The Polyploidy Effect on Leaf Size and Stomata of Chilli (Capsicum annum L

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The Polyploidy Effect on Leaf Size and Stomata of Chilli (*Capsicum annuum* L.)

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Abstract

Polyploidization is the multiplication of the number of chromosome sets in a cell. The existence of polyploidization will change the sets of chromosome to aneusomy or euploidy. Increasing the number of these chromosomes can cause changes in morphological characters, such as an increase in size and shape changes in plant parts. Objective of this research was to analyse the effect on leaf size and stomata of chilli as a result of colchicine polyploidyzation. Chilli seeds were treated by 0.0, 0.05, 0.1, 0.2% colchicine for 4, 18, and 24 hours before planting. Stomata size was determined by the replica method while leaf size was determined by the constant method from the 30 dap (days post planting) chilli. The data were analysed using Anova. The results showed that colchicine concentration and time soaked before planting caused increasing the leaf size. Statistically, the optimum treatment was 0.1% for 18 hours but not significant difference with 0.1% for 24 hour. Colchicine treatments also affect the size of the stomata, which 0.2% for 24 hour was the highest but not significant difference with 0.1% for 24 hour. The conclusion was 0.1% colchicine with 24 hour soaked seed before planting would increase the leaf and stomata size.

Key words : *colchicine, polyploidy, stomata, chilli*

INTRODUCTION

Polyploidization is a doubling of the number of chromosome sets during mitosis or meiotic division (Leitch and Leitch, 2008). Polyploidization can be induced by colchicine (Winaryo et al., 2016). Colchicine inhibits cell division by stopping mitosis at the metaphase stage. This compound prevents the polymerization of tubulin into microtubulin. The formation of microtubulin does not cause the bobbin thread to form so that the anaphase stage for chromosome separation does not occur and the separation wall fails to form so that the chromosomes and their duplicates remain in the same cell. As a result, cell division begins with diploid cells and if complete division ends with the formation of tetraploid cells (Nasir, 2002).

Polyploidization is generally followed by morphological changes or morphology size such as the shape and size of flowers, stems and leaves (Yanhong et al., 2016), increase in fruit size (Wang et al., 2015), and number or length of roots (tuwo and Indriyanto, 2016). Sari et.al. (2017) successfully developed Bombay silk medicinal plants using colchicine. Other polyploid plants are sweet sorghum (Ghaffari, 2006), mung beans (Haryanti et al., 2009), and Chrysanthemum flowers (Daryono and

Rahmadani, 2009). Polyploidization techniques in curly chilli plants produce larger flowers (Murni, 2010). How polyploidization effect on stomata size has not been reported.

METHOD

Materials

This study used Branang cultivar from Indonesian Seed Centre, Yogyakarta Indonesia, and pure Colchicine from Merck. Other materials were ethanol (70%), humidity soil, water, fertilizer, pots, and water sprayer.

Colchicine Preparation

Colchicine was diluted with absolute ethanol to 0.05, 0.1, and 0.2% voume/volume.

Planting

Chilli seeds were soaked in colchicine 0.05, 0.1, and 0.2% and in water as control treatment (0% colchicine) for 4, 18, and 24 hours. The treatment was designed as:

Time (hour) / Colchicine (%)	4 (T1)	18 (T2)	24 (T3)
0 (K0)	K0T1	K0T2	K0T3
0,05 (K1)	K1T1	K1T2	K1T3
0,1 (K2)	K2T1	K2T2	K2T3
0,2 (K3)	K3T1	K2T3	K3T3

Then the seeds were rinsed with water and growing onto a humidity soil.

Leaf size was calculated by measuring the length and width of the third leaf from the tip of the plant. Leaf area is determined by the conversion method according to Chaudary et.al., 2012 with the formula: Area = length x width x constant. The constant for chili leaves is 0.54 (Susilo, 2015).

Stomata measurements were performed at one month of age using the replica method. The leaves of the abaxial (bottom surface) are smeared with transparent nail polish and covered with tape for 5 minutes. Then the tape was removed from the leaf and taped to a glass object. Microscopic observations were carried out at 400x magnification.

RESULTS AND DISCUSSION

Chilli seeds grew faster for all of the treatment and looked like no effects of colchicine on the early of plant growth. One month after planting the effect was seen, especially on the leaf and stomata size. The effects of colchicine were shown in Figure 1 and 2.

