In vitro chromosome doubling of tomato var. Improved Pope (*Lycopersicon esculentum* Mill) via colchicine

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ABSTRACT

Prevailing biotic and abiotic stresses disrupt tomato seedling establishment, which may lead to yield and quality reduction. Colchicine, a common mutagen, prevents the development of microtubules during cell division resulting in chromosome doubling. Doubled chromosome plants have enhanced germination, shoot formation, leaf structure, and fruit size. Hence, *in vitro* mutation via colchicine was employed on tomato towards improved seedling characteristics for successful establishment. Seeds were treated with 0.05, 0.10, and 0.15% colchicine for four hours in completely randomized design. Seeds were cultured *in vitro* using Murashige and Skoog medium. Plant height ($p < 0.001$) was increased by 2.00 cm (SD 0.85) when applied with 0.05% colchicine. A higher dosage (0.10 and 0.15%) generally resulted in shorter plants wherein the shortest plant height was recorded on plants treated with 0.15% colchicine at 5.94 cm (SD 0.12), about half the height compared to plants not treated with colchicine at all. On root length ($p < 0.001$), all colchicine treated plants produced longer roots with the application of 0.05% colchicine producing the longest roots at 6.58 cm (SD 0.61). On stomata ($p < 0.001$), size (length and width) was generally increased with colchicine application where 0.05% colchicine application increased stomatal size at 17.99 mm (SD 0.62) and 16.42 mm (SD 0.62), respectively. The same was also recorded on chromosome size ($p < 0.001$) in which length and width was recorded at 21.28 mm (SD 0.66) and 15.20 mm (SD 0.50), respectively when exposed to 0.05% colchicine. Other parameters like percentage of germination and leaf size were also enhanced with the application of colchicine. Taken together, these improved characteristics can be implied to enhance the tomato seedling capacity for better field establishment.

Keywords: Chromosome doubling, morphology, stomatal size, chromosomal size, polyploidy

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INTRODUCTION

In 2019, from October to December, the production of tomato reached 28,130 metric tons (PSA, 2020). It is reported to be the most important crop in terms of total vitamins and minerals available in the total diet (Bhowmik *et al*., 2012). Studies have implicated tomato as a fighting agent against cancer, protects the liver from cirrhosis, reduces the risk of cardiovascular diseases, prevents arteries from hardening, and reduces high blood pressure. Farmers have long been struggling in tomato seed establishment. It was many times reported that it is widely susceptible to several biotic and abiotic stresses that lead to yield and quality reduction (Ewas *et al*., 2017). Studies show that tomato cultivars have reduced germination, growth, and both quality and quantity of fruit under drought

(Zhai *et al*., 2015). Drought significantly affects the process of photosynthesis which ultimately decease other important metabolic pathways. It also affects the antioxidant and osmoregulation pathways (Chaves *et al*., 2009). Adaptation to environmental changes nowadays is an important fitness trait for crop development.

Colchicine is an important mutagen that prevents the formation of the microtubules and doubles the number of chromosomes. The colchicine effect would block cell division at the early metaphase stage. A wide range of concentrations to this mutagen has been used for the induction of polyploidy in other plant species. In the field of horticulture, polyploidy is very important for the development of new varieties with desirable morphological traits like plant size, leaf thickness, and root length to survive harsh environmental conditions. This made a greater economic impact on food in developing and developed countries (Kharkwal and Shu, 2009).

In most plants, polyploidy is unavailable, but it can be induced synthetically with the help of mitotic inhibitors (Castro *et al.,* 2018). Polyploidy in plants may occur due to the formation of unreduced gametes or because of the artificial induction in doubling the number of chromosomes in somatic cells. The prevailing multiple biotic and abiotic stresses that affect, tomato seedling establishment *in–vitro* chromosome doubling via colchicine needs to be studied.

