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Investigation on the effect of benzyladenine on the germination, radicle growth and meristematic cells of *Nigella sativa* L. and *Allium cepa* L.

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ABSTRACT

The effect of different benzyladenine (BA) treatments which is a phyto-regulator or plant hormone on the cytology and growth of *Nigella sativa* L. (Black seed) and *Allium cepa* L. (onion) were investigated. Six concentrations of benzyladenine ranging from 5 to 55 ppm were applied for 6, 12, 18, 24, 36 and 48 h. The treatments elevate the germination percentages of *Nigella sativa* L. and *Allium cepa* L. and increase the root growth of both plants. In contrast concentrations higher than 60 ppm for 48 h were caused an inhibition effect for both plant. The root growth initiation was concentration and/or time dependent. The applied concentrations of BA showed a promotor effect on cell division in root tips of both plants and caused an increase in their mitotic index values (MI). The elevation in MI values in root tips of *Nigella sativa* L. was more evident than that of *Allium cepa* L. All treatments changed the frequency of mitotic phases as compared with the control values. All the applied concentrations of BA significantly induced a number of chromosomal aberrations in root tip cells of *Nigella sativa* L. and *Allium cepa* L. The total percentages of abnormalities in *Nigella sativa* L. root tip cells were more than that in *Allium cepa* L. with all concentrations of BA. The most dominant types of observed abnormalities were stickiness, bridges, and C-mitosis. BA treatments produced a number of mitotic abnormalities in dividing cells in root tips of both plants resulting from its action on the spindle apparatus such as C-mitosis, lagging chromosomes and multipolar at ana-telophases. Also, BA induced vacuolated nuclei and irregular prophases. The induction of chromosomal stickiness and chromosomal aberrations such as bridges indicates its action on the chromosome. Also, the induced chromosomal bridges at ana-telophases indicates true clastogenic potential of this chemical. It may be concluded that BA causes toxic effect on root tip cells of *Nigella sativa* L. and *Allium cepa* L. and this toxicity induces different types of genic and chromosomal variations.

Introduction

Phyto-regulators or plant hormones are substances to have fundamental role in the regulation of the life cycle of the plants (Trewavas, 1981). The most used frequently cytokinins are N-(Phenyl methyl)-7H-purin-6-amine (benzyladenine; 6-Benzyladenine or 6-Benzylaminopurine or BAP) and kinetin (Kn) or 6-furfurylaminopurine. The cytokinins regulate growth and effect on germination rate in a variety of ways in different plants. Following the treatment of the oat seeds with 10 ppm and 100 ppm of BA, seed germination percentage were enhanced and recoded a value of 44% and 57%, respectively, compared to control value of 28% after 15 days (Sharma et al., 1976).

Seeds of zinnia (*Zinnia elengas*) were subjected to pre-sowing treatments using BA at 10, 20, and 30 ppm, and distilled water (control). Observations were recorded for germination percentage, speed of germination and root and shoot length. BA at 30 ppm recorded the highest germination percentage (86.66%), speed of germination (5.3) and the highest value of root length 4.63 cm (Singh, 2004).

Cytokinins, N^6-substituted adenine derivatives, are a class of plant hormones that were first identified as cell division promoter factors (Miller et al., 1956). Cytokinins have been found in almost all higher plants as well as mosses, fungi, bacteria, and also in tRNA of many prokaryotes and eukaryotes (McGaw, 1987). Application of exogenous cytokinin to some organs that normally lack this hormone has been shown to induce cell division (Riou-Khamlichi et al., 1999). The cytokinins effect not only cell division but also many other aspects of plant growth and developmental processes including seed germination, shoot initiation and growth, apical

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dominance, senescence and abscission (Mok, 1994; Dewitte et al., 1999; Werner et al., 2001).

Cytokinin (BA) promote cell division in apple tissue and cell division is still underway in fruits is applied for thinning. Root tips of *Allium cepa* treated by 50 and 100 ppm of BA for 3, 6, 12 h showed different mitotic abnormalities such as anaphase and telophase bridges, laggards, multipolar spindle formation, C-mitosis, micronuclei and endoreduplication (Soh and Yang, 1993). BA affected chromosomal behavior and caused abnormalities such as un oriented metaphase, chromosome bridges, 2 nuclear cells, and micronuclei formation (Huyluoglu et al., 2008). In contrast, in outer tissue culture of *Cymbidium* explants, cell division activity was high in the BA treatment, the cell size was smaller in BA-treated ones and the nuclear DNA contents were stable and constant on BA hormone-free and BA supplemented media (Fujii et al., 1999). Chromosomal aberrations were not observed in root tip cells of seeds germinated in distilled water and also, no chromosome abnormalities were encountered in grains treated with BA (Tabur and Demir, 2010). Plant growth regulator absorbed by the leaves and roots, with translocation in the xylem and phloem inhibits cell division in the meristematic regions, but not cell extension (Hartley and Kidd, 1987). Numerous reports assign a stimulatory or inhibitory effect of cytokinins in different development processes such as root growth and branching, control of apical dominance in the shoot, chloroplast development and leaf senescence (Mok, 1994).

