Brassinosteroids Root Drenching as an Effective Method of Cadmium Induced Oxidative Stress Amelioration in *Solanum Lycopersicum*

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***Abstract*—**Two varieties of tomato i.e. K-25 and Sarvodya were grown in the soil amended with differential concentration of cadmium (0, 3, 6, 9 or 12 mg kg-1 soil) and were subsequently followed by the hormone treatment through root dipping method, to evaluate the role of BRs analogues in the amelioration of oxidative stress generated by the metal and also in protection of general metabolism of plant in terms of photosynthesis. Out of the four tested concentrations of cadmium, 12 mg kg-1 was found to be most toxic for both the varieties and out of two BR analogues, EBL was found to be most effective in amelioration than HBL. Variety K-25 behaved in a more resistant manner against all contamination levels of cadmium than Sarvodya.

***Keywords*—** Antioxidant system, Brassinosteroids, Cadmium, Photosynthesis, Tomato.

I. Introduction

CADMIUM is one of the most abundant and highly toxic non-essential heavy metal pollutant debilitating three major domains of environment i.e. soil, air and water [1] The contaminant enlists the sources from anthropogenic emissions, phosphate fertilizers, industrial effluents, and agricultural application of sewage sludge. This accelerates the soil Cd contamination from non-contaminated (0-1 mg/kg) to slight contaminated level (1-3 mg/kg) and in highly disturbed ecosystems reaching up to 3-10 mg/kg of soil [2]. Although highly toxic level above this range, has also been reported in the urban outskirts of India and over globe. Of all trace metal elements Cd shows the maximum tendency to accumulate in plants growing in contaminated soil [3]. Besides an array of plant physiological processes, Cd debilitate particularly photosynthesis, inhibiting its efficiency, decreasing indigenous level of pigments per unit area [4], calvin cycle and activity of Rubisco [5]. On the other hand the role of brassinosteroids have been well recognized during last few decades in enhancing photosynthetic efficiency in different crop species [6,7] regulating antioxidant system [8] interacting with multiple hormones [9]. This pot experimental set up is a practical approach to assess the amendment of soil Cd stress (in nursery plants as tomato) through root drenching method of BR application (HBL and EBL).

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II. Material And Methods

A. Plant Material

The seeds of Solanum lycopersicon var. K-25 and Sarvodya were purchased from National Seed Corporation Ltd, New Delhi. Healthy seeds were surface sterilized with 0.01% aqueous solution of mercuric chloride followed by repeated washings with DDW and were sown in earthen pots containing 0, 3, 6, 9 or 12 mg Cd kg-1 soil. These earthen pots were filled with sandy loam soil and farm yard manure mixed in the ratio of 9:1 to create the nursery. At 20 day stage, the seedling were percolated with the aqueous solution of HBL (10-8M) or EBL (10-8M) through their roots for 15 min at the rate of 20 cm3 per seedlings, the treated seedlings were subsequently transplanted to the maintained pots, under similar conditions as in case of nursery pots. The plants were grown in a net house under natural environmental conditions. The average temperature, humidity and day/night photoperiods were 25±2oC, 65±5% and 14/10 h respectively. The plants were sampled at 60 DAS to assess the various photosynthetic attributes and antioxidant enzymatic activity

B. Chlorophyll and photosynthesis measurements

The SPAD chlorophyll in the fresh leaf samples was measured by using chlorophyll meter (SPAD-502; Konica Minolta Sensing Inc, Japan) whereas the transpiration rate (E), internal CO2 concentration (Ci), stomatal conductance (gs) and net photosynthetic rate (PN) were measured by portable photosynthesis system (LI-COR 6400, Lincoln, NE, USA).

The activity of nitrate reductase (NR) activity was measured by [10] and that of carbonic anhydrase (CA) by [11].

