Aperito Journal of Advanced Plant Biology

Received: Feb 02, 2016
Accepted: Feb 24, 2016
Published: Feb 27, 2016

http://dx.doi.org/10.14437/2381-4993-2-107 Research Naser A Anjum, Aperito J Adv Plant Biol 2016, 2:1

Assessment of Divalent Metal-Toxicity and the Role of 24-Epibrassinolide in Triticum Aestivum Seedlings

Dmitry I Bashmakov[1], Kristina A Gruznova[1], Alexander S Lukatkin[1], Naser A Anjum[2*] and EDuarda Pereira[2]

[1]Department of Botany, Physiology and Ecology of Plants, N.P. Ogarev Mordovia State University, Bolshevistskaja Str., 68. Saransk, 430005, Russia

[2]CESAM-Centre for Environmental and Marine Studies & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Abstract

Reports on the metal-toxicity are extensive; however, literature is scarce on the role of sustainable stress-mitigation approaches in this context. This work investigated the significance of growth regulator 24-epibrassinolide (24-EpiBL; 1.0 µM) in the mitigation of consequences of low (10 µM) or high (1.0 mM) concentrations of plant-beneficial (Cu^{2+}, Zn^{2+}, and Ni^{2+}) and plant-non-essential (Pb^{2+} and Sr^{2+}) divalent metals in wheat (*Triticum aestivum* L.) seedlings. Apart from studying the changes in root and shoot growth, the status of oxidative stress and its metabolism (in terms of superoxide anion, $\bullet O_2^-$; lipid peroxidation, LPO; catalase, CAT) was assessed as major markers of Cu^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+} and Sr^{2+}-accrued consequences. At high concentrations, plant-beneficial divalent metals (particularly Cu^{2+} and Ni^{2+}) and plant-non-essential divalent metals (Pb^{2+} and Sr^{2+}) significantly enhanced $\bullet O_2^-$-generation, LPO and CAT activity in *T. asetivum* leaves and also inhibited the axial growth. Pretreatment of seeds with 24-EpiBL protected *T. aestivum* seedlings by differentially minimizing the impacts of particularly Zn^{2+}, Ni^{2+}, and Pb^{2+} (up to 10 µM), and diminished oxidative stress and improved overall growth. 24-EpiBL-mediated elevation in CAT activity and subsequent control of cellular $\bullet O_2^-$ and its consequence (LPO) can be convincing mechanism that eventually resulted into 24-EpiBL-mediated improved root and shoot growth traits in *T. aestivum* when exposed to low (10 µM) concentration of essential divalent metals (Zn^{2+}, Cu^{2+} and Ni^{2+}) as well as phytotoxic divalent metal (Pb^{2+}).

Keywords: Divalent Metals; *Triticum Aestivum*; Oxidative Stress; Growth Regulators; 24-Epibrassinolide; Metal Tolerance

***Corresponding Author:** Naser A Anjum, CESAM-Centre for Environmental and Marine Studies & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; Tel: +351 234401527; Ext. 24907; Fax: +351 234370084; E-mail: anjum@ua.pt

Introduction

Soil contamination with various metals/metalloids (hereafter termed as 'metal/s') and their impacts on plant health and productivity are extensively reported [1]. Once inside plants, even low levels of plant-non-essential metals such as lead (Pb) and strontium (Sr) are phytotoxic [2, 3]. However, at both low (deficiency) and high (excess) levels, plant-beneficial metals such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) can impair plant growth, development, and productivity. Elevation in the generation of reactive oxygen species (ROS; such as superoxide radicals, $\bullet O_2^-$; hydroxyl radicals, OH−; perhydroxyl radicals, HO_2^-; alkoxy radicals, RO^-; hydrogen peroxide, H_2O_2; singlet oxygen, $^1O_2$) has been considered as the major factor responsible for the previous responses of low and high levels of plant-beneficial metals [1]. Notably, if not metabolized, ROS can imbalance cellular redox homeostasis and oxidize lipids (lipid peroxidation, LPO) and proteins, and finally halt cellular metabolism [4-7]. Plants tend to counteract ROS-accrued consequences employing antioxidant defense system comprising antioxidant enzymes (such as superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase) and non-enzymes (such as ascorbate, glutathione, tocopherol, carotenoids, flavonoids) [5]. Exogenous application of mineral elements [8, 9] and phytohormones [10, 11] can significantly modulate both enzymatic and non-enzymatic components of the antioxidant defense system.

