Food Chemistry 185 (2015) 441–448

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Low level of selenium increases the efficacy of 24-epibrassinolide through altered physiological and biochemical traits of *Brassica juncea* plants

Fatima Salva Naz, Mohammad Yusuf *, Tanveer A. Khan, Qazi Fariduddin, Aqil Ahmad

Plant Physiology & Biochemistry Section, Department of Botany, Aligarh Muslim University, Aligarh 202002, India

ARTICLE INFO

Article history:
Received 22 December 2014
Received in revised form 14 March 2015
Accepted 7 April 2015
Available online 11 April 2015

Keywords:
Antioxidant system
Brassinosteroid
Photosynthesis
Selenium

ABSTRACT

This study was conducted to provide an insight into the effect of Se (through soil) induced changes in *Brassica juncea* plants in the presence and absence of 24-epibrassinolide (EBL; foliar). The Se treatments showed dual response, 10 μM of Se significantly increased growth, water relations, photosynthetic attributes along with carbonic anhydrase activity whereas its higher concentrations proved inhibitory in concentration dependent manner. The follow-up application of EBL to the Se stressed plants improved growth, water relations, photosynthesis and simultaneously enhanced the various antioxidant enzymes viz. catalase, peroxidase and superoxide dismutase with the excess accumulation of proline. In addition to this, 10 μM Se increases the efficacy of 10^{-8} M of EBL and both in combination showed maximum increase for the growth and photosynthetic traits of plants. On the other hand, the elevated level of antioxidant enzymes as well as proline could have conferred tolerance to the Se-stressed plants resulting in improved growth, water relations and photosynthesis.

1. Introduction

Elements that stimulate growth and may be essential to particular species are defined as beneficial elements. The five most investigated beneficial elements are Al, Co, Na, Se and Si. It is well documented that all of these elements promote growth of various taxa, under certain environmental conditions. However, the function and concentration varies for each element with plant species. Out of these, selenium (Se) is not a very abundant element whose soil levels are typically below 1 ppm (mg/kg soil), but 4–100 ppm can be found in seleniferous soils. Selenium is chemically similar to sulfur (S) and its metabolism follows the same mechanisms and the main bioavailable form of Se in soils is selenate, which can be taken up by plants via sulfate transporters and assimilated into selenocysteine (SeCys) and selenomethionine (SeMet). While Se is generally metabolized by sulfur pathways, there is some evidence that plants have evolved Se-specific enzymes that facilitate Se accumulation, perhaps to serve an ecological or physiological function (Pilon-Smits, Quinn, Tapken, Malagoli, & Schiavon, 2009). Among higher plants, the largest beneficial effects of Se on growth (up to 2.8-fold higher biomass with Se) have been observed in the Se hyperaccumulator plants, and Se has been suggested to be essential for these species (Shrift, 1969). Trace amounts of Se also stimulated growth in a variety of non-hyperaccumulator species including ryegrass, lettuce, potato, and duckweed (Hartikainen, 2005). Recently, it has been shown that selenium can regulate the water status of plants under conditions of water deficiency and thereby performs a protective role (Kuznetsov, Kholodova, Kuznetsov, & Yagodin, 2003). The mechanism of this apparent positive effect of Se on antioxidant capacity may be direct, owing to antioxidant activity of seleno-compound, or indirect, via Se-induced up-regulation of general stress tolerance mechanisms. It was suggested that Se can alleviate oxidative stress in chloroplasts. The responses of potato to Se supplementation were investigated by monitoring chlorophyll fluorescence and the transcription of antioxidative enzymes (Seppanen, Turakainen, & Hartikainen, 2003).

Brassinosteroids (BRs) is a group of naturally occurring plant steroidal compounds that are ubiquitously distributed in plant kingdom. BRs play prominent role in various physiological processes such as cell division, elongation and expansion, vascular differentiation, pollen tube growth, seed germination, proton pump activation, membrane polarization, source/sink relationships, reproductive development, ions uptake into the plant cell, regulation of gene expression, nucleic acid and protein synthesis, enzymes activation and photosynthesis (Sasse, 2003). Moreover,

* Corresponding author. Tel.: +91 9415 840 350.
E-mail addresses: yousuf.alig@hotmail.com (M. Yusuf), qazi_farid@yahoo.com (Q. Fariduddin).

http://dx.doi.org/10.1016/j.foodchem.2015.04.016
0308-8146/

they confer tolerance to plants against various abiotic and biotic stresses, including those caused by salt, chilling, heat, drought, and pathogens (Bajguz & Hayat, 2009). Exogenously applied 24-epibrassinolide (EBL) has the ability to substantially enhance wheat yield and its stress tolerance by inducing cellular changes that are related to stress tolerance, like stimulate nucleic acid and protein synthesis (Dhaubhadel, Browning, Gallie, & Krishna, 2002), activate ATPase pump (Khripach, Zhabinskii, & Khripach, 2003), increase antioxidant enzyme activities and osmoprotectants accumulation (Ozdemir, Bor, Demiral, & Turkan, 2004), induce other hormone responses (Vert, Nemhauser, Geldner, Hong, & Chory, 2005), regulate stress-responsive genes expression (Kagale, Divi, Krochko, Keller, & Krishna, 2007) and induce photosynthetic efficiency and the translocation of photosynthates to the sink (Shahbaz, Ashraf, & Athar, 2008). Great efforts have been made to develop this phytohormone as a plant growth regulator for widespread utilization in agricultural production; however, the mechanisms by which BRs influence plant productivity and stress tolerance are still poorly understood (Bajguz & Hayat, 2009). In addition to this, role of BRs in the presence of beneficial elements need to be explored through various physiological and biochemical approaches.