C. Antioxidant Enzymes and Proline Content

Peroxidase (POX) and catalase (CAT) were assayed following the procedure of [12]. The activity of superoxide dismutase (SOD) was assayed by using the method of [13]. The leaf proline content was determined by adopting the method of [14].

III. DISCUSSION

The plants exposed to cadmium stress exhibit a slowdown of the net photosynthetic rate that was accomplished by a significant decrease in stomatal conductance, intercellular CO2 concentration, water use efficiency and transpiration rate (Fig.1) As mentioned earlier Cd brings about the closure of stomata by decreasing the partial pressure of CO2 in the stroma [15]. Low CO2, decrease in photosynthetic pigment (SPAD chlorophyll value) and activity of CA (Fig. 2b) are the additional reasons to cause lower PN rate which becomes the direct cause of observed loss of gs, Ci, and E (Fig 1). The other possible reason for this decrease in the net photosynthetic rate under the influence of cadmium may be its direct impact on the metabolic reactions as the metal has a capacity to bind with specific proteins, to inactivate them [16]. Moreover, cadmium has a damaging effect on the activity of Rubisco [17] which will naturally have a negative impact on CO2 reduction. The observed decrease in NR activity is an impact of the metal on the activity of plasma membrane proton pump [18] and the fluidity of the membrane [19], restricting the uptake of nitrate, the inducer and substrate of NR [20,21]. However, the application of BRs improved all the photosynthetic attributes as well as the activity of both the enzymes (Figure 1 and 2 A, B). BRs are also known to have a positive impact on Rubisco activity [22] a key enzyme of photosynthesis and related process. The increased in the activity of NR could be an expression of the impact of BRs on translation and/or transcription [23]. The other possible reason may be the involvement of BRs in increasing the concentration of NO3 by acting at the level of the membrane [24]. The other crops have also been reported to possess higher rate of photosynthesis and related attributes in response to BRs under differential abiotic stresses [6,25]. The reasons assigned above may be the potential contributions for improving photosynthetic activity in the plants supplemented with BRs. In a natural course, the plants exposed to stress generate a large quantity of reactive oxygen species (ROS) [26] that may oxidize proteins, lipids and nucleic acids, resulting in the abnormalities at the level of the cell [27]. However, the plants are capable to counter successfully such stress conditions by inducing the synthesis of antioxidant metabolites (ascorbate, glutathione, tocopherol and proline) and enzymes (superoxide dismutase, catalase, peroxidase and glutathione reductase) that provide additional power of resistance to neutralize the toxic effects of the stress generated through ROS [26] such as superoxide radical, hydroxyl ions and H2O2. Moreover, the level of these enzymes in cadmium stressed plants increased further by a follow-up treatment with BRs (Figure 2).

The elevation in the activities of antioxidant enzymes by BRs is a gene regulated phenomenon. Cao et al. 2005[8] demonstrated on the basis of molecular, physiological and genetic approaches that this increase in antioxidant enzymes was the consequence of enhanced expression of DET-2 genes that improve the resistance capabilities to oxidative stress in Arabidopsis. Similarly BRs modified antioxidant enzyme activities in Brassica jucnea under salinity [28], Vigna radiata and Cicer arietinum under heavy metal stress [6,28] had also been reported earlier.