Brassinosteroids (BRs), a novel group of phytohormones are known to influence growth, seed germination, rhizogenesis, senescence etc., and are also extensively reported to confer abiotic stress tolerance in plants. In particular, 24-epibrassinolide (24-EpiBL) is among the three bioactive BRs those are widely used in most physiological and experimental studies, where 24-EpiBL was evidenced to differentially modulate antioxidant defense system components in stressed plants [12]. Considering both a differential phytoxicity potential of various metals/metalloids [1-3, 6], and the reported significant roles of 24-EpiBL in plants [12], it was hypothesized herein that exogenously sourced 24-EpiBL can benefit economically important crop winter wheat (*Triticum aestivum* L.) differently when it is exposed to different divalent metals.

To test the previous hypothesis, taking in to account *T. aestivum* as a model plant system, this study aimed: (a) to assess the phytotoxicity of equimolar solutions of plant-essential (Cu^{2+}, Ni^{2+}, Zn^{2+}) and plant-non-essential (Pb^{2+}, Sr^{2+}) divalent metals in terms of important growth traits, cellular $\bullet O_2^-$, membrane LPO, and CAT activity, and (b) to evaluate the significance of 24-EpiBL in the control of previous test parameters in *T. aestivum* under Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+} and Sr^{2+} exposure. Additionally, before executing the works on the aforesaid objectives, efforts were also made to determine the optimal concentration of 24-EpiBL for *T. aestivum*. The method of 24-EpiBL-treatment by spraying the plants cannot always be practical because it requires a large labor and technical costs. Hence, this study investigated the physiological and biochemical efficacy of less expensive and less labor-intensive method for reducing the negative effects of previous metals using pre-sowing seeds treatment with solutions of 24-EpiBL.

Materials and Methods

Plant culture, 24-EpiBL-dosimetric study, and metal-exposure

Commercially obtained seeds of *T. aestivum* (sub-species Lutescens cultivar Prokhorovka) were surface-sterilized with 0.5% $KMnO_4$ for 5 min before their use in the study. For the 24-EpiBL-dosimetric studies surface-sterilized *T. aestivum* seeds imbibed with 24-EpiBL (10^{-4} M to 10^{-12} M) for 12 h. The control seeds were imbibed with distilled water. Subsequently, seeds were germinated in plastic pots (50 seeds per pot) at temperature 22-24°C, photoperiod 16/8 h (day/night), PFD 80 µmol m^{-2} s^{-1} for 7 days. The optimal concentration of 24-EpiBL for *T. aestivum* was determined by the maximum elongation of axial organs in 7 day old seedlings.

For the metal-exposure experiments, the surface-sterilization of *T. aestivum* seeds were done as described above. Thereafter, these seeds were imbibed with the optimal concentration of 24-EpiBL (1.0 µM) for *T. aestivum* for 12 h. Here also, seeds imbibed with distilled water were considered as the control. *T. aestivum* seeds, imbibed with either 24-EpiBL (1.0 µM) or water (control) were germinated in plastic pots (50 seeds per pot). Subsequently, the 24-EpiBL (1.0 µM)-imbibed seeds were supplemented with low (10 µM) or high (0.5–1.0 mM) $Pb(NO_3)_2$, or $CuSO_4 \times 5H_2O$, or $NiSO_4 \times 7H_2O$ or $ZnSO_4 \times 7H_2O$, or $SrSO_4 \times 7H_2O$. *T. aestivum* seedlings were maintained at temperature 22–24°C, photoperiod 16/8 h (day/night), PFD 80 µmol m^{-2} s^{-1} for 7 days. *T. aestivum* seedlings were harvested on day 7, and all the detailed below determinations were performed.

Determinations

Growth Traits: The length of axial organs of 30 selected *T. aestivum* seedlings per treatment was measured on meter scale. Wilkins Tolerance Index (WTI) was applied to assess the metal-tolerance of *T. aestivum* seedlings employing the formula: $I_t = (l_{me}/l_c) \times 100\%$, where l_{me} indicates the increase in root growth in a metal ion solution and l_c is the increase in root growth in the control [13].

