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BRASSINOSTEROIDS: ABIOTIC STRESS TOLERANCE IN TOMATO AND WHEAT PLANTS

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Abstract: Due to rapid changes in environment, plants are likely to face a plethora of environmental stresses. Cold, salinity, drought and heavy metal stresses are the major constraints that adversely affect the plant growth and development. In fact, these abiotic stresses represent the main cause of crop failure worldwide, dipping average yields for major crops by more than 50%. Since the world population is increasing at an alarming rate, therefore, strategies must be adopted by all the nations to face the challenges of increasing demand for food. To cope with various abiotic stresses in tomato and wheat plants, one way out is use of steroidal plant hormone. Brassinosteroids (BRs) is a polyhydroxysteroidal phytohormone which plays a pivotal role in normal plant growth and development. Our studies have revealed that exogenous application of BRs protected the photosynthetic machinery of the tomato and wheat plants, enhanced antioxidants system and proline metabolism under various abiotic stresses. Moreover, the proteomics study for BRs mediated response in wheat showed that 38 differentially expressed proteins were identified under aluminium and/or salt stress, which might be functionally relevant for many biological processes. Greater understanding of the BRs signalling and response pathways conferring tolerance to various stresses in plants will provide a lead that could be applied for the development of new, higher yielding varieties of crop species.

Keywords: **a**biotic stress, antioxidants, brassinosteroids, proline, proteomics

INTRODUCTION

In the recent past, majority of research had focused on the single stress conditions for plants but unlike animals, they are facing multiple stresses at a time due to sessile in nature. It has been well documented by the various workers that the responses of plants under combination of stresses are different from the response under single stresses (Suzuki et al., 2014). Improved agriculture is vital to address global food demand, and stress-tolerant crops have provided promising solutions. This study was conducted with an aim to establish the relationship between different abiotic stresses alone and in combination and simultaneously examine the brassinosteroids mediated changes on the antioxidant and photosynthetic efficiency through varied mode of application in tomato and wheat plants. Brassinosteroids (BRs) are naturally occurring plant steroidal hormones that are present in plant kingdom with more than 70 different analogues (Fariduddin et al., 2014). It plays pivotal role in plant's life cycles and acts at cellular levels, BRs regulate cell elongation, division, and differentiation (Zhipanova et al., 2013). At whole plant levels, BRs are involved in diverse abiotic and biotic stress responses (Fariduddin et al., 2014). Most stable analogue of BR, 24-epibrassinolide (EBL) has the ability to substantially enhance the stress tolerance by inducing cellular changes that are related to stress tolerance, like stimulations of nucleic acid and protein

synthesis (Dhaubhadel et al., 2002), activate ATPase pump (Khripach et al., 2003), increase antioxidant enzyme activities and accumulation of osmoprotectants (Ozdemir et al., 2004), induce other hormone responses (Vert et al., 2005), and regulate expression of stress responsive genes (Kagale et al., 2007).

Plants are exposed to numerous biotic and abiotic stresses that may affect their normal growth, development and reproduction. In order to survive, they have evolved elaborate mechanisms to perceive and respond to each type of stress. A complex and still obscure network of interactions between hormones and genes expression allows the plant to fine tune the appropriate response. Each type of stress has received a great deal of attention but studying each response in isolation is an oversimplification. Indeed, plants are able to integrate multiple signals and respond to different stresses in a specific manner. Moreover, evidence indicates that each stress/pathway interacts with each other. Recent progress in transcriptome analysis and the construction of large databases centralizing microarray data from different laboratories, allows researchers to carry out comparative approaches. These types of approaches, give us the opportunity to understand the plant responses in a more comprehensive, integrative manner in the presence of brassinosteroids.

