Effect of 24-Epibrassinolide and 28-Homobrassinolide on Some Biochemical Parameters in Raphanus sativus L. Plants under Chromium Stress

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ABSTRACT

The effects of 24-epibrassinolide (24-EBL) and 28-homobrassinolide (28-HBL) on protein content and the activities of Polyphenol oxidase (PPO; EC 1.10.3.1) and glutathione peroxidase (EC 1.11.1.9) were studied in the 75-days old Raphanus sativus L. cv ‘Pusa Chetaki’ plants grown under chromium (Cr) metal stress. Surface sterilized seeds of R. sativus were pre-treated with different concentrations (0 M, 10⁻³ M, 10⁻¹ M and 10⁻⁷ M) of 24-EBL and 28-HBL for 8 h and grown in the field. The soil was treated with different concentrations of Cr metal. Seventy-five days old plants were harvested for further analysis of biochemical parameters. Cr metal treatment enhanced the protein content and PPO activity in both roots and leaves of radish plants. However, the activity of Glutathione peroxidase (GPOX) declined. The 24-EBL and 28-HBL treatments further increased the protein content and activities of antioxidant enzymes under Cr-stressed plants.

Keywords: brassinosteroids, antioxidants, heavy metal stress, radish, glutathione peroxidase, reactive oxygen species

Abbreviations: BR, brassinosteroid; 24-EBL, 24-epibrassinolide; GPOX, Glutathione peroxidase; 28-HBL, 28-homobrassinolide; PPO, Polyphenol oxidase; ROS, reactive oxygen species

INTRODUCTION

Plants produce numerous steroids and sterols (Geuns 1978; Jones and Roddick 1988; Janezcko and Skoczowski 2005). Brassinosteroids (BRs) are polyhydroxy steroids with significant growth-promoting activity (Bhardwaj et al. 2008; Sharma et al. 2010). In plants, BRs promote cell elongation, division, differentiation, disease resistance, stress tolerance and senescence throughout the plant life cycle (Clouse 2002; Bajguz and Hayat 2008). BRs also provide resistance to plants under biotic and abiotic stresses (Khripach et al. 2000) like high and low temperature (Dhaubhadel et al. 1999), drought (Li and Van Staden 1998), salt (Sasse et al. 1995), infection; pesticides (Sasse 1999) and heavy metals (Sharma et al. 2010) stress. Heavy metal toxicity is one of the major abiotic stresses leading to hazardous health effects in animals and plants (Maksymiec 2007). At higher concentrations, these metals are toxic and severely interfere with physiology and biochemical functions of plants (Parmar and Chandra 2005; Salvatore et al. 2008; Triantaphyllidès and Havaux 2009). These have been demonstrated to induce oxidative stress through formation of reactive oxygen species (ROS).

Chromium (Cr) phytotoxicity results in the inhibition of seed germination, degraded pigment status, nutrient balance and induces oxidative stress in plants (Panda and Choudhury 2005). Also, Cr metal toxicity triggers the formation of ROS and catalyses the Haber-Weiss reaction (Shanker et al. 2005; Halliwell and Gutteridge 2006). Over-production of ROS is highly toxic and can oxidize biological macromolecules such as nucleic acids, proteins and lipids, thereby disturbing the membrane permeability (Stohs and Bagchi 1995; Schutzendubel and Polle 2002) and causes oxidative stress. Antioxidant enzymes play an important role in protective mechanisms against ROS and like many other biochemical systems, their effectiveness varies with the type of plant and metal involved (Ozdemir et al. 2004; Almeida et al. 2005; Hayat et al. 2007). The influence of BRs on the response of antioxidant enzymes of plants under stress conditions have been studied recently (Hayat et al. 2007; Bhardwaj et al. 2008; Sharma et al. 2010).