Superoxide Anion Generation: The estimation of $\bullet O_2^-$ in leaf discs was based on the capacity of $\bullet O_2^-$, to oxidize adrenaline to adrenochrome [14]. In brief, fresh discs (300 mg) were homogenized with 15 ml of distilled water. The solution was centrifuged for 15 min at 3000 rpm. To 3.0 ml of homogenate, 0.1 ml of adrenaline solution (0.01%) was added. The mixture was incubated for 45 min at ambient temperature with illuminance of about 200 µM m^{-2} s^{-1}. The optical density of the adrenochrome formed was measured immediately after incubation on a spectrophotometer (UV-mini 1240, Shimadzu, Japan) at 480 nm. The generation of $\bullet O_2^-$ was calculated by adopting a molar extinction coefficient ($\varepsilon = 4020$ M^{-1} cm^{-1}) in µM g^{-1} min^{-1}.

Lipid Peroxidation: The detection of LPO intensity in leaf discs was done as per the method based on a storage of Malondialdehyde (MDA) [15]. *T. aestivum* leaf discs (300 mg) were homogenized in 10 ml of isolation medium (with 0.1 M Tris-HCl buffer; 0.35 M NaCl; pH 7.6). To 3.0 ml of homogenate, 2.0 ml of thiobarbituric acid (prepared in 20% trichloroacetic acid) was added. The resultant solution was heated in a boiling water bath for 30 min and filtered. After stopping the reaction by keeping the tubes with solution on ice, the optical density was recorded on a spectrophotometer 532 nm. The concentration of MDA was calculated by adopting a molar extinction coefficient ($\varepsilon = 1.56 \cdot 10^5$ M^{-1} cm^{-1}) in µM g^{-1} of fresh leaf weight.

Catalase Activity: *T. aestivum* leaf discs (1.0 g) were homogenized with 10 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was filtered and centrifuged for 10 min at 8000 ×*g*. To 25 ml of enzyme extract, 2.9 ml of phosphate buffer (pH 7.0) was added. Directly before the measurement, 90 µl of 3% H_2O_2 was added to the solution and decrease in the optical density during 1 min was measured on a spectrophotometer at 240 nm. The activity of CAT was calculated by adopting a molar extinction coefficient ($\varepsilon = 39.4$ mM^{-1} cm^{-1}) in mM g^{-1} min^{-1} [16].

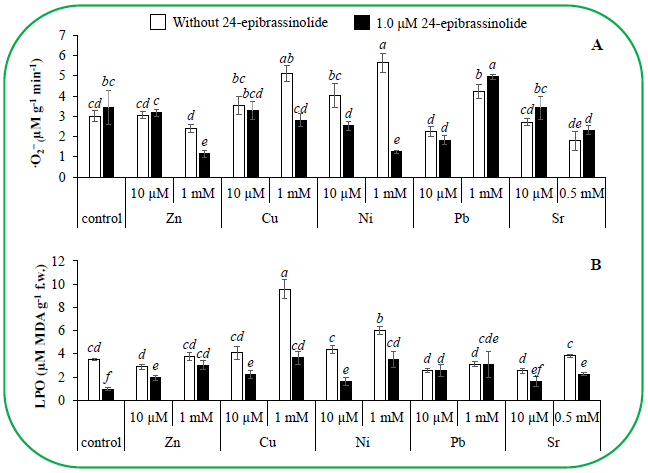
Statistical Analysis: All experiments were conducted in triplicate, and each experiment consisted of 150 seeds or seedlings. For all measurements averages and standard errors were calculated in *Microsoft Excel 2007*. Differences between means were assessed by the *Tukey's* test at $P = 0.05$.