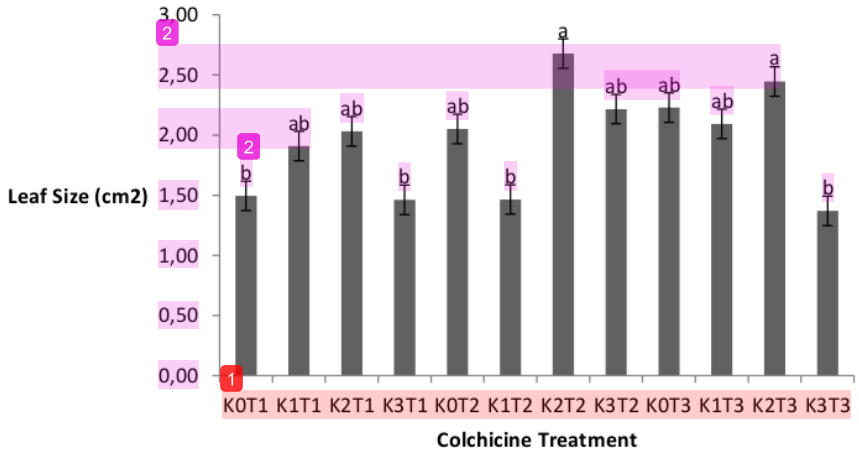

Figure 1. The effect of colchicine treatment to leaf size of *Capsicum annuum* 30 dap (days after planting). K0 – K3 : colchicine concentration (0, 0.5, 0.1, and 0.2%), T1 – T3 : time soaked before planting (4, 18, and 24 hour).

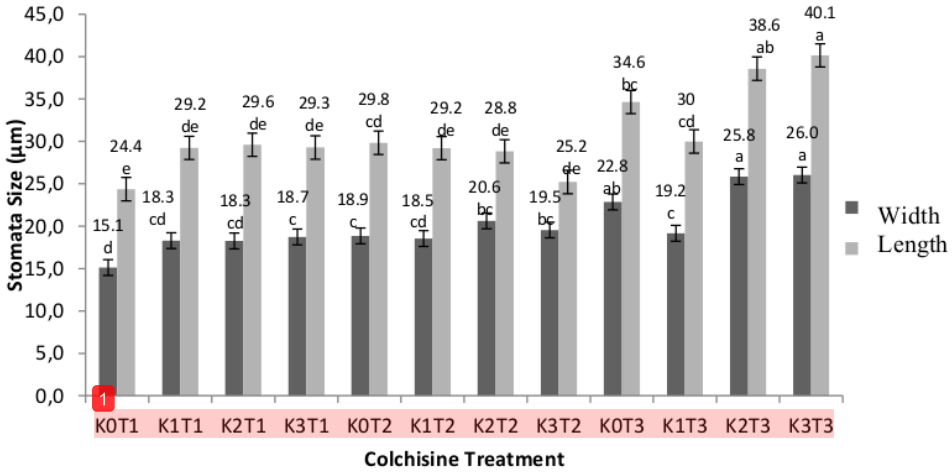

Figure 2. The effect of colchicine treatment to leaf size of *Capsicum annuum* 30 dap (days after planting). K0 – K3 : colchicine concentration (0, 0.5, 0.1, and 0.2%), T1 – T3 : time soaked before planting (4, 18, and 24 hour).

Figure 1 showed that 0.1% of colchicine with 18 hour soaked before planting was optimum treatment for increasing the leaf size, but it was no significant difference with 0.1% for 24 hour. This showed that time soaked before planting affected the growth of plant, accordance to Esmaeilpour and Van Damme (2016). The optimum time for seed soaking of chilli pepper growth was 24 hour and this result is similar to Dantas et.al. (2014). Seeds need

enough water to germinate because water plays a very important role in activating embryonic cells in the seeds, softening the seed coat, and stimulating the growth of embryo and endosperm (Ai and Ballo, 2010), and the water need time to enter the seed optimally.

Colchicine concentration affected the leaf size on the same time soaking seed. On the 4 hour soaked, increasing colchicine from 0.05 to 0.1% caused increasing of the leaf size but 0.2% caused decreasing the leaf size. This also occurred to the 18 hour and 24 hour seed soaking. It was accordance to Pirkoohi et.al. (2011), colchicine concentrations for seed treatment could range from 0.1%–0.8%. Combine with the time soaking, the optimum colchicine concentration to increasing leaf size was 0.1% and seed was soaked 24 hour before planting.

Botelho et.al. (2015) published that colchicine treatment cause "gigas effect", increasing the cell size and showed increasing size of commercial interest of plants like leaves, seeds, or flowers. Result in this study showed the hypothesis.

Colchicine concentration not affected statistically to stomata size on 4 and 18 hour time soaking but different on 24 hour. Colchicine at 0.1 and 0.2% with 24 hour soaked before planting increased the stomata size. Using colchicine at lower concentrations is advisable because a highly doses cause malformation and reduce the production of plants (Pirkoohi et.al., 2011). This result was similar to Moghbel et.al. (2015) that 0.1% colchicine for 24 hour was optimum condition for Sunflower and Lycorize to get larger stomata.

Leaf and stomata size may indicate a polyploidy level occurred in plants. According to Zuzana Mu¨nzbergov (2017),tetraploids were primarily characterized by longer stomata. The larger stomata may induce the lower frequency of stomata on the leaves surface (Manzoor et.al., 2019) due to the balancing of plant transpiration rate. This result showed 0.1 and 0.2% colchicine may cause polyploidyzation effect on the chilli plants. Chromosome analysis may be needed for proofed the polyploidyzation.

CONCLUSION

Conclusion in this study was the optimum treatment 0.1% colchicine with 24 hour soaked seed before planting would increase the leaf and stomata size.

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