Acta Biologica Hungarica 67(1), pp. 53–63 (2016)
DOI: 10.1556/018.67.2016.1.4

PLANT GROWTH REGULATORS INDUCED UREASE ACTIVITY IN *CUCURBITA PEPO* L. COTYLEDONS

Hamed M. El Shora[1] and Awatif S. Ali[2]*

[1]Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt
[2]Department of Botany, Faculty of Science, Kafr El Sheikh University, Kafr El Sheikh, Egypt

(Received: July 4, 2015; accepted: October 5, 2015)

This study is aimed to investigate the activity of urease (EC 3.5.1.5, urea amidohydrolase) that catalyzes the hydrolysis of urea in 5-day-old *Cucurbita pepo* cotyledons subjected to various concentrations of different growth regulators. The treatment of *C. pepo* cotyledons with different concentrations (100–600 µmol) of different auxins [indole-3-acetic acid (IAA), indole butyric acid (IBA), indole propionic acid (IPA) and naphthalene acetic acid (NAA)]; or with different concentrations (100–300 µmol) of different cytokinins [kinetin, zeatin and benzyladenine (6-BA)] resulted in a significant increase of urease activity, compared to control. The optimal effects were recorded for each of 500 µmol of IAA and 300 µmol of zeatin treatments. A gradual increase in urease activity was detected in cotyledons treated with various concentrations (0.2–1.0 mM) of 28-homobrassinolide (HBL), in relative to control. A substantial increase in urease activity was observed in cotyledons subjected to different concentrations of triazole (10–60 mg L^{-1}), containing either triadimefon (TDM) or hexaconazole (HEX), compared to control. The combination of 300 µmol zeatin with any of protein inhibitors, namely 5-fluorouridine (FUrd), cordycepin and α-amanitin, resulted in the alleviation of their inhibitory effect on the urease activity.

Keywords: Auxins – benzyladenine – brassinosteroids – cordycepin – triazole – urease activity-zeatin

INTRODUCTION

The growth and development of plants are mediated by coordination of different metabolic processes that are catalyzed by different enzymes such as urease [34]. Ureases are nickel-dependent enzymes [13], and have been isolated from a wide variety of organisms including plants, fungi and bacteria [40]. Urea received by plants as nitrogen source from soil, released from the ornithine cycle and derived from ureides and arginine produced in cytokinins metabolism [30]. Urea cannot be used directly in plant metabolism and its accumulation cause leaf burning [8]. Urease detoxifies the harmful effect of urea by catalyzing the hydrolysis of urea to form ammonia (NH_3) and carbon dioxide [27]. Ammonia is subsequently transformed into ammonium (NH_4^+) and nitrate (NO_3^-) ions. It may function coordinately with arginase in the utilization of seed protein reserves during germination [30].

*Corresponding author; e-mail address: awatifali95@yahoo.com

Plant hormones regulate the growth and development of plants through alteration metabolic processes. Auxins belong to chemically diverse compounds, most of which have an aromatic system such as indole, phenyl or naphthalene ring with a side chain containing a carboxyl group attached [9]. IPA stimulates plant growth and development, as it converted into active IAA in the tissues. IBA which is identical with IAA except two additional side chain methylene groups and classified as synthetic auxin is an endogenous plant compound. NAA is a synthetic plant growth regulator with high auxin activity [4]. Auxin compounds increased the activities of nitrogen assimilating enzymes as nitrate reductase (NR) and glutamate dehydrogenase (GDH) [31, 35].

Cytokinins may have role to coordinate root and shoot development, as they may receive long distance signal in the perception of nutrients like NO_3 by roots and the subsequent response of shoots [32]. Kinetin and zeatin were used to induce callus capable of bud formation [25]. Benzyladenine increased the resistance of plants against environmental stresses, for example it increased RuBPCase activity of wheat seedling under water deficit [39]. Triazole plant growth regulators are potent gibberellins biosynthesis inhibitors and shoot growth retardants and responsible for many other responses in plants [18]. For example, triazole compounds improve plant stress tolerance [17]. Triadimefon and triadimenol, early commercialized demethylation-inhibiting fungicides (DMIs), result in growth inhibition of roots, shoots and coleoptiles of barley and wheat seedlings, primary leaves and internodes [6, 28].

Brassinosteroids (BRs) have a broad spectrum of cellular and physiological effects, including stem elongation, pollen tube growth, leaf bending, root inhibition, fruit development, ethylene biosynthesis, proton pump activity, xylem differentiation, photosynthesis, and gene expression. Moreover, BRs are included in plant tolerance to a variety of biotic and abiotic stresses [45].