Results and Discussion

Divalent Metals-Phytotoxicity

Being sessile by nature, plants have a greater chance to interact and get impacted with their immediate environment potentially contaminated with various metals such Cu, Ni, Pb, Sr, Zn [1-3, 6]. As a marker of phytotoxicity, we studied herein metal-impact on growth traits (such as root and shoot length), and oxidative stress, and its consequence and metabolism (in terms of $\bullet O_2^-$, LPO and CAT activity). In particular, 1.0 mM Cu^{2+}, Ni^{2+} and Pb^{2+} stimulated $\bullet O_2^-$-production over the control; whereas, Zn^{2+} and Sr^{2+} ions in high concentrations inhibited the generation of $\bullet O_2^-$. The rate of $\bullet O_2^-$ generation significantly increased with Cu^{2+}, Ni^{2+} or Pb^{2+} concentrations in the growth medium, where the highest $\bullet O_2^-$ level was observed with 1.0 mM Cu^{2+}, Ni^{2+} and Pb^{2+}. Notably, inhibition in the $\bullet O_2^-$ level was perceptible with higher concentrations of Zn^{2+} (1.0 mM) and Sr^{2+} (0.5 mM) (Figure 1a). The status-analysis of ROS-consequence such as membrane LPO unveiled increasing LPO with increasing concentrations of plant-beneficial divalent metals such Cu^{2+}, Ni^{2+} and Zn^{2+}; whereas Pb^{2+} and Sr^{2+} caused the maximum LPO at 1.0 and 0.5 mM respectively (Figure 1b). Earlier, the level of cellular $\bullet O_2^-$ and its consequences (such as LPO) were considered as major biomarkers of oxidative stress in metal-exposed plants [1, 6, 7, 17]. Elevation in cellular $\bullet O_2^-$ with Pb^{2+} and Sr^{2+} is obvious since these metals have no physiological function in plants. However, as evidenced herein in terms of elevated cellular $\bullet O_2^-$, deficiency or excess of plant-beneficial divalent metals such as Zn^{2+} and Cu^{2+} and Ni^{2+} can exhibit phyto toxicity [1].

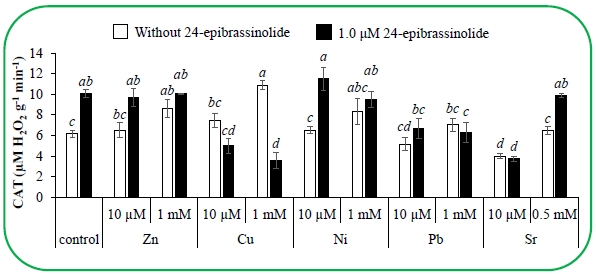
Figure 1: Effect of seed-pretreatment with 24-epibrassinolide (1.0 µM) on $\bullet O_2^-$-generation (**A**) and lipid peroxidation (LPO) (**B**) in *Triticum aestivum* seedlings exposed to various concentrations of divalent metals. The different letters next to the number indicate significant differences among treatments at $P = 0.05$ level as determined by the Tukey's test. *Fresh weight, f.w.*



Once the dismutation of the cellular $\bullet O_2^-$ into H_2O_2 is performed by SOD, the cellular pool of H_2O_2 is kept under control by CAT that does not require any reducing equivalent for metabolizing H_2O_2 into water and oxygen [7, 18]. In the present study, CAT activity was significantly higher than the control in leaves of *T. aestivum* exposed to 1.0 mM plant-beneficial divalent metals (Cu^{2+}, Ni^{2+} and Zn^{2+}), and 1.0 and 0.5 mM of phytotoxic divalent metals Pb^{2+} and Sr^{2+} respectively (Figure 2). The elevation in the CAT, as reported herein can be obvious because plants tend to metabolize ROS (such as $\bullet O_2^-$ into H_2O_2) in order to avert their potential consequences in cells [7, 5]. However, CAT response to tested divalent metals can be implied differently when CAT response is cross-talked with the level and

