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CHEMICAL STRESS PROMOTES THE TRANSIENT REDUCTION OF THE LEVELS OF 2-CYS PEROXIREDOXIN IN *Ricinus communis* PLANTS*

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ABSTRACT

Jasmonates are signaling molecules that play a key role in the regulation of metabolic processes, reproduction and defense against insects and pathogens. This study investigated the effects of methyl jasmonate on the protein pattern of *Ricinus communis* plants and the activity of guaiacol peroxidase, an antioxidant enzyme. Methyl jasmonate treatment caused a transient reduction in guaiacol peroxidase activity. A similar response was observed for the levels of 2-Cys peroxiredoxin protein. Moreover, the levels of the small and large chains of Rubisco were also reduced. The transient reduction of the levels and activity of antioxidant enzymes could account for the increase in the levels of H_2O_2, an important signaling molecule in plant defense.

INTRODUCTION

Plants respond to wide range of environmental stresses (such as high and low temperatures, drought, salinity, UV or ozone stress and pathogen infections) through synthesis of an array of defense proteins that facilitate wound healing and provide protection against further wounding or pathogen attacks [3]. A common aspect of all these adverse conditions is the enhanced production of jasmonates and reactive oxygen species (ROS) within several sub cellular compartments of the plant cell [5,10]. Aside from their destructive nature, ROS can also be used in a beneficial way by the plant [11]. ROS play an important role in inducing protection mechanisms during both biotic and abiotic stresses. The term jasmonate is used for the cyclopentanone compound jasmonic acid (JA), its methyl ester (JAME) and derivatives such as JA amino acid conjugates. The production of JAs leads to the induction of many genes [12], including those for vegetative storage proteins, a thionin, and plant defensin. JAs also induce transcription of genes that regulate JA synthesis [4]. The understanding of jasmonate signaling is complicated by the presence of multiple acyclic or cyclic oxidation products derived from the catabolism of fat acids. The activity of jasmonates as signal for defense suggests that host responses to attackers may be regulated by a complex mix of signals, which has been termed the oxylipin signature-3. It has been proposed that JA is formed through octadecanoid pathway, which is initialized by systemin, a plant hormone [2]. Recently, it was demonstrated that systemin potentiates the oxidative burst in cultured tomato cells [3]. These results permitted Orozco-Cárdenas et al. [3] to conclude that JA activates the signal pathway genes (early genes) in the vascular bundles, whereas H_2O_2, produced by cell wall–derived oligogalacturonides released by PG is a second messenger that activates defense genes (late

genes) in mesophyll cells. Excessive levels of ROS result in damage to the photosynthetic apparatus (photoinhibition) ultimately leading to severe cellular damage and chlorosis of the leaves. In this study, we report that methyl jasmonate treatment promotes a degradation of Rubisco enzyme, a transient reduction of the levels of 2-Cys peroxiredoxin, as well as transient reduction of the levels of guaiacol peroxidase activity that may be involved in the removal of H_2O_2.

MATERIALS AND METHODS

Castor bean (*Ricinus communis* L., cultivar IAC-226) seeds were germinated in plastic pots containing a mixture of soil and vermiculite (3/1, v/v) under controlled conditions in a greenhouse. Plants at the three-leaf were sprayed with 0.125 (v/v) of methyl jasmonate (MJ) in 0.1% of triton X-100. For control plants, MJ was omitted. At specific intervals after MJ treatment (1, 2, 6, 12, 24 and 48 h), primary leaves of each plant were harvested, frozen immediately in liquid N_2 and stored at –70ºC until extracted. These leaves were ground in the presence of 10% (w/w) polyvinylpolypirrolidone and proteins were extracted with phosphate buffer (49 mM Na_2PO_4, 22 mM K_2PO_4, 68 mM NaCl, 0.2 % β-mercaptoethanol, 1 mM PMSF, 2 mM EDTA, pH 7.0). The slurry was centrifuged at 14,000g for 30 min. The supernatant was centrifuged at 30,000g for 30 min. Protein concentration was determined according to Bradford [7] using ovalbumin as standard. Total soluble proteins extracts were resolved by SDS-PAGE 12.5% or in the presence of tricine (16% tricine-SDS-PAGE). The proteins resolved by SDS-PAGE under both conditions were transferred to PVDF membranes. The N-terminal sequences of determined protein bands on PVDF were analyzed on a Shimadzu PPSQ-10 automated protein sequencer. The sequences selected were submitted to automatic alignment. Densitometric analysis was determined [6]. **Peroxidase activity**, (Guaiacol peroxidase, GPX; EC 1.11.1.7) with guaiacol and H_2O_2 as substrate, was assayed by following the increase in absorbance at 470 nm using a modified procedure of Cakmak and Horst [1]. The assay mixture contained 25 mM phosphate buffer (pH 6.0), 2.58 mM guaiacol, 10 mM H_2O_2 in a total volume of 1 mL. The reaction was initiated by the addition of 10 μg of total protein and the increase in absorbance at 470 nm was measured for 5 min. All determinations were made in triplicate. For gel activity, proteins extracts were resolved by native-continuous-PAGE pH 8.8 using 25 mM Tris, 192 glycine, pH 8.3 as running buffer. After electrophoresis, the gel was washed three times for 5 min with 25 mM phosphate buffer (pH 6.0) and incubated with the assay mixture described above. The colored bands (due guaicol tetramerization) were identified.

