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Brassinosteroids and Plant Responses to Heavy Metal Stress. An Overview

Miriam Núñez Vázquez*, Yanelis Reyes Guerrero, Lisbel Martínez González, Walfredo Torres de la Noval
Department of Plant Physiology and Biochemistry, National Institute of Agricultural Sciences, San José de las Lajas, Cuba
Email: *mnunez@inca.edu.cu

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ABSTRACT

Soil contamination with heavy metals has become a world-wide problem, leading to the loss in agricultural productivity. Plants have a remarkable ability to take up and accumulate heavy metals from their external environment and it is well known that high levels of heavy metals affect different physiological and metabolic processes. Brassinosteroids are considered as the sixth class of plant hormones and they are essential for plant growth and development. These compounds are able of inducing abiotic stress tolerance in plants. In this paper, information about brassinosteroids and plant responses to heavy metal stress is reviewed.

Keywords: Heavy Metal Stress; Brassinosteroids; Tolerance

1. Introduction

Soil contamination with heavy metals has become a world-wide problem, leading to the loss in agricultural productivity and hazardous health effects as they become a part of the food chain [1]. However, their availability in the soil is determined by natural processes, especially the lithogenic and pedogenic ones, and by anthropogenic factors [2]. Plants have a remarkable ability to take up and accumulate heavy metals from their external environment and it is well known that some of these metals such as Cu, Zn, Mn, Fe are required for normal plant growth and development at trace levels [3], since they are structural and catalytic components of proteins and enzymes. However, high concentrations of them and others such as Al, Cd, Cr, Pb affect different physiological and metabolic processes at cellular and organism levels.

Toxicity mechanisms include the blockade 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 organellar membrane integrity [4].

Heavy metal toxicity can elicit a variety of adaptive responses in plants. These responses are based on mechanisms that lowering metal uptake and accumulation by plants. A common mechanism for heavy metal detoxification is the chelation of the metal ion by a ligand. Such ligands include organic acids, amino acids, peptides and polypeptides. Peptide ligands include the phytochelatins (PC), which detoxifies intracellular metals by binding them through thiolate coordination [4].

Plant hormones such as auxins (indole-3-acetic acid, IAA), abscisic acid (ABA) and brassinosteroids (BRs) have been recently found to work as vital components of stress management. Auxins have been observed to ameliorate the intensity of various stresses such as salinity, drought [5], chilling [6], heat and heavy metal stress [7]. Similarly, ABA triggers plant responses to adverse environmental stimuli [8]. Enhanced synthesis of PC contents by exogenous ABA has been reported in *Prosopis juliflora* under Cu, Zn and Cd stress [9].

BRs are steroidal hormones which play a critical role in a range of developmental processes and they have also been implicated in plant responses to abiotic stress. Their ability to improve antioxidant system by elevating the activities and levels of enzymatic and non-enzymatic antioxidants has made them a favorite tool to increase resistance potential of important agricultural crops against various abiotic stresses such as heavy metal excess

*Corresponding author.

[10,11].

2. Brassinosteroids and Plant Responses to Heavy Metal Stress

BRs are able to regulate the uptake of ions into the plant cells and can be used to reduce the accumulation of heavy metals [12], because they can reduce the metal uptake by roots [13] and can also stimulate the synthesis of some ligands such as the phytochelatins, which are combined with metal ion [14,15]. They also increase the activities of some antioxidant enzymes detoxifying the increased production of reactive oxygen species (ROS) generated by heavy metal stress [16-18] and so exogenous applications of BRs improve the growth and metabolic activity in plants under heavy metal stress.

2.1. Responses to Copper (Cu) Stress

Copper is an essential transition metal required for normal plant growth and development at trace levels [3,19]. It is an indispensable component of diverse plant metabolic reactions, such as a structural element in regulatory proteins and its participation in photosynthetic electron transport, mitochondrial respiration, oxidative stress response, cell wall metabolism and hormone signaling are well established [20]. Among pollutants of agricultural soils, Cu has become increasingly hazardous due to its involvement in fungicides, fertilizers and pesticides [21].

However, excess of Cu metal catalyzes the formation of ROS [22,23]. These ROS are highly toxic and oxidize important macromolecules such as nucleic acids, proteins and lipids, thereby disturbing cell stability and membrane permeability [24,25]. Also, the reduced shoot and root growth, decline in photosynthetic pigment formation have been observed in plants under Cu stress [26].