Aim of the work

Study the phytotoxicity, cytotoxicity and genotoxicity of Benzyladenine by using two plants model as bio indicators.

Materials and methods

Seed germination test

Healthy and uniform size seeds of *Allium cepa* and *Nigella sativa* were obtained from the Agricultural Research Center, Giza, Egypt. Benzyladenine (BA) are supplied from Sigma. BA is dissolved in redistilled water and the applied concentrations were 5, 15, 25, 35, 45, and 55 ppm after a preliminary test which showed that concentrations higher than 60 ppm applied for 48 h exerted toxic effect on cells. To evaluate the rate of seed germination of both plants under the stress of BA, seeds of both plants presoaked in distilled water for 2 h, then placed directly in different concentrations of BA, ranging from 5 to 55 ppm and then incubated for different times (6, 12, 18, 24 36 and 48 h). For each treatment, a triplicate of 25 seeds was used. The treated seeds (25 for each treatment) were washed carefully with distilled water then transferred to Petri-dishes containing filter paper moistened with distilled water and allowed to germinate at room temperature 25 ± 1 °C for 5 days. Control seeds were treated with distilled water. Seed with radicale length of 5 mm was considered as germinated. The germinated seeds were counted and the percentages were calculated.

Root growth investigation

To study the effect of BA on root growth, the seeds were germinated in distilled water till appearance the radicle. Thirty of the germinated seeds were immersed in suitable amount of each tested concentration of 5, 15, 25, 35, 45, and 55 ppm of BA for the different times of 6, 12, 18, 24, 36 and 48 h. Similarly, 30 seedling roots were soaked in distilled water for the same period was run with each treatment as the control. Following the treatments, the treated seedlings together with each control samples were kept in the dark at 25 ± 1 °C, in order to minimize the fluctuation in the rate of cell division. At the end of each treatment time after 7 days, the length of the radicale was measured. The relative change of root length was calculated as a percentage of the variance from the control or expressed as percent of controls and expressed as T/C ratio (root length of seed treated divided by the root length of control).

Cytological examination

Ten germinated seeds, with radicle 2–3 cm long, were treated with different concentrations of BA for different times (6, 12, 18, 24 36 and 48 h). Control germinated seeds were placed in distilled water. After each treatment, the roots were cut off and immediately fixed in glacial acetic acid: absolute ethyl alcohol (1:3 v/v) for overnight. The root tips were stained by using the Feulgen squash technique (Ostergreen and Heneen, 1962). At least three slides for each treatment were examined to determine the mitotic index (MI) which was calculated as the percentage of dividing cells to the total number of cells examined. The frequency of each mitotic phase was calculated as the percentage of dividing cells in that stage to the total number of dividing cells examined. The same slides were analyzed for the percentages and types of chromosomal abnormalities in cells at each mitotic phase as well as non-dividing cells.

Statistical analysis

The significance of differences between treatments and control on both mitotic index and the frequency of chromosomal abnormalities was evaluated statistically. Each treatment was made in three replicates. For statistical analysis, one-way ANOVA (Sigma Plot 13.0 software) SPSS was used to determine significance at $p \leqslant 0.05$ and $p \leqslant 0.01$.

Results

The results given in Table 1 showed that the germination percentages in both tested plants were increased by all applied concentrations as compared with the control values. The elevation in the germination percentages increased with increasing the BA concentrations and treatment durations. Thus, the treatment with 48 h is characterized by the highest rate of germination with the six tested concentrations compared to the control values. The highest concentration of benzyladenine (55 ppm) used in this study increased the germination percentage of *Nigella sativa* seeds from the control value 80.0 ± 0.02% to 89.3 ± 0.03% after 6 h' treatment and to 98.7 ± 0.03% after 48 h. In *Allium cepa* the minimum initial percentage of germination attained is 87.3 ± 3.3% following the treatment with (5 ppm) for 6 h, then the rate sharply raised to a value of 100.0 ± 0.01% following the treatment with (55 ppm) of benzyladenine for 48 h. Thus, the results showed that increasing in the percentage of seeds germination at different concentrations indicating that seeds are sensitive to the high concentrations applied for all treatment times.

Also the results in Table 1 showed that the effects of BA on radicale growth of both tested plants varied with both concentrations applied and treatment times {the results expressed as T/C ratio (root length of seed treated divided by the root length of control)}. The initiation of root growth for each treatment time increased with increasing BA concentration from 5 to 55 ppm. After 6 h treatment, the roots were more or less normal throughout the whole range of BA concentrations from 5 to 55 ppm, while the treatment for 36 and 48 h with 55 ppm was the most effective in elevating root growth, which was statistically significant ($p \leqslant 0.01$).

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