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Physiological and anthocyanin biosynthesis genes response induced by vanadium stress in mustard genotypes with distinct photosynthetic activity

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ABSTRACT

The present study aimed to elucidate the photosynthetic performance, antioxidant enzyme activities, anthocyanin contents, anthocyanin biosynthetic gene expression, and vanadium uptake in mustard genotypes (purple and green) that differ in photosynthetic capacity under vanadium stress. The results indicated that vanadium significantly reduced photosynthetic activity in both genotypes. The activities of the antioxidant enzymes were increased significantly in response to vanadium in both genotypes, although the purple exhibited higher. The anthocyanin contents were also reduced under vanadium stress. The anthocyanin biosynthetic genes were highly expressed in the purple genotype, notably the genes TT8, F3H, and MYBL2 under vanadium stress. The results indicate that induction of TT8, F3H, and MYBL2 genes was associated with upregulation of the biosynthetic genes required for higher anthocyanin biosynthesis in purple compared with the green mustard. The roots accumulated higher vanadium than shoots in both mustard genotypes. The results indicate that the purple mustard had higher vanadium tolerance.

1. Introduction

Heavy metal pollution of agricultural soils is one of the major environmental concerns, adversely affecting the yield and the quality of crops. Vanadium (V) is the 5th most abundant transition metal and is widely distributed (~0.01%) in the earth' crust (Amorim et al., 2007). Vanadium naturally exists in several mineral forms such as carnotite, patronite, chileite and vanadinite, with varied distribution ranges (3-310 mg kg^{-1}) in soils. Recently, accumulated evidence of the increasingly higher vanadium level in the atmosphere has raised concerns over its potential ecological hazard due to vanadium associated anthropogenic activities (Teng et al., 2011). Vanadium is considered as an active component of the vanadium biogeochemical cycle in the surface environment, due to its high mobility. Recent ecotoxicological reports

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have placed vanadium among the dangerous pollutants, in the same class as mercury and lead (Naeem et al., 2007; Baken et al., 2012).

The significance of vanadium for various biological functions, such as nitrogen-fixing bacteria and various soil fungi, has been identified very recently (Anke, 2004). Moreover, vanadium-associated toxicity affects living organisms, including plants (Qian et al., 2014; Tian et al., 2014a). Vanadium toxicity can seriously impair root and leaf morphology that may lead to complete growth inhibition and even plant death (Saco et al., 2012; Imtiaz et al., 2015). A recent study demonstrated that vanadium pollution of industrial red mud was responsible for noticeable chromosomal damages in pollen assembly and root cells of food crops (Misik et al., 2014).

Heavy metals induce formation of reactive oxidation species (ROS), superoxide anion (O^{2-}), hydroxyl (OH-) radicals, and H_2O_2 that in turn damage the chloroplast and the photosynthetic efficiency. The presence of H_2O_2 in the chloroplasts restricts the Calvin-cycle enzymes and reduces carbon assimilation (Takeda et al., 1995). These changes disrupt key cell membrane functions, such as the regulation of the peroxidation process of polyunsaturated fatty acids (De Vos et al., 1993) and the reduction in the oxidative damage caused by the formation of oxygen free radicals and/or by the reduction in the status of enzymatic and non-enzymatic antioxidants (Somashekaraiah et al., 1992; Shaw, 1995). Various plants exhibit a wide array of defense mechanisms in order to protect chloroplast and cellular membranes from ROS (Foyer and Harbinson, 1994). The plants have evolved an antioxidant defense system against stressors, including heavy metals. Superoxide dismutase (SOD) activates primary cellular defense response in the stressed plant. It catalyzes partitioning of superoxide radicals (O^{2-}) into H_2O_2 and O_2, whilst the accumulation of H_2O_2 in the cell membrane is restricted by catalase (CAT) (Noctor et al., 2002).

Anthocyanins are a flavonoid subclass abundantly present in several plant tissues. These water-soluble pigments are initially produced in the cytoplasm and are later transported into the vacuole (Jaakola et al., 2002; Gould, 2004). Anthocyanins are produced by plants in response to various stresses. They are often associated with enhanced stress tolerance in the plant and upregulation of the antioxidant defense system under stressful conditions (Neill et al., 2002; Merzlyak et al., 2008).

