ISSN:0976-4933
Journal of Progressive Science
A Peer-reviewed Research Journal
Vol.12, No.01 & 02, pp 57-66 (2021)

Review research article

Effect of Brassinosteroids on heavy metal toxicity in plants-A Review

Nisha Raghava*, Vijaylaxmi Gupta and Ravindra Pratap Raghava
PG Department of Botany, S. M. M. Town PG College, Ballia (UP), India
* Corresponding author- nisharaghava62@gmail.com

Introduction

Growth is an organized, well-coordinated complex process where metabolism provides the energy and the building blocks. Plants require light, water, oxygen, minerals and other nutrients for their growth and development. Apart from these external requirements, plants also depend on certain organic compounds to signal, regulate and control the growth of plants. These are collectively called Plant Growth Regulators or Plant Growth Hormones.

However, it is the relative hormone level that regulates the pace of growth of each individual plant part, to produce a form that is recognized as a plant. Earlier, only five groups of hormones (Auxins, Gibberellins, Cytokinins, Abscissic acid and Ethylene) were designated as regulators of plant growth. However, in the recent past, compelling evidences have been put forward to classify an additional group of steroidal substances (brassinosteroids, first isolated from rape *(Brassica napus* L.) pollen), as a new class of phytohormones (phytosteroids). It was in 1970, when Mitchell and co-workers screened the pollens of nearly sixty species, out of which the extract from about thirty species generated growth in bean seedlings. This growth promoting substance was called "Brassin".

The search for its active factor(s) was collectively approached in 1974 by the USD, scientists working at Northern Regional Research Centre (NRRC), Peoria; Eastern Regional Research Centre (ERRC), Philadelphia and Beltsville Agricultural Research Centre (BARC), Maryland. Bee-collected pollens (500 lb) were processed through a pilotplant-size solvents (2-propanol) extraction procedure at ERRC and succeeded inpartial purification at BARC. However, it was crystallized at NRRC and was subjected to x-ray analysis to establish its structure. This biologically active plant growth promoter was found to be steroidal lactone ($C_{28}H_{48}O_6$) and was named as "Brassinolide" which was renamed as "Brassinosteroid". All natural Brassinosteroids have a common 5-choleston skeleton and its structural variants come from the type and the orientation of functionalities on the skeleton. Their low level in plants is not uniform throughout its body but young growing tissues have comparatively a larger share than the mature tissues (Yokota and Takahashi, 1986; Clouse and Sasse, 1998).

BRs are present in the pollen and immature seeds varying its concentration from 1–100 ng g^{-1} FM, while shoots and leaves typically have lower concentrations ranging from 0.01–0.1 ng g^{-1}

FM (Takatsuto, 1994).Up to now, approximately 70 different kinds of BR- linked compounds from plants have been characterized (Haubrick and Assmann, 2006). Out of which, three (brassinolide, 24-epibrassinolide and 28-homobrassinolide) are being largely applied to have an economical impact on plant metabolism, growth and productivity. Grove, *et al.*(1979) have studied them extensively since they were detected and characterized from the pollen of the rape plant, *Brassica napus*. BRs are normally present at comparatively low doses in plants. Moreover, endogenous BR levels could vary between plant tissues, like immature growing tissues which have greater BR levels than mature tissues.

Brassinosteroids are intricately involved to control different physiological and metabolic processes including elongation of stem, growth of pollen tube, maturation of xylem, synthesis of proteins, and improvement of photosynthetic rate (Farazi, *et al*., 2015; Rajewska, *et al.,* 2016). Seed germination and seedling growth are important parameters for healthy crop production. Seeds are very delicate during germination and can be easily damaged by minor external stimuli (stress) (Lapik, *et al*., 2003; Raghava, *et al.,* 2014). BR enhanced germination rate and ultimately increase seedling growth under stress condition (Ozdemir, *et al*., 2004; Mahesh, *et al*., 2013). BR control the key genes G-protein α-subunit 1 (GPA1) and G-protein coupled receptor 1 (GCR1) that are responsible for seed germination. GPA1 is a subunit of the heterotrimeric G protein and GCR1 is a putative G protein-coupled cell surface receptor (Lapik, *et al*., 2003). GPA1 (G-protein α-subunit 1) is a key gene that regulates various biological processes, including biotic and abiotic stresses, growth and developmental processes, biosynthesis of flavonoids, as well as activating transcription factors and nutrient transporters (Chakraborty, *et al.,* 2015). GPA1 interacts with Pirin1 and regulates early seed germination and seedling growth (Lapik, *et al*., 2003). Over the past decade, remarkable progress has been made in understanding the biosynthesis of BRs, and signalling and their multifaceted roles in plant growth and metabolic processes (Wang *et al*., 2014; Zhabinskii *et al*., 2015).Brassinosteroids are also involved in the adaptation of plants to various ecological cues including Heavy metal toxicity (Nazir, *et al*., 2019, 2020), salinity (Mir *et al*., 2015), temperature extremes (Niu, *et al*., 2016), drought (Talaat and Shawky, 2016), and pathogen infection (Zhu, *et al*., 2015). BRs modify the plant defense system to counter negative influences of stress on the plant.