MATERIALS AND METHODS

Collection and Treatments

Tomato (*Lycopersicon esculentum* Mill var. Improved Pope) seed was used in this study. The seeds were collected directly from the fruit of Improved Pope tomato and were fermented for two days. These were washed with tap water and were sterilized afterward with bleach (0.01% sodium hypochlorite) for 5 min and with 70% (v/v) ethanol for 5 min. The seeds were then washed with sterilized distilled water three times. These were soaked with colchicine at different concentrations, i.e., 0.05, 0.10, and 0.15% for 4 hr, and analyzed in a completely randomized design with three replications. The treatment range was based on Eng and Ho (2019).

In Vitro Culture

The seed coat of the seeds was removed during inoculation. Seeds were then cultured on Murashige and Skoog culture media. Two seeds were inoculated per bottle. The plantlets were allowed to grow and observed for four weeks after inoculation.

Cytological Examination

The root tips of the tomato were macerated and stained with safranin and glycerol. The sample slides were then viewed under the microscope with a magnification of 400x. Chromosomes were measured using the image ruler application.

Morphological Examination

Seed germination percentage, plant height (cm), root length (cm), size of stomata and chromosome and morphological characteristics of tomato were gathered in this study. Seed germination percentage was accounted as the number of shoots germinated per treatment. Plant height was measured using a ruler at the base to top part of the plant. A ruler was used in measuring the whole root per plant. Sizes of stomata and chromosome were collected from the leaf sheaths of the plants and examined under a compound microscope using an image ruler application for measuring the length. Morphological characteristics included notable appearances of the plantlets were recorded including growth behavior and leaf sizes.

Data Analysis

The experiment was laid out in a completely randomized design. Each treatment was replicated three times. The data were subjected to analysis of variance (ANOVA) using SPSS. Tukey's test at $p < 0.05$ was utilized to obtain multiple comparisons among treatment means.

RESULTS AND DISCUSSION

Germination and Growth

As shown in Figure 1, the lowest dose of colchicine (0.05%), attained the highest germination percentage of 60%. This is followed by 0.10 and 0.15% at 53 and 46%, respectively. This shows that colchicine application improved germination, but germination percentage decreases as the dosage was increased. Colchicine usually reduces the germination rate in plants, but in the study of Kobayashi *et al*. (2008), treated seeds have a higher percentage of seed germination than control in *S. coccinea* and *S. patens* that obtained 80% germination. It was because colchicine inhibition for germination was not expressed until the initiation of root elongation. However, seedling growth was inhibited.

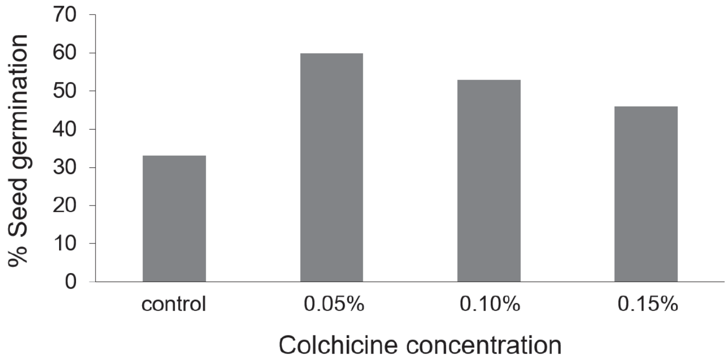

Figure 1 Seed germination percentage of *Lycopersicon esculentum* affected by colchicine treatment

In the current study, the stunting of plants is more severe in higher mutagen dose treatment. Specifically, plants treated with 0.15% dose exhibited severe stunting one week after inoculation (Figure 2). This growth behavior was related to the signs of doubling of the chromosome, as demonstrated in the study conducted by Wang *et al*. (2017) that compared with the diploid plant, the leaves of the polyploid plant were longer and broader, and the upper epidermis, lower epidermis, and palisade layer of the leaves were thicker. Mutagen can cause biological damage like a seedling injury, which worsens with the increase in dosage. It has been suggested that colchicine inhibition in some plants is not expressed until the initiation of root elongation. Development of other seeds is inhibited usually at this stage, while the growth of some seedlings usually halted after the emergence of cotyledons.

