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The defensive role of silicon in wheat against stress conditions induced by drought, salinity or cadmium

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ABSTRACT

In the crust of earth, silicon (Si) is one of the two major elements. For plant growth and development, importance of Si remains controversial due to the widely differences in ability of plants to take up this element. In this paper, pot experiments were done to study Si roles in improving salt, drought or cadmium (Cd) stress tolerance in wheat. Up to full emergence, all pots were watered at 100% field capacity (FC) every other day with nutrient solution without any treatments. Fifteen days after sowing, pots were divided into four plots, each with 40 pots for no stress (control) and three stress treatments; drought (50% FC), salinity (200 mM NaCl) and cadmium (2 mM Cd). For all plots, Si was applied at four levels (0, 2, 4 and 6 mM). Under no stress condition, Si applications increased Si content and improved growth as a result of reduced electrolyte leakage (EL), malondialdehyde (MDA) and Na^+ contents. Under stress conditions, Si supplementation conferred higher growth, gas exchange, tissue water and membranes stabilities, and K^+ content, and had limited MDA and Na^+ contents and EL compared to those obtained without Si. Compared to those without Si, enzyme (e.g., superoxide dismutase, catalase and peroxidase) activity was improved by Si applications, which were linked with elevated antioxidants and osmoprotectants (e.g., free proline, soluble sugars, ascorbic acid and glutathione) contents, might providing antioxidant defense against abiotic stress in wheat. The level of 4 mM Si was most effective for mitigating the salt and drought stress conditions, while 6 mM Si level was most influentially for alleviating the Cd stress condition. These results suggest that Si is beneficial in remarkably affecting physiological phenomena and improving wheat growth under abiotic stress.

1. Introduction

As major abiotic stresses globally, salinity and drought cause restrictions of crop productivity due to their adverse influences on plant morphology and physio-biochemistry, reflecting on growth and development (Anjum et al., 2011; Kusvuran et al., 2016; Rady and Hemida, 2016). Another abiotic stress factor that has received a massive attention is the metal toxicity. Large metal amounts such as lead, cadmium, arsenic and mercury are released into the environment and constitute dangerous threats to living organisms including crops and human (Dinakar et al., 2008; Kusvuran et al., 2016). Cadmium (Cd) is a toxic heavy metal, a non-redox metal helpless to take part in Fenton type reactions. In normal, Cd naturally exists in low concentration; however its amounts can be considerably increased by anthropogenic activities. It causes an imbalance in status of cellular redox due to displacing the essential metals and/or cofactors at enzyme active site. Uptake of Cd drastically affects living cells by stimulating oxidative stress; therefore cell death may occur depending on the dose of metal and time-length of exposure (Yadav, 2010; Yan et al., 2015; Vaculík et al., 2015). Cadmium affects environment and plant yield, where exposing plants to Cd stress in their growth environments causes undesired changes in several physiological, biochemical and structural attributes (Yan et al., 2015; Rady and Hemida, 2015).

Silicon (Si) is a second major element in the Earth's crust, and is considered a non-essential (or quasi-essential) for growth and development of plant (Luyckx et al., 2017). According to soil type, Si amount varies considerably from 1% to 45% (Sommer et al., 2006; Parveen and Ashraf, 2010). Silicon is helpful for growth of plant due to its pivotal physico-mechanical role in most plants. In spite of Si deposition on cell walls, it activates a plenty of physiological and metabolic processes (Marschner, 1995; Moussa, 2006; Parveen and Ashraf, 2010). Silicon, in the growth medium, alleviates the effects of various stresses including

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drought, salinity and Cd toxicity (Hajiboland et al., 2016). It has been reported several beneficial effects of Si such as it improves photosynthetic activity, reduces mineral toxicity, modifies nutrient imbalance and enhances abiotic stress tolerance (Ma, 2004).

Silicon enhances water contents in drought-stressed plants, stimulating the formation of double layer of silica cuticle under leaf epidermis that decreases loss of water (Gong et al., 2003; Luyckx et al., 2017). Because of Si deposition, it modifies cell wall properties and decreases stomatal conductance in connection with turgor loss of guard cells (Luyckx et al., 2017). Under salt stress, Si reduces Na^+ uptake, increasing K^+: Na^+ ratio to alleviate ion toxicity effect in plant (Hajiboland et al., 2016). In addition, it participates in overcoming heavy metal toxicity in plants by different mechanisms. Decrease of Cd uptake into plant roots and reduction of its translocation from root to shoots is a mechanism of Cd detoxification. This leads to alleviating the adverse influences of Cd on photosynthetic machinery (Kabir et al., 2016).

Exposing to different abiotic stresses and may be to multifaceted stresses occurs to plants during their life cycle, causing excessive production of reactive oxygen species (ROS). Singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) are of ROS generated in plant cells (Kim et al., 2017). Within a crop, plant species and/or cultivars differ greatly in their response to environmental stress according to their contents of antioxidants. High contents of either constitutive or induced antioxidants enable plants to be greater resistance to oxidative damage (Kusvuran et al., 2016). According to Kim et al. (2017), Si can regulate ROS overproduction in abiotic stressed-crops. They have elucidated this result that plants with Si shows resistance to abiotic stress because Si contributes to decrease of ROS production by improving enzymes activities; particularly catalase and ascorbate peroxidase that are involved in H_2O_2 conversion into H_2O, and/or by reducing the activity of MDA.

Wheat is a moderately salt resistant crop and Si-accumulating species (Broadley et al., 2011; Hajiboland et al., 2016). Many reports demonstrated that Si decreases wheat leaf and root concentrations of Na^+, leading to enhanced wheat salt resistance (Tahir et al., 2006; Tuna et al., 2008; Ali et al., 2012; Hajiboland et al., 2016). Also, Broadley et al. (2011) indicated that wheat has a high tendency to uptake and accumulation of Si.

