ALLEVIATION OF COPPER STRESS WITH HOMOBRASSINOSTEROID IN GERMINATING SUNFLOWER ROOTS

Article *in* Fresenius Environmental Bulletin · November 2016

CITATIONS
0

READS
62

3 authors, including:

Sevgi Marakli
Amasya University
19 PUBLICATIONS **44** CITATIONS
SEE PROFILE

Nermin Gozukirmizi
Istanbul University
130 PUBLICATIONS **1,168** CITATIONS
SEE PROFILE

© by PSP Volume 25 – No. 11/2016, pages 4872-4878 Fresenius Environmental Bulletin

ALLEVIATION OF COPPER STRESS WITH HOMOBRASSINOSTEROID IN GERMINATING SUNFLOWER ROOTS

Kevser Betul Burun[1], Sevgi Marakli[2]*, Nermin Gozukirmizi[2]

[1]Bartin University, Faculty of Science, Department of Molecular Biology and Genetics, 74100, Bartin/TURKEY
[2]Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, 34118, Vezneciler Street-Istanbul/TURKEY

ABSTRACT

We investigated potential alleviation effects of homobrassinosteroid (HBR) hormone on sunflower (*Helianthus annuus* L. cv. TR003) grown under copper (Cu) stress conditions. Seeds were grown under Cu (30 µM and 40 µM) and also HBR (2 µM) treatments to analyse primary root lengths, protein contents, antioxidant system enzymes' activities (superoxide dismutase-SOD and catalase-CAT) and expressions of these enzymes at 48 h and 72 h. When compared to control, Cu applications decreased primary root lengths. On the other hand, HBR application increased primary root lengths of Cu-stressed samples at 48 h and 72 h. Depending on concentration and timing, protein contents and enzyme activities showed varying results in Cu-treated and Cu+HBR-applied samples. In addition to enzyme activities, SOD and CAT expression profiles were also investigated by RT-PCR. Expected band sizes for SOD (309 bp) and CAT (248 bp) were observed in all samples. These results indicate a positive role of HBR on Cu-stressed sunflower for agricultural applications.

KEYWORDS:
Helianthus annuus L., heavy metal, plant hormone, root growth, antioxidant enzyme activity.

INTRODUCTION

Cu is an essential micronutrient for most biological organisms and affects photosynthesis, electron transport chain, respiration, cell wall metabolism and hormone signaling [1, 2, 3]. Cu toxicity in plants is also associated with the production of reactive oxygen species (ROS) leading to oxidative stress in plants [4, 5].

Plants have developed complex mechanisms to avoid the accumulation of free Cu ions in cells [2, 6]. These mechanisms could be morphological, physiological and molecular level. Among these mechanisms, the reorganisation of the root system architecture (RSA) also shows a high degree of plasticity [7, 8] and stress conditions cause remodelling of the RSA characterised by an inhibition of primary root (PR) growth [9, 10]. In addition, plants also provide a balance between the production of activated oxygen species and scavenging capacity of antioxidants [12, 13]. In addition, plants activate gene expression mechanisms to alleviate abiotic stresses [13, 14].

Phytohormones also play an important role in plant growth, development and even yield. Among the phytohormones, brassinosteroids (BRs) form a group of steroidal lactones structurally similar to animal and insect steroid hormones. BRs regulate various developmental and physiological processes, including cell elongation, morphogenesis, tissue differentiation and reproduction [15, 16]. Various studies show that BRs have also the ability to enhance the capacity of plants to cope with various stresses like heavy metal, water, salt etc. [9, 17, 18]. The aim of this study was to investigate the alleviation effect of exogenously applied HBR on Cu-stressed sunflower roots in terms of morphologic studies (primer root lengths), physiologic studies (SOD and CAT enzyme activities) and molecular studies (SOD and CAT expression profiles).