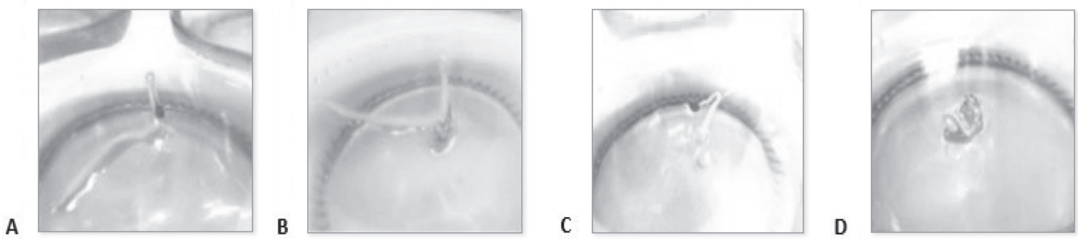

Figure 2 Growth behavior after one week from inoculation of tomato explants from untreated and colchicine-treated seeds: control (A), 0.05% (B), 0.10% (C) and 0.15% (D) colchicine

The result showed that 0.15%, which is the highest dose of colchicine, resulted in the shortest plant at 5.94 cm (SD 0.12), followed by 0.10% at 9.87 cm (SD 0.17) (Figure 3). The data in this study indicated that plant height decreases as the dosage of mutagen were increased. Shorter plants, as reported by Berry (2012), have their advantages with seed establishment, which also prevent lodging and yield loss. Lodging limits yield potential and reduce grower profits, but it is difficult to control because it is a complicated process that is influenced by many factors, including wind, rain, topography, and soil type. In addition, as the plant would bear fruit, a shorter plant would have less possibility of falling to the ground as the fruit gets heavier.

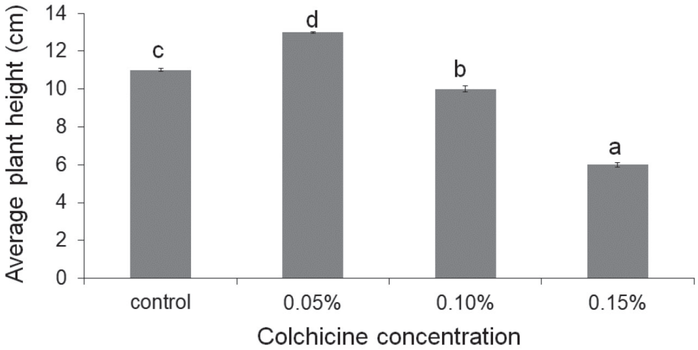

Figure 3 Average plant height after four weeks from inoculation of *Lycopersicon esculentum* affected by colchicine treatment. Different small superscript letters indicate significant differences (Tukey HSD, α = 0.05)

Leaf size is based on appearance where it can be seen that amount of colchicine dosage is directly proportional to the increase in leaf size (Figure 4). These observations are in agreement with Amit and Vikas (2015) where polyploidy generally leads to thicker leaves and expanded width-to-length ratio of leaves, larger and heavily textured flower, and a more compact growth pattern. Thicker leaves mean higher heat tolerance, and this is very advantageous most especially on the Philippine setting where drought is inevitable during the summer season. In the study of Leigh *et al*. (2012), it was demonstrated that an increase in the thickness of only fractions of a millimeter could prevent excursions to damaging high temperatures. It was concluded in the study that leaf thickness is beneficial in reducing the incidence of too much heat stress and, in some species, in enhancing long-term survival. Aside from thickness, it can be observed in the study that all treated plants have bigger leaf sizes. The size of leaves can affect the reception of light and alter leaf photosynthetic activity by adjusting light-harvesting efficiency (Sarlikioti *et al*., 2011).

Figure 4 *Lycopersicon esculentum* in terms of their leaf thickness from the colchicine untreated and treated plants: control (A), 0.05% (B), 0.10% (C) and 0.15% (D) colchicine

In Figure 5, lower colchicine resulted in longer root and this reduces as the colchicine dosage increased. Overall, colchicine applied improved root length. Treatment 0.05% attained the longest root length at 6.58 cm (SD 0.06). The untreated plants had the shortest root length at 3.00 cm (SD 0.10). Mensah *et al*. (2007) reported the same reduction of root length at increased mutagen concentration hence reduce dry weight.

