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Cysteine-induced upregulation of nitrogen metabolism-related genes and enzyme activities enhance tolerance of maize seedlings to cadmium stress

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ABSTRACT

We aimed to determine the effects of cysteine (Cys) on nitrogen metabolism of maize seedlings exposed to cadmium (Cd) stress at the biochemical and molecular levels. Cd stress significantly reduced root-leaf lengths and dry weight, whereas Cys application partially mitigated these reductions. Correspondingly, the declines occurring in chlorophyll content under the stress were also markedly mitigated with Cys supplementation. When compared to the control, although significant reductions were recorded in the activities of nitrate reductase, nitrite reductase, glutamine synthase (GS) and glutamate synthase (GOGAT) in Cd-applied seedlings, a marked rise was determined in glutamate dehydrogenase (GDH) activity. Similarly, while nitrate and nitrite contents dropped with the application of Cd, a remarkable elevation of ammonium content took place. Cd-induced declines detected in enzyme activity and nitrate and nitrite contents were reversed with Cys application, and this was in contrast to the decrease in ammonia content. Versus the other enzymes studied, the Cd-induced enhancement recorded in GDH activity was reversed by Cys application. Therefore, an important positive correlation was established between activity and gene expression levels of all enzymes studied. The reductions noted in oxidant content and membrane damage with Cys application demonstrated that the soothing effect of Cys arises from mitigation of oxidative damage through the direct and/or indirect influence of cysteine. Taken together, it is possible to say that the repressive effect of Cd on nitrogen metabolism at the level of the enzyme and gene was significantly alleviated by Cys, and hence an important contribution to enhancing plant tolerance in face of Cd stress was noted.

1. Introduction

At present, heavy metals accumulating throughout the ecosystem are a massive environmental problem threatening the lives of all organisms. Cadmium (Cd) poses a particularly serious risk because it has highly reactive features (Astolfi et al., 2004; He et al., 2011). This element, found abundantly in soil and water although it is not an essential element for the growth and development of plants (Prasad, 1995); is absorbed easily by the roots and carried to the body and leaves (Lux et al., 2011; Gill et al., 2012).

Cd toxicity causes serious inhibitions in plants, such as growth inhibition, oxidative stress, changes in the concentrations of macro- and micro-elements and disruption of basic metabolic functioning, including photosynthesis, respiration, and nucleic acid production (Deng et al., 2010; Ali et al., 2015). In order to prevent or minimise these issues, plants are continuously developing new defence strategies. Immobilisation, vacuolar compartmentalisation, exclusion, synthesis of phytochelatins (PCs), and activation of antioxidant systems are just a few such strategies (Bai et al., 2015). In addition to these, various growth regulators and chemical materials applied externally can also make a distinct contribution to increasing the resistance of the plants under stress conditions. One of the materials with noted antioxidant features applied to the plants, especially in recent years, is cysteine (Cys). Cys, an essential amino acid, has various vital functions, from joining the structure of proteins to sustaining redox stability (Romero et al., 2014). As a result of containing thiol groups, Cys can easily react with reactive oxygen species besides bonding to metals, and can therefore inactivate them and protect

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plants against the exposed stress (Cooper et al., 2002; Genisel et al., 2015).

In the literature, there are a limited number of studies focusing on the effect of Cys applied exogenously to plants exposed to abiotic stress. In one study conducted by Genisel et al. (2015), it was stated that oxidative stress caused by salinity in barley was soothed with Cys application. In another investigation, N-acetylcysteine (NAC) application modulated Cd-based inhibitions in *Solanum nigrum* (Deng et al., 2010). Similarly, in the study conducted by Sun et al. (2014) it was reported that NAC application to two different types of barley genotypes exposed to Cd toxicity could remove detrimental effects of the stress by showing protective effect on the plants. In both of these works, the effect of Cys was generally attributed to its stimulating effect on the antioxidant system in addition to its own antioxidant features, however no study has been conducted on its influence on nitrogen metabolism, one of the most important metabolic processes in plants.

Nitrogen, joining the structures of various organic molecules, like amino acids and proteins, is one of the macro-essential elements affecting the growth and development of plants In the case of this element's deficiency, a decrease in plant productivity is observed along with restrictions in plant growth and development (Crawford, 1995; Zhang et al., 2011; Luo et al., 2013a, 2015). Plants generally obtain nitrogen through nitrate and ammonium ions (Hassan et al., 2008; Luo et al., 2013b). Nitrate ions taken in by plant are degraded to ammonium ions that then join the structure of organic molecules through catalysis by nitrate reductase (NR) and nitrite reductase (NiR) enzymes, key factors in nitrate metabolism (Fontaine et al., 2006; Mishra and Dubey, 2011). Ammonium ions are either integrated directly into the structure of amino acids via the GS/GOGAT circle or used as substrates to form glutamate via the reversible amination of 2-oxoglutarate through the catalytic effect of GDH enzyme (Mishra and Dubey, 2011; Luo et al., 2013a). Extensive reports are available on Cd-mediated regulation of enzymes and metabolites involved in nitrogen metabolism (Wang et al., 2008; Chaffei-Haouari et al., 2009). In these studies, it is generally put forth that nitrogen concentration decreases, nitrate intake is inhibited, and nitrogen assimilation-related enzymes are negatively influenced (Chaffei-Haouari et al., 2009; Khan et al., 2016). Therefore, modulation of nitrogen metabolism is crucial for the control of plant responses under conditions of Cd toxicity. That said, Cd-mediated changes are connected to the disruption of the carbon/nitrogen balance based on the inhibition of photosynthesis as Cd is a potential inhibitor of the photosynthesis process (He et al., 2013; Koc et al., 2013; Khan et al., 2016).