MATERIALS AND METHODS

Helianthus annuus L. cv. TR003 seeds were obtained from Directorate of Trakya Agricultural Research Institute and used as plant material. Three different experiment groups were prepared at 48 h and at 72 h. The first group was control group. Cu-stressed samples were in the second group. For this purpose, copper II sulfate ($CuSO_4$) was used for generating Cu stress (30 µM and 40 µM) sunflower seeds. In the last group, HBR (2 µM) supplemented with 30 µM and 40 µM Cu was applied on sunflower seeds. Seeds were placed randomly in Petri dishes (9 cm diameter) containing filter paper soaked in (1) only H_2O (control), (2) 30 µM Cu, (3) 30 µM Cu+2 µM HBR, (4) 40 µM Cu, (5) 40

µM Cu+2 µM HBR. All cultures were kept in dark in a controlled growth chamber (26ºC). Each concentration with three separate experiments was evaluated. Totally 45 sunflower seeds for 48 h and 45 sunflowers for 72 h were used (3 seeds in each petri dish, three replicates for 5 different applications). Primary root lengths were statistically evaluated. We observed Cu and HBR treatments were more effective at 72 h than at 48 h. Therefore, protein contents, enzyme activities and molecular analyses were carried out for only 72 hour-samples.

Determination of protein content and antioxidant enzyme activities. Control and treated-roots at 72 h were homogenized in mortar and pestle with liquid nitrogen, subsequently 1 ml extraction buffer (50 mM PBS [0.2 M monobasic sodium phosphate, 0.2 mM dibasic sodium phosphate pH 7.0], 0.1 mM EDTA, 4% polyvinylpyrrolidone) was used per 0.01 mg plant material. The homogenate was centrifuged at 14000 g for 20 min at 4ºC [19]. The supernatant was used for protein content and enzyme activity analyses [20]. Characterization of protein profiles was carried out using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For this purpose, supernatant for each experimental group was denatured by heating at 100ºC for 5 minutes and loaded in 15% acrylamide slab gel containing 10% SDS. The run was performed at 120 V for 75 minutes till the tracing bromophenol blue dye reached the gel bottom. Protein bands were visualised by staining the gels with 0.1% Coomassie Brilliant Blue R-250.

SOD activity was analysed by measuring the ability of enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), as described by Cakmak and Marschner (1992) [21], which measures inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm. One unit of activity was determined as amount of enzyme required to inhibit the photoreduction of NBT to blue formazan by 50% and was expressed as SOD unit's mg^{-1} protein. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 100 µM EDTA, 50 mM Na_2CO_3, 13 mM L-methionine, 75 µM p-nitrobluetetrazolium chloride (NBT) and 2 µM riboflavin. Reactions were carried out at 25ºC, under light intensity of about 300 μmol^{-1} $m^{-1}s^{-1}$ through 7 min and spectrophotometric readings at 560 nm were recorded. The experiments were repeated at three times.

Catalase activity was determined according to Cho et al. (2000) [22], who measured the decline of the extinction of H_2O_2 at the maximum absorption at 240 nm. About 5 µl of samples were added to the reaction mixture containing 130.5 µl H_2O, 20 µl phosphate buffer (50 mM, pH 7.0) and 44.5 µl hydrogen peroxide (35%). Spectrophotometric readings were obtained after addition of enzyme extract for 120 seconds of 10 second intervals and results were presented as ΔA240/min/mg protein. Experiments were repeated at three times.

Statistical analysis. Variance was analysed statistically between control and treatment groups by using T-test (Graphpad). The data were obtained from three independent experiments. For the statistical evaluation of the results, the significance was accepted at the probability level of $P<0.05$.

Analyses of SOD and CAT expressions by RT-PCR. Gene expression profiles of SOD and CAT enzymes were investigated by RT-PCR. Total RNA of all samples at 72 h was extracted by TRI Reagent® (Sigma, T9424) and cDNAs were synthesized from RNA samples with the following steps: DNase I (RNase-free, AM224, Ambion) was added to the RNA samples to prevent DNA contamination and then cDNA synthesis was carried out using cDNA synthesis kit (Transcriptor High Fidelity cDNA Synthesis Kit, 05091284001, Roche) according to the manufacturer's instructions.

