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Excess sulfur supplementation improves photosynthesis and growth in mustard under salt stress through increased production of glutathione

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ABSTRACT

Sulfur (S) assimilation leads to synthesis of reduced glutathione (GSH), a thiol compound known for its involvement in abiotic stress tolerance. In this study, the influence of sufficient-S (100 mg S kg^{-1} soil) and excess-S (200 mg S kg^{-1} soil) was studied on photosynthesis and growth of mustard (*Brassica juncea* L.) plants grown with or without 100 mM NaCl. Both the S levels equally promoted photosynthesis and growth of plants under no salt stress, while excess-S more conspicuously alleviated the negative effects of salt stress and improved photosynthesis and growth compared to the control plants. Excess-S mediated increase in photosynthesis and growth of salt grown plants was through increased production of GSH. The exogenous 1 mM GSH treatment and excess-S produced similar results on photosynthesis and growth of plants establishing that excess-S improves photosynthesis and growth under salt stress through increased GSH production. Thus, excess-S/GSH may be used as potential tool for alleviation of salt stress in mustard plants.

1. Introduction

Soil salinity is one of the major environmental constraints to agricultural production. According to an estimate by Food and Agricultural Organization (2008), over 6% of the world's land is salt affected; and increased salinization of arable land is expected to have devastating global effects with average agricultural land loss of up to 50% by the mid of this century (Manchanda and Garg, 2008). Salt stress-induced toxicity averts the realization of full genetic potential of plants due to limitation of photosynthetic capacity in the presence of enhanced reactive oxygen species (ROS) production. It has been shown that salt stress negatively affects plant photosynthetic functions and various cellular functions by disturbing the homeostasis of Na^+ and Cl^- ions and disturbance in the uptake of major essential nutrients (Munns, 2002; Ashraf and Harris, 2004; Munns and Tester, 2008; Khan et al., 2009a).

Sulfur (S) has now been recognized to regulate photosynthesis under optimal and stressful environments and play an important role in mitigation of salt-induced oxidative stress (Nazar et al., 2011a). It has significant role in the formation of photosynthetic apparatus and electron transport system (Marschner, 1995). The importance of S as a plant nutrient has been recognized for a long time, but active research started when widespread S deficiencies were reported. The deficiency of S obstructs with plant metabolism (Honsel et al., 2012) and decreases chlorophylls content and photosynthesis in sugar beet (Kastori et al., 2000) and photosynthetic efficiency in rice by their influence on the content and activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Lunde et al., 2008). Sulfur has been found in major metabolic compounds such as amino acids [methionine (Met), cysteine (Cys)], antioxidant [reduced glutathione (GSH)] proteins, sulfo-lipids, iron–sulfur (Fe–S) clusters, glucosinolates, vitamins (biotin and thiamine) coenzyme A (CoA) and thioredoxin system; which have potential to modulate physiological processes of plants to enhance the salt tolerance competence (Khan et al., 2014). Further, S-containing compounds are involved in ROS metabolism and reduction of oxidative stress by improving potassium/sodium ions (K^+/Na^+) selectivity and antioxidant system (Fatma et al., 2013). Moreover, the degree of oxidative cellular damage in plants

Abbreviations: ANOVA, analysis of variance; AsA-GSH, ascorbate-glutathione; APX, ascorbate peroxidase; ATPS, ATP-sulfurylase; CAT, catalase; CoA, coenzyme A; Cys, cysteine; Ci, intercellular CO_2 concentration; DAS, days after sowing; GR, glutathione reductase; LSD, least significant difference; (F_v/F_m), maximal PS II photochemical efficiency; Met, methionine; Pn, net photosynthesis; OASTL, O-acetylserine (thiol) lyase; GSSG, oxidized glutathione; ROS, reactive oxygen species; GSH, reduced glutathione; gs, stomatal conductance; S, sulfur; TBARS, thiobarbituric acid reactive substances; WUE, water use efficiency.

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exposed to salt stress is controlled by the capacity of the antioxidative systems (Turkan and Demiral, 2009). Plants up-regulate productions of non-enzymatic antioxidants, ascorbic acid and GSH and activity of enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) under stressful environments that help in neutralizing or scavenging ROS (Noctor et al., 2012).

Reduced glutathione (γ-Glu-Cys-Gly) is a major source of non-protein thiols and acts as an important non-enzymatic antioxidant. The induction of antioxidant system participates in stress resistance, adaptation along with fulfilling other roles related to redox sensing and signaling and regulates the protective reaction of plants against salt stress (Nazar et al., 2011a). Glutathione content in plants is strongly influenced by S supply, as it is involved in the regulation of uptake, assimilation, transport and storage of reduced S (Noctor et al., 2012). Ruiz and Blumwald (2002) reported that S assimilation rate and biosynthesis of GSH were greatly increased in *Brassica napus* plant exposed to saline conditions. Recently, studies on barley (Astolfi and Zuchi, 2013) and mustard (Nazar et al., 2014) have shown that S supply protects plants from adverse effects of salt stress by increasing GSH content. However, the studies concerning the availability of S in the protection of photosynthetic potential under salt stress are few and need elucidation of the mechanism by which S alleviates negative effects of salt stress on photosynthesis. In particular, reports on the association between exogenous GSH and S for salt stress are not available in the literature. We have studied the mechanisms of antioxidant metabolism and S-assimilation in protection of excess-S/GSH-mediated photosynthesis of mustard plants under salt stress. In the present work, the effectiveness of sufficient-S (100 mg S kg^{-1} soil) was compared with the excess-S (200 mg S kg^{-1} soil) in the alleviation of salt stress and the role of GSH was studied in S-mediated alleviation of salt stress in mustard (*Brassica juncea* L.). We have shown earlier that 100 mg S kg^{-1} soil and 200 mg S kg^{-1} soil are sufficient-S and excess-S, respectively (Khan et al., 2005; Masood et al., 2012).

