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Simultaneous exposure of sulphur and calcium hinder As toxicity: Up-regulation of growth, mineral nutrients uptake and antioxidants system

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ABSTRACT

The current study was carried out to investigate the role of exogenous sulphur (K_2SO_4: S; 60 mg S kg^{-1} sand) and calcium ($CaCl_2$: Ca; 250 mg Ca kg^{-1} sand) individually as well as in combination (S + Ca) in ameliorating the inhibitory effect of As ($Na_2HAsO_4{\cdot}7H_2O$: As_1; 15 mg As kg^{-1} sand and As_2; 30 mg As kg^{-1} sand) by analyzing biomass accumulation, mineral nutrients uptake, photosynthetic pigments content, redox status of the cell, enzymatic and non-enzymatic defense system in *Brassica juncea* L. seedlings. Biomass accumulation, uptake of mineral nutrients, photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) content and the activity of proline dehydrogenase (ProDH) declined with increasing accumulation of As in root as well as leaves in As dose dependent manner. Contrary to this, exogenous application of S, Ca and S + Ca, markedly reduced the negative impact of As on above captioned traits except ProDH activity. On the other hand, ROS and their biomarkers (superoxide radical; $O_2^{\cdot-}$, hydrogen peroxide; H_2O_2, malondialdehyde; MDA equivalents content and membrane damage; electrolyte leakage), activities of enzymatic (superoxide dismutase; SOD, peroxidase; POD, catalase; CAT and glutathione-*S*-transferase; GST) and non-enzymatic antioxidant i.e. proline (Pro) content and its enzyme pyrroline-5-carboxylate synthetase; P5CS activity were increased in root and leaves under As stress. While, exogenous application of S, Ca and S + Ca, further enhanced the activities of above mentioned enzymes and Pro content thereby causing considerable reduction in $O_2^{\cdot-}$, H_2O_2, MDA equivalents content and electrolyte leakage. This study suggests that exogenous application of S and/or Ca efficiently (particularly S + Ca) lowered the negative impact of As on biomass accumulation in *Brassica* seedlings by improving the uptake of essential mineral nutrients', content of photosynthetic pigments, activities of enzymatic and content of non-enzymatic antioxidants.

1. Introduction

Arsenic ranked as 20th most occurring element in the earth's crust. It is released in the environment from natural sources like pyrite ores and anthropogenic activities (Bundschuh et al., 2011). In recent decades, As contamination has become a global health issue especially in 21 countries of South and South-East Asia including West Bengal (India) and Bangladesh (Singh et al., 2015a), where much higher levels of As i.e. more than permissible limit (20 mg kg^{-1} soil) has been reported (Kabata-Pendias and Pendias, 1992). Arsenic mainly exists in two oxidation states: arsenate (As^V) and arsenite (As^{III}) and former is comparatively more stable in the environment (Ma et al., 2008). Arsenate enters in root through phosphate channels (Singh et al., 2016); while As^{III} is through aquaporin channels (nodulin 26-like intrinsic proteins: NIPs; Ma et al., 2008) and silicon entrance pathway, where it rapidly accumulates in different parts of the plant thus, enters to the food chain (Kumar et al., 2015). It hampers growth and development of plant by beating array of metabolic processes like uptake of essential mineral nutrients, chlorophyll biosynthesis, reduction in photo fixation of CO_2 and PSII activity (Stoeva et al., 2005; Gupta and Ahmad, 2014). Impediment in photosynthetic electron transport processes under As stress along with the conversion of As^V to As^{III} results into excess generation of reactive oxygen species (ROS), which affects enzyme activities, metabolic pool and plant biomass (Singh et al., 2016; Abbas et al., 2018). The elevated ROS levels damage cellular biomolecules even cell death in severe stress conditions (Kader and Lindberg, 2010). To cope up the deleterious effect of metals, plant cells are a companied with antioxidant defense system consisting of enzymatic and non-enzymatic components (Singh et al., 2016; Abbas et al., 2018) that facilitate the reduction of As^V to As^{III}, chelation of As with ligands and its dumping into vacuoles (Kumar et al., 2015). Nevertheless, plant always suffers from As toxicity when its accumulation exceeds a threshold limit