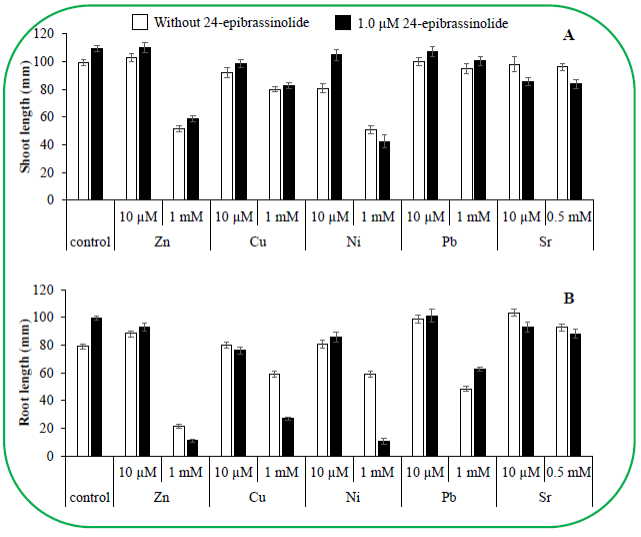
consequence of cellular $\bullet O_2^-$. Notably, the absence of a fine coordination was perceptible between $\bullet O_2^-$-dismutation by SOD (data not shown) and the metabolism of $\bullet O_2^-$-dismutation product (i.e. H_2O_2) by CAT. However, the response of CAT was very well-coordinated with the status of LPO where elevation in CAT significantly kept LPO under control perhaps by efficient H_2O_2-metabolism (Figures.1a-b and 2).

Figure 2: Effect of seed-pretreatment with 24-epibrassinolide (1.0 µM) on Catalase (CAT) activity in *Triticum aestivum* seedlings exposed to various concentrations of divalent metals. The different letters next to the number indicate significant differences among treatments at *P = 0.05* level as determined by the Tukey's test.



Plant growth is known as the outcome of the coordination of major physiological/biochemical processes in plants that in turn can be influenced by plant's immediate environmental conditions [19]. Nevertheless, because changes in plant growth traits can be easily visible and analyzed in metal-exposed in plants, the evaluation of plant growth traits has been used as an easily measurable parameter for monitoring the effects of various stressors including metals [20]. Considering these points, the response of major growth traits (such as root and shoot length, and axial growth) was studied in the current investigation. Herein, root and shoot differently responded to the considered herein divalent metals. In particular, a strong inhibition in growth of shoots was observed at 1.0 mM of essential metals Ni^{2+}, Cu^{2+} and Zn^{2+} (Figure 3a). At the same time, there was a tendency to stimulate of shoots elongation by 10 µM of Zn^{2+} and Pb^{2+}. On the other hand, all the divalent metals studied stimulated the growth of roots at 10 µM as well as Sr^{2+} at 0.5 mM; whereas, there was a strong inhibition in the root growth at 1.0 mM of essential metals and Pb^{2+} (both 10 µM and 1.0 mM) (Figure 3b). The inhibited growth of shoot with 1.0 mM Pb^{2+} and that of root with both 10 µM and 1.0 mM Pb^{2+} is possible because of known phytotoxicity of Pb^{2+} [2] (Pinho and Ladeiro 2012). Apart from that, our observation on the 10 µM of Zn^{2+}-mediated stimulation in shoot growth and the strongly inhibited shoot growth (with 1.0 mM) and root growth (with both 10 µM and 1.0 mM) of essential metals Ni^{2+}, Cu^{2+} and Zn^{2+} can be obvious since both deficiency and excess concentrations of the previous plant-beneficial metals (such Ni^{2+}, Cu^{2+} and Zn^{2+}) were extensively reported to impair plant growth and development [1]. Additionally, the root response to the studied divalent metals confirmed the earlier reports where compared to shoots, a high tolerance of roots to metals was evidenced [1, 2, 20]. However, the stimulation of the growth of shoot (with 10 µM of Pb^{2+}; 10 µM and 0.5 mM of Sr^{2+}) and that of root (with 0.5 mM Sr^{2+}) is debatable. Increased *Allium cepa* dry weight by low doses of Pb^{2+} (1.0 and 3.0 mg dm^{-3}) was argued as a result of increased synthesis of cell wall polysaccharides [21]. In another report, 10 µM Pb^{2+} was evidenced to increase chlorophyll content either in the PS II core or LHC II in cucumber and poplar plants [22]. On the hand, radicle growth-stimulating tendency of 0.1 mM Sr^{2+} has also been evidenced without convincing mechanisms in maize (*Zea mays*) [23].

