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Sequenced ascorbate-proline-glutathione seed treatment elevates cadmium tolerance in cucumber transplants

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ABSTRACT

During its life cycle, plant has to cope with a number of abiotic stresses including cadmium stress. Cadmium (Cd) is highly toxic to plant and greatly influences its growth and entire metabolism. Antioxidants have to enable plant to beat such stresses. Therefore, effects of ascorbate (AsA), proline (Pro) and glutathione (GSH) applied, as seed soaking solutions, singly or in a sequence on cucumber transplant growth, physio-biochemical attributes and antioxidant defense system activity were investigated under 2 mM Cd stress. Adding Cd to transplants in irrigation water reduced photosynthetic efficiency, and nutrient (K^+ and Ca^{2+}) contents, while increased the activity of defense systems (non-enzymatic and enzymatic antioxidants) and Cd^{2+} contents in roots and leaves. Exogenous AsA, Pro and GSH applied singly or in a sequence improved transplant growth (e.g., shoot length, leaf area, shoot fresh weight and shoot dry weight), photosynthetic efficiency (i.e., SPAD chlorophyll, Fv/Fm and PI), transplant health (i.e., increased leaf MSI and RWC, and decreased root and leaf Cd^{2+} contents), antioxidant defense systems activity (enzymatic; superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase, and non-enzymatic; Pro, AsA and GSH antioxidants) and nutrient (K^+ and Ca^{2+}) contents. These positive results were obtained under irrigation with or without Cd, AsA. Sequenced AsA-Pro-GSH was the best treatment of which this study recommends to use, followed by GSH treatment, for growing cucumber transplants under Cd stress.

1. Introduction

Environmental pollution by heavy metals has abundantly increased in recent decades. Adversely affecting soil-plant environment system, various toxic metals are inserted into agricultural soils due to many reasons including the manifold industrial activities, soil waste management, sewage waste water irrigation, extensive use of chemical fertilizers and agricultural practices improvements.

Over thousands years, cadmium (Cd) is considered a major environmental bother to agricultural system (Kumar, 2013), ranking seventh among the top toxins. It has been reported that elevated insertions of Cd into plants cause several changes in physio-biochemical attributes and organelles structures related to the oxidative stress resulted from overproduction of reactive species of oxygen (ROS) (Feng et al., 2010; Yadav, 2010; Rady and Hemida, 2015; Yan et al., 2015). Plants possess a group of antioxidant mechanisms for ROS scavenging through inducing enzymatic (e.g., catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase, etc.) and non-enzymatic (e.g., proline; Pro, ascorbate; AsA, reduced glutathione; GSH, etc.) antioxidants (Parida and Das, 2005; Rady and Hemida, 2015, 2016). Along with the endogenous defense mechanisms, plants need some exogenous supports, such as antioxidants, to further improve their defense systems against stresses.

Antioxidants that have low molecular weight are synthesized within the stroma of chloroplast and cytosol in plants including glutathione (GSH) and ascorbic acid (AsA). These antioxidants buffer the redox interactions through interacting with several cellular components, leading to positive influences on cell elongation and plant growth (Sharma et al., 2012). Further, they may affect gene expression related to environmental stresses responses to maximize defense mechanisms in stressed plants. Depending on its thiol group (–SH), GSH acts as an effective electron acceptor/donor for many biological reactions. The –SH nucleophilic nature is also needful for mercaptide bonds formation with metals and it is important in reacting with select electrophiles (Xiang, 2001). GSH considers as an ideal biomolecule for a crop plant against abiotic stress due to its high reactivity, stability and water solubility. It involves in many cellular processes including xenobiotics detoxification (Dixon et al., 1998), heavy metals sequestration

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(Cobbett, 2000) and defense against ROS (Foyer and Noctor, 2005). It also functions as a substrate for glutathione peroxidase and glutathione S-transferase, which scavenge the ROS (Noctor et al., 2002).

As a strong antioxidant and abundantly occurs in plant, AsA plays many important roles in various cellular processes (Akram et al., 2017). It is involved in cell division and cell wall expansion, regulating plant growth and development (Pignocchi and Foyer, 2003). Many reports have suggested that, AsA plays a significant role in plant protection against several environmental stresses including stress of heavy metals (Rady and Hemida, 2015).

Another low molecular weight antioxidant, accumulation of proline (Pro) is one of different adaptive strategies to fight Cd stress (Ashraf and Foolad, 2007; Islam et al., 2009; Rasheed et al., 2014). Higher levels of Pro in plant cells strongly correlates to an enhanced tolerance to stress in plants (Habibi, 2012; Sharma and Dietz, 2006). Along with its potential as osmoprotectant, Pro considers more crucial in maintaining the redox homeostasis and detoxifying/scavenging the ROS (Hong, 2000). A lot of plant species accumulates more Pro in response to a heavy metal stress. Many works have assumed that, Pro copes with metal stress, while still others have considered that Pro accumulates as a stress symptom (Ashraf and Foolad, 2007). In plant cells, Pro protects cells from osmotic and metal-induced oxidative damages and enzyme denaturation, regulates the ratio of $NAD(P)^+$/ NAD(P)H, buffers pH in cytosols, acts as a source of nitrogen and carbon, and detoxifies accumulated ROS (Gill and Tuteja, 2010; Rady and Hemida, 2016). Although mechanism of plant tolerance to metal toxicity by Pro is vague, it has been reported that it could alleviate the stress of metals by stimulating the antioxidant defense systems in plant cells or by direct ROS scavenging (Rasheed et al., 2014).