2. Materials and methods

2.1. Plant material, growth conditions and experimental design

The experiment was carried out in the greenhouse of the Botany Department, Aligarh Muslim University, Aligarh, India. Mustard (*Brassica juncea* L. Czern & Coss. var. Varuna) seeds were surface sterilized with 0.01% $HgCl_2$ followed by repeated washing with distilled water and sown in 23-cm earthen pots containing soil with peat and compost (4:1, w/w) mixed with sand (3:1, w/w). The pots were kept under natural day/night conditions with photosynthetically active radiation (PAR) ~ 640 μmol m^{-2} s^{-1} and average day/night temperature of 22/14 ± 3 °C and relative humidity 62–70%.

The appropriate elemental S was used for obtaining 100 mg S kg^{-1} soil (100 S) and 200 mg S kg^{-1} soil (200 S) by applying 10 days before sowing, while 100 mM NaCl was given for salt stress at the time of sowing and its application was done at alternate days for 15 days from the sowing time. In addition, there was a control group of plants. The native soil S concentration was 100 mg S kg^{-1} soil. After seeds germination, three plants were maintained in each pot and each treatment was replicated four times. Based on the results of this experiment that 200 S more potentially alleviates salt stress than 100 S, and that GSH is involved in alleviation of salt stress by 200 S, we performed another experiment in which the effectiveness of 200 S and 1 mM GSH was compared for the alleviation of salt stress. For this, plants were grown with 0 (control), 100 mM NaCl, 200 S, 1 mM GSH or with combined treatment of 200 S with NaCl and 1 mM GSH plus NaCl. GSH was added to the plants at 10 days after sowing (DAS). The arrangement of treatments in both the experiments was completely randomized block design. At 30 DAS in both the experiments, determinations were made for content of Na^+ and Cl^-, H_2O_2, thiobarbituric acid reactive substances (TBARS), S, GSH, redox state, Cys, ATP-sulfurylase activity (ATPS) and antioxidant enzymes. In addition, photosynthesis and growth attributes and SDS-PAGE for Rubisco protein were done.

2.2. Determination of contents of ions

For the determination of Na^+ and Cl^- contents, plant samples were digested in Tri acid mixture (TAM), containing mixture of HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 10:5:4. The content of ions was extracted in distilled water by boiling for 30 min twice. The filtered extract thus obtained was used to measure specific ions. The Na^+ content was estimated using flame photometer (Khera-391: Khera Instruments, New Delhi), whereas Cl^- content was determined by titration against 0.02 N silver nitrate solution using 5% K_2CrO_4 as indicator as described by Nazar et al. (2011b).

2.3. Determination of content of H_2O_2 and lipid peroxidation

The content of H_2O_2 was determined following the method of Okuda et al. (1991). Fresh leaf tissues were ground in ice-cold 200 mM perchloric acid followed by centrifugation at 1200 × *g* for 10 min. After centrifugation, perchloric acid of the supernatant was neutralized with 4 M KOH and the insoluble potassium perchlorate was eliminated by further centrifugation at 500 × *g* for 3 min. The reaction mixture contained 1 ml of the eluate, 400 μl of 12.5 mM 3-(dimethylamino) benzoic acid in 0.375 M phosphate buffer (pH 6.5), 80 μl of 3-methyl-2-benzothiazoline hydrazone and 20 μl of peroxidase (0.25 unit) in a final volume of 1.5 ml. The reaction was started by the addition of peroxidase at 25 °C and the increase in absorbance was recorded at 590 nm.

The level of lipid peroxidation in leaves was determined by estimating the content of TBARS as described by Dhindsa et al. (1981). Fresh leaf tissues 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 quickly 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 5% TBA were added. The absorbance of the supernatant was read at 532 nm and corrected for nonspecific 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}).

2.4. Assay of antioxidant enzymes

Fresh leaf tissue was homogenized with an extraction buffer containing 0.05% (v/v) Triton X-100 and 1% (w/v) polyvinylpyrrolidone (PVP) in potassium-phosphate buffer (100 mM, pH 7.0) using chilled mortar and pestle. The homogenate was centrifuged at 15,000 × *g* for 20 min at 4 °C. The supernatant obtained after centrifugation was used for the enzyme assays. For APX acitivity, extraction buffer was supplemented with 2 mM ascorbate.

The activity of CAT was measured by the method of Aebi (1984) with slight modification by monitoring the disappearance of H_2O_2 at 240 nm. Activity was calculated by using extinction coefficient 0.036 mM^{-1} cm^{-1}. One unit of enzyme is the amount necessary to decompose 1 μmol of H_2O_2 per min at 25 °C.

The activity of APX was determined according to Nakano and Asada (1981) by the decrease in absorbance of ascorbate at 290 nm. The assay mixture contained phosphate buffer (50 mM, pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2, and enzyme extract. The activity of APX was calculated by using the extinction