SOD and CAT primers (Table 1) were used in RT-PCR analyses designed by Fernández-Ocaña et al. (2011) [13] and Pena et al. (2011) [14]. RT-PCR was performed in a total volume of 20 µL, containing 11.4 µl of sterile distilled water, 2 µl of 10X buffer (1X), 2 µl of 25 mM $MgCl_2$ (2.5 mmol/L), 0.4 µl of 10 mM dNTP mixture (0.2 mM), 1 µl of 10 pmol/µl of each primer (0.4

TABLE 1
Primer sequences for the amplification of SOD and CAT.

No	Primer	Sequence (5'→3')	Ta (ºC)	Target size (bp)
1	*CuZn-SOD-F*	GCTCCTAAGCCGCTTACGGTTGTCG	58	309
2	*CuZn-SOD-R*	CACGCCATCGGCATTGGCAATTATG	58	309
3	*CAT-F*	CTTCCCGCTTGAATGTGAAG	61	248
4	*CAT-R*	CCGATTACATAAACCCATCATC	61	248

TABLE 2
Primary root lengths (cm) of *Helianthus annuus* L. cv. TR003 after 48 h and 72 h germination between filter papers at dark.

	Control	30 µM Cu	30 µM Cu+2 µM HBR	40 µM Cu	40 µM Cu+2 µM HBR
Primary root lengths (cm) after 48 h germination	0.96 ± 0.14	0.85 ± 0.29	0.89 ± 0.03	0.81 ± 0.3	1.15 ± 0.33
Primary root lengths (cm) after 72 h germination	2 ± 0.38	1.44 ± 0.28	2 ± 0.38	1.61 ± 0.47	2.1 ± 0.76

pmol/µl), 2 µl of 25 ng/µl template cDNA (2 ng/µl), 0.2 µl of 5 U/µl High Fidelity PCR Enzyme Mix (K0192, Fermentas). The values given in parentheses were the final concentrations. PCR conditions were as follows: initial denaturation at 95°C (3 min) followed by 30 cycles of denaturation at 94°C (30 sec), annealing at different temperatures which are 58°C for SOD and 61°C for CAT (30 sec) and extension at 72°C (1 min).The reaction was completed by additional extension at 72°C for 10 min. Twenty microliters of PCR products were mixed with 4 µl 6X loading buffer and were resolved in an agarose gel (1 % concentration) at 70 V for 40 min in 1X TAE buffer. A molecular weight marker (GeneRuler™ DNA Ladder Mix, SM0331, Fermentas) was also loaded to determine the size of the amplicons. After running, the gels were photographed on a UV transilluminator.

RESULTS

Sunflower seeds grown under (1) only H_2O (control), (2) 30 µM Cu, (3) 30 µM Cu+2 µM HBR, (4) 40 µM Cu, (5) 40 µM Cu+2 µM HBR showed different root lengths at 48 h and at 72 h (Figure 1, A and B, respectively). 2 µM HBR application alleviated the negative effects of Cu on sunflower roots (3, 5 in Figure 1, A and B).

Cu caused negative effects on primary root lengths (Table 2). As compared to control (0.96 ± 0.14 and 2 ± 0.38 for 48 h and 72 h, respectively), the lengths of primary root decreased in 30 µM Cu application (11.4% and 28%, 48 h and 72 h, respectively) and 40 µM Cu application (15.6% and 19.5%, 48 h and 72 h, respectively). When compared to only Cu treatment, root lengths increased in Cu+HBR-applied samples (5% for 48 h and %38.8 for 72 h at 30 µM Cu+2 µM HBR; 42% for 48 h and 30% for 72 h at 40 µM Cu+2 µM HBR).

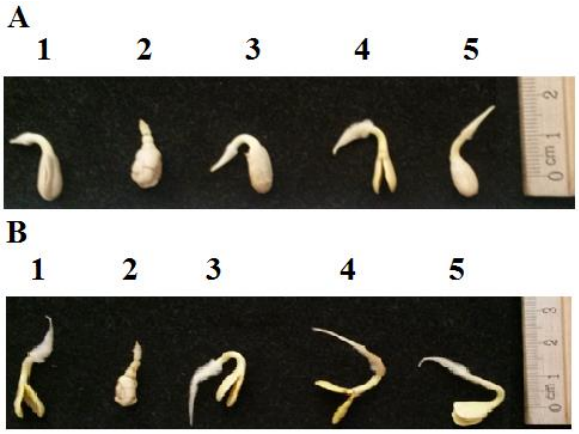

FIGURE 1
Morphological view of *Helianthus annuus* L. cv. TR003 primary roots germinated for A. 48 h, B. 72 h. 1, control; 2, 30 µM Cu; 3, 30 µM Cu+2 µM HBR; 4, 40 µM Cu; 5, 40 µM Cu+2 µM HBR, respectively.

