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Does methyl jasmonate modify the oxidative stress response in *Phaseolus coccineus* treated with Cu?

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ABSTRACT

The contribution of methyl jasmonate (MJ) as a signal molecule able to take part in the defense mechanism against copper (Cu)-imposed oxidative stress was studied in the leaves and roots of runner bean (*Phaseolus coccineus*) plants. Roots of plants cultivated hydroponically were preincubated in MJ (10 μM) for 1 h or 24 h and subsequently exposed to Cu (50 μM) for 5 h (short-term experiment) or 5 days (long-term experiment). Enzymatic (activity of superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; guaiacol peroxidase, POX) and non-enzymatic (accumulation of malondialdehyde, MDA; homoglutathione, hGSH; proline; anthocyanins; low molecular weight organic acids, LMWOAs) responses were determined in the leaves and roots. The antioxidative defense mechanism was significantly activated after Cu supplementation. In most cases, activities of ROS (reactive oxygen species) scavenging enzymes like SOD, CAT, APX, POX, as well as MDA, hGSH and proline concentrations increased following Cu exposure. MJ showed a time-dependent effect on antioxidative enzymes activity. In the short-term experiment, MJ elevated CAT, APX and POX activities in the roots, and POX activity in the leaves of non-Cu-treated plants. In the long-term experiment, MJ not only decreased POX and partially CAT activity in the roots, but also increased the MDA level and partially CAT activity in the leaves of the control plants. In Cu-treated plants, MJ reduced APX, but elevated POX activity in the leaves after 5-h exposure. After 5-day-Cu treatment, MJ inhibited POX activity in the leaves and mainly reduced SOD and CAT activities in the roots. Moreover, in the long-term experiment, MJ reduced tartrate and pyruvate in the leaves of Cu-stressed plants, but mostly elevated tartrate and malate in the roots comparing with Cu alone treatment. MJ alone and under Cu excess did not alter accumulation of MDA, hGSH and proline comparing with Cu alone, but partially elevated anthocyanin concentration. The results indicated that MJ was both partially potent in modifying the antioxidative enzymes activity and metabolites accumulation in non-stress and Cu-stress conditions.

1. Introduction

Under normal environmental conditions, reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$) are generated mainly in the chloroplasts, mitochondria, peroxisomes, plasma membranes and cell walls as important signaling intermediates, which regulate stress response in plants (Demidchik, 2015). However, exaggerated production of ROS, generated by various stress factors including heavy metals, cause oxidative stress resulting in disturbance of metabolic pathways and damage of nucleic acids, carbohydrates, proteins and lipids (Demidchik, 2015). ROS excess influences also the integrity of cellular membranes (increasing the secondary products, such as malondialdehyde, MDA, the cytotoxic product of polyunsaturated fatty acids disintegration) and finally leads to the cell and the whole plant dysfunctions (Strubińska and Hanaka, 2011).

Plants developed sophisticated defense mechanism of enzymatic antioxidants and oxidative stress-related metabolites, to fight against elevated ROS levels and scavenge them from the cells. The most important enzymatic antioxidants include: superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and guaiacol peroxidase (GPX, EC 1.11.1.7). SOD is a metalloenzyme divided into three classes on the

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; H_2O_2, hydrogen peroxide; hGSH, homoglutathione; hPC, homophytochelatin; LMWOAs, low molecular weight organic acids; MDA, malondialdehyde; MJ, methyl jasmonate; $O^{2\bullet -}$, superoxide anion radical; POX, guaiacol peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid

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basis of metal cofactor (Mn, Fe, Cu/Zn) and is localized in chloroplasts, mitochondria, peroxisomes and cytosol (Demidchik, 2015). SOD is believed to play the first line of defense via catalyzing the dismutation of $O_2^{\bullet -}$ into H_2O_2 and O_2. Then, the level of toxic H_2O_2 is degraded to water and molecular oxygen by concerted action of CAT and peroxidases (Prxs). CAT is a tetrameric homoprotein, it exists in multiple isozymes and is located mostly in peroxisomes and glyoxisomes (Soares et al., 2010). Prxs are homoproteins and exist in multiple isozymes found in many cell compartments. APX is primarily located in chloroplasts, mitochondria and cytosol, and GPX in vacuoles, cytosol and the cell wall (van Doorn and Ketsa, 2014). Unlike CAT, Prxs require a reducing substrate, such as ascorbate or guaiacol, to work.

The most important oxidative stress-related metabolites include: glutathione (GSH) or its homolog homoglutathione (hGSH), proline, anthocyanins and low molecular weight organic acids (LMWOAs). The presence of GSH and/or hGSH is plant species-dependent and in *Phaseoleae* only hGSH is found (Tukendorf et al., 1997). hGSH is a low-molecular weight tripeptide (γ-Glu-Cys)-β-Ala, which is one of the sources of non-protein tiols in plant cells and it is also the direct substrate for homophytochelatins (hPCs) formation, for example under Cd-treatment (Tukendorf et al., 1997). An amino acid proline can take part in signaling, metal binding, antioxidant defense and eliminating ROS imposed damages caused by heavy metals (Szafrańska et al., 2011). Environmental stresses, such as wounding, drought, nutrient deficiency, light of high intensity stimulate accumulation of anthocyanins, a class of flavonoids, which fulfill a protective function for the cell structures (Mancinelli, 1984). Organic acids, especially malate, oxalate and citrate, as cellular chelators are involved in metal detoxification (Clemens, 2001). They modulate plant adaptation and usually increase in the adverse environmental conditions.