Brassinosteroids under heavy metal stress-

Plants have a remarkable ability to take up and accumulate heavy metals from their exterior, for example aquatic environment. Metal contamination of the aquatic environment occurs as a result of human activities and affects organisms at the biochemical, cellular, community and population level. Aquatic plants are primary producers of most aquatic food chains and account for much of the production base of freshwater and marine ecosystems. They have been used to remove heavy metals from aquatic environments since they have a high capability for accumulating dissolved metals without dying. High concentrations of all metals, including those essential for growth and metabolism, exert toxic effects on the metabolic pathways of plants. Toxicity mechanisms include the blocking of functional groups of important molecules, e.g. enzymes, polynucleotides, transport systems for essential nutrients and ions, displacement and/or substitution of essential ions from cellular sites, denaturation and inactivation of enzymes and disruption of cell and organelle membrane integrity. Heavy metal toxicity can elicit a variety of adaptive responses in plants. A ubiquitous mechanism for heavy metal detoxification is the chelation of the metal ion by aligned. Such ligands include organic acids, amino acids, peptides and polypeptides. Peptide ligands include the phytochelatins (PC), small gene-encoded, cysteine rich polypeptides. They are heavy metal-binding peptides derived from glutathione and have the general structure (g-Glu-Cys) n-Gly, where n has been reported as being as high as 11, but is generally in the range of 2-5. PC detoxifies intracellular metals by binding them through thiolate coordination (Cobbett and Goldsbrough, 2002). Similarly Brassinosteroids is a plant steroidal hormone like animal that plays an important role in a variety of plant physiological processes and adaptation to different kinds to abiotic and biotic stresses (Li, *et al.,* 2013a; Wei *et al*., 2015).

The cultures of *Chlorella vulgaris* treated with BRs and heavy metalsaccumulated a lower level of heavy metals than those cultures treated with metalsalone. A stimulatory effect of BRs after blocking the accumulation of heavy metalson the growth and development of *C. vulgaris* occurs. Application of BRs to *C. vulgaris* cultures reduced the impact of heavy metals stress on growth, prevented chlorophyll, sugar and protein loss and increased PC synthesis. Concentration-dependent stimulation was observed with increasing concentration of BR and decreasing concentration of heavy metals (Bajguz, 2000). BRs stimulated the synthesis of PC in *C. vulgaris* cells treated with lead. Thestimulatory activity of BRs on PC synthesis may be sequenced as: brassinolide (BL) >24-epiBL > 28-homo-BL >castasterone (CS) > 24-epiCS > 28-homoCS (Bajguz and Tretyn, 2003).

The accumulation of heavy metals (cadmium, copper, lead and zinc) under the influence of BR has been studied for different agricultural plants such as barley, tomato, radish and sugar beet. It was found that the application of 24-epiBL significantly reduced themetal absorption; for example, the content of lead in beet roots was more than 50% lower than in the control culture (Khripach, *et al,* 1999). Brassinosteroids have the ability to regulate the uptake of ions into the plant cells and they can be used to reduce the accumulation of heavy metals and radioactive elements in plants. Moreover, BRs also minimize the toxic effects and symptoms generated by excess quantity of heavy metals (Bajguz, 2010). Brassinosteroids (BRs) regulate a wide range of plant growth in where double brassinolide stimulate seed germination and different seedling growth parameters and chlorophyll content in moongbean (Shagun, *et al.,* 2011; Srivastava, *et al.*, 2011).