Determination of protein content and antioxidant enzyme activities. Compared with control (5.45 ± 2.25), protein content of 30 µM Cu-treated samples increased (8.3%). In addition, protein content of 40 µM Cu-treated samples (23.6%) was also higher than control. 30 µM Cu+HBR application decreased protein content (9%) as compared to only 30 µM Cu-applied samples. On the other hand, protein content increased in 40 µM Cu+HBR treatments (%6) compared to only 40 µM Cu-applied samples. This result showed that HBR application stimulated reverse effect on low Cu stress (30 µM) in terms of protein concentration (Table 3).

TABLE 3
Protein contents and antioxidant enzyme activities of *Helianthus annuus* L. cv. TR003 roots after 72 h of germination.

	Control	30 µM Cu	30 µM Cu+2 µM HBR	40 µM Cu	40 µM Cu+2 µM HBR
Protein content (mg/ml)	5.45 ± 2.25	5.9 ± 1.76	5.36 ± 1.53	6.74 ± 2.95	7.14 ± 1.14
Superoxide dismutase activity ΔA_{560} min^{-1} mg^{-1} protein	0.014 ± 0.03	0.015 ± 0.01	0.014 ± 0.08	0.013 ± 0.01	0.012 ± 0.01
Catalase activity ΔA_{240} min^{-1} mg^{-1} protein	0.019 ± 0.01	0.016 ± 0.02	0.023 ± 0.01	0.033 ± 0.033	0.035 ± 0.035

Total proteins were extracted from experimental groups after 72 h treatment and analysed by SDS-PAGE. As visualised from SDS-PAGE, Cu and HBR treatments caused a significant change in the electrophoretic protein band profiles at 72 h (Figure 2).

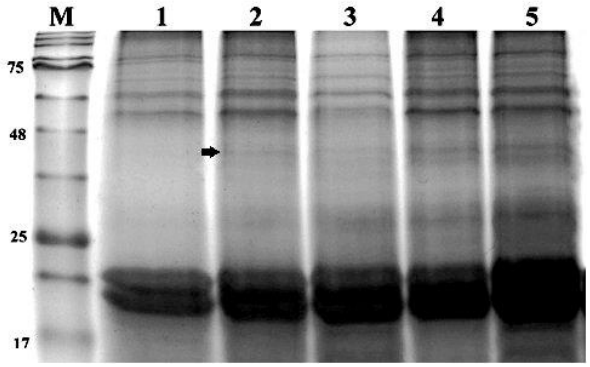

FIGURE 2
SDS-PAGE analysis of 72 h germinated root samples. 1, control; 2, 30 µM Cu; 3, 30 µM Cu+2 µM HBR; 4, 40 µM Cu; 5, 40 µM Cu+2 µM HBR, respectively. The arrow shows the polymorphic band.

In addition to protein analyses, SOD and CAT enzyme activities were also investigated in 72-hour-samples. There were also slightly differences among samples. Only Cu application (0.015 ± 0.01 for 30 µM Cu) enhanced enzyme activities as compared to control (0.014 ± 0.03). However, high Cu concentration (40 µM) showed opposite result. Comparison with control (0.014 ± 0.03), SOD enzyme activity decreased (0.013 ± 0.01) in high Cu concentration. Cu+HBR applications indicated that SOD enzyme activity declined as a result of HBR treatment when compared with only Cu application (0.014 ± 0.08 for 30 µM Cu+2 µM HBR; 0.012 ± 0.01 for 40 µM Cu+2 µM HBR) (Table 3). Different results were obtained as a result of CAT enzymes activities. Low Cu application (0.016 ± 0.02 for 30 µM Cu) decreased CAT activity as compared with control (0.019 ± 0.01). On the other hand, HBR application increased activity (0.023 ± 0.01 for 30 µM Cu+2 µM HBR). Other Cu application (40 µM) showed a distinct result. Both Cu and Cu+HBR application increased CAT enzyme activity (0.033 ± 0.033 for 40 µM Cu and 0.035 ± 0.035 for 40 µM Cu+2 µM HBR).