To our knowledge, there are no reports done to determine the optimum level of Si to use for plants (under soil pH of 6.0 – 6.2), particularly wheat under each of three stresses; drought, salinity and Cd, besides the normal conditions. Therefore, the main purpose of this research was to determine the defensive effects of Si application and the optimum level of Si for plant growth, leaf photosynthetic gas exchange, tissue health (uptake of water and ions, and membrane stability), osmoprotectants and antioxidants contents, and antioxidative enzyme activities in wheat grown under the three mentioned stresses.

2. Material and methods

2.1. Material, conditions of growth and experimental treatments

The surface sterilization of wheat seeds was done using 0.1% $HgCl_2$ for 1 min. Seeds were then rinsed in sterilize-deionized water and were sown after air-drying in plastic pots (40 cm in diameter, 35 cm depth) filled with sand free from any anions or cations as a growth medium. Experiments were conducted in a an open greenhouse conditions with 19 ± 3/10 ± 2 °C as day/night temperature and humidity of 62.0–65.1%. Plants were watered using ½-strength Hoagland's nutrient solution (Hoagland and Arnon, 1938). Up to full emergence, the nutrient solution without any of stress treatments was supplied at 100% field capacity (FC) every two days to all pots. Fifteen days after sowing (DAS) pots were divided into 4 plots; no stress, drought (50% FC), salinity (200 mM NaCl) and cadmium (2 mM Cd), and 40 pots were allocated for each plot. Four treatments; 0, 2, 4 and 6 mM silicon (Si in potassium silicate; $K_2SiO_3.nH_2O$) were also allocated for each plot. For different stresses, our preliminary studies exhibited that NaCl salt level of 200 mM, drought of 50% FC and Cd of 2 mM were greatly affected wheat seedling growth, therefore, were selected. Throughout the duration of experiments, control plants (with no stress and received 0, 2, 4 or 6 mM Si) were watered by ½-strength Hoagland's nutrient solution without any stress. Soil pH was adjusted back to the control pH of 6.0–6.2 with diluted H_2SO_4.

A completely randomized design was the experimental layout. Ten replicates/pots for each treatment under each plot were used. The observation was terminated after 60 DAS. At this time, seedlings were collected from each treatment under each plot for various measurements.

2.2. Experimental methods

2.2.1. Analysis of plant growth

Sixty-d-old wheat seedlings were removed from each pot and the adhering sand particles were removed by distilled water. Shoot lengths were taken with a meter scale. Leaf areas were assessed using a digital meter (LI-3000 Portable Area meter Produced by LI-COR Lincoln, NE, USA). After fresh seedlings were weighed, they were placed in an oven at 70 °C until reach a constant dry weight (DW).

2.2.2. Assessments of physio-biochemical and non-enzymatic antioxidant attributes

Leaf net photosynthetic rate (*Pn*), rate of transpiration (*Tr*) and stomatal conductance (*Gs*) were assessed for photosynthetic parameters by using a portable photosynthesis system (LF6400XTR, LI-COR, USA). The assessments were taken at 09:00–11:00 a.m. on the second full expanded leaf. The WUE was calculated by dividing the value of *Pn* on the value of *Tr* for each treatment under each plot.

Assessments of relative water content [RWC; Weatherly (1950) with some modifications by Osman and Rady (2014)], membrane stability index [MSI; Premchandra et al. (1990) with some modifications by Rady (2011)] and electrolyte leakage [EL; Sullivan and Ross (1979)], using fresh fully-expanded leaves excluding the midrib were done.

As detailed in Zhang and Qu (2004) thiobarbituric acid (TBA) reaction method, malondialdehyde (MDA) content was determined using a sample (0.5 g) of fresh leaf. Homogenization using 5% trichloroacetic acid, and then centrifugation at 4.000 × *g* for 10 min was conducted. A 2 ml extract was mixed with 2 ml 0.6% TBA and placed in a boiling water bath for 10 min. Thereafter, 532, 600 and 450 nm were used to read the absorbances to apply for a formula [e.g., MDA content $= 6.45 \times (A_{532}–A_{600})–0.56 \times A_{450}$].

To extract and assess total soluble sugar content, the method of Irigoyen et al. (1992) was utilized. Homogenization for a dried leaf sample (0.2 g) was done in 5 ml of 96% (v/v) ethanol, and then washing in 5 ml 70% (v/v) ethanol was exercised. Thereafter and prior to measurements, extract centrifugation was done at 3500 × *g* for 10 min and supernatant storage was applied at 4 °C. To assess soluble sugar content, 0.1 ml of the ethanolic extract was reacted with 3 ml of reagent of freshly-prepared anthrone [150 mg anthrone + 100 ml of 72% (v/v) sulphuric acid] utilizing a boiling water bath for 10 min. Absorbances were read, after cooling, at 625 nm with a Bauschand Lomb-2000 Spectronic Spectrophotometer (Thermo Spectronic, Mercers Row, Cambridge, UK).

The rapid colorimetric Bates et al. (1973) method was applied to assess contents of proline in 0.5 g-dried leaf samples. Extraction in 10 ml of 3% (v/v) sulphosalicylic acid was done, and then extract centrifugation was conducted at 10.000 × *g* for 10 min. Supernatant (2 ml) was received 2 ml of freshly prepared acid-ninhydrin solution into a test-tube, and then the incubation was applied in a water bath at 90 °C for 30 min. Using an ice-bath, the reaction was terminated and the extraction was done with 5 ml of toluene by vortex-mixed for 15 s. In dark at room temperature, separation of the toluene and aqueous