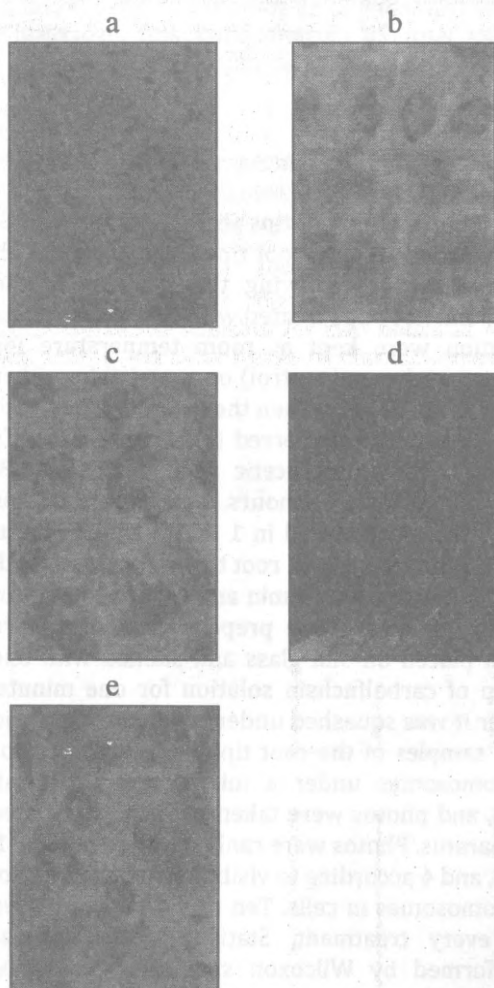


Figure 1: These root tips were pre-treated with 0.01% colchicine solution; a, two hours at room temperature; b, one hour freezing at 4°C; c, one and half hours freezing at 4°C; d, two hours kept in refrigerator at 4°C and e, two and half hours kept in refrigerator at 4°C

Conclusion

It was observed that *Exacum ritigalensis* is a diploid plant with 28 chromosomes. Results

revealed that two hours at 4°C was the best to observe chromosomes compared to other treatments.

Table 1: Visibility and clearness of chromosomes in photographs

Freezing time (hrs)	Rank
01.00	02 ^c
01.30	01 ^d
02.00	04 ^a
02.30	01 ^d
00.00	03 ^b

Column values followed by the same letter are not significantly different

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