Tomato seedlings (prepared by nursery) and wheat grains were sown in pots containing sand and allowed to grow under environmentally controlled conditions, these pots were irrigated with deionized water and nutrient solution (Hewitt, 1966) on alternate days till the termination of the experiment under randomized block design experiment. Tomato plants at 20 days stage of growth, exposed to day/night temperature of 10/3, 12/7, 20/14, or 25/18 °C (control) for 24 h in a plant growth chamber with a 12-h photoperiod, 750±50 µmol m^{-2} s^{-2} of PAR from cool white fluorescent (CWF) and 400-W high-pressure sodium (HPS) lamps (Philips Light Company, Lynn, MA) above plants and ambient carbon dioxide (CO_2). At 30 DAS, plants were sprayed with 0 or 10^{-8} M of epibrassinolide (EBL). The plants were harvested at 60 days of growth stage for assessment of various growth, photosynthetic, and biochemical parameters. On the other hand, wheat seedlings at 10 days stage of growth, treated with Al (10 mM) and/or NaCl (150 mM) for 3 days under pH ≤ 5.5 of sand and allowed to grow till 21 days stage of growth. On the same day, wheat plants were sprayed with 0 or 10^{-8} M of EBL for 5 days. The plants of all the sets were harvested at 45 days stage of growth to assess various growth biomarkers, physiological traits, antioxidants and enzymes of proline metabolism.

Overview of the results revealed that tomato plants exposed to varied low temperatures resulted in adaptive responses. BR tested against a range of low temperature stress in tomato plants, EBL emerged as an effective anti-stressor, which elevated the antioxidant activity. Therefore, it is suggested that foliar spray of EBL is more favorable for yield and quality improvement of tomato cultivars under low temperature stress. In another experiment, aluminum and salt stress alone showed the similar deleterious response in terms of growth biomarkers, and photosynthetic attributes whereas, in combination the deleterious effect was more pronounced in wheat plants. However, EBL improved tolerance against combination of Al and salt stress through modulation in enzymes related to proline metabolism and enhanced antioxidants system.

METHODS

Growth biomarkers were measured by using meter scale after removing soil particles from roots with the use of distilled water and then the plants were blotted and then placed in an oven, run at 70 °C for 72 h. The samples were weighed after allowing them to cool at room temperature to record their dry mass. The leaf water potential (LWP) was monitored with the Psypro water potential system (Wescor, Inc. 370 West

1700 South Logan, Utah 84321, USA) in the third fully expanded leaves of the plant. Net photosynthetic rate and stomatal conductance were determined on the third fully expanded leaves between 11:00 and 12:00 h by using an infra-red gas analyzer (IRGA) portable photosynthetic system (LI-COR 6400, LI-COR, and Lincoln, NE, USA). Maximum quantum yield i.e. chlorophyll fluorescence (Fv/Fm) was measured by using a leaf chamber fluorometer (LI-COR 6400-40, LI-COR, Lincoln, NE, USA). Activities of catalase (CAT), peroxidase (POX) and super-oxide dismutase (SOD) were analyzed as described in our previous study (Naz et al., 2015). Accumulation of proline was analyzed as per the method followed by our previous study (Yusuf et al., 2011).

The activity of Rubisco was determined by the method described by Usuda (1985) with slight modifications in which oxidation of NADH at 30°C was observed spectrophotometrically at 340 nm. Leaf samples were homogenized in a chilled mortar with ice-cold cocktail of extraction buffer (0.25 M Tris-HCl (pH 7.8), 0.05 M $MgCl_2$, 0.0025 M EDTA, and 37.5 mg dithiothreitol (DTT). The homogenate was centrifuged at 10,000 ×g at 4°C for 10 min. The supernatant was used for the enzyme assay. The reaction mixture constitutes 100 mM Tris-HCl (pH 8.0), 40 mM $NaHCO_3$, 10 mM $MgCl_2$, 0.2 mM NADH, 4 mM ATP, 0.2 mM EDTA, 5 mM DTT, and 1 U of 3-phosphoglycerate kinase. The activity was estimated after the addition of enzyme extract and 0.2 mM ribulose-1,5-bisphosphate (RuBP).