Effects of exogenous applications of BRs (24-epibrassinolide, EBL) have been studied on mustard and radish plants under copper stress. Sharma and Bhardwaj (2007) observed an improvement in the shoot emergence and plant biomass production under Cu stress when mustard seeds were soaked for 8 hours in EBL (10^{-7}, 10^{-9} and 10^{-11} M) solutions. In addition, EBL blocked Cu metal uptake and accumulation in these plants [12].

In radish seedlings, EBL-treated seeds (10^{-7} M) showed a reduced copper toxicity by stimulating the root and shoot growth [15]. This growth response was associated with enhanced IAA and ABA concentrations, lowered oxidative stress (a major increase of antioxidant enzyme activities, a major decrease of MDA and increased contents of antioxidant metabolites such as proline, ascorbic acid and total phenols) and increased phytochelatin content in the seedlings. Effects of EBL on antioxidant capacity and free radical scavenging activity of radish seedlings were also studied [27].

2.2. Responses to Nickel (Ni) Stress

Nickel is one of the most abundant heavy metal contaminants of the environment due to its release from mining and smelting practices. It is classified as an essential element for plant growth [28]. However, at higher concentrations, nickel is an important environmental pollutant. Ni^{2+} ions bind to proteins and lipids such as specific sub-sequences of histones [29] and induce oxidative damage. Excess of nickel affects chlorophyll biosynthesis, since it affects both the synthesis of δ-aminolevulinic acid and protochlorophyllide reductase complex [30]. Ni^{2+} also replaces the Mg^{2+} ion of chlorophyll pigment [31], causes the inhibition of enzymes of chlorophyll biosynthesis [32] and stimulates chlorophyllase [33], ultimately leading to a decline in the level of chlorophyll pigment. Nickel also causes a significant inhibition in the activities of enzymes associated to carbon fixation in plants [34].

EBL and 28-homobrassinolide (HBL) protect plants under nickel stress conditions. Mustard (*Brassica juncea*) plants treated with Ni_2Cl and later, with a HBL foliar spray showed no nickel toxic effect on growth, net photosynthetic rate, chlorophyll content and nitrate reductase and carbonic anhydrase activities. Moreover, HBL treatment stimulated the activities of some antioxidant enzymes such as peroxidases and catalases and the level of proline [35]. Similar results were obtained in this specie by Ali *et al*. (2008) using EBL foliar spray and by Sharma *et al.* (2008) with HBL seed treatment for 8 hours [36,37]. EBL enhanced the level of antioxidant system (superoxide dismutase, catalase, peroxidase and glutathione reductase and proline), under stress conditions. The influence of EBL on antioxidant system, at least in part, increased the tolerance of mustard plants to $NiCl_2$ stress and thus protected the photosynthetic machinery and the plant growth.

Recently, a research was performed in which various concentrations of the isolated EBL from *Brassica juncea* leaves were given as pre-sowing treatment to the seeds of this same species for 8 h. The pre-treated seeds were subjected to nickel stress. Results showed that pre-treatment of isolated EBL lowered the Ni ion uptake in plants and improved growth. The amelioration of Ni toxicity was also observed by the activities of antioxidant enzymes [28].

The results described above associate the BR protection to Ni stress in plants, particularly in mustard, with the lowering of oxidative stress, because of the increase of antioxidant enzyme activities and the proline level. It may explain the protection to photosynthetic machinery and the plant growth stimulation.

2.3. Responses to Cadmium (Cd) Stress

Cd is not an essential nutrient and it is one of the heavy metals that are known to generate toxicity even at a very low concentration. It accumulates in plants during growth in edible parts, thereby, endangering crop yield and their quality. This causes a potential hazard to human and animal health. Cd is known to cause enzyme inactivation and damages cells by acting as antimetabolite or forms precipitates or chelates with a number of essential metabolites [38]. Cd inhibits plant growth [39], retards the biosynthesis of chlorophyll [40], alters water balance [41], decreases the activities of various enzymes [42], stimulates stomatal closure [43] and controls photosynthesis [34,44]. Cadmium stress reduces the uptake of essential mineral nutrients and also affects the activity of ATPase of plasma membrane [45].

Various researchers have demonstrated that BRs reduce the adverse effects induced by Cd stress in plants. Thus, Janeczko *et al*. (2005) found that EBL reduced the toxic effect of Cd on photochemical processes by diminishing the damage of photochemical reaction centers and the activity of O_2 evolving centers as well as maintaining efficient photosynthetic electron transport [46]. Probably, the effectiveness of the protective action of EBL increases in older tissue, which is more susceptible to damage by Cd. Later, Anuradha and Rao (2009) reported that EBL stimulated photosynthetic activity in radish plants under Cd stress [11].

