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Potassium starvation induces oxidative stress in *Solanum lycopersicum* L. roots

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ABSTRACT

The relationship between potassium deficiency and the antioxidative defense system has received little study. The aim of this work was to study the induction of oxidative stress in response to K^+ deficiency and the putative role of antioxidants. The tomato plants were grown in hydroponic systems to determine the role of reactive oxygen species (ROS) in the root response to potassium deprivation. Parameters of oxidative stress (malondialdehyde and hydrogen peroxide (H_2O_2) concentration), activities of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and glutathione reductase (GR)) and antioxidant molecules (ascorbate (ASC) and glutathione) were investigated. H_2O_2 was subcellularly located by laser confocal microscopy after potassium starvation in roots. During the first 24 h, H_2O_2 induced the cascade of the cellular response to low potassium, and ROS accumulation was located mainly in epidermal cells in the elongation zone and meristematic cells of the root tip and the epidermal cells of the mature zones of potassium starved roots. The activity of the antioxidative enzymes SOD, peroxidase and APX in potassium deprivation significantly increased, whereas CAT and DHAR activity was significantly depressed in the potassium starvation treatment compared to controls. GR did not show significant differences between control and potassium starvation treatments. Based on these results, we put forward the hypothesis that antioxidant molecule accumulations probably scavenge H_2O_2 and might be regenerated by the ASC–glutathione cycle enzymes, such as DHAR and GR.

Introduction

Potassium is an essential macronutrient involved in plant development and growth, acting as a cofactor in several key enzymes of different metabolic pathways (Marschner, 1995; White and Karley, 2010). It has been proposed that potassium starvation signaling is mediated by many components, including hormones (Armengaud et al., 2004; Jung et al., 2009), Ca^{2+} permeable ion channels (White et al., 2002; White and Broadley, 2003), Ca^{2+} sensors (Amtmann and Blatt, 2009; Luan et al., 2009), membrane potential (Nieves-Cordones et al., 2008) and reactive oxygen species (ROS) (Wang and Wu, 2010; Aleman et al., 2011; Shin et al., 2005; Schachtman and Shin, 2007). Under potassium starvation, hydrogen peroxide (H_2O_2) is produced and it accumulates in roots (Shin and Schachtman, 2004; Shin et al., 2005; Kim et al., 2010), and probably leads to oxidative stress. However, increasing evidence indicates that H_2O_2 functions as a signaling molecule in plants. The generation of H_2O_2 because of potassium starvation seems to be mediated by a PM-bound NADPH oxidase complex (Shin and Schachtman, 2004). If H_2O_2 generation is prolonged by potassium starvation, oxidative stress can be induced, altering the cellular redox homeostasis. ROS accumulation induces oxidative damage of membrane lipids, nucleic acids and proteins (Mittler, 2002). Therefore, tight control of steady-state of concentration of ROS appears to be necessary to avoid oxidative damage at subcellular levels, while simultaneously allowing ROS to perform useful functions such as signaling under stress (Hernandez et al., 2010). H_2O_2 is a versatile molecule that may be involved in several cell processes under normal and stress conditions (Quan et al., 2008).

The antioxidative system includes antioxidant compounds such as carotenoids, ascorbate (ASC), reduced glutathione (GSH), α-tocopherol and several enzymes that are involved in the detoxification of ROS. These enzymes include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX) and

Abbreviations: APX, ascorbate peroxidase; ASC, ascorbate; CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; POX, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.

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glutathione reductase (GR). The primary scavenger in the detoxification of ROS in plants is SOD, which converts superoxide to H_2O_2 and molecular oxygen (Scandalios, 1993). Superoxide radicals are toxic by-products of oxidative metabolism. The SOD protein family can be divided into Cu, Zn-SOD, Mn-SOD or Fe-SOD isoforms, depending on the metal present in the active site. APX is the most important POX in detoxifying H_2O_2, catalyzing the reduction of H_2O_2 to water (Foyer, 1996). APX, together with monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and GR, remove the H_2O_2 through Foyer–Halliwell–Asada pathway (Foyer and Halliwell, 1976; Halliwell, 1987). CAT can also reduce H_2O_2 to water, but it has a lower affinity for H_2O_2 than APX (Graham and Patterson, 1982). POXs are involved in several cellular processes. It is well known that POXs participate in lignin biosynthesis (Lee et al., 2007), but they can also act as a plant defenses, eliminating H_2O_2 under oxidative stress conditions (Olmos and Hellin, 1996). Interestingly, a POX has been described to contribute to ROS production in response to potassium deficiency (Kim et al., 2010).

On the other hand, antioxidant molecules like ASC and reduced GSH are involved in many metabolic cell pathways (Noctor and Foyer, 1998). ASC can react with ROS, as ($O_2{}^1$), ($HO^\bullet$) and ($O_2{}^-$) and can act as the substrate for the enzyme APX (Noctor and Foyer, 1998). GSH acts as a cell redox regulator and may act as a ROS scavenger. The balance between GSH and oxidized glutathione (GSSG) is critical for keeping a favorable redox status for the detoxification of H_2O_2. Once the generation of ROS exceeds these avoidance and scavenging mechanisms, oxidative stress occurs and causes damage to cellular components, such as lipids and proteins.

