Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Physiology and Biochemistry 43 (2005) 1082–1088
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Original article

Response of ascorbate peroxidase isoenzymes and ascorbate regeneration system to abiotic stresses in *Cucumis sativus* L

Xing Shun Song [a], Wen Hai Hu [a], Wei Hua Mao [a], Joshua Otieno Ogweno [a], Yan Hong Zhou [a], Jing Quan Yu [a,b,*]

[a] *Department of Horticulture, Huajiachi Campus, Zhejiang University, Kaixuan Road 268, Hangzhou, China 310029*
[b] *Key Laboratory of Horticultural Plants Growth, Development and Biotechnology, Agricultural Ministry of China, Kaixuan Road 268, Hangzhou, China 310029*

Received 9 June 2005
Available online 05 December 2005

Abstract

Ascorbate peroxidase (APX) isoenzymes, distributing in at least four distinct cell compartments, the chloroplastic stroma (sAPX) and thylakoid membrane (tAPX), microbody (mAPX) and cytosol (cAPX), catalyze the reduction of H_2O_2 to water by using ascorbic acid (AsA) as specific electron donor. In order to better clarify the response of APX isoenzymes and AsA regeneration enzymes to abiotic stresses, the activities of APX isoenzymes as well as monodehydroascorbate reductase (MDAR), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) were investigated in cucumber plants after heat, methyl viologen (MV) and H_2O_2 treatments. The activities of cAPX, sAPX, mAPX increased after a slight decline throughout the experiment. Consistent closely with sAPX activity, the expression of sAPX followed a similar change pattern, indicating that sAPX was regulated at the transcriptional level. In contrast, constitutive expression was observed in tAPX activity and no significant changes in tAPX activity were found throughout the experiment. The increases in MDAR and GR were accompanied with enhanced level of AsA/DHA, implying that the AsA regeneration system plays an essential role in compensating AsA degradation.

Keywords: Ascorbate peroxidase; Ascorbate regeneration system; Isoenzyme; Stress

1. Introduction

Molecular oxygen is essential for the existence of aerobic organisms including plants. However, toxic reactive oxygen species (ROS) such as the superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, are formed in all aerobic cells as by-products of normal metabolic processes [2], especially under environmental stresses, resulting in oxidative damage at the cellular level. Meanwhile, plants contain a series of nonenzymatic antioxidants such as ascorbate, glutathione, flavonoids and carotenoids and enzymatic antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX; EC 1.11.1.11), and catalase. These antioxidants function properly to interrupt the cascades of uncontrolled oxidation in some organelles and to scavenge the toxic ROS and then protect the plants from ROS damage [21].

APX plays an important role in eliminating H_2O_2 by utilizing ascorbate (AsA) as its specific electron donor to reduce H_2O_2 to water with the concomitant generation of monodehydroascorbate (MDHA), which is converted to AsA by MDHA reductase (MDAR) or disproportionated nonenzymatically to AsA and dehydroascorbate (DHA). DHA is reduced to AsA by the action of dehydroascorbate reductase (DHAR), using reduced glutathione (GSH) as the reducing substrate [21]. This reaction generates oxidized glutathione (GSSG), which is in turn re-reduced to GSH by NADPH, a reaction catalyzed by glutathione reductase (GR). Thus, APX in combination with the effective AsA regeneration functions to prevent the accumulation of toxic levels of H_2O_2.

APX are distributed in at least four distinct cell compartments, the stroma (sAPX) and thylakoid membrane (tAPX) in

Abbreviations: APX, ascorbate peroxidase; AsA, reduced ascorbate; cAPX, cytosol APX; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; FW, fresh weight; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; mAPX, microbody APX; MDAR, monodehydroascorbate reductase; MDHA, monodehydroascorbate; MV, methyl viologen; ROS, reactive oxygen species; sAPX, stromal APX; tAPX, thylakoid membrane-bound APX.

* Corresponding author. Tel.: +86 571 8697 1120; fax: +86 571 8604 9815.
E-mail address: jqyu@zju.edu.cn (J.Q. Yu).

0981-9428/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.plaphy.2005.11.003

chloroplasts, the microbody (mAPX), and the cytosol (cAPX) [2,12]. These APX isoenzymes respond differently to environmental stresses such as low temperature, high-light stress, pathogen infection, paraquat and salinity [10,13,17,22,29,30]. For example, the steady-state level of transcripts of cAPX is dramatically induced during the hypersensitive response of tobacco plants infected with TMV [17]; *Arabidopsis* lines overexpressing tAPX showed an increased resistance to treatments with paraquat [20]. Overexpression of DHAR, the enzyme responsible for regenerating AsA can increase the AsA content of plants [7], indicating the probable involvement of AsA regeneration system in AsA homeostasis. Until now, however, relatively few studies have been carried out to characterize the different isoenzymes by measurement of their activities [30]. Furthermore, to our knowledge, no studies have been carried out to study the relationship between APX isoenzymes activities and AsA regeneration capacity in plants exposed to different abiotic stresses. In this regard, assay of the activity of APX isoenzymes would supply a valuable tool in evaluating the role of different APX isoenzymes in scavenging H_2O_2.