Benzyladenine obviated the effect of inhibitors of RNA and protein synthesis was dependent on transcriptional and translational processes [29]. Imbibition of *Arabidopsis* seeds in water containing urease inhibitor delayed germination by 36 h and completely blocked germination of aged seeds. This inhibition could be abolished by supplying nitrogenous compounds into the imbibition medium [48]. Plant systems have not been amenable to the use of amatoxins for the selection of RNA polymerase II mutants, although all of the RNA polymerase II activities which have been purified from plants show a sensitivity to inhibition by α-amanitin similar to the enzyme from animal sources [10].

This study aimed to investigate the stimulatory effects of different plant growth regulators [auxins (IAA, IBA, IPA and NAA), cytokinins (kinetin, zeatin, 6-BA), HBL and triazoles (TDM and HEX) on urease activity of 5-day-old *C. pepo* cotyledons. The alleviation of harmful inhibitory effects of protein inhibitors (FUrd, cordycepin and α-amanitin) on urease activity via combination with zeatin was studied.

MATERIAL AND METHODS

Plant materials

Pure strain of *Cucurbita pepo* L. seeds (marrow, family *Cucurbitaceae*) was obtained from Egyptian Ministry of Agriculture.

Seed germination and growth conditions

Seeds were germinated according to El-Shora and ap Rees [15]. Seeds of *C. pepo* were surface sterilized in 10% sodium hypochlorite for 10 min, soaked in running tap-water for 24 h, and then germinated between paper towels, moistened with distilled water in sterilized plastic trays. The trays were covered and incubated in darkness at 25 °C for 48 h. Seeds with well-grown roots were then supported on plastic bowls containing continuous and vigorously aerated 0.2 mM $CaCl_2$ solution. Seeds were grown for further 48 h in light at 25 °C.

Auxins treatments

Five-day-old *C. pepo* seedlings were incubated in Petri dishes, with 20 ml of distilled water (control) or with 20 ml of different concentrations (100–600 μM) of each of IAA, IBA, IPA and NAA for 72 h at 25 °C.

Cytokinins treatments

Five-day-old *C. pepo* seedlings were incubated with 20 ml of distilled water (control) or with 20 ml of each of kinetin, zeatin and 6-BA at various concentrations (100, 200 and 300 mM) for 72 h at 25 °C.

HBL treatment

Five-day-old *C. pepo* seedlings were incubated with 20 ml of distilled water (control) or with 20 ml of various concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mM) of HBL for 72 h at 25 °C.

Triazole treatments

Five-day-old *C. pepo* seedlings were incubated with 20 ml of distilled water (control) or with 20 ml of each of TDM and HEX at various concentrations (10–60 mg L^{-1}) for 72 h at 25 °C.

Protein inhibitors treatments

Five-day-old *C. pepo* seedlings were incubated with 20 ml of distilled water (control) or with 20 ml of each of 5 µmol FUrd, 5 µmol cordycepin and 20 µg ml^{-1} α-amanitin in absence or in presence of 300 µmol zeatin for 72 h at 25 °C.

Enzyme extraction and assay

After the incubation period, the cotyledons and roots of 8-day-old plants were excised with a razor blade and kept on ice to be used for enzyme extraction immediately. The tissue was ground with a pestle and mortar at 0–4 °C using 50 mM sodium acetate buffer (pH 6.0). The homogenate was centrifuged at 5000 *g* for 15 min, and the supernatant was collected and represents as the crude extract. urease activity was assayed according to El-Shora [14]. The reaction mixture contained 0.1 M-Tris-HCl buffer (pH 8.0), 20 mM urea, 2 mM $CaCl_2$, and the enzyme was incubated for 30 min at 30 °C. The reaction was stopped by adding 10 ml of 1 N H_2SO_4 to the mixture. The samples were centrifuged at 3000 *g* and treated with Nessler's reagent. The ammonia concentration was determined on the basis of a standard curve previously obtained with ammonium sulfate. One unit (U) of urease activity was defined as the amount of enzyme liberating one µmol NH_3 in one minute at 40 °C at the above specified conditions. Specific activity of urease is defined as the unit per mg protein.

Determination of protein content

Total protein in the extract was determined by the method of Lowry et al. [26].

Statistical analysis

All the data are expressed as mean ± SE obtained from three measurements. The data were subjected to one-way ANOVA, tests at the 5% level ($p \leq 0.05$) of significance using the SPSS version 22.0 for Windows. The post-hoc Turkey test for mean comparison was performed, for a 95% confidence level, to test for significant differences among treatments.