On the other hand, EBL foliar spray enhanced the level of antioxidant system (superoxide dismutase, catalase, peroxidase and glutathione reductase, and proline) of bean plants under Cd stress conditions [17]. This author suggested that the elevated level of antioxidant system, at least in part, increased the tolerance of bean plants to $CdCl_2$ stress, thus protected the photosynthetic machinery and the plant growth. Similar results had been reported in mustard [47] and chickpea [16] plants using EBL and HBL, respectively.

The application of BRs (EBL and HBL) improved the chlorophyll content and photosynthesis efficiency of Cd-stressed tomato plants applied as shotgun approach [48]. Besides, BR treatment significantly increased the number of fruits, fruit yield and lycopene and *β*-carotene contents in the fruits from plants grown under Cd stress.

2.4. Responses to Other Heavy Metal Stresses

Zinc (Zn) is an essential microelement, the second most abundant transition metal after iron (Fe) and plays a pivotal role in many metabolic reactions in plants [49,50]. However, high concentrations of Zn are toxic, induce structural disorders and cause functional impairment in plants. At organism level, Zn stress causes reduced rooting capacity, stunted growth, chlorosis and at cellular level alters mitotic activity [51,52], affects the membrane permeability, the electron transport chain, the uptake and translocation of nutrient elements [53,54] and induces oxidative stress by promoting the generation of ROS. Since Zn is a non-redox metal, it cannot generate ROS directly through Haber-Weiss reactions, over production of ROS and occurrence of oxidative stress could be an indirect consequence of Zn toxicity.

Recently, Ramakrishna and Rao (2012) reported that the application of EBL significantly alleviated the zinc induced oxidative stress. EBL had a protective role on lipid peroxidation, protein oxidation and membrane integrity in radish seedlings [55]. This BR induced lowering of ROS levels, MDA and carbonyl levels could be attributed to the increased activities of ROS scavenging enzymes and the decreased activities of lipoxygenase (enzyme which catalyzes the oxygenation of polyunsaturated fatty acids into lipid hydroperoxides), and NADPH oxidase (enzyme which catalyzes the formation O^{2-} that is converted to more stable H_2O_2 via complex reaction). A similar response of Zn induced oxidative stress had been reported in *Brassica juncea* plants [56].

In lower organisms like the alga *Chlorella vulgaris*, Bajguz (2000) demonstrated that EBL blocked the heavy metals accumulation in the cells. The inhibitory effect on heavy metal accumulation was arranged in the following order: Zn > Cd > Pb > Cu [57]. The author associated BR protection to heavy metal stress to EBL-induced pH increase in the medium, since lower pH increased the toxicity of heavy metals in *C. vulgaris* cells.

On the other hand, aluminum (Al) and chromium (Cr) are not essential nutrients. Al toxicity is the major growth-limiting factor for crop cultivation on acidic soil [58] that generates oxidative stress indirectly, mediated by its influence on membrane lipids and other peroxidants such as iron [59]. Al ions are capable of binding with lipid components of the plasma membrane [60] causing the rigidification of plasma membrane [61] and to DNA [62], and therefore, impairs cell division [58]. Al is reported to inhibit the plant growth [63], mainly that of root [64,65]. Besides this, Al also alters water relations [41], reduces stomatal opening, decreases photosynthetic activity and causes chlorosis and necrosis of leaves [58].

Ali *et al*. (2008) reported that EBL or HBL treatment improved the response of mung bean seedlings to Al stress [66]. This response was associated to the ameliorated level of antioxidant system, suggesting that, at least in part, it was responsible for the development of resistance against Al stress in these seedlings. The increase in the degree of resistance due to the application of BRs was reflected in the improvement of plant growth, photosynthesis and related processes, in the presence of Al.

Cr metal pollution in the biosphere has increased sharply over the last few decades, mainly due to its anthropogenic release from leather, electroplating, catalytic manufacturing, chromic acid and refractory steel industries [67]. However, in nature, Cr is usually found in trivalent (Cr III) and hexavalent (Cr VI) oxidation states, of which Cr (VI) is more phytotoxic, owing to its greater mobility. The entry of Cr into a plant system occurs through roots using the specialized uptake systems of essential metal ions (Fe, S), required for normal plant metabolism [67]. Reduced seed germination, disturbed nutrient balance, with decreased rate of photosynthesis, inactivation of Calvin cycle enzymes, and chloroplast disorganization have been documented in plants under Cr stress [67]. The oxidative stress under Cr excess is caused by the over production of ROS.