Analyses of SOD and CAT by RT-PCR. SOD and CAT gene expressions were analysed with RT-PCR by using samples' cDNAs as a template. PCR products were then resolved on a 1% agarose gel (Figure 3 and 4).

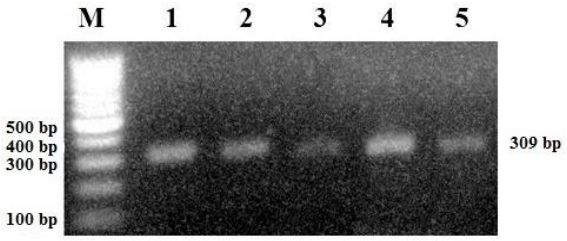

FIGURE 3
PCR results for SOD. M, marker; 1, control; 2, 30 µM Cu; 3, 30 µM Cu+2 µM HBR; 4, 40 µM Cu; 5, 40 µM Cu+2 µM HBR, respectively.

Expected band size for SOD (309 bp) was observed in all samples (Figure 3). SOD gene expression decreased with 30 µM Cu+2 µM HBR and 40 µM Cu+2 µM HBR applications. This result was supported by SOD enzyme activity analysis.

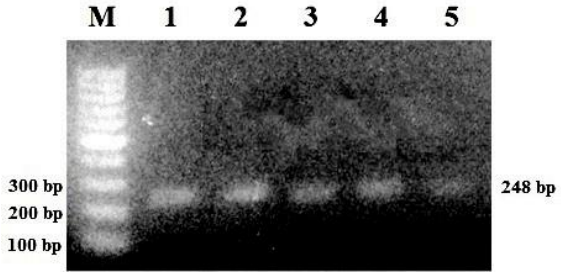

FIGURE 4
PCR results for CAT. M, marker; 1, control; 2, 30 µM Cu; 3, 30 µM Cu+2 µM HBR; 4, 40 µM Cu; 5, 40 µM Cu+2 µM HBR, respectively.

CAT expression profile was also analysed among samples. We observed expected band size (248 bp) in 1% agarose gel. There were no different expression profiles among samples (Figure 4).

DISCUSSION

The present study indicates the effects of HBR on primary root lengths, protein content, antioxidant system enzymes (SOD and CAT), expression profiles of SOD and CAT in sunflower seeds (*Helianthus annuus* L. cv. TR003) germinated under different Cu concentrations (30 µM and 40 µM) at 48 h and 72 h. Root growth was analysed at 48 h and 72 h but other experiments (protein content, enzyme activities and molecular analyses) were performed only at 72 h. Cu stress reduced primary root lengths. Janas et al. (2010) [23] reported the same result. They studied lentil (*Lens culinaris* Medic.) seedlings and found that Cu application decreased root elongation. In another study, five-week-old *Arabidopsis* plants growing in hydroponics were exposed to different Cu^{2+} concentrations (up to 5 µM) and results showed that primary root elongation reduced with increasing Cu concentrations [25]. Moreover, Thounaojam et al. (2012) [26] applied three different Cu concentrations (10, 50 and 100 µM) on rice for 5 days. They reported that decreasing in root growth was observed with the increase of Cu concentration and duration of treatment. Results indicated that Cu+HBR applications alleviated Cu stress both 48 h and 72 h. This result was concordant with Fariduddin et al. (2009) [27] who studied on *Brassica juncea*. They cultivated *Brassica juncea* plants with 50, 100 and 150 mg/kg Cu concentrations and then they applied to these plants in 10^{-10},10^{-8} and 10^{-6} M HBL (28-Homobrassinolide). They reported that Cu reduced root growth but HBL application alleviated this stress (especially Cu50 + 10^{-10} HBL application). In another study, Choudhary et al. (2012) [4] supported these results. They studied the effect of EBR (24-epibrassinolide) on *Raphanus sativus* plants. They concluded that Cu application reduced root elongation by 2 fold. When compared to only Cu treatment, EBR increased root growth by 2 fold. The growth-promoting effects of BRs on seedlings under Cu stress may be related to the general ability of BRs to promote cell elongation and cell cycle progression [28, 29, 30].