The mechanisms induced by BRs to ameliorate the deleterious impacts of heavy metals such as (arsenic, lead , cadmium,copper, nickel, cobalt , manganese) were thoroughly examined and well explored in literature(Fariduddin, *et al.,* 2015; Hussain, *et al.,* 2019 ; Nazir, *et al.,* 2019, 2020). BR signifcantly increased tomato seedling growth under low temperature and weak light stress (Shu, *et al*., 2016), cucumber seedling growth under Ca $(NO_3)_2$ stress (Yuan, *et al*., 2012), pepper shoot growth, fresh and dry weight under stress (Abbas, *et al*.,2013). Take other evidence illustrated that the BR signaling pathway played a fundamental role in plant cell diferentiation and increased induced stress tolerance (Xu, *et al*., 1994; Xia, *et al*., 2009a; Rehman, *et al*., 2016; Hou, *et al*., 2017). Chlorophyll is highly sensitive to external stimuli (stress) that decrease total chlorophyll a, b, and carotenoid contents in leaves (Rehman, *et al*., 2016). BR promote accumulation of chlorophylls and photosynthetic capacity by regulating a variety of enzymes including chlorophyllase and Rubisco, transcript levels of encoded genes involved chlorophyll and photosynthesis under stress (Xia, *et al*., 2009a,b; Hasan, *et al*., 2011; Li, *et al.,* 2016; Zhao, *et al*., 2017). BR played a positive role in fruit ripening and fruit growth of mango, and in the quality of pitaya (Zaharah, *et al*., 2012; Li, *et al*., 2013b).

Brassinosteroids response to Lead (Pb) metal stress-

Lead (Pb) is a major heavy metal pollutant in both terrestrial and aquatic ecosystems. Significant increase in the Pb cause sharp decrease in crop productivity thereby posing a serious problem for agriculture. Enhancement of 24-epiBL reduced Pb toxicity and enhanced the growth in radish (*Raphanus sativus* L.) seedlings by increasing the activities of antioxidant enzymes like CAT, APX,GPX ,SOD and reducing POD activity. Mitigation of the consequences of Pb (100 or 200µM) was reported in tomato plants as a result of 24-epiBL-mediated increases in the activities of SOD, CAT, APX and GR and the contents of AsA and GSH (Radyand Osman, 2012). Kohli, *et al.* (2018) revealed that the treatment of EBL had improved all gas exchange characteristics including cellular CO_2,stomatal conductance, net photosynthetic rate, and transpiration rate in Pb stressed mustard plants. Binary treatment of 24-EBL and SA, further elevated the content of osmoprotectants. The study revealed that co-application of combined treatment of 24-EBL and SA led to dimination of toxic effects of Pb in *B. juncea* seedlings (Kohli, *etal.,* 2019).Swamy, *et al.*(2014) confirmed that seed priming treatment with low concentration of HBL accelerated the net photosynthetic rate, and content of bio molecules such as carbohydrates, proteins, and nucleic acid in *Trigonella foenugraecum* plants in response to Pb stress.

Brassinosteroids response to Cadmium (Cd) metal stress-

BR eliminates the toxic effect of cadmium on photochemical pathways in rape cotyledons, mainly by diminishing the damage in reaction centres and O2 evolving complexes as well as maintaining efficient photosynthetic electron transport (Janeczko, *et al.,* 2005). Hasan, *et al.* (2008) also reported that homo BL mediated elevated activity of CAT, POD, and SOD in *Cicer arietinum* against Cd (50, 100, or150µM). Application of BRs (10−8 M) to Cd (3.0, 6.0, 9.0, and 12 mgkg−1) stressed *Solanum lycopersicum* plants enhanced antioxidant system activity and improved fruit yield and quality (Hayat, *et al*., 2012). In Cd (0.5, 1.0, and 1.5mM)-exposed *Raphanus sativus*, adiminished oxidative stress via 24-epiBL (10^{-7}, 10^{-9} and 10^{-11} M) supplementation mediated elevation in the activity of GST and PPO enzymes was reported (Sharma, *et al.,* 2012). Plants developed from the foliar spray treatment of EBL have been reported to show enhanced growth as well as total chlorophyll content under metal exposure. Also, exogenous application of EBL had improved photosynthetic pigments in radish plants subjected to Cd stress (Kapoor, *et al.,* 2016).

Brassinosteroids response to Copper (Cu) and Nickel (Ni) metal stress

BRs also reduced the content of Cu in Indian mustard (Sharma and Bhardwaj, 2007). Spraying of24-epiBL (1.0µM) to Ni-exposed *Brassica juncea* was reported to detoxify Ni-impacts (Ali, *et al*., 2008a). Elevated CAT, POD, and SOD activity via the spray of 0.01µM of 28-homo BL was argued to protect five wheat (*Triticum aestivum*) cultivars (PBW-373, UP-2338, DL-LOK-01, DL-373andHD-2338) against micro concentrations (50 and 100µM) (Yusuf, *et al*., 2011). Earlier, these authors reported BRs mediated significant mitigation of Ni (0.2, 0.4 and 0.6 mM) stress in *Brassica juncea* plants by elevating the activity of ROS metabolizing enzymes (and also via lowering the metal uptake) (Kanwar, *etal*., 2012).Application of 24-epiBL ameliorated Ni-stress in *Brassica junce*a by enhancing mainly the activity of antioxidant enzyme (Kanwar, *et al*., 2013).It was revealed that EBL treated plants had increased translocation of Ni to shoots than roots which could have mediated through synthesis of organic molecules implicated in their transport and better translocation of water to apex cells through xylem, thus maintaining cell elongation (Hu, *et al.,* 2013).

