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The relationship between photooxidative stress tolerance and levels of antioxidants involved in leaf age of higher plants

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Introduction

Photosynthetic cells are prone to oxidative stress via active oxygen species (AOS) caused by the highly energetic reactions and abundant O_2 supply in photosynthesis. On the other hand, AOS have an important role in plant tissues during the senescence process, leading to irreparable metabolic dysfunction and cell death. Higher plants contain antioxidants and antioxidant enzymes that play a role in regulating or modulating senescence dynamics of plant tissues (Hodges and Forney, 2000). Many changes resembling those associated with senescence can be induced when a plant is exposed to one or more abiotic stresses such as drought (Irigoyen *et al*., 1992, Olsson, 1995), ozone (Pell *et al*., 1997) or excessive levels of exogenous compounds such as NaCl (Lutts *et al*., 1996). It seems likely that the capacity to tolerate oxidative damage depends on the balance between AOS-generation and defense systems in the cells. Here, we studied the relationship between the oxidative stress tolerance and levels of antioxidants in plant leaves of different ages.

Materials and methods

Nicotiana tabacum cv. Xanthi was cultured for eight weeks in a growth chamber under a 12-h light/12-h dark regime with a moderate light intensity (400 µmol $m^{-2}s^{-1}$), 60% relative humidity, and day/night temperature of 25/20°C. Methyl viologen (MV) treatment was carried out according to a modified method of Sen Gupta *et al*. (1993). Leaf discs of 1 cm in diameter were punched out from leaves of wild-type plants using a cork borer. Ten leaf discs were floated on a solution containing 0.5 µM MV and 0.1% Tween-20 in 6cm-diameter petri dishes and preincubated in the dark for 12h at 25˚C. They were exposed to light (150 µmol $m^{-2}s^{-1}$) for 1h at 25˚C and were then harvested, frozen in liquid N_2 and stored at -80˚C. The conductance of the floating solutions was determined by using HORIBA ES-12 conductivity meter as a measurement of ion leakage from the leaf discs due to the lipid peroxidation of the cell membranes. The activities of ascorbate peroxidase (APX) isoenzymes, glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydoroascorbate reductase (DHAR) were assayed as described previously (Shigeoka *et al*., 1987). The levels of ascorbate (AsA) and glutathione (GSH) were determined as described previously (Miyagawa *et al*., 2000). The CO_2 fixation was measured with the portable photosynthesis system LI-6400 (Li-Cor, Lincoln, NE) at 1000 µmol $m^{-2}s^{-1}$, 360 ppm CO_2.

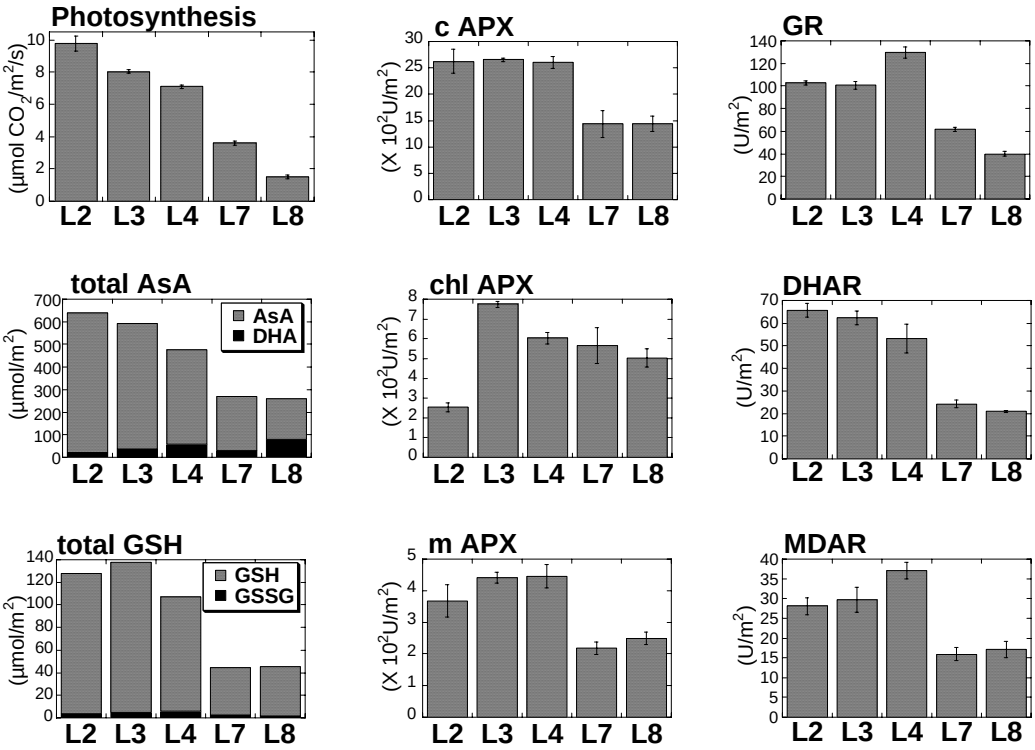

Fig. 1 The photosynthetic capacity, the levels of antioxidant enzymes (APX isoenzymes, GR, MDAR, DHAR) and antioxidants (AsA, GSH) in leaves of different ages.

Results

Figure 1 shows the levels of antioxidant enzymes (APX isoenzymes, GR, MDAR, DHAR) and antioxidants (AsA, GSH) in leaves of different ages. Activities of cytosolic APX and chloroplastic APXs in the older leaves (the eighth leaves counting from the top) of tobacco plants were 54% and 65% compared to those in younger leaves (the third leaves counting from the top) under normal conditions, respectively. The enzyme activities of MDAR, DHAR and GR in older leaves were 58%, 34%, and 39% of those in younger leaves, respectively. The levels of total AsA and GSH in older leaves were 44% and 33% of those in younger leaves, respectively. The levels of chlorophyll content were scarcely changed regardless of their age. The chloroplastic APXs activities in the older leaves remained relatively high among the antioxidant enzymes.