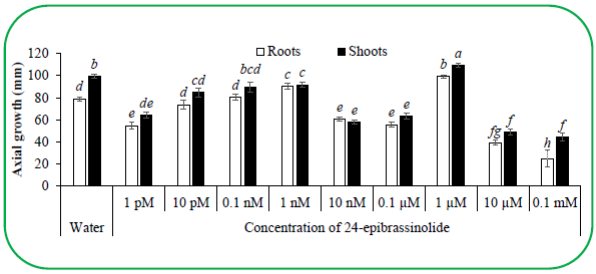
Figure 3: Effect of seed-pretreatment with 24-epibrassinolide (1.0 µM) on the length of shoot (**A**) and root (**B**) in *Triticum aestivum* seedlings exposed to various concentrations of divalent metals.



Divalent metal-phytotoxicity-mitigation potential of 24-EpiBL

Reports on the metal-phytotoxicity are extensive; however, literature is meager on the role of sustainable stress-mitigation approaches in this context. Considering the highlighted lacunae, effort was made herein to assess the significance of exogenously sourced 24-EpiBL in divalent metals-exposed *T. aestivum*. Because the efficiency of 24-EpiBL for the amelioration of metal/metalloid-impact largely depend on the plant and metal/metalloid types, and most importantly on the concentration of 24-EpiBL [10-12], we performed a dosimetric study on *T. aestivum* seedlings to determine the concentration of 24-EpiBL optimal for maintaining optimal growth of *T. aestivum*. Notably, maximum elongation in the length of both roots and shoots was found at 1.0 µM 24-EpiBL (Figure 4).

Figure 4: Concentration effects of 24-epibrassinolide on the axial growth of 7 day old *Triticum aestivum* seedlings. The different letters next to the number indicate significant differences among treatments at $P = 0.05$ level as determined by the Tukey's test.



The metal-impact-ameliorative role of 1.0 µM 24-EpiBL in the control of oxidative stress and its metabolism (in terms of $\bullet O_2^-$, LPO and CAT activity), and eventually the *T. aestivum* growth depended on the level and type of the tested divalent metals (Figs. 1a-b and 2). Seed-pretreatment with 24-EpiBL significantly reduced the level of $\bullet O_2^-$ in *T. aestivum* seedlings exposed to 10 µM Ni^{2+} and 1.0 mM Cu^{2+}, Ni^{2+} or Zn^{2+} (in the latter two cases it was below the water control). Against 1.0 mM Sr^{2+} or Pb^{2+} seed pretreatment of 24-EpiBL tended to increase the rate of superoxide generation (compare to untreated plants). In variants 24-EpiBL+1.0 mM Pb^{2+}, $\bullet O_2^-$ anion level was significantly higher the water control. Thus, there was metal-specific selectivity in the 24-EpiBL-effects on $\bullet O_2^-$generation rate, especially in relation to essential divalent metals (Zn^{2+}, Ni^{2+} and Cu^{2+}). Moreover, 24-EpiBLbrought a significant reduction in LPO in the leaves (vs. control), when exposed to essential metals (such as Ni^{2+} and Cu^{2+}) but not with 10 µM Zn^{2+}, 10 µM and 1.0 mM Pb^{2+} and 10 and 0.5 mM Sr^{2+} where 24-EpiBL tended to further enhance these metal-accrued $\bullet O_2^-$-generation. The previous response of $\bullet O_2^-$-generation to the tested divalent metals corresponded well with the CAT-mediated ROS-scavenging and subsequent ROS ($\bullet O_2^-$)-consequence such as LPO. The previous results together imply that seed-pre-sowing treatment with 24-EpiBL maintained a fine tuning between $\bullet O_2^-$-dismutation via SOD (data not shown) and the enhanced CAT activity-mediated scavenging of $\bullet O_2^-$-dismutation product (i.e. H_2O_2). Our previous observations are in concurrence with the earlier studies where BRs (including 24-EpiBL) were reported to make plant life easier under metals/metalloid stress conditions via BRs/24-EpiBL-mediated control of antioxidant defense system [12]. Our observation on the studied plant growth traits (such as) also supports the previously discussed 24-EpiBL-mediated control of oxidative stress and its metabolism (in terms of $\bullet O_2^-$, LPO and CAT activity). In particular, pretreatment of seeds with 24-EpiBL significantly enhanced the growth of shoots at 10 µM of essential metals (Zn^{2+}, Cu^{2+} and Ni^{2+}) as well as Pb^{2+}. However, 24-EpiBL failed to improve shoot growth in Sr^{2+} (10 µM and 0.1 mM) or 10 µM Ni^{2+}-exposed *T. aestivum*. Similarly, 24-EpiBL significantly improved root growth under 10 µM concentrations of the studied essential divalent metals (Fig. 3a-b). Earlier, 24-EpiBL has widely been reported to control plant growth and development by activating the cell cycle during seed germination [24], controlling progression of cell cycle [25], inducing exaggerated growth in hydroponically grown plants [26], and also by controlling proliferation of leaf cells [27]. In addition to the potential role of 24-EpiBL-controlled previous processes, 24-EpiBL-mediated elevation in CAT activity and subsequent control of ROS (such as $\bullet O_2^-$) observed herein can be convincing mechanism that eventually resulted into 24-EpiBL-mediated improved growth traits in *T. aestivum* under different divalent metals.