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however, it varies from plant species, growth stage as well as concentration of As in soil, soil types and other variables. Further, United States Department of Agricultural (USDA), European Union (EU) and WHO suggested 0.15, 0.5 and 1.0 mg As kg^{-1}, respectively the threshold limit for rice and according to European Food Safety Authority (EFSA) this limit is 1.0 mg As kg^{-1} for wheat, oat and corn.

Arsenic toxicity could be improved using physiological tools. Sulphur (S) and calcium (Ca) are recognized as chemical signaling substances, and have been intensively investigated for their roles in plant adaptation to changing environments. Sulphur is required at 0.1–1.0% (on dry weight basis) for plant growth and development (Gaafar et al., 2012). The increasing reduction in the emission of S in natural system, use of fertilizers (NPK) lacking S such as urea, di-ammonium phosphate instead of ammonium superphosphate, in addition to leaching of sulphate ions deeper into the soil profile cause limited phytoavailability of this building block. In present scenario, S deficiency seems to be an emerging problem that resulted into decrease in quality and quantity of crop. In plants, about 90% S is present as cysteine (Cys) and methionine (Met), which is essential in the formation of sulfhydryl (-SH) and disulfide bonds (S-S) of proteins and enzymes possess thiol groups at their active centers (Saito, 2000; Yu et al., 2018). Moreover, it also serves as S donor for enhanced synthesis of thiol peptides, i.e. reduced glutathione (GSH) and phytochelatins (PCs), that are considered to be an important defense compounds against heavy metal stress (Tuli et al., 2010; Gaafar et al., 2012; Batista et al., 2014; Zhou et al., 2018).

Besides this, nutrient enrichment (N, P, K, Mg and Ca) may also be considered another approach to minimize the effects of As toxicity in plants. Calcium has been shown to stabilize cell membrane surfaces, prevent solute leakage from the cytoplasm, maintain water status, photosynthesis, transpiration rate and regulate plant hormone metabolism (Ahmad et al., 2016; Naeem et al., 2018; Singh et al., 2018). Calcium not only regulates the cell metabolism but is also a well-known secondary messenger that transduces signals to carry out the basic metabolism of plants (Price et al., 1994; Li et al., 2016; Aldon et al., 2018; Gao et al., 2018). In recent years, considerable interest has been focused on the role of Ca in protecting plants under adverse environmental conditions (Siddique et al., 2012; Ahmad et al., 2015, 2016; Li et al., 2016; Aldon et al., 2018; Gao et al., 2018) by inducing antioxidant enzyme activities and reducing lipid peroxidation of cell membranes (Naeem et al., 2018).

Rapeseed-mustard contributes 28.6% in the total oilseeds production and ranked second after groundnut sharing 27.8% in India's oilseed economy. *Brassica* is also known as hyper-accumulator of heavy metals but is facing a risk of being exposed to As in a very large coverage area of Ganga–Meghna–Brahmaputra (GMB) basin of India and Bangladesh. Most studies dealing with S or Ca involvement in As tolerance only considered either S or Ca; however, their putative interactions received little attention, a condition which prevails in field. Beside this, to the best of our knowledge, no study has been conducted on this aspect with *Brassica juncea*. Therefore, in the present communication an attempt have been made probably for the first time to trace the coordinated action of S and Ca (S + Ca) in alleviating As toxicity in *B. juncea* L. seedlings. Further, to achieve the above objectives: growth, photosynthetic pigments content, status of important mineral nutrients, As accumulation, oxidative biomarkers, antioxidant capability, proline (osmolyte) and its metabolism in plant parts of *B. juncea* L. under As stress were analyzed.