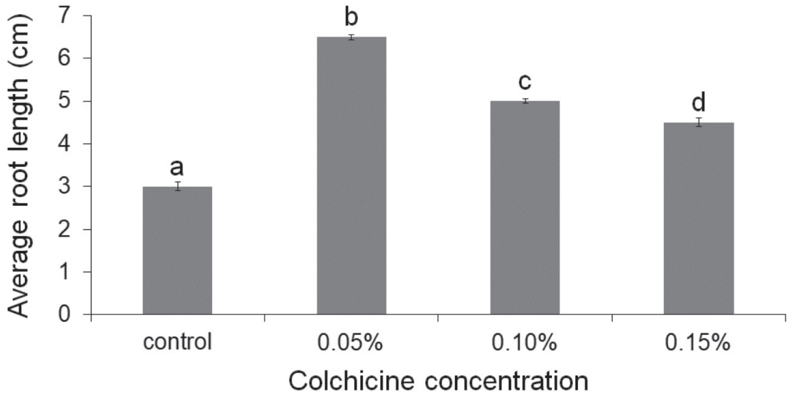

Figure 5 Average root length of *Lycopersicon esculentum* affected by colchicine treatment. Different small superscript letters indicate significant differences (Tukey HSD, α = 0.05)

Stomatal Size

The results revealed that all colchicine-treated seeds resulted in seedling with increased stomatal sizes (Figure 6A). However, stomatal sizes resulted in length and width total reduction as the colchicine dosage was increased. The application of 0.05% colchicine produced the largest stomatal length and width at 17.99 mm (SD 0.62) and 16.42 mm (SD 0.62), respectively. This is consistent with the result of Nahid *et al*. (2015), wherein increased concentration of colchicine can lower the size of the stomata of *Glycyrrhiza glabra* var. *glandulifera* and *Carthamus tinctorius* L. Larger stomata is essential in plants during photosynthesis. This controls the volume of CO_2 entering the intercellular air spaces of the leaf for photosynthesis, which was supported by the study of Doheny-Adams *et al*. (2012), where plants with reduced density and larger stomata showed reduced transpiration, greater growth rates, and larger biomass. Researchers ascribed the improved growth rate to a combination of improved water status and higher metabolic temperatures associated with the development of guard cells.

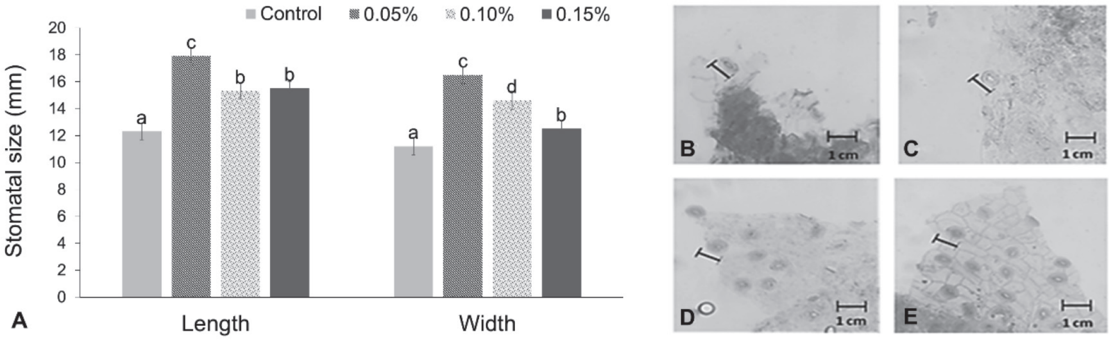

Figure 6 Stomatal sizes' length and width of *Lycopersicon esculentum* affected by colchicine treatment (A) and stomatal morphology of *Lycopersicon esculentum* from control (B), treated by 0.05% (C), 0.10% (D) and 0.15% (E) colchicine. Different small superscript letters indicate significant differences (Tukey HSD, α = 0.05)