RESULTS

The response of urease activity in 5-day-old *C. pepo* cotyledons subjected to different concentrations of auxins, cytokinins, triazole, HBL and protein synthesis inhibitors for 72 h was conducted in this study. The results showed a positive response of urease activity for IAA, IBA, IPA and NAA treatments as displayed in (Fig. 1A, B, C and D).

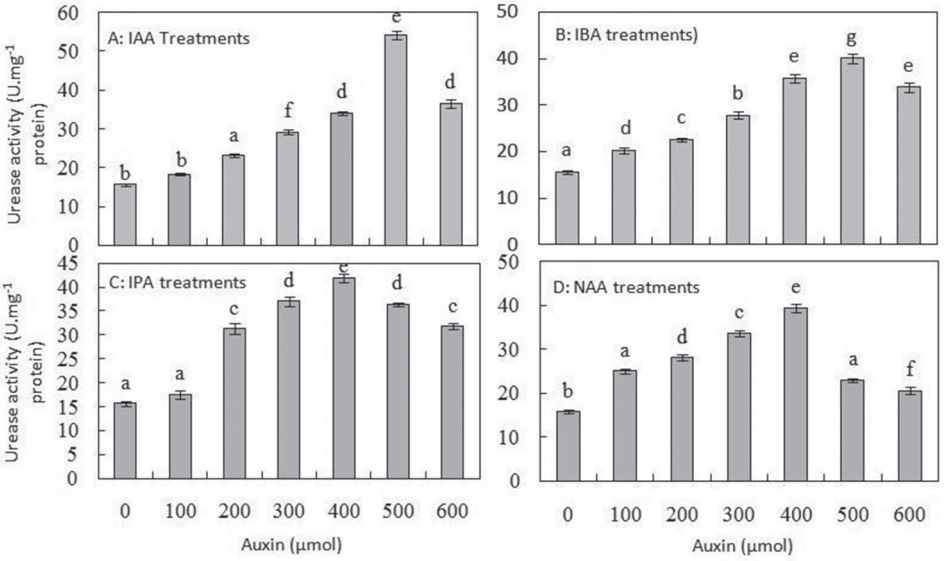

Fig. 1. Effect of different concentrations of IAA, A; IBA, B; IPA, C and NAA, D on urease activity of 5-day-old *C. pepo* cotyledons. Vertical bars represent standard errors (n = 3). Values with the same letters are not significantly different ($p \leq 0.05$, Tukey test)

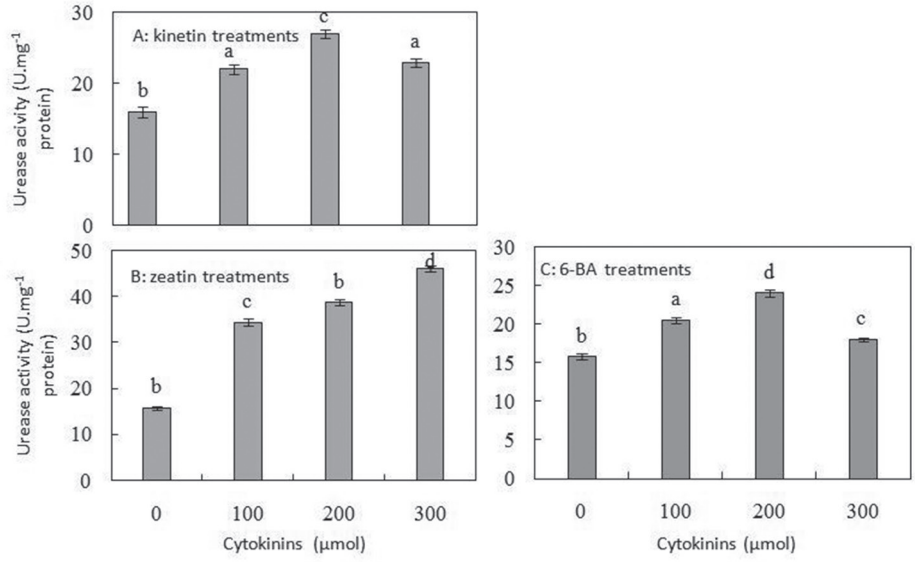

Fig. 2. Effect of different concentrations of kinetin, A; zeatin, B and 6-BA, C on urease activity of 5-day-old *C. pepo* cotyledons. Vertical bars represent standard errors (n = 3). Values with the same letters are not significantly different ($p \leq 0.05$, Tukey test)

The greatest increase in urease activity was recorded when applying 500 µmol IAA and IBA treatments respectively. The activity was 54.14 and 40.04 U·mg^{-1} protein representing about 3.5- and 2.6-fold increase, compared to the control, respectively. However, the greatest significant increase of urease activity in cotyledons subjected to different concentrations of IPA and NAA respectively, was recorded with 400 µmol IPA and NAA-treatments respectively. The activity was 41.9 and 39.34 U·mg^{-1} protein respectively representing about 2.7- and 2.5-fold increase, compared to the control.

