South African Journal of Botany 118 (2018) 120–128

Contents lists available at ScienceDirect

South African Journal of Botany

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

24-Epibrassinolide supplemented with silicon enhances the photosynthetic efficiency of *Brassica juncea* under salt stress

H. Siddiqui, M. Yusuf, A. Faraz, M. Faizan, F. Sami, S. Hayat *

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

ARTICLE INFO

Article history:
Received 13 December 2017
Received in revised form 4 May 2018
Accepted 3 July 2018
Available online xxxx

Edited by M Vaculik

Keywords:
Catalase
Chlorophyll
Growth
Peroxidase
Photosynthesis

ABSTRACT

Silicon (Si) and 24-Epibrassinolide (EBL) were exogenously applied to assess the photosynthetic efficiency in *Brassica juncea* under salt stress and to correlate it with the antioxidant system. Seeds of *B. juncea* were sown in pots and supplemented with NaCl at 15-day stage. Si and EBL treatments were given at 20 and 25-day stage, respectively. Net photosynthetic rate and stomatal conductance reduced significantly in the presence of NaCl. The spray of Si and EBL alone or in combination significantly increased the growth and photosynthetic traits in the presence/absence of NaCl stress. The antioxidant activity (catalase, peroxidase and superoxide dismutase) and proline content got enhanced by salt stress which was further enhanced upon follow-up treatment with 24-epibrassinolide and silicon alone or in combination. A combined effect of Si and EBL counters the damaged caused by the salt stress.

1. Introduction

Plants are often exposed to various factors like air, temperature, water and soil salinity and may cause stress in a minutes or days to weeks (Taiz and Zeiger, 2006). Soil salinity is an important restraining factor for the growth and productivity of plants. Salt stress significantly hinders the proper functioning of photosynthetic machinery and alters respiration and protein synthesis which ultimately results in the retarded growth and development in plants (Kawasaki et al., 2001; Meloni et al., 2003; Pal et al., 2004). Excess production of reactive oxygen species (ROS) like hydroxyl radicals ($OH^{\cdot}$), superoxide anion ($O^{\cdot}_2$), and hydrogen peroxide (H_2O_2) occurs in the presence of salt stress (Mittler, 2002; Azevedo et al., 2009).

Mineral elements are taken up by the plants from the soil and atmosphere for the production of organic matter. Thus, for the normal functioning of plant these elements become highly essential. Overall 17 essential elements are required by plants to complete their life cycle, deprivation of even one element results in physiological disorder, e.g. B deficiency in tobacco plants leads to cell death (Koshiba et al., 2009). Elements having potential of stimulating growth are found to be essential for a particular species are regarded as beneficial elements. Al, Co, Na, Se and Si are few beneficial elements which are highly examined (Pilon-Smits et al., 2009). Silicon (Si), a beneficial element comprises of more than 25% of the earth's crust (Sommer et al., 2006). Si is generally available to plants as monosilicicate, $[Si(OH)_4]$, at a particular concentration (0.1–0.6 mM) in the soil water (Epstein and Bloom, 2005). After absorption, Si is deposited as amorphous silica (SiO_2–nH_2O) in the cell walls of the plant and promotes cell wall rigidity and strength (Currie and Perry, 2007). Despite the major component of plants its essentiality has been established only in members of Equisetaceae (horsetail, *Equisetum arvense*) and Poaceae (paddy rice, *Oryza sativa*) (Chen and Lewin, 1969; Richmond and Sussman, 2003). According to the definition of essentiality given by Epstein and Bloom (2005), Si may be regarded as 'quasi essential' element for plants as its deficiency might result in various deformities related to growth, development and reproduction of plants. Si when applied exogenously proves to be beneficial for the growth and yield of several plant species by enhancing leaf exposure to light, resistance to lodging, pathogens and root parasites, and mitigating abiotic stresses (Marschner, 1995; Fauteux et al., 2006; Liang et al., 2007).

Phytohormones improve the ability of plants to adjust the environmental adversities by modulating growth, development, nutrient allocation and source/sink transition. Brassinosteroids (BRs) have emerged as a new class of plant hormones eliciting photosynthesis and other wide range of physiological processes in plants under normal conditions as well as in presence of stress (Bajguz and Hayat, 2009; Siddiqui et al., 2018). Out of a wide range of BR analogues, three natural brassinosteroids, brassinolide, 24-epibrassinolide and 28-homobrassinolide, are identified to have an economic impact on plant growth and productivity and providing stability under field conditions (Khripach et al., 2000). These analogues are involved in various

* Corresponding author.
E-mail address: hayat_68@yahoo.co.in (S. Hayat).

https://doi.org/10.1016/j.sajb.2018.07.009
0254-6299/

physiological processes such as stem elongation, ethylene induction, photosynthetic enzymes and net photosynthetic rate; biosynthesis of nucleic acids and proteins (Hayat and Ahmad, 2003; Khripach et al., 2003; Sasse, 2003; Yu et al., 2004). Apart from these features, BRs also provide tolerance against various abiotic stresses (salts, water, drought, low/high temperature and heavy metals) (Bajguz and Hayat, 2009). The responses triggered by BRs in the presence of silicon in plants exposed to NaCl stress remain unexplored.

