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Effect of 24-epibrassinolide on ROS content, antioxidant system, lipid peroxidation and Ni uptake in *Solanum nigrum* L. under Ni stress

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ABSTRACT

The present work aimed to evaluate the effects of exogenous application of 24-epibrassinolide (24-EBL) in the physiological and biochemical responses of *Solanum nigrum* L. exposed to nickel (Ni). After the seedling stage, 24-EBL treated and untreated plants were grown hydroponically for 28 days in the presence of 100 μM $NiSO_4 \cdot 6H_2O$. The exposure of *S. nigrum* to high levels of Ni resulted in a decrease of biometric parameters in both shoots and roots, with a partial recovery of both fresh weight and length in the 24-EBL pre-treated plants. Higher levels of Ni were found in roots, regardless of the pre-treatment with the brassinolide. Older leaves of Ni-exposed plants exhibited cell death symptoms, manifested in the form of chlorotic and necrotic spots. A decrease in photosynthetic pigments, soluble protein and relative RuBisCO contents were also observed in Ni-treated plants, however Ni-mediated toxicity was partially reverted by 24-EBL pre-treatment. Lipid peroxidation was chosen as a stress biomarker and malondialdehyde (MDA) levels did not change neither in roots nor in shoots. Soluble proline levels increased in response to Ni in both organs, but the pre-treatment with the phytohormone seems to mitigate the differences observed from the control shoots and roots. When ROS accumulation is concerned, generally Ni-exposed plants exhibited decreases in $O_2^{\bullet-}$ and H_2O_2 levels regardless of being or not treated with 24-EBL. The Ni treatment led to a positive response of the plant's enzymatic antioxidant system. In shoots of Ni-stressed plants, an enhanced activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX), accompanied by a decline of catalase (CAT) activity were observed. In roots, increases in SOD and CAT activities were detected in response to Ni, whilst APX was not. 24-EBL pre-treatment caused a decline in APX and CAT activities, while SOD activity was positively affected. Reverse transcriptase-PCR analysis revealed that mRNA transcript levels do not correlate with total enzymatic activity for SOD, CAT and APX, suggesting that these enzymes are regulated posttranscriptionally. Overall, the results suggested that Ni did not induce a severe oxidative stress in *S. nigrum*, yet the exogenous application of the brassinolide enhanced the plant tolerance to Ni.

1. Introduction

Heavy metals (HM) such as cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn), are important environmental pollutants and their presence in the soil is a worldwide problem, since all of them affect soil quality and fertility, both characteristics of fundamental importance for agricultural productivity. Even in trace amounts, with the exception of those naturally used by the plant metabolism and within the concentrations normally found in plant cells, HM can cause serious damages to all organisms as they

Abbreviations: EBL, 24-EBL, 24-epibrassinolide; HM, heavy metals; Cd, cadmium; Ni, nickel; Pb, lead; Zn, zinc; Cu, copper; ROS, reareactive oxygen species; $O_2^{\bullet-}$, superoxide anion; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; AsA, ascorbate; GSH, glutathione; PCs, phytochelatins; BRs, brassinosteroids; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; GR, glutathione reductase; DHAR, dehydroascorbate reductase.

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become part of the food chain (Awasthi and Sinha, 2013; Salt et al., 1995).

Ni is considered a large-scale pollutant due to its elevated concentrations in soils all over the world (Hussain et al., 2013). This metal is released to the soil by natural and anthropogenic inputs. The natural source includes mainly weathering of ultrabasic igneous rocks, while human activities include those from industrial and urban activities (Hussain et al., 2013; Yusuf et al., 2011). On average, the total Ni content in soil varies from 2 to 750 mg kg^{-1}. Critical toxicity levels of Ni for crop species are >10 mg kg^{-1} dry mass (DM) in sensitive species, >50 mg kg^{-1} in moderately tolerant species and >1000 mg kg^{-1} in hyperaccumulator plant species (Hussain et al., 2013; Yusuf et al., 2011). It is well documented that Ni is an essential micronutrient and plants cannot complete their life cycle without adequate levels of this metal (0.01 to 10 μg g^{-1} dry wt.) (Gratão et al., 2008). Ni is a component of nine metalloenzymes and it is associated with nitrogen metabolism due to the presence of two Ni atoms in the active center of urease; it also promotes root nodule growth and hydrogenase activation (Harasim and Filipek, 2015; Khoshgoftarmanesh et al., 2014; Polacco et al., 2013). The fact that several enzyme activities depend on the presence of Ni may explain the benefit effects of this ion on plant growth and development (Dalton et al., 1985; Kazemi, 2012). However, similarly to other elements, excess concentrations of Ni become toxic for most plant species (Dourado et al., 2015; Gratão et al., 2008;). The anatomical symptoms of Ni toxicity in plants include growth inhibition, chlorosis, necrosis and wilting (Gajewska et al., 2009; Yusuf et al., 2011). Ni also causes disturbances in several physiological processes including photosynthesis, respiration, mineral nutrition, transport of assimilates and water relations (Gajewska et al., 2009; Hussain et al., 2013).