Raphanus sativus is a widely used plant with culinary and medicinal importance, and has a protective role against environmental mutagens and their eventual use as therapeutics (Ghayura and Ćilani 2007; Alquasoumi et al. 2008). Raphanus plants facing stressful conditions viz. heavy metal stress show reduced growth and development (Sharma et al. 2010). Recently, considerable efforts have been given to find out the possible roles of two most biologically active BRs viz. 24-epibrassinolide (24-EBL) and 28-homobrassinolide (28-HBL) in stress protection mechanisms (Khripach et al. 2000; Krishna 2003; Hayat et al. 2007; Bhardwaj et al. 2008). Keeping this in view, the importance of R. sativus in diverse ways and the role of BRs in stress amelioration, the present investigation was designed to study the effects of 28-HBL and 24-EBL on protein content and specific activity of Polyphenol oxidase (PPO) and Glutathione peroxidase (GPOX) enzyme in roots and leaves of 75-days-old R. sativus plants.

MATERIALS AND METHODS

Field experiment

To study the modulative effects of 28-HBL and 24-EBL on plant responses to oxidative burst produced due to heavy metal toxicity, a seasonal field experiment was carried out from December, 2008 to February, 2009 in the Experimental Fields of the Botanical Garden, Guru Nanak Dev University, Amritsar, India. Certified seeds of R. sativus cv ‘Pusa Chetaki’ were procured from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. The seeds were surface sterilized with 0.4% sodium hypochlorite.
Table 1. Effect of 28-HBL and 24-EBL on protein content (mg/g f.w.) in leaves and roots of *Raphanus sativus* L. plants under chromium metal stress (mean ± S.E.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Protein content in leaves</th>
<th>Protein content in roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (DW)</td>
<td>0.5 mM Cr</td>
</tr>
<tr>
<td>Control (DW)</td>
<td>8.106 ± 0.951</td>
<td>8.958 ± 0.58</td>
</tr>
<tr>
<td>Effect of</td>
<td>10⁻¹¹ M 28-HBL</td>
<td>10.488 ± 1.787</td>
</tr>
<tr>
<td>28-HBL</td>
<td>10⁻¹ M 28-HBL</td>
<td>12.99 ± 1.305</td>
</tr>
<tr>
<td>24-EBL</td>
<td>10⁻¹¹ M 24-EBL</td>
<td>26.68 ± 2.148</td>
</tr>
<tr>
<td>Effect of</td>
<td>10⁻¹¹ M 24-EBL</td>
<td>34.32* (7.130)</td>
</tr>
<tr>
<td>24-EBL</td>
<td>F value (HSD)</td>
<td>4.488 ± 0.489</td>
</tr>
<tr>
<td>24-EBL</td>
<td>F value (HSD)</td>
<td>7.308 ± 0.0529</td>
</tr>
<tr>
<td>24-EBL</td>
<td>F value (HSD)</td>
<td>4.8652* (5.750)</td>
</tr>
</tbody>
</table>

* Indicates statistically significant values at P ≤ 0.05, DW indicates distilled water.

RESULTS

Cr metal stress significantly affected the protein content and activities of GPOX and PPO in both roots and leaves of radish plants. Seed-presoaking treatment with 28-HBL and 24-EBL significantly enhanced the protein content and activities of antioxidant enzymes under Cr-stressed plants (Tables 1-3). Overall, in both roots and leaves, 28-HBL alone was a more effective treatment than 24-EBL alone in comparison to untreated plants.

**Effect on protein content**

Protein content increased significantly with an increase in Cr metal concentration in both roots and leaves of *R. sativus* plants (Table 1). 24-EBL and 28-HBL significantly enhanced the levels of protein under Cr stress. 28-HBL was a more effective treatment than 24-EBL alone, compared to the control.

**1. Protein content in leaves**

Maximum increase in protein content was observed at 1 mM Cr stress in leaves (17.106 mg/g fresh weight (FW)). However, seed-presoaking with 28-HBL at 10⁻⁷ M (15.357 mg/g FW) and 10⁻¹¹ M (18.438 mg/g FW) at 1 mM stress further enhanced the total soluble proteins significantly. In contrast, 10⁻⁷ M of 24-EBL alleviated the protein content and it was found to be maximum (16.98 mg/g FW) at 1 mM Cr. To conclude, 10⁻¹¹ M of HBL and 10⁻¹¹ M of 24-EBL were the most effective concentrations to enhance protein content alone and in combination with Cr stress in leaves of radish plants. Overall, 28-HBL alone increased the protein content approximately 3.5 times more than the control whereas 24-EBL treatment showed a 1.4-fold increase. Hence, 28-HBL was more effective than 24-EBL when compared to the control.