The Pyrroline-5-carboxylate synthase (P5CS) activity was determined by monitoring the consumption of NADPH and measuring the increase in absorbance at 340 nm. Reaction mixture constitutes 75 mM Glutathione, 100 mM Tris-HCl (pH 7.2), 20 mM $MgCl_2$, 5 mM ATP, 0.4 mM NADPH and 0.5 mL enzyme extract was incubated at 37°C for 20 min and then the absorbance was read at 340 nm (Stines et al., 1999). P5CS was expressed as unit per mg protein (one unit is defined as an increase in 0.001 A340 per min). The activity of Delta-Ornithine Amino Transferase (δ-OAT) was assayed according to Vogel and Kopac (1960). The reaction mixture constitutes 0.1 mL enzyme extract and 100 mM potassium phosphate buffer (pH 8.0) containing 50 mM L-ornithine, 20 mM α-ketoglutarate and 1mMpyridoxal-5'-phosphate. The reaction medium was incubated at 37 °C for 30 min. The reaction was stopped by adding 10% TCA and the color was developed by incubating the reaction mixture with 0.5% o-aminobenzaldehyde for 1 h. After centrifugation at 12000×g for 10 min, the clear supernatant fraction was collected to measure the absorbance at 440nm. Activity of δ-OAT was expressed as unit per mg protein (one unit is defined as an increase in 0.001 A440 per min).

The activity of Proline dehydrogenase (ProDH) was assayed by directly measuring the NAD^+ reduction at 340 nm as described by Rena and Splittstosser (1975). Reaction mixture constitutes 100 mM Sodium carbonate-bicarbonate buffer (pH 10.3) containing 20 mM L-proline, 10 mM NAD+ and 0.1 mL enzyme extract was incubated at 25 °C for 5 min, and the absorbance was measured at 340 nm. ProDH was expressed as unit per mg protein.

Data were statistically analyzed using SPSS, 17.0 for windows (SPSS, Chicago, IL, USA). Standard error was calculated and analysis of variance (ANOVA) was performed on the data to determine the least significance difference (LSD) between treatment means with the level of significance at $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth biomarkers significantly decreased due to exposure of tomato plants to low temperatures. Chilling caused tissue discoloration and increased water loss, which was the result of suppressed expression of genes active at normal temperatures (Saltveit and Morris 1990). On the other hand, the

damage caused by low temperature was significantly improved by EBL treatment, an active analogue. BRs are recognized to mediate growth through the regulation of gene expression (Fellner 2003) and BRU1 and TCH4 genes encoding xyloglucan endotransglycosylase (XET) and expansins (Cosgrove 1997), respectively. These enzymes are responsible for loosening of cell wall. Moreover, BRs also maintain a healthy metabolic state in cell and organs (Ali et al. 2006), which fulfills the demand for additional structural material and the energy needed for the growth of the cell wall as well as that of the plant as a whole.

Net photosynthetic rate (PN) and their related parameters as well as chlorophyll content decreased significantly in the low temperature-stressed plants. The reduction in the net photosynthetic rate under low temperature stress might be largely due to disruption of all major components of photosynthesis, including thylakoid electron transport, carbon reduction cycle, and stomatal control of CO_2 supply (Allen and Ort 2001). Chilling stress has been shown to induce changes in the ultrastructure of chloroplast, and the chloroplast may lose its capacity to capture light energy after a long chilling period (Yang et al. 2005). Maximum quantum yield of PSII (Fv/Fm), chlorophyll level (SPAD value), and carbonic anhydrase (CA) activity decreased with the increasing level of low temperature. The activity of the enzyme CA, that catalyzes the interconversion of CO_2 and HCO_3^-, is regulated by photonflux density, CO_2 concentration, the availability of Zn (Tiwari et al. 2005), and the expression of genes encoding CA protein. Better CA activity can also be accomplished by the application of BRs to plants as shown in *Cucumis sativus* under chilling stress (Fariduddin et al. 2011). It is reported that tomato plants grown under stress conditions that also received BRs exhibited higher CA activity which could be an expression of the impact of BRs on translation and/or transcription (Khripach et al. 2003). The most likely reason for supporting the increase in chlorophyll content is that BRs might directly or indirectly encourage chlorophyll biosynthesis (Hayat et al. 2011). Plants exposed to low temperatures showed higher level of antioxidant enzymes i.e., catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) as well as proline accumulation in tomato plants. The common consequence of most stresses is that they result, at some stage of stress exposure, in an increased production of reactive oxygen species (ROS), that could oxidize proteins, lipids, and nucleic acids resulting in abnormalities at the level of the cell (Sanita di Toppi and Gabbrielli 1999). In order to counter excess ROS, plants stimulate the synthesis of antioxidant enzymes (CAT, POX, and SOD) and osmoprotectant (proline) that scavenge excess ROS. However, low temperature stress showed enhanced production of ROS and malondialdehyde (MDA) content, used as an indicator of lipid peroxidation (Liu et al. 2013). Application of BRs resulted in a significant decline in O_2^- production rate (Liu et al. 2009) and the MDA content related to the chilling-stressed plants (Fariduddin et al. 2011). Treatment of plants with BRs both in the absence and presence of stress enhanced the activities of antioxidant enzymes. Therefore, maximum values were noted in the plants exposed to lowest temperature, followed by the application of BRs. The elevation in antioxidant enzymes by BRs was the consequence of enhanced expression of DET2 gene, which enhanced the tolerance to oxidative stress in Arabidopsis (Cao et al. 2005). The application of EBL increased activities of SOD and POX and decreased lipid peroxidation of the membrane in rice (Chen et al. 2007).