Conclusions

In divalent metals-exposed *T. aestivum* seedlings generated from non-24-EpiBL-imbibed seeds: high concentrations of the tested plant-beneficial metals (Cu^{2+} and Ni^{2+}) and that of known phytotoxic metal Pb^{2+}, and 0.5 mM Sr^{2+} divalent metals differentially impacted growth traits as a result of inefficiency of enhanced CAT activity in the control of oxidative stress (in terms of generation of $\bullet O_2^-$ and its consequence LPO). In divalent metals-exposed *T. aestivum* seedlings generated from 24-EpiBL-imbibed seeds: 24-EpiBL-mediated elevation in CAT activity and subsequent control of ROS (such as $\bullet O_2^-$) can be convincing mechanism that eventually resulted into 24-EpiBL-mediated improved root and shoot growth traits in *T. aestivum* when exposed to low (10 µM) concentration of essential divalent metals (Zn^{2+}, Cu^{2+} and Ni^{2+}) as well as phytotoxic divalent metal (Pb^{2+}).

Acknowledgements

DIB, KAG and ASL thank to the Ministry of Education and Science of Russia for financing their present research through project number 6.783.2014K. Financial support received from the Portuguese Foundation for Science and Technology (FCT) is gratefully acknowledged by NAA (SFRH/BPD/64690/2009; SFRH/BPD/84671/2012), and EP ("COMPETE", Project n.º FCOMP-01-0124-FEDER-02800; FCT PTDC/AGR-PRO/4091/2012).

References

1. Anjum NA, Singh HP, Khan MI, Masood A, Per T, Negi et al. (2015a). Too much is bad - an appraisal of phytotoxicity of elevated plant-beneficial heavy metal ions. Environ Sci Pollut Res. 22:3361-3382.

2. Pinho S, and Ladeiro B. (2012). Phytotoxicity by lead as heavy metal - focus on oxidative stress. J Bot doi:10.1155/2012/369572

3. Moyen C, and Roblin G. (2013). Occurrence of interactions between individual Sr^{2+}-and Ca^{2+}-effects on maize root and shoot growth and Sr^{2+}, Ca^{2+} and Mg^{2+} contents, and membrane potential: Consequences on predicting Sr^{2+}-impact. J Hazard Mater 260:770-779.

4. Anjum NA, Umar S, and Chan MT. (2010). Ascorbate-Glutathione Pathway and Stress Tolerance in Plants. Dordrecht: Springer.

5. Anjum NA, Umar S, and Ahmad A. (2012a). Oxidative Stress in Plants: Causes, Consequences and Tolerance. New Delhi: IK International Publishing House Pvt. Ltd.

6. Anjum NA, Sofo A, Scopa A, Roychoudhury A, Gill SS, Iqbal M et al. (2015b). Lipids and proteins – major targets of oxidative modifications in abiotic stressed plants. Environ Sci Pollut Res 22:4099-4121.