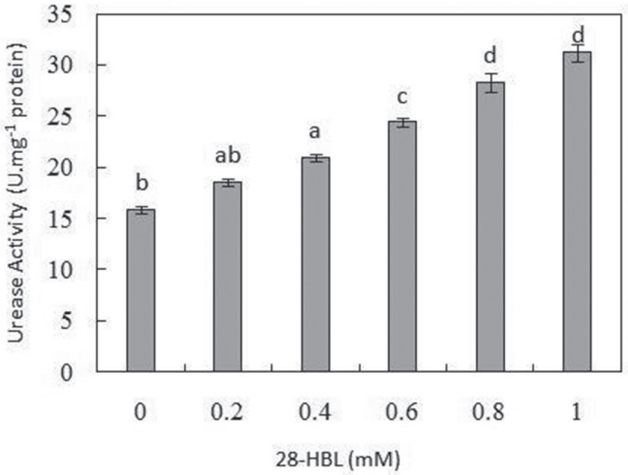

Fig. 3. Effect of different concentrations of HBL on urease activity of 5-day-old *C. pepo* cotyledons Vertical bars represent standard errors (n = 3). Values with the same letters are not significantly different ($p \leq 0.05$, Tukey test)

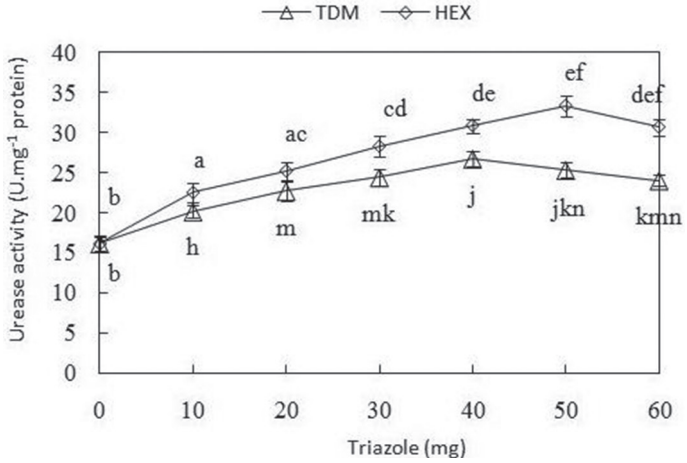

Fig. 4. Effect of different concentrations of triazole (TDM and HEX) on urease activity in 5-day-old *C. pepo* cotyledons. Vertical bars represent standard errors (n = 3). Values with the same letters are not significantly different ($p \leq 0.05$, Tukey test)

The experimental results revealed that all applied concentrations (100, 200 and 300 µmol) of kinetin, zeatin and 6-BA caused significant increase in the urease activity of *C. pepo* cotyledons (Fig. 2A, B and C), compared to control. The greatest significant increases in urease activity were detected in cotyledons treated with 200 µmol kinetin, 200 µmol 6-BA and 300 µmol zeatin, which resulted in up to 1.7-, 1.52- and 2.9-fold enhancements, compared to the control, respectively.

The results represented in Figure 3 shows the stimulatory effect of HBL on urease activity of 5-day-old *C. pepo* cotyledons. Urease activity was increased gradually with the increase of HBL concentration. The optimal increase in urease activity was observed in *C. pepo* cotyledons subjected to 1.0 mM HBL, when it increased up to 31.2 $U \cdot mg^{-1}$ protein representing about 2-fold the control.

Results in Fig. 4 show the response of urease activity of *C. pepo* cotyledons subjected to different concentrations of TDM and HEX respectively. All applied TDM and HEX treatments enhanced the urease activity, compared to control. The most effective TDM-treatment was 40 mg L^{-1} that caused the maximum significant increase in urease activity up to 26.73 $U \cdot mg^{-1}$ protein, about 1.66-fold the control. However, 50 mg L^{-1} HEX treatment was more effective and enhanced the urease activity up to 33.3 $U \cdot mg^{-1}$ protein about 2.1-fold the control.