RESULTS AND DISCUSSION

Recent biochemical and genetic studies confirm that hydrogen peroxide is a signaling molecule in plants that mediates responses to abiotic and biotic stresses. Thus, in order to investigate the effects

of MJ upon antioxidant enzymes of *R. communis* plants, we determined the peroxidase guaiacol activity in crude leaf extracts. Figure 1 shows that the exposure to MJ vapors decreases the guaiacol peroxidase activity in leaf extracts, when compared with untreated control plants. This effect was stronger during the first hour of treatment and the enzymatic activity returned to ~60% of the original activity after12 h of treatment. A similar behavior was also observed when the guaiacol activity was assayed in a gel after native PAGE (Fig.1 inset). The leaf protein patterns from untreated and treated plants were also investigated in order to obtain any clue about the possible biological functions of the MJ-induced proteins. Therefore, partial amino acid sequences were determined from some proteins. The best resolution of proteins smaller than M_r 20,000 was achieved using a 16% Tricine-SDS-PAGE system (Fig. 2) and the following results were obtained: a) increase of some proteins with molecular weight larger than 20 kDa; b) increase of a protein with 13.5 kDa, denoted RC-MJ13; c) reduction of a protein with 11 kDa (called RC-MJ11) .The N-terminal partial sequence of RC-MJ11 showed a high degree of homology to Rubisco –Small Chain from several sources. A conventional Laemmli denaturing gel system was applied to resolve larger proteins (Fig. 3 A). This figure shows that the levels of some proteins with ~50 kDa were reduced. A particular behavior was observed for one protein of 21 kDa (called RC-MJ21), since its level in control plants was 3.6% of the total protein content and after 1 and 6 h exposure to MJ, its level was reduced to 2.0% and 1.5% respectively. However, the level of this protein began to increase after 12 h of treatment as indicated by densitometric analysis (Fig. 3 B). The N-terminal partial sequence of RC-MJ21 showed high degree of homology with 2-Cys peroxiredoxin, a thiol-specific antioxidant protein, from several sources.

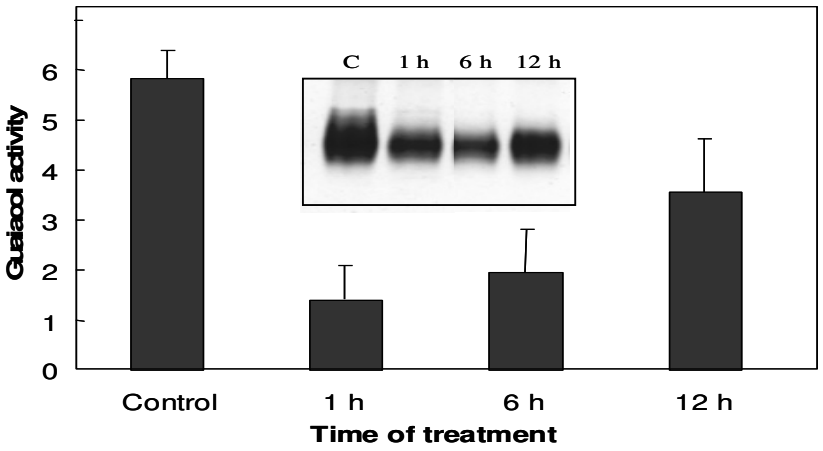

Figure 1. Effect of methyl jasmonate exposure on guaiacol peroxidase activity in *R. communis* leaves. Peroxidase specific for guaiacol is defined as ΔABS 470nm.min^{-1} μg^{-1} of protein. Inset: native gel stained for guaiacol peroxidase of R. *communis* leaves. Equal amounts of protein (50 μg) were loaded onto the gel. Lane C – extracts from control leaves. Extracts from plants exposed

to methyl jasmonate during different times (lanes 1 h, 6 h and 12 h).