We have reported that BR had ability to confer tolerance against individual aluminum and salt stress (Fariduddin et al., 2014). Moreover, we have found that both aluminum and salt stress alone decreased the growth traits (shoot and root length and dry mass of plant) in similar trend whereas, their combinatorial stress showed adverse toxicity and generated maximum damage to the growth biomarkers. Contrary to this, treatment of EBL to the stressed or non-stressed plants significantly increased the growth traits in comparison to control plants. Excess salt in soil leads to various physiological and metabolic dysfunction and ultimately inhibits crop growth and development (James et al., 2011). Depending on the severity and duration of the salt stress, initially it is believed that salinity represses growth in the form of

osmotic stress (physiological drought) which is then followed by ion toxicity (James et al., 2011). This is because of acidic nature of soil due to drought conditions (Joris et al., 2013). The relationship between excess salt stress and Al toxicity further strengthened our findings in which combinatorial stress of both salt and Al stress lowered the leaf water potential. The loss of plants growth under stress was recovered by BR treatment and genome-wide transcriptional profiling and microarray analysis revealed multiple effects of BRs, including the coordinated growth and development as well as interaction with other hormones and environment (Gudesblat and Russinova, 2011). In addition to this, findings of Catterou et al. (2001) revealed involvement of EBL in cell elongation, regulation of genes encoding enzymes responsible for the activity of cell wall modification and enlargements, cellulose synthase under stress as well as stress free conditions.

Presence of excess Al and salt individually and in combination significantly decreased the rate of net photosynthesis and maximum quantum yield of PS II (Fv/Fm) along with the activities of Rubisco. It is reported by Pereira et al. (2000) that the Al-induced decrease in CO_2 assimilation was associated with structural damage to the thylakoids, as shown by a decrease in the ratio of variable fluorescence (Fv) to initial fluorescence (Fo). Moreover, Moustakas et al. (1995) revealed that Al caused a decline in photosynthesis as a result of the closure of PSII reaction centers and a reduction in PSII electron transport rate. Excess salt limits photosynthesis through induction of stomatal closure leading to a reduction in intercellular CO_2 concentration (Brugnoli and Lauteri, 1991) and inhibition of non-stomatal factors such as chlorophyll synthesis, photosynthetic electron transport reactions, quenching ability of excessive energy through chlorophyll fluorescence (Lee et al., 2004), efficiency of ribulose-1,5-bisphosphate carboxylase/oxygenase for carbon fixation (Megdiche et al., 2008). However, the treatment of EBL significantly countered the damage caused by the combined stress of Al and salt through enhanced photosynthesis rate, maximum quantum yield of PS II (Fv/Fm) and activity of Rubisco. Xia et al. (2009) reported that BRs had potential to positively activate the Rubisco by increasing Rubisco carboxylation rate (Vc, max), total Rubisco activity and, to a greater extent, initial Rubisco activity induced by an enhanced expression of genes encoding other calvin cycle genes. The BRs treatment might have also played a positive role in RuBP regeneration (Jmax), thereby increasing maximum carboxylation rate of Rubisco (Vc,max). Therefore, BRs promote photosynthesis by positively regulating synthesis and activation of a variety of photosynthetic enzymes including Rubisco (Xia et al., 2009). In addition to this, increase in net photosynthetic rate by BRs might be the result of the activation of Rubisco activity.

