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Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat

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SUMMARY

We have studied the influence of selenium (Se) and sulfur (S) in the protection of photosynthetic capacity of wheat (*Triticum aestivum*) against cadmium (Cd) stress. The involvement of ethylene and its interaction with proline and antioxidant metabolism in the tolerance of plants to Cd stress was evaluated. Application of Se or S alleviated Cd-induced oxidative stress by increasing proline accumulation as a result of increased activity of glutamyl kinase (GK) and decreased activity of proline oxidase (PROX). These nutrients also induced the activity of ATP-sulfurylase and serine acetyl transferase and the content of cysteine (Cys), a precursor for the synthesis of both reduced glutathione (GSH) and ethylene. Further, application of Se and S to plants under Cd stress reduced ethylene level and increased the activity of glutathione reductase (GR) and glutathione peroxidase (GPX), reduced oxidative stress and improved photosynthesis and growth. The involvement of ethylene in Se and S-mediated alleviation of Cd stress was substantiated with the use of ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG). The use of AVG reversed the effects of Se and S on ethylene, content of proline and GSH and photosynthesis. The results suggested that Se and S both reversed Cd-induced oxidative stress by regulating ethylene formation, proline and GSH metabolism. Thus, Se or S-induced regulatory interaction between ethylene and proline and GSH metabolism may be used for the reversal of Cd-induced oxidative stress.

Introduction

Cadmium (Cd) is a powerful pollutant due to its long half life in soil and greater solubility in water. Plants grown under excess Cd show reduced growth and metabolism. Elevated levels of Cd induce excessive production of reactive oxygen species (ROS). These ROS cause damage to photosynthetic apparatus resulting in adverse effects on photosynthetic potential of plants (Mobin and Khan, 2007; Astolfi et al., 2012; Dias et al., 2013; Asgher et al., 2014). Several efforts have been made to counteract Cd-induced toxicity and restore the photosynthetic ability of plants. Supplementation of plants with mineral elements is one of the strategies adopted. The added mineral elements benefit plants because of their known biological role in metabolism and also help to reduce the toxicity generated by Cd. It has been reported that inputs of essential or beneficial nutrients such as nitrogen (N), sulfur (S), iron (Fe) and selenium (Se) restore photosynthetic ability, improve antioxidants capacity and productivity of crop plants (Iqbal et al., 2011; Hasanuzzaman and Fujita, 2011; Hasanuzzaman et al., 2011; Astolfi et al., 2012; Asgher et al., 2014).

Sulfur is an essential mineral nutrient element and an integral part of certain amino acids (cysteine, Cys and methionine), antioxidant (reduced glutathione; GSH), co-enzymes, prosthetic groups, vitamins, secondary metabolites, phytochelatins (PCs) and lipids (Khan et al., 2014a,b). The role of S in detoxification of Cd-induced oxidative stress was correlated with adequate S availability in *Hordeum vulgare* and *Brassica juncea* plants with increased GSH production (Astolfi et al., 2012; Masood et al., 2012; Asgher et al., 2014).

Abbreviations: ACS, 1-aminocyclopropane carboxylic acid synthase; ATP-S, adenosine triphosphate sulfurylase; AVG, aminoethoxyvinylglycine; Cd, cadmium; Cys, cysteine; DAS, days after sowing; ETR, electron transport rate; GK, glutamyl kinase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; NPQ, non-photochemical quenching; NR, nitrate reductase; PROX, proline oxidase; PS, photosystem; qP, photochemical quenching; ROS, reactive oxygen species; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; S, sulfur; SAT, serine acetyltransferase; Se, selenium; TBARS, thiobarbituric acid reactive substances.

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We have shown earlier that S application reduced the adverse effects of Cd and improved photosynthesis in *B. juncea* plants by enhancing the activity of antioxidant enzymes (Masood et al., 2012; Asgher et al., 2014).

Recent studies on Se have shown that this beneficial element also reduces the adverse effects of abiotic stress. The low concentration of Se stimulates growth and alleviates effects of Cd stress. While it promotes oxidant activity, it causes damages to plants at high concentration (Feng et al., 2013; Hawrylak-Nowak et al., 2014; Saidi et al., 2014). It has been reported that Se regulates ROS metabolism and induces activity of antioxidant enzymes (Djanaguiraman et al., 2010) resulting in reduced damage to growth of plants (Hasanuzzaman et al., 2011; Hawrylak-Nowak et al., 2014; Saidi et al., 2014). Recently, Hasanuzzaman et al. (2012) have shown that exogenous application of Se increases tolerance of *Brassica napus* plants to Cd-induced oxidative damage by enhancing the enzymatic and non-enzymatic antioxidant systems. Hawrylak-Nowak et al. (2014) have shown that Se reduced PCs accumulation in roots, but did not change its concentration in leaves of *Cucumis sativus* plants. Selenium has been found to increase GSH content in *B. napus* plants (Hasanuzzaman et al., 2012). In higher plants, Se metabolism is closely related to S because of its similar chemical properties. Selenium is considered a chemical analog of S and competes for the same transporters during uptake by roots (Mikkelsen and Wan, 1990; Zayed and Terry, 1992). Moreover, Cd up-regulates sulfate transporters (Sultr1;1 and Sultr2;1) (Takahashi et al., 2000; Herbette et al., 2006). Selenite resistant mutants of *Arabidopsis thaliana*, caused by a mutation in the high-affinity sulfate transporter Sultr1;2, showed decreased uptake of both Se and S (Shibagaki et al., 2002; El Kassis et al., 2007). Pilon-Smits et al. (1999) reported that Se and S were both involved in ATP-sulfurylase overexpression.

