The effects of selected substances on polyploidization and the regulation of the cell cycle

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ABSTRACT
Oryzalin, a dinitroaniline herbicide, applied to the plants inhibited microtubule polymerization from free tubulin subunits. The roots of plants treated with Oryzalin were club-shaped and the root tips were swollen. Oryzalin impeded both root and shoot growth and elongation. When Olomoucine was applied to an asynchronous *Hibiscus esculentus* cell suspension culture, the cells were blocked both in G1 and G2, but this effect was reversible. 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; Olomoucine, Oryzalin, Polyploidy; Chromosomes; Stomata density

1.0 INTRODUCTION

The cell cycle of a growing cell is the period between the formation of the cell by division of its mother cell and the time when the cell itself divides to form two daughter cells. Cyclin-dependent Kinases (CDKs) play a central role in the initiation, ordering and completion of cell cycle events (Mirnov. et. al., 1999). Inhibitors of CDK activity block the cell cycle and show anti-tumour activities (Meyer, 1996). Binding of CDK inhibitors to CDK/Cyclin complexes modulates the kinase activity. Colchicine inhibits the process of mitosis in many varieties plant cells as it interferes with the structure of the mitotic spindle (Eigsti and Dustin (1955). It also interferes with the orientation of newly deposited cellulose fibrils in plant cell walls (Green, 1962). The high frequency of mitosis is caused by an arrest of the mitoses at metaphase because of the absence of the mitotic spindle. It is quite evident that the essential effect of colchicine is a destruction of the spindle. Olomoucine is a specific inhibitor of cell cycle regulating cdc2/cdk2 kinases. Olomoucine acts as a competitive inhibitor for ATP binding (Vesely et al., 1994) and preliminary analysis of cdk2/ olomoucine crystal shows that the purine group of olomoucine is located in the pocket where ATP binds to cdk2. Most cell division inhibitors act upon the tubulin

to stop their polymerization and thus interrupt nuclear material movement, or they affect the microtubules in the process of forming the new cell wall. 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).

2.0 MATERIALS AND METHOD

In this study, Colchicine, Oryzalin, and Olomoucine were used to their effects on the cell cycle. 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, Oryzalin and Olomoucine treatments and the Control respectively). A total of four hundred (800) 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, 15µM Oryzalin, 10µM Olomoucine 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, one hundred (100) healthy seedlings each were selected from the Control and treatments with colchicine, oryzalin, or olomoucine 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 or 50ml 15µM Oryzalin, 50ml 10µM Olomoucine were 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

Oryzalin, a dinitroaniline herbicide, applied to the plants inhibited microtubule polymerization from free tubulin subunits. The roots of plants treated with Oryzalin were club-shaped and the root tips were swollen. Oryzalin impeded both root and shoot growth and elongation. When Olomoucine was applied to an asynchronous *Hibiscus esculentus* cell suspension culture, the cells were blocked both in G1 and G2, but this effect was reversible. 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. 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). 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. Oryzalin, a dinitroaniline herbicide, applied to the plants inhibited microtubule polymerization from free tubulin subunits. Microtubules are protein strands that are involved in cell division and the formation of new cell walls. This inhibition of the microtubule polymerization led to the loss of spindle and kinetochore microtubules. It also resulted in the inability of the chromosomes to migrate to the poles during mitosis. The cells showed an arrested prometaphase configuration. The work of Vaughn and Lbnen, (1991) reported a similar result and further that nuclear membranes re-formed around the chromosomal masses to form lobed nuclei. Cortical microtubules, which influence cell shape, were also absent, and, as a result, the cell expands isodiametrically. The roots of plants treated with Oryzalin were club-shaped and the root tips were swollen. Oryzalin impeded both root and shoot growth and elongation. The results of this study are congruent with those reported by Vaughn (1986) were roots of susceptible plants were club shaped after treatment with dinitroaniline herbicides. The root tip was swollen and the zone where the root hairs are formed is closer to the tip than in untreated controls. The

bases of grass shoots are also swollen, giving a bulbous appearance to this area. According to Vaughn and Lhnen, (1991) without these microtubules, cells cannot elongate but rather expand isodiametrically (square- shaped, rather than rectangular). Thus, the swollen or club- shaped root tip results from the production of isodiametric cells, because of the loss of cortical microtubules. When Olomoucine was applied to an asynchronous *Hibiscus esculentus* cell suspension culture, the cells were blocked both in G1 and G2, but this effect was reversible. This result points to the possibility that cdc2 and cdk2 related kinases are involved in both G1/S and G2/M transitions. Olomoucine was applied to protoplasts, with only active G1 cells with no initiation of DNA synthesis, after 12 hours of their isolation. Within two days after the application of Olomoucine, the cells showed an increase in size and about fifty percent of the cells were observed to be at the G2 phase. On the third day, about 96 percent of all cells had divided at least once.

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.

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