Unlike animals, plants are exposed to different stress factors in combination or in alone at a single time. Plants exposed to Al or salt stress causes oxidative stress (Sharma et al., 2012) due to the overproduction of reactive oxygen species (ROS), hydrogen peroxide (Sharma et al., 2012). It is believed that excess ROS accumulation leads to lipid peroxidation and therefore causes the damage of cell membrane stability, photosynthetic apparatus and chlorophyll biosynthesis (Smirnoff, 1993). To maintain the homeostatic of ROS accumulation under abiotic stresses, plants possess effective and systematic scavenging systems for reactive oxygen species that strengthened the plants to counter the destructive oxidative reactions (Arora et al., 2002). In the present study, the presence of excess Al and/or salt showed significant increase in antioxidant systems (CAT, POX and SOD) and this increase was further boost up by the treatment of EBL but the maximum activities of CAT, POX and SODwere reported in the plants exposed to Al and salt stress in combination and subsequently treated with EBL. Findings of various workers revealed that gene expression analysis demonstrated that Al-induced stress *activatedseveral* genes responsible for increased activities of POX, SOD and GSH S-transferase (Ezaki et al., 2000). Darko et al. (2004) showed that Al treatment significantly increased SOD, POX and APX activities in *Triticum aestivum*. Similar to our results, Ma et al. (2012) showed that two maize and rice cultivars with different tolerance capacity to Al,

respectively, showed that the improvement in protection against Al toxicity was obtained by an increase in the activity of the antioxidant system. It is believed that higher antioxidant enzymes could be interpreted as marker of oxidative stress or damage and at the same time also provide tolerance to oxidative stress (Abogadallah, 2010). Our findings further supported by the reports of Fariduddin et al. (2014). In plants, it is believed that proline metabolism could be exploited to provide stress tolerance by maintaining NADPH/$NADP^+$ balance, GSH levels, and during pathogen infection, drive the oxidative burst of the hyper sensitive response (Ben Rejeb et al., 2014). In addition to this, Szabados and Savoure (2010) reported that proline acts as a signaling molecule to moderate mitochondrial functions, and triggers pacific gene expression that could be essential for plant to recover under various abiotic stresses. In agreement with these reports, present study revealed that combination of Al and salt stress in the presence of EBL significantly increased the proline accumulation and activity of P5CS (Pyrroline-5-Carboxylase Synthetase) which catalyzes the synthesis of proline from glutamate. Analysis of OAT enzymatic activity in extracts of salt stressed Arabidopsis showed that OAT activity doubled in the first 24 h of salt stress and continued to increase up to 72 h after the start of salt treatment (Verslues and Sharma, 2010). Moreover, overexpression of OAT resulted in enhanced proline accumulation (Verslues and Sharma, 2010). Overall, proline metabolism modulates various developmental and stress responses and its accumulation plays pivotal role for conferring tolerance against various abiotic stresses (Szabados and Savoure, 2010). EBL treatment through foliage significantly increased activities of enzymes related to proline metabolism under Al and/or salt stress and Ozdemir et al. (2004) and Zeng et al. (2010) reported that BR has the ability to induce accumulation of proline under stress conditions through expression of genes related to proline biosynthesis.

Aluminum and salt stress alone showed the similar deleterious response in terms of growth biomarkers, and photosynthetic attributes whereas, in combination the deleterious effect was more pronounced in wheat plants. However, EBL improved tolerance against combination of Al and salt stress through modulation in enzymes related to proline metabolism and enhanced antioxidants system.