The roles of proline metabolism, GSH synthesis and ethylene formation have been identified in tolerance of plants to stress. The independent study on *Arabidopsis* (Yoshida et al., 2009) and *B. juncea* plants (Asgher et al., 2014) has shown that GSH production and ethylene formation are linked with ozone and Cd tolerance. Similarly, it has been shown that there exists an interaction between proline metabolism and ethylene formation for heat stress tolerance in *Triticum aestivum* (Khan et al., 2013). It is therefore, assumed that Se and S may modulate ethylene formation and proline and antioxidant metabolism for Cd tolerance. The inhibition of Cd-induced stress ethylene and oxidative stress by Se and S may result in the increase in sensitivity of plants to ethylene and promote proline and antioxidant metabolism. In the present study, we compared the effectiveness of Se and S in Cd stress tolerance and studied the tolerance induced through ethylene-mediated modulation of proline and GSH metabolism in *T. aestivum*.

Materials and methods

Plant material and growth conditions

Healthy seeds of wheat (*Triticum aestivum* L.) cv. WH 711 were surface sterilized with 95% ethyl alcohol followed by repeated washings with double distilled water. Seeds were sown in 23 cm diameter earthen pots filled with reconstituted soil (peat and compost, 4:1 (v/v); mixed with sand, 3:1 (v/v)). The treatments were 0 (control), 200 mg Cd kg^{-1} soil, 2 mg Se kg^{-1} soil, 200 mg S kg^{-1} soil or Cd combined with Se or S. The sources for Cd, Se and S were cadmium chloride ($CdCl_2$), sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) and elemental sulfur, respectively. An amount of 326.2 mg $CdCl_2$ and 6.7 mg sodium selenite was taken for 200 mg Cd kg^{-1} soil and 2 mg Se kg^{-1} soil, respectively. Elemental sulfur was used to obtain 200 mg S kg^{-1} soil. The required amounts for 2 mg Se kg^{-1} soil and 200 mg S kg^{-1} soil were mixed thoroughly with soil and left for 15 days. After 15 days the pots were filled with the soil and seed sowing was done. The Cd treatment was given at the time of seed sowing. The pots were kept in a naturally illuminated green house of the Botany Department, Aligarh Muslim University, Aligarh, India with day/night temperatures at 21 °C/17 °C (±3 °C), photosynthetically active radiation (PAR; 680 μmol m^{-2} s^{-1}) and relative humidity of 68 ± 5%. Another experiment was conducted to substantiate the information that ethylene is involved in Se or S-induced Cd stress tolerance. For this, plants grown with Cd in the presence of Se or S were given foliar treatment of 50 μL L^{-1} of aminoethoxyvinylglycine (AVG), an ethylene biosynthesis inhibitor. Earlier, independent experiments on Se and S have shown that 2 mg Se kg^{-1} soil and 200 mg S kg^{-1} soil have reduced oxidative stress (Chu et al., 2010; Masood et al., 2012). Treatments were arranged in a factorial randomized complete block design. The number of replicates for each treatment was four ($n = 4$). The measurements in the experiments were done at 30 DAS.

Determination of H_2O_2 content and lipid peroxidation

The content of H_2O_2 was determined following the method of Okuda et al. (1991). Fresh leaf tissues (500 mg) were ground in ice-cold 200 mM perchloric acid. After centrifugation at 1200 × *g* for 10 min, perchloric acid of supernatant was neutralized with 4 M KOH. Insoluble potassium perchlorate was eliminated by centrifugation at 500 × *g* for 3 min. In a final volume of 1.5 mL, 1 mL of the eluate, 400 μL of 12.5 mM 3-(dimethylamino) benzoic acid (DMAB) in 0.375 M phosphate buffer (pH 6.5), 80 μL of 3-methyl-2-benzothiazoline hydrazone (MBTH) and 20 μL of peroxidase (0.25 U) were added. The reaction was started by the addition of peroxidase at 25 °C and the increase in absorbance was recorded at 590 nm on a UV–Vis spectrophotometer.

Lipid peroxidation in leaves was determined by estimating the content of TBARS as described by Dhindsa et al. (1981). Fresh leaf tissues (500 mg) were ground in 0.25% 2-thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA) using mortar and pestle. After heating at 95 °C for 30 min, the mixture was rapidly cooled on ice bath and centrifuged at 10,000 × *g* for 10 min. To 1 mL aliquot of the supernatant 4 mL 20% TCA containing 0.5% TBA was added. The absorbance of the supernatant was read at 532 nm and corrected for non-specific turbidity by subtracting the absorbance of the same at 600 nm. The content of TBARS was calculated using the extinction coefficient (155 mM^{-1} cm^{-1}).

Measurements of gas exchange parameters, Rubisco activity, chlorophyll and growth

Gas exchange parameters (net photosynthesis, stomatal conductance and intercellular CO_2 concentration) were measured in fully expanded uppermost leaves of plants using infrared gas analyzer (CID-340, Photosynthesis System, Bio-Science, USA). The measurements were done on a sunny day at light saturating intensity; PAR; 720 μmol m^{-2} s^{-1} and at 370 ± 5 μmol mol^{-1} atmospheric CO_2 concentrations. Chlorophyll was measured in intact leaves with the help of SPAD chlorophyll meter (502 DL PLUS, Spectrum Technologies, USA).

The activity of ribulose 1,5-bisphosphate carboxylase (Rubisco; EC 4.1.1.39) was determined adopting the method of Usuda (1985) by monitoring NADH oxidation at 30 °C at 340 nm during the conversion of 3-phosphoglycerate to glycerol 3-phosphate after addition of enzyme extract to the assay medium. For enzyme extraction, leaf tissue (1.0 g) was homogenized using a chilled mortar and pestle with ice-cold extraction buffer containing 0.25 M Tris–HCl (pH 7.8), 0.05 M $MgCl_2$, 0.0025 M EDTA and