**2. Protein content in roots**

A similar trend was followed in roots: maximum increase in protein levels was observed at 1 mM Cr (6.636 mg/g FW). Protein content decreased significantly to a minimum...
(4.866 mg/g FW) by 0.5 mM Cr treatment and increased by 1 mM Cr treatment showing an overall enhancement in content. 28-HBL treatment at 10⁻⁷ M significantly enhanced the protein content to a maximum in the control (7.086 mg/g FW) while 10⁻¹¹ M 28-HBL was effective in ameliorating the stress caused by Cr (Table 1). The protein content was increased 1.25- and 1.2-fold by 28-HBL or 24-EBL, respectively as compared to the control. Overall, 24-EBL at 10⁻⁷ M improved protein levels in roots of R. sativus plants most under Cr metal stress (Table 1).

Effect on PPO activity

Specific activity of PPO decreased as Cr stress in leaves increased whereas in roots an increasing trend was observed under Cr stress in R. sativus plants (Table 2). Furthermore, seed-presoaking treatments of both BRs effectively modulated the activity of PPO in radish plants under Cr stress. In leaves, 24-EBL was more effective than 28-HBL compared to the control whereas in roots, the opposite trend was observed.

1. Activity of PPO in leaves

The activity of PPO was observed to decrease to lowest decline at 1 mM Cr (0.0214 unit activity/mg protein). Further treatment of 28-HBL at 10⁻⁷ M concentration, significantly increased the specific activity of PPO to the maximum (0.083 unit activity/mg protein) at 0.5 mM Cr. Similarly, 10⁻⁹ M of 24-EBL significantly enhanced the specific activity of PPO to the maximum (0.083 unit activity/mg protein) at 0.5 mM Cr metal stress. 10⁻³ M 28-HBL Overall, 10⁻⁹ M treatment of both 28-HBL and 24-EBL was observed to have maximum significant enhancing effect in specific activity of PPO in radish leaves. Treatment of 24-EBL alone resulted in 3.9-fold (approximately) increase in PPO activity whereas 28-HBL showed a 1.1-fold increase compared to the control.

2. Activity of PPO in roots

At 0.5 mM Cr concentration maximum increase (0.075 unit activity/mg protein) in the specific activity of PPO was observed. A similar increasing trend was followed in roots when 24-EBL and 28-HBL treatments were given to radish plants under Cr stress. It was observed that 10⁻⁷ M 28-HBL and 10⁻⁹ 24-EBL were most effective concentrations in alleviating the PPO activity in R. sativus roots. When effect of both BRs was compared, 28-HBL showed 3-fold increase whereas 24-HBL resulted in 2.2-fold increase as compared to the control.

Effect on GPOX activity

With increase in Cr stress, the specific activity of GPOX was observed to decrease significantly in both the roots and leaves in radish plants. However, both 24-EBL and 28-HBL significantly enhanced the activity of GPOX under Cr stress in both roots and leaves of radish plants (Table 3). When compared together both BRs (24-EBL and 28-HBL alone) were found effective in altering activity of GPOX as compared to the control.

1. Activity of GPOX in leaves

Maximum decline in specific activity of GPOX was observed at 0.5 mM Cr (0.0008 unit activity/mg protein) in leaves. It was observed that 10⁻⁷ M of 28-HBL and 10⁻⁹ M of 24-EBL significantly increased the specific activity of GPOX effectively in leaves. When both the treatments of BRs were compared, there was no significant change (in GPOX activity) caused by both 24-EBL and 28-HBL alone as compared to the control.

2. Activity of GPOX in roots

Similarly, lowest activity of GPOX was noticed at 0.5 mM Cr (0.0024 unit activity/mg protein) in roots. The treatment of 10⁻⁷ M of both 28-HBL and 24-EBL were noticed to be most effective in control whereas 10⁻¹¹ M of both 28-HBL and 24-EBL showed maximum enhancing effect in 0.5 mM Cr and 1 mM Cr. There was no significant increase/decrease in activity of GPOX was observed when treatments of 24-EBL and 28-HBL alone were evaluated as compared to the control.
DISCUSSION