7. Gill SS, and Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909-930.

8. Anjum NA, and Lopez-Lauri F. (2011). Plant Nutrition and Abiotic Stress Tolerance III. Ikenobe: Global Science Books.

9. Anjum NA, Gill SS, Umar S, Ahmad I, Duarte AC, and Pereira E. (2012b). Improving growth and productivity of oleiferous Brassicas under changing environment: significance of nitrogen and sulphur nutrition, and underlying mechanisms. Sci World J doi:10.1100/2012/657808.

10. Khan NA, Nazar R, Iqbal N, and Anjum NA. (2012). Phytohormones and Abiotic Stress Tolerance in Plants, Springer, Berlin, Heidelberg

11. Asgher M, Khan MIR, Anjum NA, and Khan NA. (2015). Minimizing toxicity of cadmium in plants - role of plant growth regulators. Protoplasma 252:399-413.

12. Vardhini BV, and Anjum NA. (2015). Brassinosteroids make plant life easier under abiotic stresses mainly by modulating major components of antioxidant defense system. Front Environ Sci 2:67. doi:10.3389/fenvs.2014.00067

13. Wilkins DS. (1978). The measurement of tolerance to edaphic factors by means of root growth. New Phytol 80:623-633.

14. Lukatkin AS. (2002). Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants: 2. The activity of antioxidant enzymes during plant chilling. Russ J Plant Physiol 49:782-788.

15. Lukatkin AS, and Golovanova VS. (1988). Lipid peroxidation in cooled leaves of thermophilic plants. Soviet J Plant Physiol 35:773-779 (in Russian).

16. Patterson BD, Payne LA, Chenl LY, and Graham D. (1984). An inhibitor of catalase induced by cold in chilling-sensitive plants. Plant Physiol 76:1014-1018.

17. Garmash EV, and Golovko TK. (2009). Effect of cadmium on growth and respiration of barley plants grown under two temperature regimes. Russ J Plant Physiol 56:343-347.

18. Gill SS, Anjum NA, Gill R, Yadav S, Hasanuzzaman M, Fujita M et al. (2015). Superoxide dismutase – mentor of abiotic stress tolerance in crop plants. Environ Sci Pollut Res 22:10375-10394.

19. Bhattacharya A, and Vijaylaxmi. (2008) Physiology and biochemistry of disease resistance. In: Bose B, Hemantranjan A (ed), Developments in Physiology, Biochemistry and Molecular Biology of Plants, Volume 2, New India Publishing Agency, New Delhi, India, pp 33-93.

20. Shah FUR, Ahmad N, Masood KR, Peralta-Videa JR, and Ahmad FUD. (2010). Heavy metal toxicity in plants. In: Ashraf M, Ozturk M, Ahmad MSA (eds), Plant Adaptation and Phytoremediation, Dordrecht: Springer, pp 71-97.

21. Wierzbicka M. (1998). Lead in the apoplast of Allium cepa L. root tips-ultra structural studies. Plant Sci 133:105-119.

22. Sárvári E, Gáspár L, Fodor F, Cseh E, Kröpfl K, Varga A, and Baron M. (2002). Comparison of the effects of effects of Pb treatment on thylakoid development in poplar and cucumber plants. Acta Biol Szeged 46:163-165.

23. Moyen C, and Roblin G. (2010) Uptake and translocation of strontium in hydroponically grown maize plants, and subsequent effects on tissue ion content, growth and chlorophyll a/b ratio: comparison with Ca effects. Environ Exp Bot 68:247-257.

24. Zadvornova YV, Alekseichuk GN, Laman NA, Khripach VA, and Grut S. (2005). Effect of brassinosteroids on activation of the cell cycle during germination of *Brassica oleracea* L. seeds. Doklady Natsional'noi Akademii Nauk Belarusi 49:70-73.

25. González-Garcia MP, Vilarrasa-Blasi J, Zhiponova M, Divol F, Mora-Garcia S et al. (2011). Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. Development 138:849-859.

26. Arteca JM, and Arteca RN (2001). Brassinosteroid-induced exaggerated growth in hydroponically grown Arabidopsis plants. Physiol Plant 112:104-112.

27. Nakaya M, Tsukaya H, Murakami N, and Kato M. (2002). Brassinosteroids control the proliferation of leaf cells of *Arabidopsis thaliana*. Plant Cell Physiol 43:239-244.