The 24-epi BL-mediated up-regulation of antioxidant enzyme activity and the elevated level of proline (osmolyte) were argued to confer Ni-tolerance and improve growth, nodulation and yield attributes (Yusuf, *et al*., 2012). Another experiment was carried out by (Filova *et al*., 2013) regarding the effect of BR on the induction of physiological changes in sunflower (*Helianthus annus* L.) against Cu stress. (Yusuf, *et al*., 2014) reported BRs-mediated improved antioxidant defense (and also nitrogen metabolism) in two contrasting cultivars of *Vigna radiata* under different levels of Ni. EBL was also found effective in maintaining proper health of egg plants and B. juncea plants against salinity and Cu stress respectively (Ding, *et al*., 2012; Poonam, *et al*., 2014) by enhancing the activities of antioxidative enzymes and antioxidant contents, thus leading to decrease in oxidative stress.In addition, EBL- triggered protective mechanisms in *Solanum nigrum* L. subjected to Ni stress(Soares, *et al*., 2016). Application of 10-6M 24-epi BL as shotgun approach (pre-sowing seed soaking) to the Ni-stressed T-44(Ni-tolerant) and PDM-139 (Ni-sensitive) varieties of *Vigna radiata* plants improved biological yield, number of nodules, leghemoglobin content, and the activity of CAT, POD and SOD enzymes. In addition, Ni and Cu acquisition in tomato plants has been shown to decrease significantly following BR treatment, thereby decreasing the harmful effects of Ni and Cu (Nazir, *et al.,* 2019, 2020).

Brassinosteroids response other heavy metal stress-

Ali, *et al*. (2008 a, b) also found amelioration of aluminium stress by applying BRs in *Vigna radiata* L. Wilczek. Plants which were provided aluminium treatment showed a steep decline in growth parameters, relative water content, carbonic anhydrase activity, water use efficiency, photosynthetic rate and chlorophyll content whereas proline content and some antioxidative enzyme activities were found increased in Al stressed plants. Plants which were supplied EBL or HBL spray exhibited an improvement in the above said parameters along with a further increase in proline content as well as antioxidative enzyme activities. Another experiment was carried out by (Arora, *et al*., 2010) where chromium (Cr) toxicity was reduced in *B. juncea* L. plants under the effect of 24-epibrasssinolide. Another study was done by (Choudhary, *et al*., 2012) regarding the antistress properties of Polyamine-Brassinosteroid application against Chromium (Cr) metal stress in radish (*Raphanus sativus* L). Sharma, *et al*. (2016) studied the regulation of stress tolerance by 24- epibrassinolide in rice (*Oryza sativa* L.) seedlings by modulating the antioxidant defense expression against Cr metal toxicity. Overall, BR significantly alleviate the harmful effects of heavy metals and improve plant growth. Furthermore, supplementation of BRs alleviated the Mn- induced alterations in the photosyntheticand biochemical attributes of *Brassica juncea* (Hussain, *et al.,* 2019a).

Plants under various metal stresses, generate large amounts of ROS which may oxidize lipids, proteins, and nucleic acids, resulting in disturbances at the cellular level which lead to apoptosis. The toxic influence of metals on plants also depends on its absorption and bioaccumulation, which in turn is associated with the bioavailability, uptake route, storage, degradation, immobilization, and excretion of the metal, as well as avoidance/tolerance mechanisms. Studies concerning the molecular mechanism of BRs' activity will allow for better and more thorough understanding of anti-stress activities of these phytohormones and facilitate the development of appropriate strategies of protecting plants from metal stress. BR signal transduction may result in activation of NADPH oxidase to ROS production, which probably initiates a cascade of protein phosphorylation through MAPKs to activate transcription factors to target specific genes participating in cellular protection (Xia, *et al*., 2009 b). Owing to the importance of BRs in the crop improvement, further investigations are needed to identify the key regulatory elements in BR signalling pathway and the underlying mechanism of BR-modulated growth and developmental responses in major crop plants to design optimal strategies to enhance crop yield and improve their performance under stress conditions.