Results in Table 1 show that both FUrd, cordycepin and α-amanitin caused a substantial and significant decrease in the urease activity in *C. pepo* cotyledons in relative to control. However, the most harmful effect was that of α-amanitin, when urease activity decreased to 35.6% in relative to control. The combination of 300 µmol zeatin that resulted in optimal increase in urease activity (about 2.9-fold the control) with each of the protein synthesis inhibitors caused an improvement in urease activity all over. Zeatin combination was more effective with cordycepin as it improved urease activity from 8.4 $U \cdot mg^{-1}$ protein (relative activity about 53.5%) to 32.4 $U \cdot mg^{-1}$ protein (relative activity about 206.3%).

Table 1

Effects of different protein synthesis inhibitors on the urease activity in 5-day-old *C. pepo* cotyledons in absence or in presence of zeatin. Data are the mean ± SE (n = 3)

Treatments	Urease activity (U mg^{-1} protein)	Relative activity (% Control)
Control	15.8 ± 0.4^{b}	100
300 µmol Zeatin	46.7 ± 0.8^{a}	299.3
5 µmol FUrd	11.4 ± 0.6^{c}	72.6
300 µmol Zeatin + 5 µmol FUrd	29.6 ± 0.5^{d}	184.7
5 µmol Cordycepin	8.4 ± 0.4^{f}	53.5
300 µmol Zeatin + 5 µmol Cordycepin	32.4 ± 0.9^{g}	206.3
20 µg ml^{-1} α-amanitin	5.6 ± 0.2^{h}	35.6
300 µmol Zeatin + 20 µg ml^{-1} α-amanitin	20.6 ± 0.5^{e}	131.2

Values with the same letters are not significantly different ($p \leq 0.05$, Tukey test).

DISCUSSION

In the present investigation, the treatment of 5-day-old *C. pepo* cotyledons with different concentrations of each of IAA, IBA, IPA and NAA resulted in a significant increase in the urease activity, compared to control. IAA was the most effective in stimulating urease activity. Optimal urease activity was observed in cotyledons subjected to 500 µmol IAA, it was about 3.5-fold higher than the control. In agreement, with it IAA, NAA, IPA, IBA and indole lactic acid enhanced the activity of GDH in pea plants and NR in leaves of a tree legume [31, 35], as well as sucrose synthase and sucrose phosphate synthase in chickpea cotyledons [36]. In contrast, NR activity in IAA-treated *Hordeum vulgare* showed no response [24]. In previous studies, auxins as IBA, 2,4-D, and NAA induced the rapid cell proliferation when they were used in tissue culture. But long time exposure to high concentrations of IBA, 2,4-D and NAA caused the suppression of morphogenesis [7]. The stimulatory effect of auxin compounds on urease activity may be resulted from the enhancement in the protein synthesis through increasing ammonium assimilating potential [3]. Other important mechanisms could explain the stimulatory action of auxins such as phosphorylation mechanism which is confirmed by higher sensitivity of *in vitro* NR to magnesium [43].

The treatment of *C. pepo* cotyledons with different concentrations of each of kinetin, zeatin and 6-BA revealed that zeatin treatments were more effective in stimulating the activity of urease (Fig. 2). The optimal urease activity was recorded in 300 µmol zeatin-treated cotyledons, it was about 2.9-fold higher than that of the control. In agreement with this, exogenous supply of benzyladenine enhanced NR activity in *Hordeum vulgare* [24] and in wheat leaves [21]. In addition, it was stated that BA induced the formation of protocorm like bodies (PLBs) and shoot formation from leaf segments of *Aerides odorata* [20]. Kinetin treatment improved seed germination and seedling growth in pea under salinity stress [38].

Cytokinins such as 6-BA, zeatin and 2-isopentyladenine were efficient elicitors in stimulating phosphoenolpyruvate carboxylase (PEPCase), the key enzyme in crassulacean acid metabolism (CAM); proline and pinitol accumulation and an osmotin-like protein in *Mesembryanthemum crystallimum* [37, 42], a halophyte, and PEPCase and carbonic anhydrase in maize, a glycophyte [41]. The stimulatory effects of cytokinins compounds exerted on urease activity may be realized via an increase in protein synthesis by stimulating the RNA polymerase activity [2], and/or via delaying the protein degradation by inhibiting ribonucleases [44]. Under stress conditions, cytokinins enhanced chloroplast development and synthesis of photosynthetic enzymes, prevents leaf senescence through the promotion of protein synthesis [12].