We also observed that application of both low Cu concentration (30 µM) and high Cu concentration (40 µM) increased protein content when compared to control. Protein content analyses were performed only 72-hour-samples. This period of time might not be enough to observe Cu effect. Moreover, sunflowers could increase protein content to ameliorate this stress. Körpe and Aras (2011) [31] reported opposite results. They studied with *Solanum melongena* L. plants under four different Cu concentrations (30, 60, 120 and 240 mg/l) and application of 240 mg/l Cu concentration reduced protein content. In this study, we used 30 µM and 40 µM Cu concentrations. These concentrations were lower than Körpe and Aras (2011) [31]. Therefore, 30 µM and 40 µM Cu concentrations might not be affected and even positively effect on protein content. Moreover, HBR treatment (40 µM Cu+2 µM HBR) enhanced protein content when compared with only Cu-application. However, low Cu concentration (30 µM)+HBR treatment showed different result. These results were supported by Poonam et al. (2014) [18]. They studied the role of ameliorative effect of 24-EBL under Cu stress and concluded that Cu treatment lowered the protein content, while at the same time, application of 24-EBL improved the protein content. This decrease in the protein content under Cu stress might be possibly due to decrease in the metabolism of amino acids [32]. On the other hand, exogenous application of HBR enhanced protein content. This increase could be explained on the basis of well-documented effect of BRs on gene expression. Increase in the protein content was observed by the exogenous application of EBL under various metal stresses [33].

SOD enzyme activities increased only low Cu application (30 µM). On the other hand, high Cu concentration (40 µM) decreased SOD activity. Posmyk et al. (2009) [34] also analysed SOD activity and concluded that SOD activity increased in 2.5 mM Cu-treated red cabbage seedlings but there was no change in 0.5 mM Cu-treated plants when compared to controls. In another study, rice plants under Cu stress (10, 50 and 100 µM) were analysed and it was observed that SOD activities enhanced with increasing Cu concentrations [26]. Concentration and application time are important to observe effect of Cu on plants. We analysed 72-hour-samples and this period might not be adequate to show the effect of Cu. When compared to controls, CAT activities showed different results related to Cu concentration. Only high Cu concentration improved CAT activity. Depends on plant species and Cu concentrations, CAT activities might be changed. Choudhary et al. (2012) [4] reported that Cu stress enhanced SOD and CAT activities. Wang et al. (2011) [35] also concluded that CAT activity was improved under Cu stress. On the other hand, Jouili and El Feriani (2003) [36] found different results. They were studied with sunflower and showed that CAT activities decreased under Cu stress. In addition, there are studies that reported no change in CAT activity under Cu stress [26]. In our study, CAT enzyme activities were higher in Cu+HBR applications than

only Cu treatments. Same results were found in different studies [18, 27]. Increased levels of antioxidant enzymes with application of hormones result in better oxidative stress management in plants [3]. Some studies showed that BR also enhanced gene expressions of antioxidant enzymes [33]. In our study, expected band size (309 bp for SOD and 248 bp for CAT) were observed in all samples. Expressions of SOD and CAT were analysed in different studies. One of this was carried out by Fernández-Ocaña et al. (2011) [13]. They determined CuZn-SOD gene expression in 9-days old sunflower. In another study, Pena et al. (2011) [14] indicated that sunflower seeds under 5 and 10 µM Cu stress increased CAT expression.

CONCLUSION

In this study, we showed that snegative effects observed on sunflower roots under heavy metal stress (Cu) could be alleviated by HBR. Depending on concentrations and duration, HBR improved growth parameters and increased or decreased the level of antioxidant enzymes (SOD and CAT) under stress and stress-free conditions when compared to only Cu-treated samples. Moreover, SOD and CAT enzyme expression were also found in all samples. These findings are expected to contribute to understand how HBR affects sunflower roots grown under different Cu concentrations.