References

1. Abbas, S., Latif, H.H, and Elsherbiny, E.A. (2013). Effect of 24-epibrassinolide on the physiological and genetic changes on two varieties of pepper under salt stress conditions.Pak J Bot. 45:1273-284.
2. Ali, B., Hassan, S.A., Hayat, S., Hayat, Q., Yadav, S., Fariduddin, Q. and Ahmad, A. (2008a). A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (VignaradiataL. Wilczek). Environmental and Experimental Botany62: 153-159.
3. Ali, B., Hayat, S., Fariduddin, Q. And Ahmad, A. (2008b) .24-Epibrassinolide protects against the stress generated by salinity and nickel in Brassica juncea. Chemosphere, 72, 1387-1392.
4. Arora, P., Bhardwaj, R. and Kanwar, M.K. (2010). 24-Epibrassinolide regulated diminution of Cr metal toxicity in Brassica juncea L. plants. Brazilian Journal of Plant Physiology, 22: 159-165.
5. Bajguz, A. (2000). Effect of brassinosteroids on nucleic acid and protein content incultured cell of Chlorella vulgaris. Plant Physiol. Biochem., 38: 209-215.
6. Bajguz, A. (2010). An enhancing effect of exogenous brassinolide on the growth and antioxidant activity in Chlorella vulgaris cultures under heavy metals stress. Environ. Exp. Botany, 68: 175–179.
7. Bajguz, A. and Tretyn, A. (2003).The chemical structures and occurrence of brassinosteroids in plants. In Brassinosteroids: Bioactivity and CropProductivity (eds. S. Hayat, A. Ahmad) Kluwer Academic Publishers, The Netherlands, pp 1-44.
8. Chakraborty, N., Sharma, P., Kanyuka, K., Pathak, R.R., Choudhury, D., Hooley, R. And Raghuram, N. (2015). G-protein α-subunit (GPA1) regulates stress, nitrate and phosphate response, flavonoid biosynthesis, fruit/seed development and substantially shares GCR1 regulation in Arabidopsis thaliana.Plant Mol Biol.,89: 559–76.
9. Choudhary, S.P., Kanwar, M., Bhardwaj, R., Yu, J.Q. and Tran, L.S.P. (2012). Chromium stress mitigation by polyamine-brassinosteroid application involves phytohormonal and physiological strategies in Raphanussativus L. PLoS ONE 7: e 33210.
10. Clouse, S.D. and Sasse, J. M. (1998). Brassinosteroids: essential regulators of plant growth and development. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 427-451.
11. Cobbett, C. and Goldsbrough, P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis, Annu. Rev. Plant Biol. 53:159-182.
12. Ding, H.D., Zhu, X.H., Zhu, Z.W., Yang, S.J., Zha, D.S. and Wu, X.X. (2012). Amelioration of salt- induced oxidative stress in egg plant by application of 24-epibrassinolide. Biologia Plantarum, 56: 767-770.
13. Farazi, E., Afshari, H. And Abadi, H.H. (2015). Effect of different concentrations of brassinosteroid on physio morphological characteristics of five pistachio genotypes (Pistacia vera L.). J.

Nuts.,6: 143-153.

