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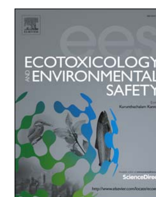

The humic acid-induced changes in the water status, chlorophyll fluorescence and antioxidant defense systems of wheat leaves with cadmium stress

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ABSTRACT

The using of bio-stimulant in plants grown under stress conditions for enhancing nutrition efficiency and crop quality traits is an effective approach. One of the bio-stimulants, humus material, is defined as humic acid (HA). HA application as a promotion of plant growth to plants grown in the heavy metals-contaminated soils has promised hope in terms of effects on plants but the its limiting effect is the application dose. Therefore, the wheat seedlings were grown in hydroponic culture for 21 d and the various concentrations of humic acid (HA; 750 or 1500 mg L^{-1}) were treated alone or in combination with cadmium (Cd) stress (100 or 200 µM) for 7 d. The results showed that after Cd stress treatment, water content (RWC), osmotic potential (Ψ_{Π}) and chlorophyll fluorescence parameters decreased and proline content (Pro) increased for 7 d. In spite of activated peroxidase (POX) and ascorbate peroxidase (APX), stress induced the toxic levels of hydrogen peroxide (H_2O_2) accumulation. Cd stress triggered lipid peroxidation (TBARS content). HA application successfully eliminated the negative effects of stress on RWC, Ψ_{Π} and photosynthetic parameters. In the presence of HA under stress, the increased activation of superoxide dismutase (SOD), catalase (CAT) and NADPH-oxidase (NOX) enzymes and ascorbate, glutathione and GSH/GSSG ratio observed. Only 750 mg L^{-1} HA under stress conditions induced the activities of monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR), and dehydroascorbate (DHA) content. After the combined application of HA and Cd stress, the low contents of H_2O_2 and TBARS maintained in wheat leaves. Hence, HA successfully eliminated the toxicity of Cd stress by modulating the water status, photosynthetic apparatus and antioxidant activity in wheat leaves.

1. Introduction

Due to the increasing number of populations in the world, the need for food is increasing (Clair and Lynch, 2010). It is expected that in order to meet this demand agricultural productivity will be increased. In the last century, agricultural intensification was driven by inputs derived from non-renewable energy sources such as synthetic fertilizers (Canellas et al., 2015). However, this solution can result in adverse situations such as deforestation, soil erosion, industrial pollution, declines in surface- and ground-water quality and loss of biodiversity. One of the measures to be taken against these problems is the use of plant bio-stimulants based on humic substances. Humic substances can occur from plant or animal remains through biological or chemical transformations in the soil and are known as the pool of organic carbon (Canellas et al., 2015). Humus materials are also described as humic acids (HA) by soil scientists (Hayes, 2006). HA has important roles in growth, the regulation of carbon and nitrogen cycling, stability of soil structure and transport of heavy metals in plants (Piccolo et al., 1996). Excess accumulation of heavy metal concentrations such as cadmium (Cd) in soils is very phytotoxic for plants (Groppa et al., 2007). This stress disturbs membrane permeability, enzymatic activity, peroxidative metabolisms of polyunsaturated fatty acids and basic metabolic process such as germination, growth, photosynthesis, cellular respiration and protein synthesis (Pizzeghello et al., 2013). Plants can be scavenged reactive oxygen species (ROS) produced under cadmium-induced oxidative stress by enzymatic/non-enzymatic antioxidant systems including superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR),

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ascorbate, glutathione, carotenoids and α-tocopherol (Dixit et al., 2001).

Besides the protection by antioxidant compounds against stress conditions in plants, HA can also eliminate the adverse effects of heavy metals. In the contaminated soils with heavy metals, HA regulates mobilization or transformation of toxic metals, the activated antioxidant enzyme system, hormone balance such as cytokinins, proline accumulation which functions as an osmolyte, increased uptake of minerals, cell elongation and cell division in leaves (Aydin et al., 2012; Bunluesin et al., 2007; Pizzeghello et al., 2013). In the literature, the articles about the interactions between HA and stress are mostly mentioned its effects on plant growth. This effect of HA can be connected with increasing activity of H-ATPase in roots (Leonard and Hodges, 1973). This enzyme is responsible for the formation of the electrochemical gradient, which is the energy required to transport protons to the cell apoplast and to transport the minerals to the opposite side of the cell membrane. So, accelerating the intake of minerals can encourage growth in roots. Tahir et al. (2011), Cordeiro et al. (2011) and García et al. (2012, 2016) reported that there is a protective role of humic acid application in rice and maize plants under PEG-mediated osmotic and water stress by increases in the antioxidant systems such as SOD and POX. Besides the beneficial effects of HA, depending on plant species or the range of concentration, HA can cause phytotoxicity or can exhibit no-response in plants. For example, Asli and Neumann (2010) detected that HA decreased growth in maize shoots. Also, Kirn et al. (2010) and Calvo et al. (2014) noted that no-response to bio-stimulant application induced on growth or in fruits per plant. Some previous studies have shown that after HA treatment, it lowered antioxidant enzyme activities when compared to the heavy metal treated plants (Haghighi et al., 2010; Sergiev et al., 2013). Therefore, there are contradictory results in terms of its growth under control conditions and antioxidant effects in stress-treated plants. To resolve this uncertainty, the general aim of the present study was to help in understanding the importance of HA application in stress acclimation processes, particularly regarding the interaction among water status, photosynthetic efficiency and antioxidant defense system in wheat leaves with/without HA treatment under Cd toxicity.