This study was conducted to investigate the response of 24-epibrassinolide on the selenium induced changes in *Brassica juncea* in terms of physiological and biochemical characterization under different levels of selenium and also explore the possibility to establish the selenium as quasi-essential or essential elements for the growth and productivity of *B. juncea*.

2. Materials and methods

2.1. Plant materials

The seeds of *B. juncea* cv. Krishna Kranti were procured from National Seed Corporation Ltd., New Delhi, India. The healthy looking and uniform size seeds were surface sterilized with 1% sodium hypochlorite solution for 10 min, followed by repeated washing with double distilled water (DDW).

2.2. Hormone preparation

24-Epibrassinolide (EBL) was obtained from Sigma–Aldrich Chemicals Pvt. Ltd. India. A stock solution of EBL (10^{-4} M) was prepared by dissolving required quantity of the EBL in 5 ml of ethanol in a 100 ml volumetric flask and final volume was made up to the mark by using double distilled water (DDW). The desired concentration of EBL i.e. 10^{-8} M was prepared by the dilution of stock solution and the concentration of EBL was based on the study of Hayat, Ahmad, Mobin, Hussain, and Fariduddin (2000). Tween-20 was added as surfactant prior to the foliar application.

2.3. Source of selenium (Se)

Sodium selenate (Na_2SeO_4) was used as the source of Se. A stock solution of Se (1.0 mM) was prepared by dissolving the required quantity of Na_2SeO_4 in 10 ml of DDW in a 100 ml volumetric flask and final volume was made up to the mark by using deionized water. The required concentrations (10, 20, 40, 80 μM) of Se were prepared by the dilution of stock solution.

2.4. Treatment pattern and experimental design

The experiment was conducted under randomized block design with 50 earthen pots (10-in. in diameter), filled with sandy loam soil and farmyard manure (3:1). The surface sterilized seeds were sown in pots and allowed to germinate under natural environmental conditions in the net house of Department of Botany, Aligarh Muslim University, Aligarh, India. Fifty pots were divided into 10 sets of 5 pots each (replicates) representing one treatment. The treatments pattern are as follows:

- Set I: served as control (-EBL and -Se) and foliage at 21 and 22 days stage of growth sprayed with deionized water.
- Set II: foliage at 21 and 22 days stage of growth sprayed with 10^{-8} M of EBL.
- Set III: at 10, 12 and 14 days stage, plants exposed to 10 μM of Se solution through soil.
- Set IV: at 10, 12 and 14 days stage, plants exposed to 20 μM of Se solution through soil.
- Set V: at 10, 12 and 14 days stage, plants exposed to 40 μM of Se solution through soil.
- Set VI: at 10, 12 and 14 days stage, plants exposed to 80 μM of Se solution through soil.
- Set VII: a combination of set II and set III (10^{-8} M EBL + 10 μM Se).
- Set VIII: a combination of set II and set IV (10^{-8} M EBL + 20 μM Se).
- Set IX: a combination of set II and set V (10^{-8} M EBL + 40 μM Se).
- Set X: a combination of set II and set VI (10^{-8} M EBL + 80 μM Se).

The foliage of each plant was sprinkled thrice. The nozzle of the sprayer was adjusted in such a way that it pumped out 1 ml (approx.) in one sprinkle. Therefore, each foliage of plants received 3 ml EBL solution. The plants in all the sets were harvested at 30 days stage of growth to assess various growth and leaf gas exchange traits as well as biochemical parameters. These assays were repeated 5 times with the utilization of 15 plants per treatment (03 plants per pot).

2.5. Morphological traits and leaf water potential

The study of morphological traits i.e. root-shoot length, dry mass of plant, leaf area and leaf water potential were followed as described in our previous study Parashar, Yusuf, Fariduddin, and Ahmad (2014).

2.6. Chlorophyll content (SPAD level)

The SPAD values of chlorophyll in the leaf was measured, under natural conditions by using the SPAD chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan).

2.7. Photosynthetic traits

Photosynthetic traits were determined on the third fully expanded leaves between 11:00 and 12:00 h by using an infrared gas analyzer (IRGA) portable photosynthetic system (LI-COR 6400, LI-COR, and Lincoln, NE, USA). To measure net photosynthetic rate (P_N) and its related attributes [stomatal conductance (gs), internal CO_2 concentration (Ci), water use efficiency (WUE)], the air temperature, relative humidity, CO_2 concentration and PPFD were maintained at 25 °C, 85%, 600 μmol mol^{-1} and 800 μmol mol^{-2} s^{-1}, respectively.

2.8. Determination of carbonic anhydrase activity

The activity of carbonic anhydrase (CA) in the leaves was measured following the method described by Dwivedi and Randhawa (1974). The leaf samples were cut into small pieces in cysteine