The anthocyanin biosynthesis pathway is one of the key components of flavonoid metabolism, and has been extensively studied in several plant species. The biosynthesis of anthocyanins is initiated by sequential enzyme reactions involving chalcone synthase (CHS), chaclone isomerase (CHI) and flavanone 3-hydroxylase (F3H) (Holton and Cornish, 1995; Winkel-Shirley, 2001). However, the enzyme dihydroflavonol 4-reductase (DFR) catalyzes the NADPH-mediated reduction of dihydroflavonols to leucoanthocyanidins as an immediate precursor of anthocyanidins (Lo Piero et al., 2006). In view of their importance in the regulation of plant stress, certain studies have examined genes that are related to the anthocyanin biosynthesis. Notably, CHS and DFR genes have been isolated from several higher plants and are relatively well characterized. The induction of CHS and DFR gene expression as well as that of other genes involved in anthocyanin biosynthesis has been reported under stress conditions, including stress caused by metal exposure (Zhang et al., 2014). However, a paucity of studies are available on the expression levels of genes involved in *Brassica* plants under heavy metal stress conditions, particularly vanadium exposure.

The ability of plants to accumulate heavy metals in their roots and aerial plant parts depends upon the type of metal and its concentration in the growth medium as well as the plant species (Sobukola and Dairo, 2007). The level of metal accumulation into different plant parts is fundamental in order to screen for their metal hypertolerance and/or exclusion (Sekhar et al., 2001). Mustard is a well-known model hyperaccumulator plant that effectively removes various metal contaminants. The diverse photosynthetic capacity of the mustard plant to detoxify ROS and to protect key chloroplast functions against metal induced oxidative stress highlights its wider applicability in metal contaminated soil. The mechanism of anthocyanin biosynthesis and composition, which is considered a key stress adaptive measure, in the mustard plant under vanadium stress is unknown.

To date, the dynamics of vanadium in plants are not fully known. The major objective of the present work was to study the physiological performance of genotypes that exhibited different levels of anthocyanins. Therefore, the objectives of the present study were to (1) to evaluate the influence of vanadium stress on anthocyanin accumulation in leaves of purple mustard plants, (2) to investigate the mechanism of the anthocyanin biosynthesis in the purple mustard, the induction of the expression of structural and regulatory genes in two genotypes, namely purple genotype (Ziyejiecai) and green genotype (G19). In addition, the current study aimed (3) to evaluate the effect of vanadium stress on the net photosynthetic rate (*P*n), stomatal conductance (*g*s), transpiration rate (*T*r) in mustard genotypes differing in photosynthetic capacity, and finally (4) to evaluate the expression levels of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in mustard genotypes with different photosynthetic activity.

2. Materials and methods

2.1. Plant material and growth conditions

Mustard (*Brassica juncea* var. gracilis) was used as a test plant in this experiment. Seeds of purple genotype (Ziyejiecai) and green genotype (G19) that differed in photosynthetic capacity were used. The seeds of selected mustard genotypes were surface sterilized and sown into the peat moss mixed plastic pots (170 mm–220 mm). At five leaf stage, seedlings of uniform size were selected and transferred into small plastic pots with plate holes. A total of 5 plants were maintained in each pot supplemented with half strength Hoagland nutrient solution. The pH of the nutrient solution was adjusted at 5.5 with NaOH and/or HCl. The hydroponic system was provided with continuous air flow using a pump equipped with a pre-filtered membrane. The hydroponic nutrient solution was renewed once a week. The plants were kept in a growth chamber at 22 ± 2 °C and relative humidity of 60–70%. A photoperiod of 14 h light with an average photon flux density of 820 μmol m^{-2} s^{-1} was maintained by an assembly of cool–white fluorescent lamps. The experimental design was conducted following completely randomized design (CRD) with three replicates.

Following an acclimatization period of 7 days, vanadium as ammonium metavanadate (NH_4VO_3) was added into full strength Hoagland nutrient solution according to the following 5 treatments: (1) control (without vanadium), (2) vanadium @ 20 mg L^{-1}, (3) vanadium @ 40 mg L^{-1}, (4) vanadium @ 80 mg L^{-1}, (5) vanadium @ 100 mg L^{-1}. Following 60 days, the plants were harvested for plant biochemical and molecular analysis, while prior to harvesting, photosynthetic parameters were recorded. The plants were washed and rinsed with Milli-Q water prior to separation into leaves, shoots, and roots. Fully expanded leaves were harvested, and placed in liquid nitrogen, and stored at −80 °C for further analysis. For the determination of vanadium, the plants were dried at 100 °C for 24 h, and then at 60 °C till constant weight.

2.2. Photosynthetic activity measurement

The photosynthetic parameters [net photosynthetic rate (*P*n), stomatal conductance (*g*s) and

transpiration rate (*T*r)] were measured using the portable photosynthesis system (LiCor-6400 LI−COR Inc., Lincoln, NE, USA). These measurements were recorded on the upper leaf of the main branch that was expanded to the maximum length. The growth conditions of the cabinet at the time of measurement were as follows: photosynthetic active photon 1000 μmol m^{-2} s^{-1}, relative humidity 60 ± 3%, temperature 20 ± 2 °C. A total of 3 readings per treatment were taken