Urease activity of 5-day-old *C. pepo* cotyledons subjected to different concentrations of HBL exhibited a gradual increase with the increase in HBL concentration. The greatest urease avtivity was detected in 1.0 mM HBL-treated cotyledons; it was about 2-fold higher than the control value. Such enhancement effect may be associated with an increased activity/amount of other Calvin-Benson cycle enzymes. However, BRs application resulted in increasing the activity of Rubisco as well as the

activity of phosphoenolpyruvate carboxylase in soybean plants and stimulating the potential rate of RUBP regeneration in cucumber leaves [45, 47]. Also, *Cicer arietinum* plants produced from BRs pretreatmed seeds possessed higher leaf nitrate reductase and carbonic anhydrase activities, higher dry mass, higher nodule number and more nodule fresh and dry mass, compared to water soaked, control [1].

In this study, the application of different concentrations of each of TDM and HEX caused a positive response in urease activity of *C. pepo* cotyledons, compared to control. The optimal urease activity was recorded in 50 mg L^{-1} HEX-treated cotyledons where urease activity was about 2.1-fold of the activity of untreated cotyledons. In agreement with it, triazole compounds increased the activities of other plant enzymes such as amylase and invertase [5, 23]. Induction of urease activity by triazole compounds could be mediated by a reduction in free radical damage and increase in antioxidant potentials and enzyme activities [22]. Triazole compounds can inhibit GA_3 biosynthesis and in turn make substrate available for the synthesis of other isoprenoid pathway compounds like abscisic acid and or cytokinin [16].

The treatment of *C. pepo* cotyledons with 5 µmol both of FUrd and cordycepin or with 20 $\mu g \cdot ml^{-1}$ α-amanitin resulted in a sharp significant decrease in urease activity, compared to control. Interestingly, the combination of any of FUrd, cordycepin or α-amanitin with 300 µmol zeatin ameliorated their inhibitory effect for great extent. Studies showed that each of cordycepin, actinomycin D, and α-amanitin has counteractive effects including stabilization [33]. However, cordycepin was the most effective inhibitor, as inhibiting the induction of five sugar-inducible genes by sucrose [46] and increasing *GLU* and *CHN* RNA degradation [19]. Early studies revealed that FUrd reduced rRNA and DNA synthesis by 60 to 70% without affecting embryo growth to 24 h and α-amanitin application inhibited mRNA synthesis about 80% while embryo growth up to 36 h was inhibited by only 20% [11]. So, the inhibition of urease activity could be resulted from RNA degradation and consequently urease protein synthesis.

In conclusion, phytohormones [auxins (IAA, IBA, IPA and NAA); cytokinins (kinetin, zeatin and 6-BA); HBL; triazole (TDM and HEX)] stimulated the urease activity in 5-day-old *C. pepo* cotyledons. The greatest increase in urease activity in response to phytohormones was recorded in 500 µmol IAA and in 300 µmol zeatin-treated cotyledons. Combination each of FUrd, cordycepin and α-amanitin with 300 µmol zeatin resulted in high amelioration of their inhibitory effects on urease activity. Combination of zeatin with cordycepin was the best, whereas it resulted in increasing the relative activity from 53.5% up to 206.3%.

REFERENCES

1. Ali, B., Hayat, S., Ahmad, A. (2007) 28-Homobrassinosteroids ameliorates the saline stress in *Cicer arietinum* L. *Environ. Exp. Bot. 59,* 217–223.
2. Ananiev, E. D., Abdulova, G., Grozdanov, P., Karagyozov, L. (2003) Hormonal regulation of ribosomal RNA gene expression in excised cotyledons of *Cucurbita pepo* L. (zucchini). *Bulg. J. Plant Physiol*. SI. 225–231.

