The physiological effects of Colchicine in Okra, *Hibiscus esculentus L*, plant growth and development

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ABSTRACT
The Okra seed germination percentage of 79.3 % was significantly lower in plants treated with Colchicine when compared to untreated plants (i.e. Control) with 94.8%. Okra plants treated with Colchicine also exhibited reduced height as well as the mean number of leaves per plant. The mean stomata size was observed to be significantly higher in plants treated with Colchicine in comparison to the Control. The mean stomata size of the Control plants was significantly lower than those seen in plants treated with Colchicine. Plants treated with Colchicine were shorter than those of the Control. The plants treated with Colchicine also had thicker and coarse leaves which were greener in colour in comparison to the Control. Higher number of branches which portrayed a bushy growth habit, thicker roots and stems were also recorded in Colchicine treated plants. Treatment of cells with Colchicine resulted in cytologically changed cells. Examination of the internal tissues also indicated that the tissues had been polyploidised following Colchicine treatment. Flowers from treated plants exhibited enlarged pollen grains. The mitotic spindle formation was inhibited in chromosomes of colchicine-treated plants observed under the microscope using acetocarmin preparations. Polyploidy cells were observed in roots, stems, pollen, and other tissues.

KEYWORDS: Germination; Colchicine; Polyploidy; Chromosomes; Stomata density

1.0 INTRODUCTION

Colchicine was isolated in 1857 from seeds of *C. autumnale* and has subsequently been obtained from other Colchicum species and numerous other members of the family *Liliaceae* (Oberlin, 1857). Colchicine is classified as an alkaloid occurring naturally in several plants belonging to the family of *Liliaceae*, an example of which is *Gloriosa superba* (Sarin et al., 1974). Alkaloids are an important group of secondary metabolites, of which colchicine is a useful agent in the treatment of acute attacks of gout. Apart from inhibiting the assembly of microtubules, the major biological effects of colchicine include leukocyte diapedesis, lysosomal degranulation, and inhibition of proliferation of fibroblasts as well as collagen transport to the extracellular space (Ghosh and Jha, 2008). Colchicine is employed in breeding and biological research to induce polyploidy and in tubulin binding assays as a positive control (Trease and Evans, 1983). The effect of Colchicine on higher plants is dependent on the length of time the cells are exposed to the alkaloid. According to Pickett-Heaps (1967), exposure to colchicine for a short period of time results in the disassembly of the interphase microtubule network, prophase band, spindle and phragmoplast. How the tubulin containing arrays induced by colchicine are organized and where they are located in the cell vary in different plant species and by cell types (Karagiannidou et al., 1995). The use of colchicine has always been linked to chromosomal duplication, although evidence exists that this substance induces mutations in multiple points of the genome being observed in different species (Franzke, and Ross, 1957).

2.0 MATERIALS AND METHOD

In this study, the germination experiment was repeated four (4) times for treated and untreated Okra seeds (i.e. four replications of 50 seeds each; for the Colchicine treatment and the Control respectively). A total of four hundred (400) healthy seeds were used in this experiment; two hundred (200) seeds were germinated upon moist filter paper soaked with the 0.025 % of Colchicine solution, while the remaining two hundred (200) seeds were sown in moist filter paper soaked with distilled water as the Control. The seed germination was monitored weekly. Fourteen (14) days after seed germination, two hundred (200) healthy seedlings each were selected from the Control and Colchicine treatment, respectively. The seedlings were transplanted into large pots and grown in the greenhouse until fruit harvest. The roots of each plant were placed on a strip of absorbent cotton that is thoroughly wet with water and then rolled into a bundle. The cotton covered the root ends and formed a plug that fitted loosely in the pot. The bundle was then inverted and set in the pot with only the stem ends immersed in the water solution of colchicine. After transplanting seedlings to the greenhouse, 50ml of 0.5% Colchicine solution was applied to the leaves and vegetative growing tips, then at three weeks intervals until two weeks before fruit harvest. Ten (10) leaves of similar age and size were sampled from tetraploid plants (i.e. treated with Colchicine) and Control plants which were untreated. The epidermis was removed from the lower surface of the lamina using a scalpel and mounted on a drop of water between slide and cover slip. The stomata density, of 10 leaves each from Control plants and plants treated with Colchicine, was counted using 40X light microscope. Paraffin sections of treated materials were taken and observed under high-power microscope to examine the possible effect of Colchicine on the tip of vigorously growing branches. In the course of this study, the following morphological parameters were measured: number of days to germination of seeds; number of leaves per plant, plant height (cm); diameter of stomata; number of branches per plant, length of longest branch per plant (cm), number of days to flowering, pollen grain viability (%) by IKI (iodine + potassium iodide) test (Baker and Baker, 1979), seed set (%), number of pods per plant, and seed weight (g). Flowers on tetraploid (treated plants) and Control plants were used for measurement of reproductive organs. Flowers were excised from the two groups of plants just a day before their opening. The cytology of the stem and root meristems was analyzed using the acetocarmin smear method (Eigsti, 1938).