Chromosomal Size

In vitro induction of polyploids using colchicine causes a multiplication in DNA content in plants, hence an increase in the chromosome content and sizes. In this study, it was observed that there was a relative increase in chromosome size and number with colchicine application (Figure 7). It is observable based on the documented photo that this also increases the number of chromosomes as the colchicine dosage increases. Among colchicine–treated plants, application of 0.05% colchicine produced bigger chromosome (length and width) at 21.28 mm (SD 0.66) and 15.20 mm (SD 0.50) respectively. All colchicine treated plants had improved chromosomal numbers and sizes by which decreases as the colchicine dosage increases. This result was supported by Griffiths *et al*. (1999) who found that there was a correlation between the number of chromosome set and the size of the organism.

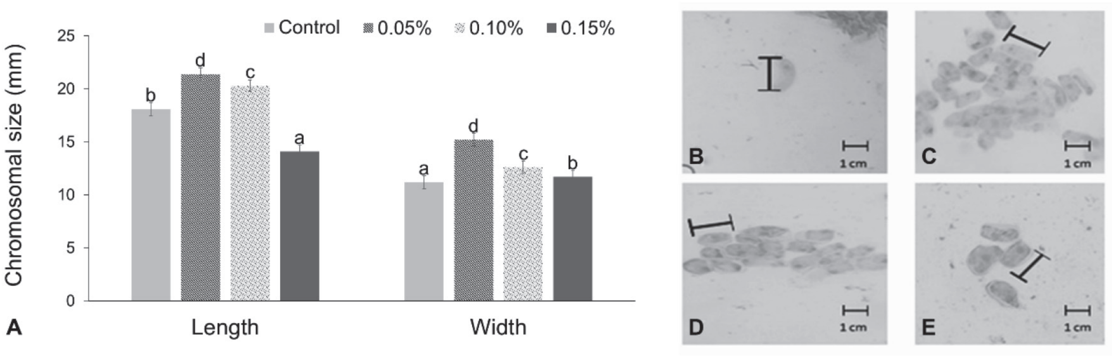

Figure 7 Chromosomal sizes' length and width of *Lycopersicon esculentum* affected by colchicine treatment (A) and chromosomal morphology of *Lycopersicon esculentum* from control (B), treated by 0.05% (C), 0.10% (D) and 0.15% (E) colchicine. Different small superscript letters indicate significant differences (Tukey HSD, α = 0.05)

Chromosome doubling is shown in Figure 8 on seeds treated with 0.10% colchicine which is the sign of polyploidy. This reportedly has a significant impact on plant physiological processes like in water relations. This would develop more extended roots and also thicker leaves that allow for retaining higher water content, which is of great help during drought period conditions and other climatic conditions. These are the reason for more developed characteristics (Manzoor *et al*., 2019).

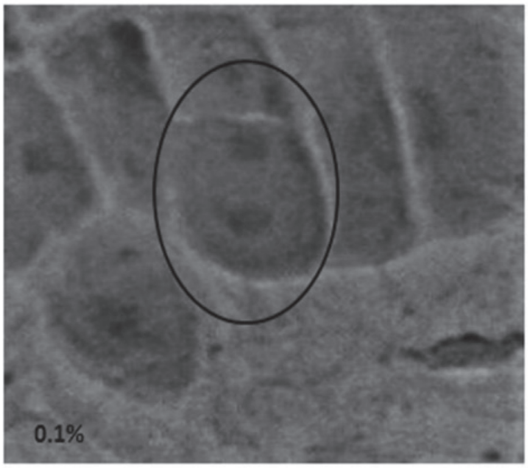

Figure 8 Chromosome doubling in 0.10% colchicine treatment

CONCLUSIONS

Colchicine treatment (0.05, 0.10, and 0.15%) was effective in inducing significant changes in the morphology and physiology of the tomato var. Improved Pope. Chromosome doubling was observed at 0.10% colchicine. These changes in characters are needed for better seedling establishment for abiotic and biotic stress conditions.

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