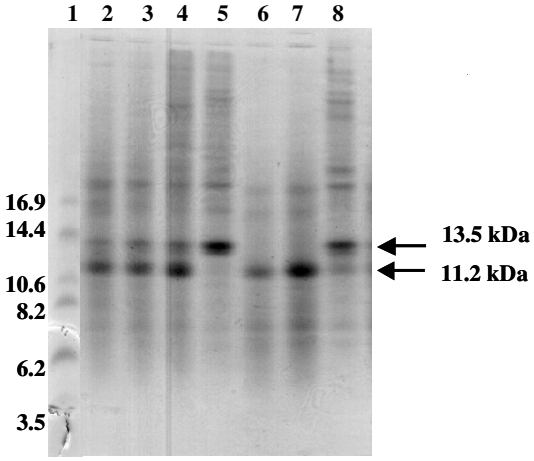

Figure 2. (SDS-Tricine-PAGE 16%) of crude protein extracts from *R. communis* leaves. Lanes 1, molecular weight markers; 2, leaves from control plants; 3- 6, leaves from methyl jasmonate treated plants after 1, 2, 6, and 12 h respectively; 7, leaves from control plants; 8, leaves from methyl treated plants after 24 h. For each lane, 100 µg of protein were loaded. The gel was stained with Coomassie-brilliant blue.

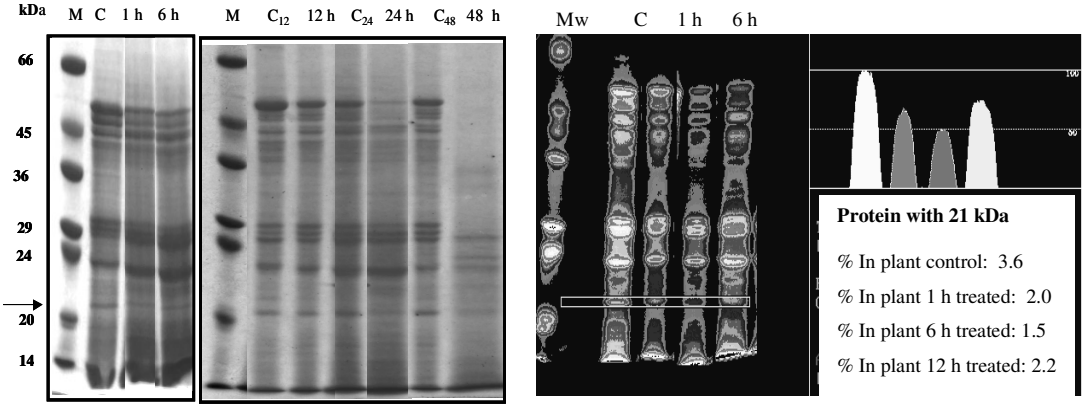

Figure 3. Analysis of protein pattern of *R. communis* plants exposed to methyl jasmonate. A, (SDS-PAGE 12%) of crude leaves protein extracts. Lanes C, C12, C24 and C48, leaves from control plants; 1 h, 6 h, 12 h, and 48 h, leaves from methyl treated plants. M, molecular weight markers. For each lane, 100 µg of protein were loaded. The gel was stained with Coomassie-brilliant blue. B, densitometric analysis of the protein with 21 kDa (boxed area). Lanes C, leaves from control plants; 1 h, 6 h and 12 h, leaves from methyl-treated plants, respectively. For each lane, 100 µg of protein were loaded. The gel was stained with Coomassie-brilliant blue.

CONCLUSIONS

- To our knowledge, this is the first identification of 2-Cys-Prx in *R. communis* plants and its physiological role needs to be investigated. However the role of 2-Cys PRX in antioxidant defense in photosynthesis, respiration, and stress response, and modulating redox signaling during development and adaptation has been observed in *Arabidopsis thaliana* , poplar and in barley;
- It is well established that MJ alters the expression of genes of several plant species. The present

investigation documents the participation of MJ in the reduction of the levels of the small chain of Rubisco. The synthesis of proteins involved in photosynthetic carbon assimilation has been demonstrated to be reduced or even shut down by treatment with MJ [8,9]. These alterations ultimately lead to characteristic senescence symptoms. As mentioned above, Prxs are known to be involved in protection against oxidative damage and, as such, we believe that degradation of Rubisco could be promoted by H_2O_2 accumulation that could be caused by the early reduction of the levels of the 2-Cys PRX and reduction of guaiacol peroxidase activity observed in *R. communis* leaves after the methyl jasmonate treatment.

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