The significant influence of EBL on the synthesis of IAA, ABA and polyamines (PAs) of radish seedlings under Cr (VI) metal stress was demonstrated by Choudhary *et al*. (2011). EBL could enhance the synthesis of IAA in order to promote normal seedling growth under Cr (VI) metal stress. On the other hand it also slightly improved the production of ABA to increase Cr (VI) stress tolerance. Altered synthesis of PAs observed under the influence of EBL may be helpful in protecting the seedlings against Cr (VI) stress by enhancing one pool of PAs (putrescine and spermidine) and decreasing the other pool (cadaverine) [68]. Increased levels of antioxidants and antioxidant enzymes activities upon EBL application with Cr (VI) metal stress also indicate its significant effect on antioxidant system of radish plants. Similarly, reduced membrane damage, enhanced proline, photosynthetic pigments, sugars and radical scavenging activities also shows a major impact of EBL on radish seedling metabolism under Cr (VI) metal stress.

Earlier, Arora *et al*. (2010) had reported the effect of EBL treatment to regulate the diminution of Cr metal toxicity in mustard plants [69].

Besides, the interaction of EBL with lead (Pb) on the growth of algae and ion accumulation in *Chlorella vulgaris* cells was studied by Bajguz (2000) [57] while the effect of BRs on oxidative stress generated by manganese stress in maize leaves was reported by Wang *et al*. (2009) [70].

3. Concluding Remarks

BRs reduced the heavy metal toxicity in plants. Firstly, this reduction was associated with lesser ion uptake and accumulation and with the increasing activity of ATPase, an enzyme responsible for acid secretion and changes in membrane level [57]. The proton pump generates an H^+ electrochemical gradient and provides a driving force for the rapid ion fluxes required for the uptake of various nutrients such as K^+, Cl^-, NO_3^-, amino acids and sucrose across the plasma membrane [71]. The regulation of H^+-ATPase activity [72] not only allows nutrient uptake in plant cells but also controls water fluxes [73]. In addition to this, proton secretion induced by BRs has been reported too. This proton secretion was accompanied by an early hyperpolarization of the plasma membrane, indicating that proton pumps could be targets of BRs [74].

On the other hand, the lesser ion accumulation induced by BRs may be explained by the ability of these compounds to increase some ligands which form chelates with metal ion as, for example, phytochelatins [14]. Further, BRs have also influence on electrical properties of membranes and transport of ions by altering their permeability and structure, stability and activity of membrane enzymes.

All the above results confirmed that phytotoxicity from heavy metals is closely related to oxidative stress and so to the production of ROS in plants. An imbalance between ROS production and ROS scavenging leads to oxidative burst. ROS can react with nucleic acids, proteins and lipids and causes membrane damage and enzyme inactivation resulting in inhibition of plant growth [75]. In plants, removal of ROS and cellular homeostasis are governed by antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and the enzymes of ascorbate-glutathione cycle and various non-enzymatic antioxidants such as carotenoids, α-tocopherol, proline, phenols, ascorbate and glutathione via scavenging and neutralization [76,77].

The elevation in the activities of antioxidant enzymes by BRs is well documented and it is known that it is a gene regulated phenomenon. Cao *et al*. (2005) demonstrated on the basis of molecular, physiological and genetic approaches the elevation in antioxidant enzymes was the consequence of enhanced expression of *det* 2 gene, which enhanced the resistance to oxidative stress in *Arabidopsis* [78]. Similarly, Xia *et al.* (2009) reported that BRs-induced stress tolerance is associated with increased expression of genes encoding antioxidant enzymes such as SOD, CAT, POD, GR and APX in leaves of cucumber [79]. Various reports have also shown that the application of BRs modified antioxidant enzymes activities under other abiotic stresses such as water deficit [80], salinity [81], high temperature [82] and chilling [83].

Plant protection induced by BRs under heavy metal stress has been related to interactions of these steroidal hormones with other plant hormones such as IAA and ABA. Recently, Choudhary *et al.* (2012) reviewed the benefits of brassinosteroid crosstalk and they concluded that the versatile role of BRs may be attributed to multilayer interactions with other plant growth regulators affecting the post-transcriptional fate of the target response [84], although they pointed out that the mechanisms behind the pleiotropic action of BRs and the execution of BR-induced responses still remain poorly understood at this time.

So, the focus of future research should be to elucidate the mechanism by which BRs confer tolerance to heavy metal stress at cellular and molecular levels.

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