The present investigation revealed that seed-presoaking treatments of both 28-HBL and 24-EBL significantly alleviated the effects of Cr stress in R. sativus plants by enhancing the levels of soluble proteins and the activities of PPO and GPOX enzymes. Cr toxicity is well-documented by Shanker et al. (2005) in terms of reduced growth and development of Cr-stressed plants. Cr-induced ROS causes plant membrane damage, ultra-structural modifications in organelles, impaired metabolic activities (enzyme activities) and growth retardations (Panda 2007). Similarly, in present study, overall decrease in activities of PPO and GPOX were noticed in R. sativus plants under Cr stress as compared to control (Tables 2, 3). To overcome ROS-induced damaging effects, the plant defence system is well-equipped with both enzymatic and non enzymatic mechanisms to scavenge free radicals (Foyer and Noctor 2003). Antioxidant enzymes like PPO and GPOX play defensive role in protecting plant against oxidative stress. They catalyze oxidation of hydroxyphenols to their quinine derivatives which then simultaneously polymerize. GPOX in plants belongs to the phosphorphenols to their quinine derivatives which then simultaneously polymerize. GPOX in plants belongs to the phospholipids hydroperoxide glutathione peroxidase family. The function of glutathione peroxidases, such as fatty acid hydroperoxides is reduction of alkyl hydroperoxides, such as fatty acid hydroperoxides (Foyer et al. 1997).

Cr-phytotoxicity resulted in overall increase in protein content, which was further increased under application of 28-HBL in both roots and leaves of R. sativus L. (Table 1). These findings are in coherence with the previous report of Hayat and Ahmad (2003) in Triticum aestivum where 28-HBL increased the protein content. Similarly, 28-HBL was reported to enhance the protein content in Oriza sativa (Anuradha and Rao 2007; Maheshwari and Dubey 2008) and Vigna radiata L. (Jaleel et al. 2007) under heavy metals stress. In Chlorella vulgaris, BRs increased the contents of DNA, RNA and protein (Bajguz 2000). It suggests the possible role of BRs-mediated regulation of certain genes at transcriptional and translational levels.

Present findings revealed that both 28-HBL and 24-EBL increased the activity of PPO in radish plants. These observations are also consistent with earlier findings of Jaleel et al. (2007) in Arabis hypogaea, Azooz et al. (2009) Vigna radiata and Hornero-Mendez et al. (2002) in G ndarray manzanilla. In the metabolism of polyphenols, the enzyme PPO is involved (Khrichap et al. 2000), therefore any alteration in activity of PPO may be considered as one of the important factor correlated with increased plant protection under stress. In assessment of GPOX, overall decrease in specific activity was observed in stressed material (Table 3). However, seed-presoaking treatments with both BRs resulted in enhanced activity of GPOX enzyme under Cr stress. These results are supported by Dixit et al. (2001) in Pisum sativum L., where GPOX activity decreased in roots and remained unmodified in leaves. Altered activities of these enzymes further suggest that 28-HBL and 24-EBL treated plants were less affected by Cr metal than the control plants (Tables 2, 3). Also, present study reveals that 28-HBL alone is more effective treatment than 24-EBL alone in comparison to untreated plants. The difference in the activities of these two important BRs may be attributed to structural differences from each other (Khrichap et al. 2000). 28-HBL differs from 24-EBL by the substitution at C-24 and its configuration at C-24.

An enhancement in the protein content and in the activity of antioxidant enzyme PPO under metal stress, suggests that higher antioxidant enzyme activity have a role in imparting tolerance against chromium metal stress. Decrease in specific activity of GPOX may be attributed to its ability to scavenge hydroperoxides. It also functions to eliminate lipid hydroperoxides from cellular membranes, where oxidative stress leads to their lipid peroxidation (Vorobets 2006). BRs also regulate the cell wall elongation by enhancing the activities of cell wall loosening enzymes via activation of H -ATPase (Khrichap et al. 2000; Haubrick and Assmann, 2006). Hence, BRs-regulated stress protection is a consequence of multifarious biochemical response regulated by both enzymatic and non-enzymatic antioxidant defence system in plants. Thus, the present findings suggest the possible ameliorative role of BRs (28-HBL and 24-EBL) in modulating the activities of key antioxidant enzymes under Chromium stress.

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