14. Fariduddin, Q., Ahmed, M., Mir, B.A., Yusuf, M. and Khan, T.A. (2015). 24-Epibrassinolidemitigates the adverse effects of manganese induced toxicity through improved antioxidant system and photosynthetic attributes in Brassica juncea. Environ. Science and Pollution Research, 22: 11349-11359.
15. Filova, A., Sytar, O. and Krivosudska, E. (2013). Effects of brassinosteroid on the induction of physiological changes in Helianthus annuus L. under copper stress. Acta Universitatis Agriculturaeet Silviculturae Mendelianae Brunensis, 61: 623-629.
16. Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N., Worley, J.F., Warthen, J.D., Steffen, G.L., Flippen-Anderson, J.L. and Cook, J.C. 1979. Brassinolide, a plantgrowth-promoting steroid isolated from Brassica napus pollen. Nature, 281:216-217.
17. Hasan, S.A., Hayat, S. and Ahmad, A. (2011). Brassinosteroids protect photosynthetic machinery against the cadmium induced oxidative stress in two tomato cultivars. Chemosphere, 84(10):1446-51.
18. Hasan, S.A., Hayat, S., Ali, B. And Ahmad, A. (2008). 28-Homobrassinolide protects chickpea (Cicer arietinum) from cadmium toxicity by stimulating antioxidants. Environ. Pollut., 151:60-66.
19. Haubrick, L. L. and Assmann, S.M. (2006). Brassinosteroids and plant function: some clues, more puzzles. Plant Cell Environ., 29: 446-457.
20. Hayat, S., Alyemeni, M. And Hasan, S. (2012). Foliar spray of brassinosteroid enhances yield and quality of Solanum lycopersicum under cadmium stress. Saudi J. Biol. Sci., 19:325-335.
21. Hou, S., Niu, H., Tao, Q., Wang, S., Gong, Z., Li, S., Weng, Y. and Li, Z. (2017). A mutant in the CsDET2 gene leads to a systemic brassinosteriod defciency and super compact phenotype in cucumber (Cucumis sativus L.).Theor Appl Genet., 130:1693-1703.
22. Hu, X., Qian, Q., Xu, T., Zhang, Y.E., Dong, G., Gao, T., Xie, Q. And Xue, Y. (2013).The U-boxE3 ubiquitin ligase TUD1 functions with a heterotrimeric G α subunit to regulate brassinosteroid-mediated growth in rice. PLoS Genet. doi: 10.1371/journal.pgen.1003391.
23. Hussain, A., Nazir, F. and Fariduddin, Q. (2019).24-epibrassinolide and spermidine alleviate Mn stress via the modulation of root morphology, stomatal behaviour, photosynthetic attributes and antioxidant defense in Brassica juncea. Physiol. Mol. Biol. Plants, 25: 905-919.
24. Janeczko, A., Koscielniak, J., Pilipowicz, M., Szarek-Lukaszewska, G., Skoczowski, A. (2005). Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica, 43: 293-298.
25. Kanwar, M.K., Bhardwaj, R., Arora, P., Chowdhary, S.P., Sharma, P. and Kumar, S. (2012). Plant steroid hormones produced under Ni stress are involved in theregulation of metal uptake anti-oxidative stress in Brassica juncea L. Chemosphere, 86: 41-45
26. Kanwar, M.K., Bhardwaj, R., Chowdhary, S.P., Arora, P., Sharma, P. And Kumar, S. (2013). Isolation and characterization of 24-epibrassinolide from Brassica juncea L. and its effects on growth, Ni ion uptake, and antioxidant defense of Brassica plants and in vitro cytotoxicity. Acta Physiol. Plant., 35: 1351–1362.
27. Kapoor, D., Rattan, A., Gautam, V. and Bhardwaj, R. (2016). Alleviation of cadmium and mercury stress by supplementation of steroid hormone to Raphanus sativus seedlings. Proc. Natl. Acad. Sci. India Sect B Biol. Sci., 86: 661-666.
28. Khripach, V.A., Zhabinskii, V.N. and Groot, A.E. (1999). Brassinosteroids a New Kumar S, Sirhindi G, Bhardwaj R, Kumar M. 2014. Brassinosteroids denigrate the seasonal stress through antioxidant defense system in seedlings of Brassica junceaL. J. Stress Physiol. Biochem., 10: 75–83.