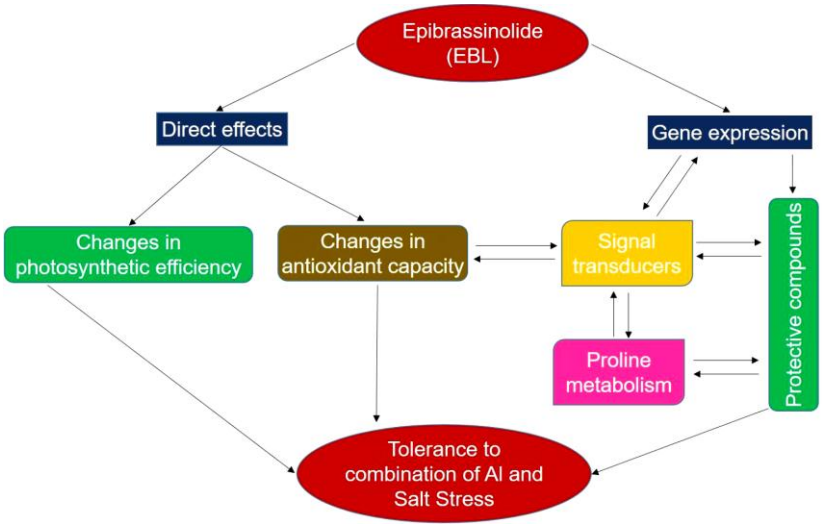

Figure 1: Schematic model of action of EBL on the induction of combine stress of aluminium and salt in wheat plants.

CONCLUSIONS

Cumulative modulation of proline metabolism and enhanced antioxidant system along with increased soluble sugar content, EBL could successfully counter the ROS mediated damage in tomato and wheat plant and increased the efficiency of both plants under combination of Al and salt stress as well as low temperature stress.

REFERENCES

Abogadallah, G.M., (2010) Antioxidative defense under salt stress. *Plant Signaling & Behavior 5*, 369–374.

Ali B, Hayat S, Hasan SA, Ahmad A (2006) Effect of root applied 28- homobrassinolide on the performance of Lycopersicon esculentum. *Scientia Horticulturae 110*, 267–273.

Allen, D.J.& Ort, D.R. (2001) Impact of chilling temperatures on photosynthesis in warm-climate plants. *Trends Plant Sciences 6*, 36–42.

Arora, A., Sairam, R.K., Srivastava, G.C. (2002) Oxidative stress and antioxidative system in plants. *Current Science 82*, 1227–1238.

Ben Rejeb, k., Abdelly, C., Savoure, A. (2014) How reactive oxygen species and proline face stress together. *Plant Physiology and Biochemistry 80*, 278–284.

Brugnoli, E. and Lauteri, M. (1991) Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant (*Gossypium hirsutum* L.) and salt-sensitive (*Phaseolus vulgaris* L.) C_3 non-halophytes. *Plant Physiology 95*, 628–635.

Cao, S., Xu, Q., Cao, Y., Qian, K., An, K., Zhu, Y., Binzeng, H., Zhao, H., Kuai, B. (2005) Loss-of-function mutations in DET2 gene lead to an enhanced resistance to oxidative stress in Arabidopsis. *Plant Physiology 123*, 57–66.

Catterou, M., Dubois, F., Schaller, H., Aubanelle, L., Vilcot, B., Sangwan-Norreel, B.S., Sangwan, R.S. (2001) Brassinosteroids, microtubules and cell elongation in Arabidopsis thaliana. II. Effects of brassinosteroids on microtubules and cell elongation in the bul1 mutant. *Planta 212*, 673-83.

Chen, K.M., Gong, H.J., Wang, S.M., Zhang, C.L. (2007) Antioxidant defense system in *Phragmites communis* Trin. Ecotypes. *Biologia Plantarum 51(4)*, 754–758.

Cosgrove, D. (1997) Relaxation in a high stress environment: the molecular basis of extensible cell walls and enlargement. *Plant Cell 9*, 1031–1041.

Darko, E., Ambrus, H., Stefanovits, B. E., Fodor, J., Bakos, F., Barnaba, B. (2004) Aluminium toxicity, Al tolerance and oxidative stress in an Al-sensitive wheat genotype and in Al-tolerant lines developed by *in vitro* microspore selection. *Plant Science 166*, 583-591.

Dhaubhadel, S., Browning, K.S., Gallie, D.R., Krishna, P. (2002) Brassinosteroid functions to protect the translational machinery and heat-shock protein synthesis following thermal stress. *The Plant Journal 29*, 681–691.