ACKNOWLEDGEMENTS

This work was supported by Scientific Research Projects Coordination Unit of Istanbul University. Project numbers: 43403, UDP-2016.

REFERENCES

[1] Bhakuni, G., Dube, B.K., Sinha, P. and Chatterjee, C. (2009) Copper stress affects metabolism and reproductive yield of chickpea. J Plant Biol 32, 703-711.

[2] Burkhead, J.L., Gogolin Reynolds, K.A., Abdel-Ghany, S.E., Cohu C.M. and Pilon M. (2009) Copper homeostasis. New Phytol 182, 799-816.

[3] Andre, C.M., Larondelle Y. and Evers D. (2010) Dietary antioxidants and oxidative stress from a human and plant perspective: a review. Curr Nutr Food Sci 6, 2-12.

[4] Choudhary, S.P., Oral, H.V., Bhardwaj, R., Yu, J-Q. and Tran, L-SP. (2012) Interactions of brassinosteroids and polyamines enhances copper stress tolerance in *Raphanus sativus*. J Exp Bot 63(15), 5659-5675.

[5] Thounaojam, T.C., Panda, P., Choudhury, S., Patra, H.K. and Panda, S.K. (2014) Zinc ameliorates copper-induced oxidative stress in developing rice (*Oryza sativa* L.) seedlings. Protoplasma 251, 61-69.

[6] Puig, S. and Peñarrubia L. (2009) Placing metal micronutrients in context: transport and distribution in plants. Curr Opin Plant Biol 12, 1-8.

[7] Potters, G., Pasternak, T.P., Guisez, Y., Palme, K.J. and Jansen, M.A.K. (2006) Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci 12, 98-105.

[8] Potters, G., Pasternaki T.P., Guisez, Y. and Jansen, M.A.K (2009) Different stresses, similar morphogenic responses: integrated a plethora of pathways. Plant Cell Environ 32, 158-169.

[9] Marakli, S., Temel A. and Gozukirmizi N. (2014) Salt stress and homobrassinosteroid interactions during germination in barley roots. Not Bot Horti Agrobo 42, 446-452.

[10] van Kleeff, P.J.M., Jaspert, N., Li, K.W., Rauch, S., Oecking, C. and de Boer, A.H (2014) Higher order *Arabidopsis* 14-3-3 mutants show 14-3-3 involvement in primary root growth both under control and abiotic stress conditions. J Exp Bot 65(20), 5877-5888.

[11] Bhaduri, A.M. and Fulekar, M.H (2012) Antioxidant enzyme responses of plants to heavy metal stress. Rev Environ Sci Bio 11(1), 55-69.

[12] Fariduddin, Q., Khalil, R.R.A.E., MIR, B.A., Yusuf, M. and Ahmad, A (2013) 24-Epibrassinolide regulates photosynthesis, antioxidant enzyme activities and proline content of *Cucumis sativus* under salt and/or copper stress. Environ Monit Assess 185(9), 7845-7856.

[13] Fernández-Ocaña, A., Chaki, M., Luque, F., Gomez-Rodrigues, M.V., Carreras, A., Valderrama, R., Begara-Morales, J.C., Hernandez, L.E., Corpas, F.J. and Barroso, J.B (2011) Functional analysis of superoxide dismutases (SODs) in sunflower under biotic and abiotic stress conditions. Identification of two new genes of mitochondria Mn-SOD. J Plant Physiol 168, 1303-1308.

[14] Pena, L.B., Azpilicueta, C.E. and Gallego, S.M. (2011) Sunflower cotyledons cope with copper stress by inducing catalase subunits less sensitive to oxidation. J Trace Elem Med Bio 25, 125-129.

[15] Clouse, S.D. (2011) Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development. Plant Cell 23, 1219-1230.