The majority of the studies on potassium deprivation have been performed at short or long starvation periods. In addition, most studies have focused on the characterization of ROS production and the changes in the expression levels of some genes that may be involved in the regulation of ROS production in response to K^+ deprivation. However, little information is available on the effect that K^+ starvation produces on the antioxidative system (Shin and Schachtman, 2004; Shin et al., 2005; Kim et al., 2010). In our study, we analyze the time course of ROS production in potassium-starved tomato seedlings (*Solanum lycopersicum* L.) and the role of the antioxidative system at short and long potassium deprivation times.

Materials and methods

Plant material and growth conditions

Tomato plants (cv. San Pedro; this cultivar is frequently grown in the south of Spain) were grown as described previously (Fernandez-Garcia et al., 2004, 2009). Seeds were pre-hydrated with continuously aerated, de-ionized water for 12 h. After this, the seeds were germinated in vermiculite at 28 °C. They were-transferred to a chamber with controlled environmental conditions: 16/8 h light/dark cycle and an air temperature of 25 °C (day) and 20 °C (night). The relative humidity was 65% (day) and 80% (night). The photosynthetically active radiation (PAR) was 400 μmol m^{-2} s^{-1}. After 7 days, the seedlings were placed in 15-L containers with a modified one-fifth Hoagland nutrient solution, which consisted of the following macronutrients (mM): 1.4 $Ca(NO_3)_2$, 0.2 KCl, 0.2 $MgSO_4$ and 0.1 $Ca(H_2PO_4)_2$ and the following micronutrients (l M): 50 $CaCl_2$, 12.5 H_3BO_3, 1 $MnSO_4$, 1 $ZnSO_4$, 0.5 $CuSO_4$, 0.1 H_2MoO_4, 0.1 $NiSO_4$ and 10 Fe-EDDHA. The pH of the nutrient solutions was adjusted daily to 5.5. In the containers in which NaCl was not added, the Na^+ concentration was around 200 μM, as determined by atomic emission spectrophotometry. Six plants were grown per container.

For K^+ starvation, 21-day-old plants were transferred for 6 and 12 h, and 1, 2, 4 and 7 days to a nutrient solution that was deprived of K^+. The nutrient solution deprived of K^+ contained (mM): 1.4 $Ca(NO_3)_2$, 0.2 $MgSO_4$ and 0.1 $Ca(H_2PO_4)_2$ and the micronutrients described above. To maintain the K^+ supply during plant growth, the K^+ concentration in the containers was determined daily by atomic absorption spectrometry and corrected as needed. For determinations, roots were detached and washed with deionized water and frozen in liquid nitrogen.

Enzyme extraction

Frozen root samples were ground in a mortar with liquid nitrogen and extracted (1:2 w/v) in 50 mM K-phosphate buffer (pH 7.8), containing 0.5% (w/v) PVP, 0.1 mM PMSF, EDTA. Na 0.1 mM and 0.2% (v/v) Trition X-100. For ascorbate peroxidase (APX) activity, 20 mM ascorbate (ASC) was added and EDTA-Na was omitted. All of the following operations were performed at 4 °C. The homogenate was centrifuged at 4800 × *g* for 10 min. The supernatant fraction was filtered on Sephadex G-25 NAP columns (Amersham Pharmacia Biotch AB, Uppasala, Sweden), equilibrated with the same buffer used for the homogenization. The samples were concentrated in centrifugal filter devices (Amicon Ultra).

Enzyme assays

Total superoxide dismutase activity was measured according to McCord and Frodovich (1969) by the ferricytochrome c method, using xanthine/xanthine oxidase as the source of superoxide radicals. The catalase activity was assayed by measuring the initial rate of hydrogen peroxide (H_2O_2) disappearance at 240 nm (Aebi, 1984). Peroxidase activity in tomato roots was determined in assays containing 50 mM Tris–acetate buffer (pH 5.0), 0.5 mM H_2O_2 and 1.0 mM 4-methoxy-ά-naphtol (ε_{595} = 21600 M^{-1} cm^{-1}). The reaction was initiated by the addition of enzyme. Controls were carried out in the absence of H_2O_2 and the presence of 5.0 mM KCN (Barceló, 1998).

APX activity was determined in a mixture containing 50 mM potassium phosphate (pH 7.0), 1.5 mM ASC, 1.0 mM H_2O_2 and enzyme extract (Saher et al., 2004). Activity was determined by following the H_2O_2-dependent decomposition of ASC at 265 nm.

Dehydroascorbate reductase was determined as described by Saher et al. (2004). Total glutathione reductase (GR) activity was determined by following the rate of NADPH oxidation, as measured by the decrease in the absorbance at 340 nm (Edwards et al., 1990). The reaction rate was corrected for the small, non-enzymatic oxidation of NADPH by glutathione (GSH). Total protein content was estimated according to Bradford (1976).

Lipid peroxidation

The level of lipid peroxides was determined as malondialdehyde (MDA) content by the thiobarbituric acid (TBA from Sigma–Aldrich) reaction, as described by Saher et al. (2004). One gram of tissue was homogenized in 5 ml 0.1% (w/v) trichloroacetic acid (TCA from Sigma–Aldrich). The homogenates were centrifuged at 10,000 × *g* for 5 min and 1.2 mL 20% TCA containing 0.5% (w/v) TBA were added to a 0.4 mL aliquot of the supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. The contents were centrifuged at 10,000 × *g* for 15 min and the absorbance was measured at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Ascorbate and glutathione extractions

Root samples were ground in a mortar with liquid nitrogen and homogenized with two volumes of cold 5% metaphosphoric acid