2. Material and methods

2.1. Plant material and experimental design

Seeds of wheat (*Triticum aestivum* L.) were surface-sterilized in 5% sodium hypochlorite for 10 min, rinsed five times with sterile distilled water and then allowed to germinate on the double-layer filter paper wetted distilled water. Germinated wheat seedlings were transferred to half strength Hoagland solution and were grown under controlled conditions (light/dark regime of 16/8 h at 25 °C, relative humidity of 70%, photosynthetic photon flux density of 350μmol m^{-2} s^{-1}). The seedlings were grown in hydroponic culture containing this solution for 21 d and the various concentrations of humic acid (HA; 750 or 1500 mg L^{-1}) were treated alone or in combination with cadmium (Cd) stress (100 or 200 μM). Plants were harvested after 7 days of treatment (7d) and then the leaves stored at − 86 °C until further analyses.

2.2. Determination of water content, osmotic potential and proline content

After harvest on 7d, six leaves were obtained from wheat and their fresh weight (FW) was determined. The leaves were floated on de-ionised water for 6 h and the turgid tissue was blotted dry prior to determining turgid weight (TW). Dry weight (DW) was determined after oven drying at 70 °C. The leaf relative water content (RWC) was calculated by the following formula (Smart and Bingham, 1974):

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

Leaves were extracted by crushing the material with a glass rod. Leaf osmotic potential (Ψ_Π) was measured by Vapro Vapor pressure Osmometer 5600. Ψ_Π was converted to MPa according to Santa-Cruz et al. (2002) by multiplying coefficient of 2.408×10^{-3}.

Pro content was done according to Bates et al. (1973). The leaves were homogenized in 3% sulfosalicylic acid and homogenate was filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, the mixture was heated at 100 °C. The mixture was extracted with toluene and the absorbance of fraction with toluene aspired from liquid phase was measured at 520 nm.

2.3. Determination of photosynthetic efficiency

A Fluorescence Monitoring System (FMS 2) was used for determination of chlorophyll fluorescence. The maximum quantum yield in the dark-adapted state (F_v/F_m), the actual quantum yield in the light-adapted steady state (Φ_{PSII}), the coefficients of photochemical quenching (qP) and the non-photochemical quenching values (NPQ) were measured.

2.4. Determination of the contents of cadmium, calcium and potassium

Samples were finely ground and 0.25 g dried plant material was digested with concentrated nitric acid (HNO_3) in a microwave system (CEM, Mars 5). The Cd^{2+}, K^+ and Ca^{2+} in extracts were analyzed by ICP-AES (Varian-Vista) (Nyomora et al., 1997).

2.5. Determination of ROS accumulation

Determination of H_2O_2 content was performed according to Liu et al. (2010). Leaves were homogenized in cold acetone and centrifuged. The supernatant was mixed with titanium reagent and then ammonium hydroxide was added to precipitate the titanium-peroxide complex. The reaction mixture was centrifuged. The pellet was washed with cold acetone and was dissolved. The absorbance of the solution was measured at 410 nm. H_2O_2 concentrations were calculated using a standard curve prepared with known concentrations of H_2O_2.

$OH^•$ scavenging activity was determined according to Chung et al. (1997) with minor changes. The reaction mixture contained 20 mM Na-phosphate buffer, 10 mM 2-deoxyribose, 10 mM $FeSO_4$, 10 mM EDTA, 10 mM H_2O_2, 0.525 mL H_2O, and 0.075 mL sample. The mixture was incubated at 37 °C. A mixture of 2.8% (w/v) trichloroacetic acid and 1.0% (w/v) thiobarbituric acid in 50 mM NaOH was added to the test tubes and boiled. After the mixture cooled, absorbance was measured at 520 nm against a blank solution. The $OH^•$ scavenging activity was calculated using the following formula:

$$OH^• \text{scavenging activity}\ (\%) = [(A_o - A_1)/A_o] \times 100$$

where A_o was the absorbance of the blank and A_1 the absorbance of the sample.

2.6. Determination of lipid peroxidation levels

The level of lipid peroxidation was determined by thiobarbituric acid reactive substances (TBARS) according to Rao and Sresty (2000). TBARS concentration was calculated from the absorbance at 532 nm, and measurements were corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. The concentration of TBARS was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.7. Enzyme extraction and determination of isozyme and/or enzyme compositions

For protein and enzyme extractions, 0.5 g of each sample was homogenized in 50 mM Tris-HCl (pH 7.8) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2% Triton X-100, 1 mM phenylmethylsulfonyl fluoride and 2 mM dithiothreitol (DTT). For APX

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