Therefore, the present study was conducted to study the role of 24-epibrassinolide (EBL) in the presence of silicon in *Brassica juncea* grown under high level of salt stress and to correlate the relationship between the silicon mediated changes in antioxidant system and degree of tolerance in terms of growth and photosynthetic traits. The hypothesis tested is that EBL and Si will mitigate the adverse effects of salt stress in Indian mustard.

2. Material and methods

2.1. Hormone preparation

A stock solution of EBL of 10^{-4} M was prepared by dissolving the required quantity of hormone in 5 mL of ethanol in a 100 mL volumetric flask and final volume was made up to 100 mL by using double distilled water (DDW). The required concentration of EBL (10^{-8} M) was prepared by the dilution of stock solution. The concentration of EBL was based on the study of Fariduddin et al. (2013).

2.2. Silicon preparation

Sodium metasilicate was used as the source of Si. A stock solution of Si (1.0 M) was prepared by dissolving the required quantity of Na_2SiO_3 in 10 mL of DDW in a 100 mL volumetric flask and final volume was made up to the mark by using DDW. The required concentrations (0.8 mM) of Si were prepared by the dilution of stock solution.

2.3. Biological material

Seeds of *B. juncea* var. T-59 were procured from the National Seed Corporation Ltd., New Delhi, India. Healthy and uniform sized seeds were sown in 24 earthen pots filled with soil and farmyard manure in a ratio of (1:6).

2.4. Experimental design and treatments

The seeds were sown in pots and allowed to germinate under natural environmental conditions (average temperature and humidity recorded were 25 °C and 55%, respectively) in the month of November–December 2014 in the net house of Department of Botany, Aligarh Muslim University, Aligarh, India. NaCl stress (150 mM) was given through soil after 15 days of sowing, foliar spray of Si (0.8 mM) was given at 20 DAS whereas 10^{-8} M of EBL was sprayed at 25 DAS. Si and EBL treatment was given alone as well as in combination to both NaCl stressed and control plants. Control plants were sprayed with DDW. 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 plant received 3 mL DDW/Si or EBL. Each treatment was replicated five times. Sampling of plants to determine various biological and biochemical parameters was done at 45 day stage of growth.

2.5. Biological determinations

2.5.1. Growth characteristics

One plant from each pot (replicate) was taken randomly and washed under running tap water and soaked in blotting sheet. The root and shoot lengths of plants were measured using a meter scale. The samples were weighed to obtain their fresh and dry mass. The rest of the samples were transferred to an oven running at 70 °C for 3–4 days for dehydration and weighed for their dry mass (Varbal 100 super, Varanasi, Balance works, Varanasi, India).

2.5.2. Leaf area of plant

Leaf area of plant was determined by portable leaf area meter (AM 350, ADC Bio Scientific Ltd. Global House, Geddings Road, Hoddesdon, Herts, EN11 0NT, UK) and was expressed in cm^2.

2.5.3. SPAD chlorophyll

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

2.5.4. Photosynthesis and its attributes

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, Lincoln, NE, USA). To measure net photosynthetic rate (P_N) and its related attributes [stomatal conductance (g_s), internal CO_2 concentration (C_i) and water use efficiency (WUE)] 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.

2.6. Biochemical determinations

2.6.1. Leaf catalase (CAT) activity

The activity of catalase was measured by permanganate titration method developed by Chance and Maehly (1955). The reaction mixture was titrated against 0.1 N (N = Normality) potassium permanganate to find the residual H_2O_2 until a purple color persists for at least 15 s. Similarly, a control set was maintained in which the enzyme activity was stopped by the addition of H_2SO_4, prior to the addition of enzyme extract.

2.6.2. Leaf peroxidase (POX) activity

The activity of peroxidase was measured by the method of Chance and Maehly (1955). To 3 mL solution of pyrogallol phosphate buffer, 0.5 mL of 1% H_2O_2 and 0.1 mL of enzyme extract were mixed in a cuvette and a change in absorbance, at 20 s intervals for a period of 3 min was read at 420 nm on a spectrophotometer (Spectronic-20D, Milton Roy, USA). The control set was prepared by using all the above reagents, except the enzyme extract.

2.6.3. Leaf superoxide dismutase (SOD) activity

The activity of SOD was measured by the method of Beauchamp and Fridovich (1971). The reaction mixture in the tubes were placed under 15 W fluorescent lamps for the initiation of reaction. After 10 min, the reaction was stopped by switching off the light. Non-illuminated reaction mixture was used as a blank. The absorbance was measured at 560 nm on a spectrophotometer (Spectronic-20D, Milton Roy, USA) and the SOD was expressed as unit g^{-1} fresh mass. One unit of SOD was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

2.6.4. Proline content

The proline content in fresh leaves was estimated following the procedure used by Bates et al. (1973). 0.5 g of fresh sample was homogenized in a mortar with 3% sulphosalicylic acid. The homogenate was centrifuged at 10,000 × *g* for 10 min in a centrifuge machine. 5 mL of sulphosalicylic acid, 2 mL each of glacial acetic acid and ninhydrin was added to 2 mL of the above supernatant. This mixture was heated in boiling water bath for 1 h and the reaction was terminated by transferring the test tubes to ice bath. 4 mL of toluene was mixed to the reaction mixture with vigorously shaking for 20–30 s. The chromophore (toluene) layer was aspirated and incubated at room temperature. The