29. Kohli, S. K., Bali, S. Tejpal, R. Bhalla, V. Verma, V. Bhardwaj, R. Alqarawi, A. A. and Ahmad, P. (2018). Interaction of 24-epibrassinolide and salicylic acid regulates pigment contents, antioxidativedefense responses, and gene expression in Brassica juncea L. seedlings under Pb stress. Environ Sci Pollut Res Int., 25(15):15159-15173.
30. Kohli, S. K., Bali, S. Tejpal, R. Bhalla, V. Verma, V. Bhardwaj, R. Alqarawi, A. A. and Ahmad, P. (2019). In-situ localization and biochemical analysis of bio-molecules reveals Pb-stress amelioration in Brassica juncea L. by co-application of 24-Epibrassinolide and Salicylic Acid. Sci Rep., 9(1):3524.
31. Lapik, Y.R. and Kaufman, L.S. (2003). The Arabidopsis cupin domain protein AtPirin1 interacts with the G protein α-subunit GPA1 and regulates seed germination and early seedling development. Plant Cell., 15:1578–90.
32. Li, P., Chen, L., Zhou, Y., Xia, X., Shi, K., Chen, Z. and Yu, J. (2013a). Brassinosteroids-induced systemic stress tolerance was associated with increased transcripts of several defence-related genes in the phloem in Cucumis sativus L. PLoS ONE, 8(6):e66582.
33. Li, H.B., Wang, J.N., Chen, Y.J. and Li, R.T. (2013b). Effects of brassinolide on fruit growth and quality of pitaya. Nanfang Nongye Xuebao., 44:1150-1153.
34. Li, J., Yang, P., Kang, J., Gan, Y. and Yu, J. (2016). Calderón-Urrea A, Jian L, Zhang G, Feng Z, Xie J. Transcriptome analysis of pepper revealed a role of 24-epibrassinolide in response to chilling. Front Plant Sci., 7:1-16.
35. Mahesh, K., Balaraju, P., Ramakrishna, B. And Rao, S.S.R. (2013). Effect of brassinosteroids on germination and seedling growth of radish under PEG-6000 induced water stress. Am J Plant Sci., 4:2305-13.
36. Mir, B.A., Khan, T.A. and Fariduddin, Q. (2015). 24-epibrassinolide and spermidine modulate photosynthesis and antioxidant systems inVigna radiata under salt and zinc stress. Int. J. Adv. Res., 3: 592-608.
37. Nazir, F., Fariduddin, Q., Hussain, A. and Khan, T.A. (2020).Brassinosteroid and hydrogen peroxide improve photosynthetic machinery, stomatal movement, root morphology and cell viability and reduce Cu-triggered oxidative burst in tomato. Ecotox. Environ. Saf., 207: 111 081.
38. Nazir, F., Hussain, A. and Fariduddin, Q. (2019).Interactive role of epibrassinolide and hydrogen peroxide in regulating stomatal physiology, root morphology, photosynthetic and growth traits in Solanum lycopersicum L. under nickel stress. Environ. Exp. Bot., 162: 479-495.
39. Niu, J.H., Ahmad, Anjum, S., Wang, R., Li, J.H., Liu, M.R., Song, J.X., Zohaib, A., Lv, J., Wang, S.G. and Zong, X.F. (2016). Exogenous application of brassinolide can alter morphological and physiological traits of Leymus chinensis (Trin.) Tzvelev under room and high temperatures. Chil. J. Agric. Res., 76: 27-33.
40. Ozdemir, F., Bor, M., Demiral, T. And Turkan, I. (2004). Effects of 24-epibrassinolide on seed germination, seedling growth, lipidperoxidation, proline content and antioxidative system of rice (Oryza sativa L.) under salinity stress. Plant Growth Regul., 42:203–11.
41. 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. Journal of Stress Physiology and Biochemistry, 10: 315-327.
42. Rady, M.M. and Osman, A.S. (2012). Response of growth and antioxidative system of heavy metal contaminated tomato plants under 24-epibrassinolide. Afr. J. Agric. Res. 7: 3249–3254.
43. Raghava, N., Srivastava, K., Srivastava, S. and Raghava, R.P. (2014). Brassinosteroids Research- A Review. In: Advances in Plant Physiology (Hemantranjan, A. ed.). Scientific Publishers (India) Jodhpur, 15: 509-604.