3.0 RESULTS AND DISCUSSION

The seed germination percentage of 79.3 % was significantly lower in plants treated with Colchicine when compared to untreated plants (i.e. Control) with 94.8%. Plants treated with Colchicine also exhibited reduced height as well as the mean number of leaves per plant. The results of this study is consistent with those of Wright (1976) and Kerr (2001) who stated that induced tetraploid seemed to grow more slowly and growth abnormalities were the first indication of successful colchicine treatment. The mean stomata size was observed to be

significantly higher in plants treated with Colchicine in comparison to the Control. The mean stomata size of the Control plants was recorded to be 18.17 µm while the Colchicine treatment gave a mean stomata size of 23.02 µm. This result indicates that Colchicine induced epidermal polyploidy in the leaves from treated plants. These results of stomatal length and frequency agreed with those obtained by Evan (1955) and Speckman, et al. (1965), who reported that stomata length was the accurate indicator of the polyploid level in many plants. Plants treated with Colchicine were shorter than those of the Control. The results of this study further indicate that the applied Colchicine inhibited formation of spindle fibres and restricted cell wall formation thereby arresting mitosis at the anaphase stage and leading to chromosome doubling in polyploidy cells. These polyploid cells were bigger than the diploid cells in untreated plants. The resulting greater cell volume also exhibited thicker tissues, resulting in large size of plant organs. This result is in agreement with that reported by Uhlik (1981) that the polyploid plants had gigantic characteristics such as thicker wider leaves, with greater stomata size and larger flowers. The plants treated with Colchicine also had thicker and coarse leaves which were greener in colour in comparison to the Control. Higher number of branches which portrayed a bushy growth habit, thicker roots and stems were also recorded in Colchicine treated plants. The increases in dimensions and area were probably due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasm to nuclear volume, and express more proteins with the presence of more genes. This increase in size may translate to an increase in plant and its organs (Raufe et al., 2006). Cytological studies revealed cells with double the number of chromosomes in plants treated with Colchicine as compared to the untreated plants. Treatment of cells with Colchicine resulted in cytologically changed cells. This result indicates that Colchicine induced polyploidy in the treated plants, perhaps by disrupting the normal process of meiosis. Examination of the internal tissues also indicated that the tissues had been polyplodised with Colchicine treatment. This result suggests that the Colchicine may have prevented the formation of microtubules during cell division, thus the chromosomes could not pull apart like in normal mitosis. Therefore, the cells from the treated plants had double the number of chromosomes that it would normally have. Flowers from Colchicine treated plants exhibited enlarged pollen grains, the same was true for root and stem sections. The results of this study are supported by those reported by Rauf, Khan and Khan (2006) that the increases in dimensions and area were probably due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasmic to nuclear volume, and express more proteins with the presence of more genes. This increase in size may translate to an increase in plant and its organs. The mitotic spindle formation was inhibited in chromosomes of Colchicine treated plants observed under the microscope using acetocarmin preparations. Polyploidy cells were observed in roots, stems, pollen, and other tissues. In this study, preliminary tests on increase in concentrations of colchicine and increase in time the seeds were exposed to colchicine treatment caused increases in both structural abnormalities and cytological abnormalities in treated plants. The colchicine treatments interrupted or inhibited certain phases of the mitotic process. The results obtained in the main study, as shown above, on the effects of

colchicine treatment suggest that Colchicine may have interfered with cell division via mitotic spindle perturbation by perhaps activating an enzyme that disrupts spindle fibres organization and function.

4.0 CONCLUSION

The use of Colchicine, an alkaloid, in plant research especially for inducing polyploidy should be properly considered, especially admitting the fact that the chemical is toxic. Proper care should be taken when handling this chemical. Higher concentrations of colchicine applied to plants can result in significant plant tissue abnormalities and the chemical is toxic to the plants at higher concentration coupled with longer exposure time. On the other hand, Colchicine remains a useful substance that can be employed to study mitosis, changes in tissue structures due to polyploidization, etc.

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