Several studies indicate that the toxicity of Ni is associated with oxidative stress in plants (Dourado et al., 2015; Gajewska et al., 2009; Gomes-Junior et al., 2006; Gratão et al., 2005; Hussain et al., 2013; Yusuf et al., 2011), although reactive oxygen species (ROS) may not be directly generated by Ni because it is not a redox-active metal. Despite this fact, research focusing on the study of antioxidant system in response to Ni stress, revealed that Ni can interfere with the antioxidant system responses. Therefore, high levels of reactive oxygen species (ROS) triggers lipid peroxidation, oxidation of proteins, degradation of chlorophyll pigments and DNA damage (Ahmad et al., 2010; Gajewska and Slodowska, 2008; Gill and Tuteja, 2010). ROS, such as the superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), are natural products of plant metabolism as by-products of numerous metabolic processes; however, under conditions of abiotic and biotic stresses their generation may be greatly increased (Ahmad et al., 2010; Gill and Tuteja, 2010).

To cope with oxidative stress plants developed a complex antioxidant defense system consisting of both antioxidant enzymes and non-enzymatic antioxidants (Gill and Tuteja, 2010; Gratão et al., 2005). Increased stress tolerance in metal exposed plants is often associated with a higher level of antioxidants, particularly the enhancement of antioxidant enzyme activity (Fidalgo et al., 2011, 2013; Gomes-Junior et al., 2006; Gratão et al., 2008). Among key antioxidant enzymes, superoxide dismutase (SOD, EC 1.15.1.1) catalyzes the dismutation of $O_2^{\bullet -}$ to H_2O_2 and O_2, and constitutes the first line of antioxidant defense; afterwards H_2O_2 may be scavenged by catalase (CAT, EC 1.11.1.6) or peroxidases such as ascorbate peroxidase (APX, EC 1.11.1.11) (Gajewska and Slodowska, 2008). Non-enzymatic antioxidants include a range of compounds such as ascorbate (AsA), glutathione (GSH), carotenoids, phenolic compounds and several nitrogenous metabolites such as amino acids, especially proline, which possesses a powerful antioxidant activity required to redress the deleterious effects of ROS (Gill and Tuteja, 2010; Hayat et al., 2012). Although the induction of the antioxidant system by HM is well established, mechanisms of Ni-induced oxidative stress and antioxidant response are not clear and need further investigation.

Brassinosteroids (BRs) are a class of steroidal hormones, which play a critical role in plant growth and development, and recent research revealed that BRs confer tolerance to a broad range of abiotic stresses (Bajguz and Hayat, 2009; Fariduddin et al., 2014; Vásquez et al., 2013), although the mechanisms underlying this tolerance have not yet been elucidated or completely understood. Several studies reported a more pronounced effect of this phytohormone when applied in plants under adverse conditions (Bajguz and Hayat, 2009; Schnabl et al., 2001). In fact, data available show no significant effect of applied BRs on crops grown under optimal conditions, but a crop subjected to stress exhibited remarkable effects of BRs application (Taiz and Zeiger, 2010). For instance, Yuan et al. (2012) observed a more prominent effect of 24-epibrassinolide (24-EBL) in plants grown under salt stress than those under normal situation. Thus, it appears that the protective ability of BRs is highly dependent upon the environmental conditions (Schnabl et al., 2001). BRs can reduce the metal uptake by roots since they are able to regulate the uptake of ions by the radicular system (Fariduddin et al., 2014). It has also been demonstrated that BRs can stimulate the synthesis of phytochelatins (PCs), small peptides that act as metal ligands enzymes as well as non-enzymatic antioxidants involved in the detoxification of ROS generated by HM oxidative stress (Bajguz and Hayat, 2009).

To minimize the impact of pollution caused by Ni several remediation strategies have been proposed, but to the date very few reports were published regarding the phytoremediation of this metal (Karn et al., 2009; Saleem et al., 2011). *S. nigrum* L. is a pioneer species with cosmopolitan distribution that exhibits a natural ability to uptake, tolerate and accumulate high amounts of diverse HM such as Pb, Zn, Cu, Cd in shoots without exhibiting visible toxicity symptoms (Lei et al., 2011). In addition, this plant species is fast growing, easily adaptable and has a large shoot biomass, which makes it a perfect candidate for phytoremediation purposes (Wei et al., 2004).

Bearing in mind the ability of *S. nigrum* to tolerate high concentrations of several HM, and taking into account that the BRs are involved in protecting plants from a wide spectrum of stresses and the important role of the antioxidant systems in plant defense, the aim of the present work was to screen a variety of biochemical and physiological parameters related with antioxidant systems in *S. nigrum* exposed to a high concentration of Ni (100 μM) and to assess whether the plant pre-treatment with 24-epibrassinolide (1 μM) affected the antioxidant system response to Ni and whether it also influenced the tolerance of *S. nigrum* to this HM.

2. Material and methods

2.1. Plant material, growth conditions and treatments

Solanum nigrum L. plants were used as the experimental material. Seeds were collected from plants grown in the location of Foz do Douro, Porto, Portugal (41°09′17,80″N and 8°39′47,91″O) and were surface-sterilized with 70% (v/v) ethanol (2 min) and 20% (v/v) commercial bleach (2 min), followed by repeated washing with sterilized deionised water. These seeds were placed in sterile Petri dishes with 50% modified Hoagland solution (HS) (Taiz and Zeiger, 2010), and were maintained at 4 °C under dark conditions for 3 days (stratification). After this period, the seeds were allowed to germinate in a plant growth chamber (16 h light/8 h dark at 24 °C) with a photosynthetically active radiation (PAR) of 60 μmol m^{-2} s^{-1}. Twenty-day-old seedlings were selected and transplanted to plastic pots with a mixture of vermiculite and perlite (2:1, v:v) as substrate and were grown hydroponically in HS. At this time,