Ezaki, B., Gardner, R.C., Ezaki, Y., Matsumoto, H. (2000) Expression of aluminum-induced genes in transgenic Arabidopsis plants can ameliorate aluminum stress and/or oxidative stress. *Plant Physiology 122*, 657–665.

Fariduddin, Q., Shaista, C., Yusuf, M., Hayat, S., Ahmad, A. (2011) 28-homobrassinolide improves growth and photosynthesis in *Cucumis sativus* L. through enhanced antioxidant system in the presence of chilling stress. *Photosynthetica 49(1)*, 55–64.

Fariduddin, Q., Yusuf, M., Ahmad, I., Ahmad, A. (2014) Brassinosteroids and their role in response of plants to abiotic stresses. *Biologia Plantarum 58*, 9–17.

Fellner, M. (2003) Recent progress in brassinosteroid research: hormone perception and signal transduction. In Hayat S, Ahmad A (eds), *Brassinosteroids: bioactivity and crop productivity*. (pp 69-86). Dordrecht: Kluwer Academic Publishers.

Gudesblat, G.E. and Russinova, E. (2011) Plants grow on brassinosteroids. *Current Opinion in Plant Biology 14*, 530–537.

Hayat, S., Yadav, S., Wani, A.S., Irfan, M., Ahmad, A. (2011) Comparative effect of 28-homobrassinolide and 24-epibrassinolide on the growth, carbonic anhydrase activity and photosynthetic efficiency of *Lycopersicon esculentum*. *Photosynthetica 49*, 397–404.

Hewitt, E.J. (1966) Sand and water culture methods used in the study of plant nutrition. *Technical Communication No. 22*. Commonwealth Bureau, London.

James, R.A., Blake, C., Byrt, C.S.,Munns, R. (2011) Major genes for Na^+ exclusion, Nax1 and Nax2 (wheat HKT1; 4 and HKT1; 5), decrease Na^+ accumulation in bread wheat leaves under saline and waterlogged conditions. *Journal of Experimental Botany 62*, 2939–2947.

Joris, H.A.W., Caires, E.F., Bini, A.F., Scharr, D.A., Haliski, A. (2013) Effects of soil acidity and water stress on corn and soybean performance under a no-till system. *Plant and Soil 365*, 409–424.

Kagale, S., Divi, U.K., Krochko, J.E., Keller, W.A., Krishna, P. (2007) Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. *Planta 225*, 353–364.

Khripach, V.A., Zhabinskii, V.N., Khripach, N.B. (2003) New practical aspects of brassinosteroids and results of their 10 year agricultural use in Russia and Belarus. In Hayat, S., Ahmad, A. (Eds.). *Brassinosteroids: bioactivity and crop productivity*. (pp 189–230). Dordrecht: Kluwer Academic Publishers.

Lee, G.J., Carrow, R.N., Duncan, R.R. (2004) Photosynthetic responses of salinity stress of halophytic seashore paspalum ecotypes. *Plant Science 166*, 1417–1425.

Liu, Y., Zhao, Z., Si, J., Di, C., Han, J., An, L. (2009) Brassinosteroids alleviate chilling induced oxidative damage by enhancing antioxidant defense system in suspension cultured cells of *Chorispora bungeana*. *Plant Growth Regulation 59*, 207–214.

Ma, B., Gao, L., Zhang, H., Cui, J., Shen, Z. (2012) Aluminum-induced oxidative stress and changes in antioxidant defenses in the roots of rice varieties differing in Al tolerance. *Plant Cell Reports 31*, 687–696.

Megdiche,W., Hessini, K., Gharbi, F., Jaleel, C.A., Ksouri, R., Abdelly, C. (2008) Photosynthesis and photosystem-efficiency of two salt-adapted halophytic seashore *Cakile maritima* ecotypes. *Photosynthetica 46*, 410–419.

Moustakas, M., Ouzounidou, G., Lannoye, R. (1995) Aluminum effects on photosynthesis and elemental uptake in an aluminum-tolerant and non-tolerant wheat cultivar. *Journal of Plant Nutrition 18*, 669–683.