The production of nitrogen-containing metabolites, including glutathione (GSH) and PCs, has an important function in Cd tolerance. It is well-documented that Cys is a precursor of GSH and a robust heavy metal chelator as well a strong antioxidant (Vadas and Ahner, 2009; Genisel et al., 2015). In addition, the critical coordination among pathways for nitrogen and sulphur improve defence systems by upregulating defence metabolites under environmental stressors (Khan et al., 2016). Cys is produced as an end-product of sulphur assimilation and can also be used as a sulphur donor. Therefore, Cys may facilitate Cd tolerance through mediating the balance between the two major processes.

Bearing in mind the vital importance of nitrogen metabolism within primary plant metabolic processes, and taking into account that Cys is involved in protecting plants from certain environmental stressors, we have focused on determining whether Cys application could mitigate growth inhibition and oxidative stress induced by Cd toxicity and whether these effects could be associated with modulation of nitrogen metabolism-related gene expression and enzyme activity in maize leaves.

2. Material and methods

2.1. Experimental procedures and growth conditions

Maize seeds were surface-sterilized with 70% ethanol (3 min) and 2% sodium hypochlorite (5 min) and washed thoroughly in running tap water and finally with deionized water. The seeds were then imbibed in distilled water for 6 h and they were planted in pots. The seeds were germinated in a growth cabinet in dark at 25° C. Two days later the germinated seeds were transferred to a hydroponic media and they were grown in a growth room under controlled environmental conditions for 10 d (16/8 h light/dark cycle, day/night temperature of 25–27/20–22° C, and relative humidity of 60/70%). The 9-day old seedlings were separated into 4 different groups as control (distilled water), Cd (100 μM), Cys (200 μM) and their combination. The prepared solutions were added into hydroponic cultures and three days later the seedlings were harvested to make the further analyses.

2.2. The quantitative analysis of plant growth

The harvested seedlings were divided into root and leaves on all samples and their lengths were measured. Secondly, calculation of plant dry weight were based on weights determined before and after oven drying whole plant samples as 70 °C for 24–48 h. Total chlorophyll content was assayed using the method of Witham et al. (1971). Leaf examples were extracted in 80% acetone and the absorbance was measured at 645 nm and 663 nm. The amount of chlorophyll was calculated using the absorption coefficient and expressed as mg g^{-1} FW.

2.3. Assay of enzyme activities involving in nitrogen metabolism

NR activity was assayed by the reduction of nitrate to nitrite as reported by Barro et al. (1991). One unit of NR was calculated as the amount of enzyme required for production of 1 μmol nitrite per hour. NiR activity was defined by the reduction of nitrite to ammonium as described by Ida and Morita (1973). One unit of the NiR was defined as the amount of enzyme required for conversion of 1 μmol nitrite to ammonium per min. GS activity was determined according to the methods of Oneal and Joy (1973). One unit of GS was expressed as an amount of enzyme catalyzing the formation of 1 μmol of glutamylmonohydroxamate per min. GOGAT activity was determined by the conversion of 2-ketoglutarate to glutamate. One unit of the GOGAT was expressed as the amount of enzyme catalyzing the oxidation of 1 μmol NADH per min (Groat and Vance, 1981). GDH activity was determined by recording reduction of NAD (deaminating GDH activity, NAD-GDH) or the oxidation of NADH (aminating GDH activity, NADH-GDH) as described by the method of Groat and Vance (1981). One unit of GDH was calculated in units of μmol of NADH oxidized/NAD reduced per minute.

2.4. Determination of nitrate, nitrite and ammonium contents

The amount of nitrate in the leaves was spectrophotometrically estimated by the reduction of nitrate to nitrite via subsequent colorimetric determination by monitoring the absorbance at 410 nm as described by Cataldo et al. (1975). Nitrate content was calculated from a standard curve obtained by known concentrations of KNO_3. Nitrite content of leaves was assayed by using a protocol reported by Barro et al. (1991). Homogenate buffer was added instead of activation buffer as a difference from nitrate reductase determination method. Ammonium content was determined by measuring the absorbance changes at 620 nm as described previously by Brautigam et al. (2007). The ammonium