[16] Gudesblat, G.E. and Russinova, E. (2011) Plants grow on brassinosteroids. Curr Opin Plant Biol 14, 530-537.
[17] Bajguz, A. and Hayat S (2009) Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol Bisoch 47(1), 1-8.
[18] Poonam, Kaur, R., Bali, S., Singh, R., Pati, P.K. and Bhardwaj R. (2014) Treatment of 24-EBL to *Brassica juncea* plants under Cu-metal stress lowers oxidative burst by activity of antioxidative enzymes. J Stress Physiol Biochem 10(2), 315-327.
[19] Pan, Y., Wu, L.J. and Yu, Z.L. (2006) Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis Fisch*). Plant Growth Regul 49, 157-165.
[20] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1-2), 248-254.
[21] Cakmak, I. and Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiol 98(4), 1222-1227.
[22] Cho, Y.W., Park, E.H. and Lim, C.J. (2000) Glutathione S-transferase activities of S-type and L-type thioltransferase from *Arabidopsis thaliana*. J Biochem Mol Biol 33(2), 179-183.
[23] Janas, K.M., Zielinska-Tomaszewska, J., Rybaczek, D., Maszewski, J., Posmyk, M.M., Amarowicz, R. and Kosinska, A. (2010) The impact of copper ions on growth, lipid peroxidation, and phenolic compound accumulation and localization in lentil (*Lens culinaris* Medic.) seedlings. J Plant Physiol 167, 270-276.
[24] Lequeux, H., Hermans, C., Lutts, S. and Verbruggen, N. (2010) Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol Bioch 48, 673-682.
[25] Thounaojam, T.C., Panda, P., Mazumdar, P., Kumar, D., Sharma, G.D., Sahoo, L. and Sanjib, P. (2012) Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol Bioch 53, 33-39.
[26] Fariduddin, Q., Yusuf, M., Hayat, S. and Ahmad, A. (2009) Effect of 28-homobrassinolide on antioxidant capacity and photosynthesis in *Brassica juncea* plants exposed to different levels of copper. Environ Exp Bot 66, 418-424.
[27] Guo, H., Li, L., Ye, H., Yu, X., Algreen, A. and Yin, Y. (2009). Three related receptor-like kinases are required for optimal cell elongation in *Arabidopsis thaliana*. PNAS 106, 7648-7653.
[28] Zhang, S., Cai, Z. and Wang, X. (2009) The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling. PNAS 106, 4543-4548.
[29] Gonzalez-Garcia, M.P., Vilarrasa-Blasi, J., Zhiponova, M., Divol, F., Mora-Garcia, S., Russinova, E. and Cano-Delgado, A.I (2011) Brassinosteroids control meristem size by promoting cell cycle progression in *Arabidopsis* roots. Development 138, 849-859.
[30] Körpe, D.A. and Aras S. (2011) Evaluation of copper-induced stress on eggplant (*Solanum melongena* L.) seedlings at the molecular and population levels by use of various biomarkers. Mut Res 719, 29-34.
[31] El-Shintinawy, F. and El-Ansary, A. (2000) Differential effect of Cd^{2+} and Ni^{2+} on amino acid metabolism in soybean seedlings. Biol Plantarum 43, 79-84.
[32] Marakli S. and Gozukirmizi, N. (2016) Abiotic stress alleviation with brassinosteroids in plant roots, In: Shanker, A., Shanker, C (eds.), Abiotic and Biotic Stress in Plants – Recent Advences and Future Perspectives, Inteq, ISBN: 978-953-51-2250-0, 373-394.
[33] Posmyk, M.M., Kontek, R. and Janas, K.M.. 2009. Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotox Environ Safe 72, 596-602.
[34] Wang, S.-H., Zhang, H., Zhang, Q., Jin, G.-M., Jiang, S.-J., Jiang, D., Jiang, Q.-Y, He. and Li, Z.-P. (2011) Copper-induced oxidative stress and responses of the antioxidant system in roots of *Medicago sativa*. J Agron Crop Sci 197, 418-429.
[35] Jouili, H. and El Feriani, E. (2003). Changes in antioxidant and lignifying enzyme activities in sunflower roots (*Helianthus annuus* L.) stressed with copper excess. C R Biol 326, 639-644.

Received: **23.02.2016**
Accepted: **07.09.2016**

CORRESPONDING AUTHOR

Sevgi Marakli
Istanbul University, Faculty of Science,
Department of Molecular Biology and Genetics,
34134, Vezneciler Street, Istanbul/TURKEY

E-mail: sevgi.marakli@istanbul.edu.tr