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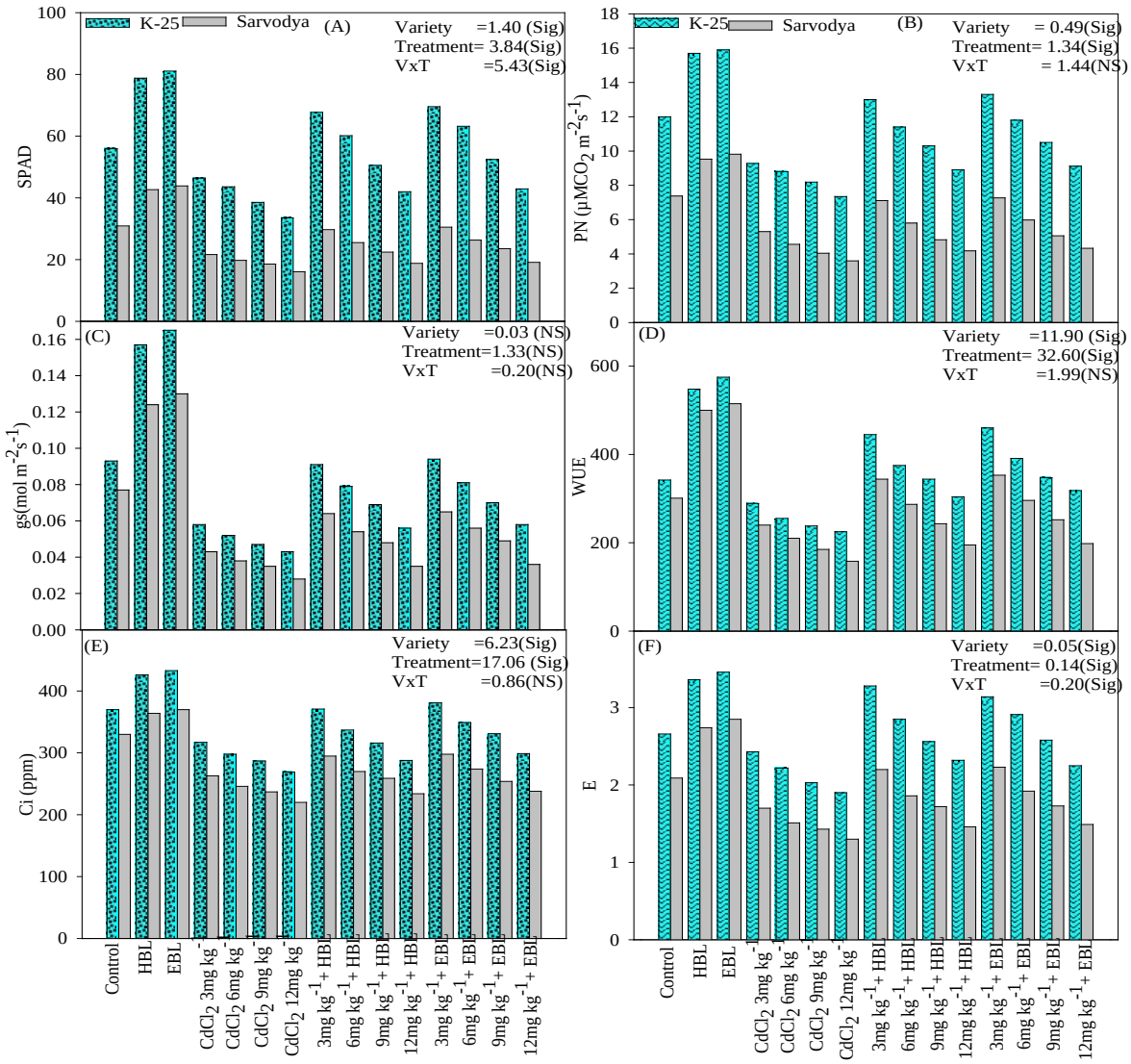

Fig. 1 Effect of dipping of root in brassinosteroids [28-homobrassinolide (HBl:10^{-8}M) and 24 epibrassinolide (EBL: 10^{-8}M)] on the cadmium induced changes in SPAD Chlorophyll content, Net photosynthetic rate (PN), Stomatal conductance (gs), Water use efficiency (WUE), Internal CO_2 concentration (CI) and Transpiration rate (E) in two different varieties of tomato.