We are interested in the response of antioxidant system in cucumber plants to environmental stress. In our early study, we found that the chill-induced decrease in the proportion of electron flux for photosynthetic carbon reduction was partly compensated by an O_2-dependent alternative electron flux driven by the water-water cycle, followed by significant increases in the activity of antioxidant enzymes (i.e. SOD, APX and GR) and antioxidants (i.e. ASA and GSH) [33]. The aim of the paper is to study the responses of four APX isoenzymes and the enzymes of AsA regeneration system in cucumber (*Cucumis sativus* L) leaves to heat, methyl viologen (MV), and H_2O_2 stress. In addition, the response of sAPX to these stresses was determined at transcriptional level.

2. Results

2.1. Effects of stresses on F_v/F_m, O_2^- and H_2O_2

The maximal photochemical efficiencies F_v/F_m, a measure of the functional status of PSII, was determined after heat, MV and H_2O_2 treatment (Fig. 1). F_v/F_m decreased at 1 d and 2 d for MV treatment and heat treatment, respectively. In comparison, F_v/F_m was little changed by H_2O_2 treatment.

Histochemical staining results showed that O_2^- level increased markedly on 2 d after MV, heat and H_2O_2 treatment (Fig. 2A). However, no apparent differences were found among the three treatments. Meanwhile, no significant increases in H_2O_2 were found after heat and H_2O_2 treatment whilst MV treatment resulted in significant increase in H_2O_2 content (Fig. 2B).

2.2. Changes in activities and transcript of APX isoenzymes in response to stresses

cAPX activity decreased by 19% and 17% at 1 d after MV and H_2O_2 treatment, respectively, but minimal changes were observed for the heat treatment. However, sharp increases in

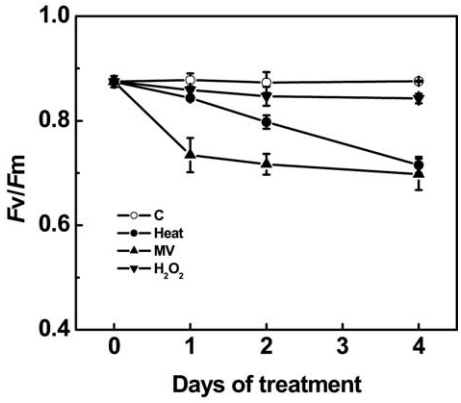

Fig. 1. Changes in maximum photochemical efficiency (F_v/F_m) in cucumber leaves as influenced by different stress treatments at different times. Data are the mean of at least three replicates with standard errors shown by vertical bars.

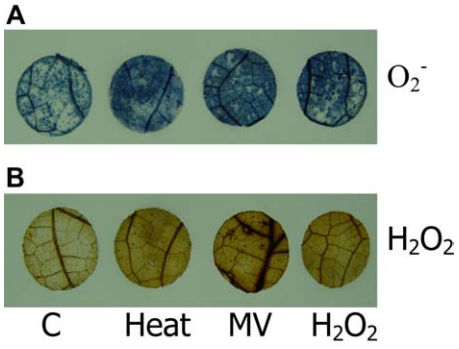

Fig. 2. Histochemical staining of O_2^- (A) and H_2O_2 (B) in leaves of heat, MV and H_2O_2 stress-treated and control (C) cucumber plants. Samples were taken after 2 d of the treatments.

the activity were found at 2 d after these treatments that increased by 22%, 15% and 11% for heat, MV and H_2O_2 treatment, respectively. The activities of sAPX and mAPX followed similar change patterns. In comparison, the heat treatment showed greatest effect on these three APX isoenzymes. However, the tAPX activity was not changed throughout the experiment despite these treatments (Fig. 3).

In agreement with the changes in sAPX activity, the transcript level of sAPX was down-regulated by all the treatments at 1 d. This reduction was especially apparent in the H_2O_2 treatment. sAPX transcript, however, increased significantly at 2 d in response to all treatments but with a larger margin especially after heat stress (Fig. 4).

2.3. Changes of AsA-regeneration enzymes activities, ascorbate and glutathione levels in response to stresses

All treatments induced a gradual increase in MDAR activity except that heat stress led to a transient decrease at 1 d (Fig. 5A). Similarly, all these stress treatments induced significant increases in GR activity from 1 d after these treatments. By the end of the experiment (4 d), GR activity of MV-treated plants was one time higher than that of control plants (Fig. 5B). The activity of DHAR significantly increased from 1 d after each treatment and thereafter declined gradually to the control