Methyl jasmonate (MJ), belonging to a class of cyclopentanone compounds, is a naturally and ubiquitously occurring plant growth regulator or phytohormone involved in a signal transduction pathway and plant response to environmental stressors (Wasternack, 2007; Yan et al., 2013; Chen et al., 2014). MJ could be a potent molecule in enhancement of plant tolerance against various stresses (Wasternack, 2007). It can influence production of antioxidant defense enzymes and secondary metabolites as well as it induces oxidative burst and H_2O_2 generation through activation of NADPH oxidase (Maksymiec and Krupa, 2006). Until now there is no consensus on the contribution of MJ to oxidative stress since on the one hand, it induces both the production of H_2O_2 and ascorbate (Jubany-Marí et al., 2010), but on the other hand it increases H_2O_2 content collaterally reducing ascorbate content (Hung and Kao, 2004). The latter could be the reason for ROS and antioxidants imbalance-mediated oxidative stress (Jubany-Marí et al., 2010). Moreover, the plants react ambiguously either increasing (Afab et al., 2011) or decreasing (Li et al., 2012) antioxidative enzyme concentrations under MJ-treatment. Copper (Cu) is an essential micronutrient for plant growth and development, but when present in excess, Cu becomes cytotoxic because of its redox properties and ability to produce ROS through Fenton and Haber-Weiss-type reaction and launch oxidative damage. Since Cu is widely spread contaminant in the environment, it is vital to broaden our understanding of the possible biochemical detoxification mechanisms. Therefore it is worth examining its relationship with other molecules, such as MJ, in antioxidative plant response.

The holistic crosstalk between enzymatic antioxidants and metabolites related to the oxidative stress in shoots and roots of plants exposed to MJ and subsequently metal-treated is still to be elucidated. To our knowledge, studies on the effect of exogenous MJ supplementation in Cu-stressed plants are still insufficient and there are no data compiling MJ influence on Cu stress, especially in plants at early growth stage. The present work was carried out to investigate the biochemical, enzymatic and metabolic responses induced by MJ under Cu excess in *Phaseolus coccineus* leaves and roots. Cu and MJ concentrations were chosen on the basis of the growth parameters, as previously published by Hanaka et al. (2015).

2. Material and methods

2.1. Plant material and experimental design

Runner bean plants (*Phaseolus coccineus* L. cv. Piękny Jaś) were grown hydroponically in pots filled with 1.4 L of Hoagland nutrient solution (5 plants per pot). The plants were cultivated in a growth chamber at 24/18 °C day/night temperature under a 16 h photoperiod and photosynthetic photon flux density of 120 μmol m^{-2} s^{-1}. MJ (95%) was used at the final concentration of 10 μM and Cu was used as $CuSO_4 \times 5$ H_2O at the final concentration of 50 μM. Both MJ and Cu were supplemented to the Hoagland solution. In the short-term experiment, 4 day-old plants were preincubated with MJ for 24 h, then transferred to the fresh Hoagland nutrient solution with or without addition of Cu^{2+} (MJ24Cu or MJ24, respectively) for 5 h. In the long-term experiment, 4 day-old plants were preincubated with MJ for 1 h or 24 h, then transferred to the fresh Hoagland nutrient solution (MJ1 and MJ24, respectively) or to the solution supplemented with Cu^{2+} (MJ1/Cu and MJ24/Cu, respectively) for 5 days. In both experiments, control (non-Cu-treated) and Cu-treated plants were cultivated under the same conditions without preincubation with MJ. After 5 h or 5 days of Cu treatment the leaves and roots of plants were harvested and fresh analyzed for hGSH, proline, anthocyanins concentrations, H_2O_2 and $O_2^{\bullet -}$ visualization, and root viability or frozen in liquid nitrogen for subsequent analyzes of SOD, CAT, APX, POX, MDA and LMWOAs.

2.2. Protein and enzymatic antioxidant determinations

Proteins were assayed according to Bradford (1976), using bovine serum for calibration. The enzymatic assays of SOD (presented as U mg^{-1} protein) and CAT, APX, POX (as $\Delta Amin^{-1}$ mg^{-1} protein) were performed spectrophotometrically at room temperature. 0.5–1 g of leaves or roots was taken for homogenization.

Determination of SOD activity was conducted as described by Verma and Dubey (2003). The plant material was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% PVPP. The homogenate was centrifuged at 16,000*g* at 4 °C for 20 min and the supernatant was applied for the SOD assay. The reaction mixture was composed of EDTA, carbonate-bicarbonate buffer (pH 10.2), epinephrine and enzyme extract. SOD activity was analyzed at 475 nm. One unit (1 U) expressed 50% inhibition of epinephrine oxidation.

Extraction for CAT, APX and POX analyzes was conducted according to Milosevic and Slusarenko (1996). The plant samples were homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1% Triton X-100 and 1% PVP-40 (w/v). The homogenate was centrifuged at 16,000*g* at 4 °C for 20 min and the supernatant was used for assays. CAT activity was measured at 240 nm according to Aebi (1984). The reaction mixture contained 10 mM H_2O_2 solution in 50 mM phosphate buffer (pH 7.0) and enzyme extract. APX activity was determined at 290 nm after Nakano and Asada (1987). The reaction mixture consisted of 25 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.2 mM H_2O_2, 0.1 mM EDTA and enzyme extract. POX activity was determined at 470 nm as described by Milosevic and Slusarenko (1996). The reaction mixture contained 100 mM phosphate buffer (pH 6.25), 0.012% guaiacol, 0.03% H_2O_2 and enzyme extract.