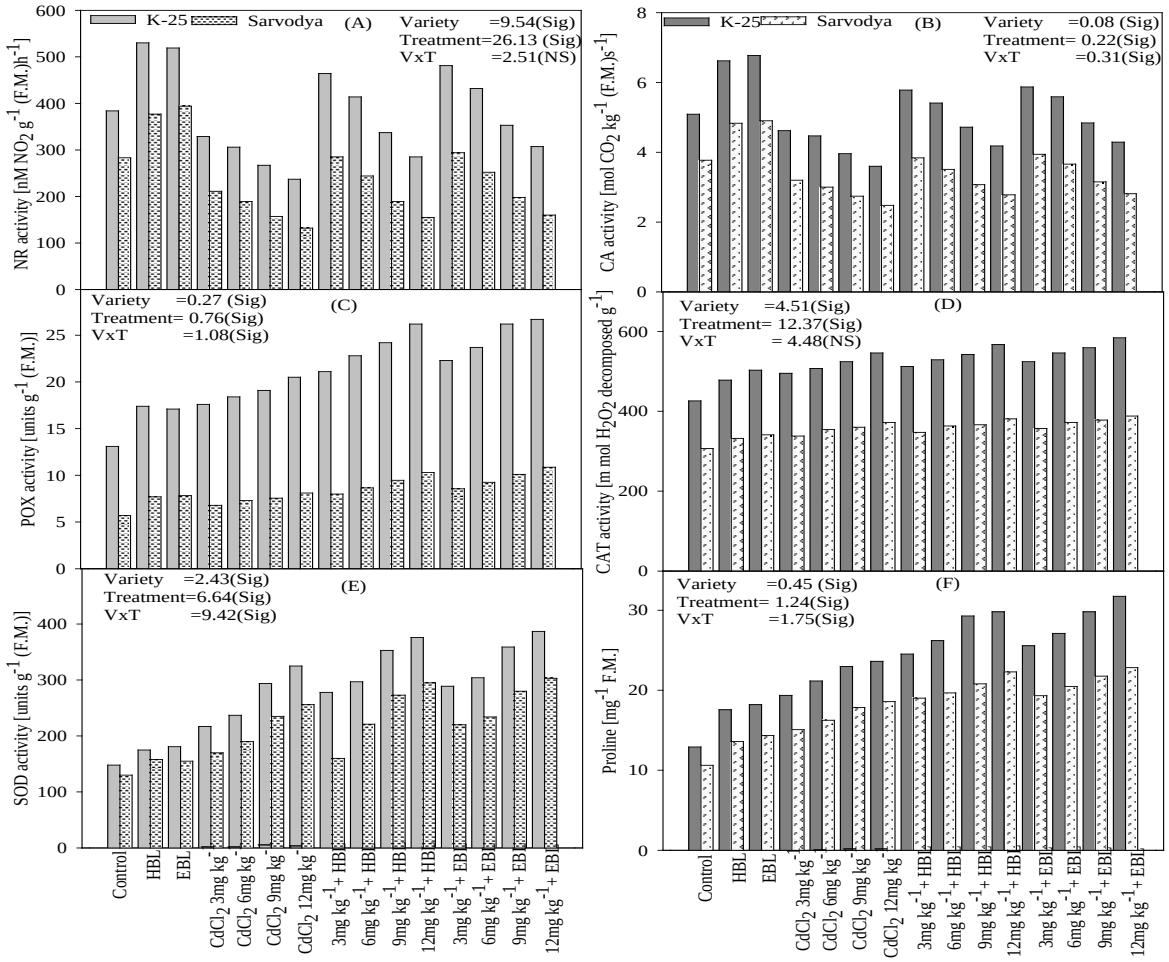

Fig. 2 Effect of dipping of root in brassinosteroids [28-homobrassinolide (HBl:10^{-8}M) and 24 epibrassinolide (EBL: 10^{-8}M)] on the cadmium induced changes in Nitratr reductase (NR) activity, Carbonic Anhydrase (CA) activity, Peroxidase (POX) activity, Catalase (CAT) activity Superoxidase (SOD) activity and Proline content in two different varieties of tomato