3. Awasthi, V., Garg, S. K. (2007) Effect of salicylic acid on enzymes of ammonium assimilation in maize seedling. *Indian J. Plant Physiol. 12,* 391–393.
4. Bajguz, A., Piotrowska, A. (2009) Conjugates of auxin and cytokinin. *Phytochemistry 70,* 957–969.
5. Buchnauer, H., Röhner, E. (1981) Effect of triadimefon and triadimenol on growth of various plant species as well as on gibberellin content and sterol metabolism in shoots of barley seedlings. *Pesticide Bioch. Physiol. 15,* 58–70.
6. Cavariani, C., Velini, E. D., Bicudo, S. J., Nakagawa, J. (1994) Avaliação dos efeitos de doses de triadimenol e de tebuconazole sobre o crescimento do mesocótilo em plântulas de trigo. *Pesq Agropec Bras. 29,* 1035–1039.
7. Chung, H. H., Chen, J. T., Chang, W. C. (2007) Plant regeneration through direct somatic embryogenesis from leaf explants of *Dendrobium*. *Biol. Plant. 51,* 346–350.
8. Ciurli, S., Benini, S., Rypniewski, W., Wilson, K. S., Miletti, S., Mangani, S. (1999) Structural properties of the nickel ion in urease: novel insights into the catalytic and inhibition mechanism. *Coordination Chem. Rev. 190–192,* 331–355.
9. Cohen, J. D., Bandurski, R. S. (1982) Chemistry and physiology of the bound auxins. *Annu. Rev. Plant Physiol. 33,* 403–430.
10. Cooke, R. M., Durand, R., Job, C., Penon, P., Mteissere, Job, D. (1984) Enzymatic properties of plant RNA polymerases. *Plant Mol. Biol. 3,* 217–225.
11. Datte, K., Marsh, L., Marcus, A. (1983) Early growth of wheat embryonic axes and the synthesis of RNA and DNA. *Plant Physiol. 72,* 394–397.
12. Davies, P. J. (2010) The plant hormones: their nature, occurrence, and functions. *Plant Hormones* A: 1–15.
13. Dixon, N. E., Gazzola, T. C., Blakeley, R. L., Zermer, B. (1975) Jack bean urease (EC 3.5.1.5). A metalloenzyme. A simple biological role for nickel? *J. Am. Chem. Soc. 97,* 4131–4133.
14. El-Shora, H. M. (2001) Effect of growth regulators and group-modifiers on NADH-glutamate synthase of marrow cotyledons. *J. Biol. Sci. 1,* 597–602.
15. El-Shora, H. M., ap Rees, T. (1991) Intracellular location of NADP-linked malic enzymes in C_3 plants. *Planta 185,* 362–367.
16. Fletcher, R. A., Arnold, V. (1986) Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. *Physiol. Plant. 66,* 197–201.
17. Fletcher, R. A., Hofstra, G. (1988) Triazoles as potential plant protectants. In: Berg, D., Plemple, M. (eds), Sterol biosynthesis inhibitors. Ellis Horwood Ltd., Cambridge, pp. 321–331.
18. Gao, J., Hofstra, G., Fletcher, R. A. (1988) Anatomical changes induced by triazoles in wheat seedlings. *Canad. J. Bot. 66,* 1178–1185.
19. Holtorf, H., Schöb, H., Kunz, C., Waldvogel, R., Meins, F. Jr. (1999) Stochastic and nonstochastic post-transcriptional silencing of chitinase and β-1,3-glucanase genes involves increased RNA turnover. A possible role for ribosome independent RNA degradation. *Plant Cell 11,* 471–484.
20. Hongthongkham, J., Bunnag, S. (2014) *In vitro* propagation and cryopreservation of Aerides odorata Lour. Orchidaceae). *Pak. J. Biol. Sci. 17,* 608–618.
21. Jain, V., Abrol, Y. P. (2005) Regulation of nitrate reductase activity by phytohormones in wheat. *J. Plant Biol. 32,* 43–48.
22. Jaleel, C. A., Gopi, R., Alagu Lakshmanan, G. M., Panneerselvam, R. (2006) Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. *Plant Sci. 171,* 271–276.
23. Kishorekumar, A., Jaleel, C. A., Manivannan, P., Sankar, B., Sridharan, R. et al. (2006) Differential effects of hexaconazole and paclobutrazol on the foliage characteristics of Chinese potato (*Solenostemon rotundifolius* Poir., J. K. Morton). *Acta Biol. Szegediensis 50,* 127–129.
24. Kumar, P. A., Lakshmikumaran, M., Abrol, Y. P. (1993) Hormonal regulation of nitrate reductase gene expression in *Hordeum vulgare*. *Indian J. Expt. Biol. 31,* 472–473.
25. Lee, S. H., Lee, D. G., Woo, H. S., Lee, K. W., Kim, D. H. (2006) Production of transgenic orchardgrass via *Agrobacterium*-mediated transformation of seed-derived callus tissues. *Plant Sci. 171,* 408–414.

