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Effect of seed pre-soaking with 24-epibrassinolide on growth and photosynthetic parameters of *Brassica juncea* L. in imidacloprid soil

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ABSTRACT

Pesticides are widely used to protect crop plants from various insect pests. However, application of pesticides causes phytotoxicity to plants which results in their impaired growth and development. Brassinosteroids are well known to protect plants under abiotic stress conditions. The purpose of the present study was to access the ameliorative role of 24-epibrassinolide (EBR) in *Brassica juncea* L. under imidacloprid (IMI) toxicity. *B. juncea* plants were raised from seeds soaked in 0.1, 1 and 100 nM of EBR, and grown in soils amended with 250, 300 and 350 mg kg^{-1} IMI pesticide, and observed for growth, pigments and photosynthetic parameters after 30, 60 and 90 days of seed sowing. The plants grown in soil treated with IMI exhibited a significant reduction in shoot length, number of leaves, chlorophyll contents and photosynthetic parameters like photosynthetic rate, stomatal conductance, inter-cellular CO_2 and transpiration rate, when compared with their respective controls. However, pigments which act as antioxidants such as carotenoids, anthocyanins and xanthophylls were increased with IMI stress. Pre-sowing seed treatment with EBR decreased the toxic effects of IMI and increased the growth, pigment biosynthesis and photosynthetic parameters of the plants grown in IMI amended soil. Maximum increase in all the growth and photosynthetic parameters was noticed in plants raised from seeds treated with 100 nM EBR and grown in IMI amended soil.

1. Introduction

Brassica juncea L., also known as Indian mustard, is cultivated as a vegetable and oil yielding crop. During its vegetative growth, it is attacked by various pests viz., soil bugs, termites, turf insects, aphids, leaf hoppers, cut worms etc. Because of their less cost, easy to use and enhanced effectiveness, pesticides are generally preferred to control the insect pests (Rabbinge and Oijen, 1997). Imidacloprid (IMI) is one of the most effective insecticides used against these insect pests (Ko et al., 2014). It is mostly applied to rape, maize and sunflower via soil and due to its systemic nature it helps in controlling both of soil insects as well as areal sucking insects hence protecting whole plant without creating aerial pesticide pollution (Heatherington, Bolton, 1992; Dewar et al., 1996; Bonmatin et al., 2005). Recommended field dose of IMI is 20–70 g a.i. ha^{-1} (Sarkar et al., 2001) and its half-life period varies from 40 to 124 days in soils without manure and with manure respectively (Gervais et al., 2010). Moreover, studies carried out by Sharma and Singh (2014) following the IMI application of 80 g a.i. ha^{-1}, the IMI residues (including its metabolites) detected after 30 and 60 days of treatment were 1.60 and 0.90 mg Kg^{-1} soil respectively. Pesticides also cause toxicity to the plants resulting in retarded growth, decreased photosynthetic pigments (Huiyun et al., 2009; Singh et al., 2016) and adversely affect their photosynthetic performance (Kaňa et al., 2004; Xia et al., 2006; Wang et al., 2016).

Brassinosteroids (BRs) are present in plants in small concentrations (Clouse and Sasse, 1998; Kanwar et al., 2015), and are known to regulate the growth and development of plants (Clouse, 2008; Kim et al., 2012; Rajewska et al., 2016). Moreover, BRs also overcome abiotic stresses like low temperature, low light (Khan et al., 2015; Shu et al., 2016), heavy metals (Sharma et al., 2015d; Yusuf et al., 2016), saltinity (Hayat et al., 2010) and pesticides (Sharma et al., 2012, 2013, 2015c) in crop plants. Exogenous application of EBR in cucumber plants under pesticide stress improves the photosynthetic machinery which gets damaged due to pesticide toxicity and reduces pesticide residues (Xia et al., 2006, 2009a, 2009b). In rice, EBR was noticed to have ameliorative role in growth and pigment system, which were negatively affected by the chlorpyrifos (CPF) and imidacloprid (IMI) pesticide toxicity (Sharma et al., 2012, 2013). Studies carried out by Zhou et al. (2015) and Yin et al. (2016) also confirmed the protective role of BRs under pesticide stress resulting in reduction of their residues in various crops. Earlier studies also showed the stress protective

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roles of EBR under IMI toxicity in *B. juncea* by enhancing various bioactive phytochemicals (Sharma et al., 2015a, 2015b), contents of non-enzymatic antioxidants (Sharma et al., 2016c) and enzymatic antioxidants along with reduction in IMI residues (Sharma et al., 2016a, 2016b). Keeping in view the physiological and abiotic stress protective roles of BRs, the present experiment was designed to observe the ameliorative role of 24-epibrassinolide (EBR) on growth, pigment system and photosynthesis of *B. juncea* L. plants grown in soil amended with IMI.

2. Materials and methods

2.1. Raising of plant material

Seeds of *B. juncea* var. RLC-1 were procured from Punjab Agricultural University, Ludhiana, India, and grown in pots containing soil amended with 0, 250, 300 and 350 mg kg^{-1} of IMI. To observe the effect of EBR on growth, pigment biosynthesis and photosynthesis in *B. juncea*, seeds were given pre-sowing treatment with 0, 0.1, 1 and 100 nM of EBR for eight h and the above mentioned parameters were studied on 30, 60 and 90 days after seed sowing.