2. Materials and methods

2.1. Plant material and growth conditions

To perform the experimental work, healthy seeds of *Brassica juncea* L. were surface sterilized with 2% (v/v) sodium hypochlorite solution and kept in dark for germination. Germinated seeds were sown in plastic pots containing 150 g acid sterilized sand and kept in darkness. After 48 h, seedlings were transferred in environmentally controlled growth chamber (CDR model GRW-300 DGe, Athens) with photosynthetically active radiation (PAR): 150 µmol photons m^{-2} s^{-1}, relative humidity: 65–70% and day-night regime: 16:8 h at 22 ± 2 °C. During growth period, seedlings were irrigated with 50% Hoagland solution (Hoagland and Arnon, 1950) on alternate days. After the emergence of secondary leaves, 30 day old seedlings were treated with different concentrations of As^V ($Na_2HAsO_4{\cdot}7H_2O$) dissolved in nutrient medium with and without additional S as K_2SO_4 and Ca as $CaCl_2$ either alone or in combination. On the basis of screening experiments, the two doses of As i.e. 15 mg As kg^{-1} sand (As_1) and 30 mg As kg^{-1} sand (As_2) were selected which correspond to LC_{16} and LC_{37}, respectively. Further it is to mention that the application of As up to 10 mg kg^{-1} sand did not show significant toxic effect on growth of the test organism. Additionally, on the basis of screening experiments, single dose of S (60 mg S kg^{-1} sand) and Ca (250 mg Ca kg^{-1} sand), which were found to be stimulatory hence, selected for the further study. The equal level of K^+ was maintained by the application of KCl in all the combinations. Treatments consisted of: Control (C)-nutrient solution alone, S, Ca and S + Ca with and without As (As_1 and As_2). After seven days of the treatment, seedlings were harvested to analyze various parameters.

2.2. Growth analysis

Biomass accumulation in *Brassica* L. seedlings was measured in terms of fresh weight (FW) and dry weight (DW) of root and shoot.

2.3. Arsenic and mineral nutrients analysis

Arsenic and mineral nutrients (S, Ca, Mn, K, Mg, Fe, Cu, Zn and P) in root and shoot tissues of test seedlings were analyzed by the method of Allen et al. (1986).

2.4. Estimation of photosynthetic pigments

The amount of chlorophylls (Chl *a* and Chl *b*) and carotenoids (Car) were calculated using the equations of Lichtenthaler (1987).

2.5. In-vitro and in-vivo estimation of reactive oxygen species (ROS) and indices of damage

2.5.1. In-vitro analysis

Superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), malondialdehyde (MDA) equivalents content (lipid peroxidation) and electrolyte leakage (membrane damage) in root and leaf tissues were estimated by the method of Elstner and Heupel (1976), Velikova et al. (2000), Heath and Packer (1968) and Gong et al. (1998), respectively.

2.5.2. In-vivo visualization

The *in-vivo* visualization of SOR, H_2O_2, MDA equivalents content and membrane damage in root and leaves was done according to Frahry and Schopfer (2001), Thordal-Christensen et al. (1997), Pompella et al. (1981) and Yamamoto et al. (2001), respectively.

2.6. Estimation of enzymatic antioxidants and analysis of isoenzymes

The activities of superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.3.6) and glutathione-*S*-transferase (GST; EC 2.5.1.18) enzymes in root and leaf were assayed by the method of Giannopolitis and Ries (1977), Zhang (1992), Aebi (1984) and Habig et al. (1974), respectively. One unit (U) activity of: SOD is the particular amount of enzyme needed for 50% inhibition of NBT, POD (U) is the amount of enzyme needed for oxidizing 1 nmol guaiacol min^{-1}, CAT (U) is equal to 1 nmol H_2O_2 dissociated min^{-1} and GST (U) is defined as 1 nmol CDNB-conjugates formed min^{-1}.