26. Lowry, O. H., Rosenbrough, N. J., Randall, R. J. (1951) Protein measurement with the Folin-phenol reagent. *J. Biol. Chem. 183,* 265–275.
27. Mérigout, P., Lelandais, M., Bitton, F. et al. (2008) Physiological and transcriptomic aspects of urea uptake and assimilation in *Arabidopsis* plants. *Plant Physiol. 147,* 1225–1238.
28. Montfort, F., Klepper, B. L., Smiley, R. W. (1996) Effects of two triazole seed-treatments, triticonazole and triadiminol, on growth and development of wheat. *Pest. Sci. 46,* 315–322.
29. Oaks, A. (1994) Efficiency of nitrogen utilization in C_3 and C_4 cereals. *Plant Physiol. 106,* 407–414.
30. Polacco, J. C., Holland, M. A. (1993) Roles of urease in plant cells. In: *Int. Rev. Cyt. – A Survey of Cell Biol. 145,* 65–103.
31. Premabatidevi, R. K. (1998) Effect of IAA, GA_3 and kinetin on nitrate reductase and nitrite reductase in the leaves of a tree legume (*Perkia javanika* Merr). *Indian J. Plant Physiol. 3,* 97–101.
32. Rahayu, Y. S., Walch-Liu, P., Neumann, G., von Wireń, N., Bangerth, F. (2005) Root-derived cytokinins as long-distance signals for NO_3^- induced stimulation of leaf growth. *J. Exp. Bot. 56,* 1143–1152.
33. Rajagopalan, L. E., Malter, J. S. (1996) Turnover and translation of *in vitro* synthesized messenger RNAs in transfected, normal cells. *J. Biol. Chem. 271,* 19871–19876.
34. Renaut, J., Hausman, J. F., Wisniewski, M. E. (2006) Proteomics and low temperature studies: bridging the gap between gene expression and metabolism. *Physiol. Plant. 126,* 97–109.
35. Sahulka, J., Gaudinova, A. (1975) Regulation on glutamate dehydrogenase level in excised pea roots by some exogenously supplied compounds with auxin activity. *Biol. Plant. 17,* 228–230.
36. Satvir, K., Gupta, A. K., Narinder, K. (2003) Priming of chickpea seeds with water and mannitol overcomes the effect of salt stress on seedling growth. *ICPN 10,* 18–20.
37. Schmitt, J. M., Piepenbrock, M. (1992) Regulation of phosphoenolpyruvate carboxylase and crassulacean acid metabolism induction in *Mesembryanthemum cystallinum* L. by cytokinin modulation of leaf gene expression by roots. *Plant Physiol. 99,* 1664–1669.
38. Setia, R. C., Narang, S. (1985) Effects of salinity stress and plant growth regulators on seed germination and seedling growth in pea (*Pisum sativum*). *Flora (Jena) 177,* 369–375.
39. Shang, Z. (2000) Effect of 6-BA and KT on photophosphorylation activity in wheat flag leaves under water stress. *Acta Agriculturae Boreali-Sinica 15,* 34–38.
40. Sirko, A., Brodzik, R. (2000) Plant ureases: roles and regulation. *Acta Biochim. Pol. 47,* 1189–1195.
41. Sugiharto, B., Burnell, J. N., Sugiyama, T. (1992) Cytokinin is required to induce the nitrogen-dependent accumulation of mRNAs for phosphoenolpyruvate carboxylase and carbonic anhydrase in detached maize leaves. *Plant Physiol. 100,* 153–156.
42. Thomas, J. C., McElwain, E. F., Bohnert, H. J. (1992) Convergent induction of osmotic stress-responses. Abscisic acid, cytokinin, and effects of NaCl. *Plant Physiol. 100,* 416–423.
43. Vuylsterker, C., Leleu, O., Rambour, S. (1997) Influence of BAP and NAA on the expression of nitrate reductase in excised chicory roots. *J. Expt. Bot. 48,* 1079–1085.
44. Wingler, A., von Schaewen, A., Leegood, R. C., Lea, P. J., Quick, W. P. (1998) Regulation of leaf senescence by cytokinin, sugars, and light. Effects on NADH-dependent hydroxypyruvate reductase. *Plant Physiol. 116,* 329–335.
45. Xia, X. J., Wang, Y. J., Zhou, Y. H., Tao, Y., Mao, W. H. et al. (2009) Reactive oxygen species are involved in brassinosteroid-induced stress tolerance in cucumber. *Plant Physiol. 150,* 801–814.
46. Yadav, U. P., Ivakov, A., Feill, R., Duan, G. Y., Walther, D. (2014) The sucrose–trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signaling by Tre6P. *J. Exp. Bot. doi*: 10.1093/jxb/ert457.
47. Zhang, Y. H., Wang, Z. M., Huang, Q., Shu, W. (2008) Phosphoenolpyruvate carboxylase activity in ear organs is related to protein concentration in grains of winter wheat. *J. Cereal Sci. 47,* 386–391.
48. Zonia, L. E., Stebbins, N. E., Polacco, J. C. (1995) Essential role of urease in germination of nitrogen-limited *Arabidopsis thaliana* seeds. *Plant Physiol. 107,* 1097–1103.