2.2. Growth parameters

Shoot length and number of leaves were observed 30, 60 and 90 days after seed sowing.

2.3. Photosynthetic pigments

Chlorophyll and carotenoid contents were estimated according to Arnon (1949) and Maclachlan and Zalik (1963) respectively. One g fresh leaves were homogenized in 4 ml of 80% acetone and centrifuged at 1500*g* for 20 min (4 °C). The supernatant was collected for the estimation of chlorophyll and carotenoid contents. For chlorophyll content, absorbance values of the supernatant were taken at 645 and 663 nm. For carotenoid content, absorbance values of supernatant were taken at 480 and 510 nm using UV–visible spectrophotometer.

Anthocyanin content was estimated according to Mancinelli (1984). Fresh leaves (1 g) were homogenized in 3 ml of extraction mixture (2.37 ml methanol, 0.6 ml water, 0.03 ml HCl). The crushed material was then centrifuged at 1500*g* for 20 min (4 °C). Absorbance of the supernatant was taken at 530 nm and 657 nm using UV–visible spectrophotometer.

Xanthophyll content was estimated by the AOAC method described by Lawrence (1990). 50 mg of dried leaf powder was transferred to 100 ml flask. Then 30 ml of extractant (10 ml hexane, 7 ml acetone, 6 ml absolute alcohol, 7 ml toluene) was poured into the flask and shaken well for 10–15 min. After this, 2 ml of 40% methanolic KOH was added and the flask was refluxed in water bath at 56 °C for 20 min followed by incubation in dark for 1 h. 30 ml hexane was added to the flask and was shaken for 1 min 10% sodium sulphate solution was added to make up the volume to 100 ml and the flask was shaken for 1 min. The flask was again kept in dark for 1 h. The upper phase was collected in 50 ml volumetric flask and the volume was made up using hexane, mixed well and absorbance was measured at 474 nm using UV–visible spectrophotometer.

2.4. Gaseous exchange parameters

Photosynthetic rate (Pn), stomatal conductance (Gs), inter-cellular CO_2 (Ci) and transpiration rate (Et) were measured at 30, 60 and 90 days of seed sowing using LI-COR LI-6400XT portable photosynthesis system.

2.5. Statistical analysis of data

Data was statistically analysed using Two-way ANOVA, Tukey's HSD, multiple linear regression (MLR) and β-regression analysis using Microsoft Excel 2010.

3. Results and discussion

3.1. Growth parameters

Seed pre-soaking with EBR significantly increased the shoot length and number of leaves, when compared to plants grown in IMI amended soil (Table 1). MLR also revealed that IMI application resulted in reduced growth of plants, whereas seed pre-soaking with EBR significantly increased the growth of *B. juncea* plants under IMI stress. Concentration of IMI in soil was negatively regressed on shoot length and number of leaves, whereas seed treatment with EBR was regressed positively on shoot length and number of leaves (Table 4). Due to its systemic nature, IMI gets accumulated in plant tissues and causes phytotoxicity. Retarded plant growth may be due to the less availability of iron, magnesium, potassium and sodium under IMI toxicity (Azzam et al., 2011). BRs help in recovering the plant under toxicity due to their ability to modulate the cell division, rate of cell expansion and biosynthesis of cellulose (Gonzalez-Garcia et al., 2011; Hacham et al., 2011; Xie et al., 2011). Enhancement of growth by EBR is also supported by the studies carried out by Zhou et al. (2015); Xia et al. (2009a, 2009b), which explain the activation of pesticide detoxification enzymes by EBR application, resulting in enhanced growth of plants.

3.2. Photosynthetic pigments

A decline in chlorophyll-a, chlorophyll-b and total chlorophyll contents was noticed with increasing concentration of IMI in soil, whereas other pigments increased with the application of IMI. However, EBR seed treatment for sowing in IMI amended soils enhanced the contents of all the pigments when compared with plants grown in soils amended with IMI only. Use of seed soaking in EBR for IMI amended soils, significantly increased the content of photosynthetic pigments, when compared to plants grown in IMI applied soils (Table 2). Moreover, MLR revealed that growth of plants in IMI treated soils resulted in reduction of chlorophyll-a, chlorophyll-b and total chlorophyll contents, whereas all other pigments were increased under IMI treatment. However, the application of EBR along with IMI significantly increased the contents of all the pigments. Soil application of IMI was negatively regressed on chlorophyll-a, chlorophyll-b and total chlorophyll contents, whereas seed treatment with EBR was regressed positively on chlorophyll contents. Plants grown in IMI amended soils with or without EBR seed soaking were positively regressed on carotenoid, anthocyanin and xanthophyll contents (Table 4). Decline in chlorophyll contents could be due to the increased activity of enzyme chlorophyllase along with chloroplast degradation and chlorophyll oxidation caused by reactive oxygen species (Kato and Shimizu, 1985; Parida et al., 2002; Harpaz-Saad et al., 2007). Moreover, studies carried out by Cheng et al. (2012) showed the down-regulation of genes involved in formation of chlorophyll protein complexes and photosynthesis under IMI toxicity. However, chlorophyll contents were found to recover after the application of EBR and it could be due to the role of BRs in up-regulation of transcription and translation processes during the biosynthesis of chlorophyll and reduction of chlorophyll breakdown