44. Rajewska, I., Talarek, M. And Bajguz, A. (2016). Brassinosteroids and response of plants to heavy metals action. Front. Plant Sci. 9: 629.
45. Rehman, S.U., Bilal, M., Rana, R.M., Tahir, M.N., Shah, M.K.N., Ayalew, H. and Yan, G. (2016). Cell membrane stability and chlorophyll content variation in wheat genotypes under conditions of heat and drought. Crop Pasture Sci. 67:712–8.
46. Shagun, Raghava, R.P., Kshitij, and Raghava, N. (2011). Influence of 28-homobrassinolide on productivity and biochemical parameters of tomato. Indian J. Applied and Pure Bio. 26(2):305-308.
47. Sharma, P. and Bhardwaj, R. (2007). Effects of 24-epibrassinolide on growth and metal uptake Brassica juncea L. under copper metal stress, Acta Physiol. Plant., 29:259-263.
48. Sharma, N., Hundal, G.S., Sharma, I. and Bharadwaj, R. (2012). Effect of 24-epibrssinolide on protein content and activities of glutathione-S-transferase and polyphenoloxidase in Raphanus sativus L. Plants under cadmium and mercury metal stress. Terrest. Aquat. Toxicol., 6:1–7.
49. Sharma, P., Kumar, A. and Bhardwaj, R. 2016. Plant steroidal hormoneepibrassinolide regulate - Heavy metal stress tolerance in Oryza sativa L. by modulating antioxidant defense expression. Environmental and Experimental Botany, 122: 1–9.
50. Shu, S., Tang, Y., Yuan, Y., Sun, J., Zhong, M., Guo, S. (2016). The role of 24-epibrassinolide in the regulation of photosynthetic characteristics and nitrogen metabolism of tomato seedlings under a combined low temperature and weak light stress. Plant Physiol Biochem., 107:344–353.
51. Soares, C., de Sousa, A., Pinto, A., Azenha, M., Teixeira, J., Azevedo, R.A. and Fidalgo, F. (2016). Effect of 24-epibrassinolide on ROS content, antioxidant system, lipid peroxidation and Ni uptake in Solanum nigrum L. under Ni stress. Environmental and Experimental Botany, 122: 115–125.
52. Srivastava,K., Raghava, N., Shagun and Raghava, R.P.(2011). Brassinosteroids stimulate seed germination parameters and chlorophyll content in moongbean, Indian J. Sci. Res., 2(3): 85-88.
53. Swamy, K.N., Vardhini, B.V., Ramakrishna, B., Anuradha, S., Siddulu, N. and Rao, S.S.(2014). Role of 28-homobrassinolide on growth biochemical parameters of Trigonella foenumgraecum L.plants subjected to lead toxicity. Int. J. Multidiscip. Curr. Res., 2: 317-321.
54. Takatsuto, S. (1994). Brassinosteroids: distribution in plants, bioassays and microanalysis by gas chromatography-mass spectrometry. Journal of Chromatography, 658: 3-15.
55. Talaat, N.B. and Shawky, B.T. (2016).Dual application of 24-epibrassinolide and spermine confers drought stress tolerance in maize (Zea mays L.) by modulating polyamine and protein metabolism. J. Plant Growth Regul., 35: 518-533.
56. Wang, W., Bai, M.Y. and Wang, Z.Y. (2014).The brassinosteroid signalling network aparadigm of signal integration. Curr. Opin. Plant Biol., 21:147-153.
57. Wei, L.J., Deng, X.G., Zhu, T., Zheng, T., Li, P.X., Wu, J.Q., Zhang, D.W., Lin, H.H.(2015). Ethylene is involved in brassinosteroids induced alternative respiratory pathway in cucumber (Cucumis sativus L.) seedlings response to abiotic stress. Front Plant Sci., 6:982.
58. Xia, X.J, Huang, L.F, Zhou, Y.H, Mao, W.H, Shi, K, Wu, J.X, Asami, T, Chen, Z. and Yu, J.Q. (2009a). Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in Cucumis sativus L. Planta, 230:1185.
59. Xia, X.J., Wang, Y.J., Zhou, Y.H., Tao, Y., Mao, W.H., Shi, K., Asami,T., Chen, Z. and Yu, J.Q. (2009b). Reactive oxygen species are involved in brassinosteroid-induced stress tolerance in cucumber. Plant Physiol. 150:801-814.
60. Xu, H.L, Shida, A., Futatsuya, F. And Kumura, A. (1994). Efects of epibrassinolide and abscisic acid on sorghum plants growing under soil water defcit. II. Physiological basis for

drought resistance induced by exogenous epibrassinolide and abscisic acid. JPN J Crop Sci., 63:676-81.

61. Yokota, T. and Takahashi, N. (1986). Chemistry, physiology and agricultural application of brassinolide and related steroids. In: Plant Growth Substances, pp 129-138.Eds. M Bopp, Springer-Verlag, Berlin.
62. Yuan, L., Yuan, Y., Du, J., Sun, J. and Guo, S. (2012). Effects of 24-epibrassinolide on nitrogen metabolism in cucumber seedlings under Ca $(NO_3)_2$ stress. Plant Physiol Biochem., 61:29-35.
63. Yusuf, M., Fariduddin, Q., Ahmad, I. and Ahmad, A. (2014). Brassinosteroid-mediated valuation of antioxidant system and nitrogen metabolism in two contrasting cultivars of Vigna radiate under different levels of nickel. Physiol. Mol. Biol. Plant., 20: 449-460.
64. Yusuf, M., Fariduddin, Q., Hayat, S., Hasan, S.A. and Ahmad, A. (2011). Protective response of 28-homobrassinolide in cultivars of Triticum aestivum with different levels of nickel. Arch. Environ.Contam.Toxicol., 60: 68-76
65. Zaharah, S.S., Singh, Z., Symon, G.M. and Reid, J.B. (2012). Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. J Plant Growth Regul., 31:363–72.
66. Zhabinskii, V.N., Khripach, N.B. and Khripach, V.A. (2015). Steroid plant hormones: effects outside plant kingdom. Steroids, 97: 87-97.
67. Zhao, G., Xu, H., Zhang, P., Su, X. and Zhao, H. (2017). Effects of 24-epibrassinolide on photosynthesis and Rubisco activase gene expression in Triticum aestivum L. seedlings under a combination of drought and heat stress. Plant Growth Regul., 81:377–84.
68. Zhu, X., Liang, W., Cui, X., Chen, M., Yin, C., Luo, Z., Zhu, J., Lucas, W.J., Wang, Z. and Zhang, D. (2015).Brassinosteroids promote development of rice pollen grains and seeds by triggering expression of Carbon Starved Anther, a MYB domain protein. Plant J., 82: 570-581.

Received on 22.07.2021 and accepted on 29.11.2021