Naz, F.S., Yusuf, M., Khan, T.A., Fariduddin, Q., Ahmad, A. (2015) Low level of selenium increases the efficacy of 24-epibrassinolide through altered physiological and biochemical traits of *Brassica juncea* plants. *Food Chemistry 185*, 441–448.

Ozdemir, F., Bor, M., Demiral, T., Tmken, I. (2004) Effect of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidant system of rice (*Oryza sativa* L.) under salinity stress. *Journal of Plant Growth Regulation 42*, 203–311.

Pereira, W.E., de Siqueira, D.L., Martinez, C.A., Puiatti, M. (2000) Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminum stress. *Journal of Plant Physiology 157*, 513–520.

Rena, A.B., Splittstosser, W.E. (1975) Proline dehydrogenase and pyrroline-5-carboxylate reductase from pumpkin cotyledons. *Phytochemistry 14*, 657–661.

Saltveit, M.E. and Morris, L.L. (1990) Overview on chilling injury of horticultural crops. In Wang C.Y. (ed). *Chilling injury of horticultural crops*. (pp 3–15). Boca Raton: CRC Press.

Sanita di Toppi, L. and Gabbrielli, R. (1999) Response to cadmium in higher plants. *Environmental and Experimental Botany 41*, 105–130.

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M. (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, http://dx.doi.org/10.1155/2012/217037.

Smirnoff, N. (1993) The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist 125*, 27–58.

Stines, A. P., Dean, J., Naylor, Peter, B. H., van Heeswijck, R. (1999) Proline accumulation in developing grapevine fruit occurs independently of changes in the levels of Δ^1-pyrroline-5-carboxylate synthetase mRNA or protein. *Plant Physiology 120*, 923-931.

Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E., Mittler, R. (2014) Tansley review Abiotic and biotic stress combinations. *New Phytologist 203*, 32–43.

Szabados, L. and Savoure, A. (2010) Proline: a multifunctional amino acid. *Trends in Plant Science 15*, 89–897.

Tiwari, A., Kumar, P., Singh, S., Ansari, S.A. (2005) Carbonic anhydrase in relation to higher plants. *Photosynthetica 43*, 1–9.

Usuda, H. (1985) The activation state of ribulose1, 5 -bisphosphate carboxylase in maize leaves in dark and light. *Plant Cell Physiology 26*, 1455–1463.

Verslues, P.E. and Sharma, S. (2010) Proline metabolism and its implications for plant environment interaction. Arabidopsis Book. doi: 10.1199/tab.0140.

Vert, G., Nemhauser, J.L., Geldnerm, N., Hong, F., Chory, J. (2005) Molecular mechanisms of steroid hormone signaling in plants. *Annual Review of Cell and Developmental Biology 21*, 177–201.

Vogel, R.H., Kopac, M.J. (1960) Some properties of ornithine-γ-transaminase from Neurospora. Biochimica et *Biophysica Acta 37*, 539–540.

Xia, X.J., Huang, L.F., Zhou, Y.H., Mao, W.H., Shi, K., Wu, J.X. (2009) Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. *Planta 230*, 1185–1196.

Yang, M.T., Chen, S.L., Lin, C.Y., Chen, Y.M. (2005) Chilling stress suppresses chloroplast development and nuclear gene expression in leaves of mung bean seedlings. *Planta 221*, 374–385.

Yusuf, M., Fariduddin, Q., Ahmad, A. (2011) 28-Homobrassinolide mitigates boron induced toxicity through enhanced antioxidant system in *Vigna radiata* plants. *Chemosphere 85*, 1574–1585.

Zeng, H., Tang, Q., Hua, X. (2010) Arabidopsis brassinosteroid mutants det2-1 and bin2-1 display altered salt tolerance. *Journal of Plant Growth Regulation 29*, 44–52.

Zhipanova, M.K., Vanhoutte, I., Boudolf, V., Betti, C., Dhondt, S., Coppens, F., Mylle, E., Maes, S., González-García, M.P., Caño-Delgado, A., Inze, D., Beemster, G.T.S., De Veylder, L., Russinova, E. (2013) Brassinosteroid production and signaling differentially control cell division and expansion in the leaf. *New Phytologist 197*, 490–502.