Cucumber; *Cucumis sativus* (L.) is a widely distributed and an economically important crop worldwide. It has been noted that, reasonable amounts of carbohydrates, proteins, vitamin C, and some mineral nutrients such as calcium, phosphorus, iron and others are found out in cucumber fruit (Lin and Mei, 2012) useful for human health and nutrition. *Cucumis sativus* (L.) is found to be sensitive to Cd stress, inhibiting plant growth and causes great losses in yield and its quality. *Cucumis sativus* (L.) plant absorbs Cd from soil and ultimately translocates it to the edible part, therefore, cucumber fruit consumption, either directly or indirectly, with high levels of Cd can be a threat of food safety.

As far as we knew, there are many reports about assessing the effects of exogenous antioxidants applied as individual treatments on various plant species growing under Cd stress conditions (Ali et al., 2013a, 2013b, 2014; Rasheed et al., 2014; Semida et al., 2015), however, no reports are conducted about evaluating the effects of exogenous antioxidants applied as sequenced treatments on plants under Cd stress, except for only one (Rady and Hemida, 2015) until now. Therefore, in the present study, the effects of exogenous AsA, Pro and GSH applied singly or in a sequence on growth, photosynthetic efficiency, tissue health (i.e., membrane stability index, relative water content and Cd^{2+} content), non-enzymatic and enzymatic antioxidant defense systems were studied.

2. Material and methods

2.1. Material of seedling and conditions of growing

Sterilized cucumber seed, hybrid Bahi®, was divided into five groups each with 80 seeds. Seeds of groups 1, 2, 3 and 4 were soaked in distilled water, ascorbate (AsA) solution (0.5 mM), proline (Pro) solution (0.5 mM) and glutathione (GSH) solution (0.5 mM), respectively for 4 h. Additionally, group 5 seed was soaked in a sequence; firstly in AsA (0.5 mM) for 90 min, then in Pro (0.5 mM) for 80 min, and finally in GSH (0.5 mM) for 70 min. A sequence of AsA-Pro-GSH and their concentrations, and soaking periods were selected due to that they conferred the best response according to our data (not shown) of preliminary studies. Each group was divided into two sub-groups (n = 40 seeds). Seeds of first sub-group were germinated and then grown with distilled water, while those of the second sub-group were germinated and then grown using 2 mM $CdCl_2$ contaminated water. The concentration of 2 mM of $CdCl_2$ was selected from several concentrations; 0.5, 1, 2 and 3 mM used in a preliminary study. The concentration of 3 mM caused death of transplants, and the concentrations 0.5 and 1 mM moderately affected transplants growth, while the concentration of 2 mM greatly affected transplants growth. Therefore, the concentration of 2 mM $CdCl_2$ was selected for this study. Seeds were sown on foam trays (209 cells) on May 28th, 2017. Seed germination media were contained peat moss:vermiculite (1:2 by volume), and were grown under black shade net greenhouse for 28 d. The greenhouse was uncontrolled but was covered by black shade net to reduce the photoinhibition. The experiment was arranged in a completely randomized design with three replicates/foam trays. Transplants were then collected for various morphological, physiological and biochemical measurements 28 days after sowing (DAS).

2.2. Measurements of growth attributes

Twenty eight DAS, seedlings (n = 20) were randomly chosen from each treatment and shoot length, leaf area, shoot FW and shoot DW were recorded.

2.3. Assessment of photosynthetic efficiency

Using leaf porometer (Decagon Devices Inc., Pullman, WA, USA), stomatal conductance (*Gs*) was determined from 7:00–17:00 h with time interval of 2 h for four times (i.e. 14, 21, 28, and 35 days after onset of Cd treatments).

Chlorophyll content of the top third and fourth leaves were measured using a chlorophyll meter (SPAD-502, Minolta, Japan). On two different sunny days, Handy PEA portable fluorometer (Hansatech Instruments Ltd, Kings Lynn, UK) was used to measure chlorophyll fluorescence parameters. Measurements of fluorescence were maximum quantum yield of PSII Fv/Fm calculated from the following formula:

Fv/Fm = (Fm – F0)/Fm (Maxwell and Johnson, 2000), and the formula of Clark et al. (2000) was used to calculate photosynthesis performance index (PI_{ABS}) based on the equal absorption.

2.4. Assessment of relative content of water (RWC) and stability index of membrane (MSI)

Excluding the midrib, twenty discs (2 cm-diameter) of a fresh fully expanded leaf were used to assess RWC as detailed in Osman and Rady (2014) method. Once fresh discs weighed (FW) were saturated by floating on double distilled water in dark for 24 h. Adhering water drops were gently dried by placing discs on filter paper and a turgid weight (TW) was taken. Thereafter, discs were dehydrated at 70 °C for 48 h to assess a dry weight (DW), and then the following formula was applied:

$$RWC\% = [(FW - DW)/(TW - DW)] \times 100$$

Excluding the midrib, Premachandra et al. (1990) method modified by Rady (2011) was used to assess the MSI with duplicate 0.2 g fully-expanded leaf. A sample (0.2 g) was placed in 10 ml of double-distilled water in a test-tube and heated using a water bath at 40 °C for 30 min, and then a solution electrical conductivity (EC_1) was recorded. The second sample (0.2 g) was boiled at 100 °C for 10 min, and a solution electrical conductivity (EC_2) was taken, and then the following formula was applied:

$$MSI\% = [1 - (EC_1/EC_2)] \times 100$$