Under photooxidative stress conditions caused by the MV treatment, a significant amount of destruction of chlorophyll was observed in the older leaves but not in the younger leaves (Fig. 2). The levels of ion leakage due to lipid peroxidation of the cell membranes indicated that significantly less damage had occurred in the younger leaves than in the older leaves (Fig. 3).

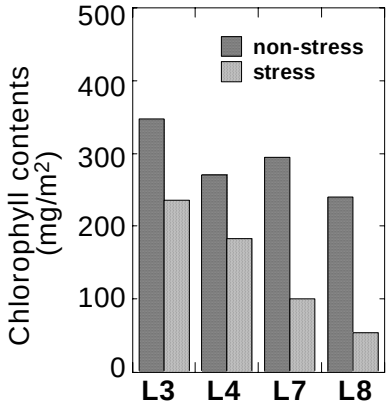

Fig. 2 The effects of leaf age on chlorophyll contents under photooxidative stress caused by MV treatment.

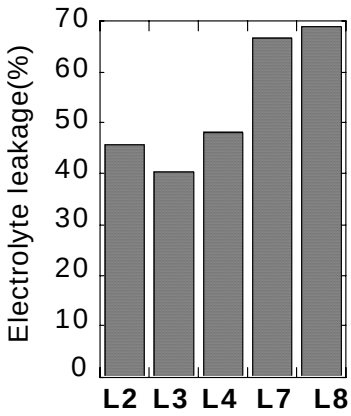

Fig. 3 The effects of leaf age on lipid peroxidation of cell membranes under photooxidative stress.

Next, to investigate the primary target molecules of photooxidative stress, we checked the sensitivity of the potential targets to MV-induced photooxidative stress in younger and older leaves (Fig. 4). Levels of chloroplastic APXs activities and AsA in the older leaves markedly decreased to 60% and 68% under photooxidative stress conditions, respectively. In younger leaves, those decreased to 85% and 80%, respectively. On the other hand, the activities of cytosolic APX, MDAR and DHAR were slightly induced or scarcely changed.

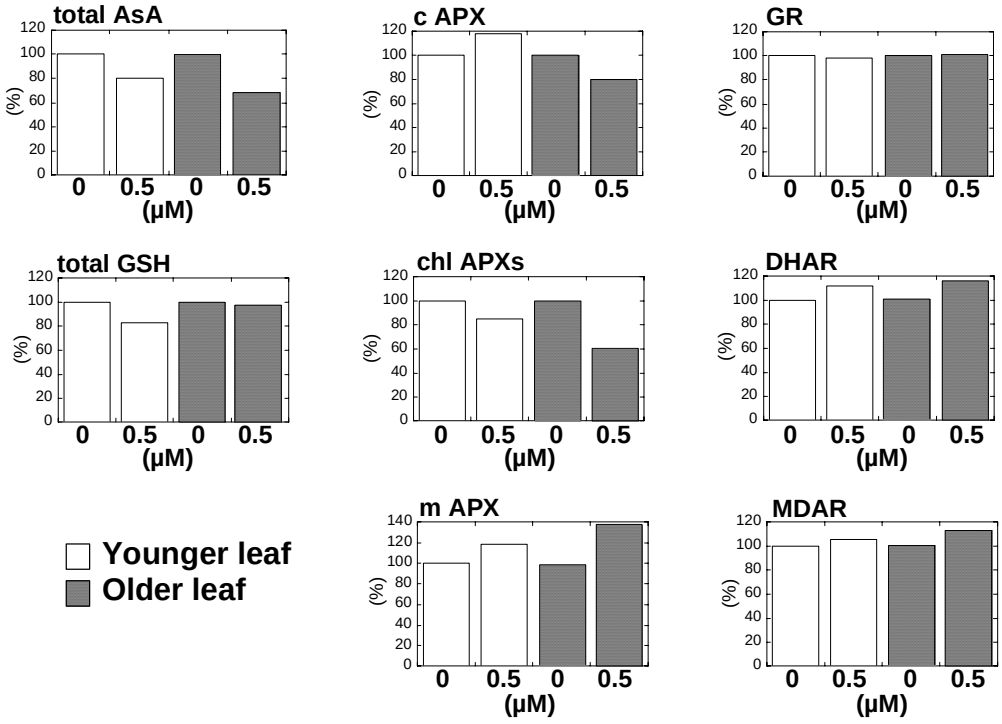

Fig. 4 The effects of leaf age on antioxidant enzymes and antioxidants under photooxidative stress.

Discussion

In the older leaves, all the levels of antioxidant enzymes and antioxidants were lower than those of the younger leaves, suggesting that the potential of the AOS-scavenging system in the older leaves is lower than that in the younger leaves. As the involvement of AOS in the senescence of plant tissue has been well reported, it is reasonable that the relationship between leaf oxidative and antioxidative potentials has been implicated in the dynamics of senescence (Kunert and Ederer, 1985, Casano *et al*., 1999, Jiménez *et al*., 1998). Indeed, the degradation of chlorophyll was significantly observed in the older leaves but not in the younger leaves under photooxidative stress conditions. In order to understand the potential of various antioxidants and antioxidant enzymes under stress conditions, we studied initial biochemical events by observing plant leaves of different ages. MV treatment causes the excess generation of AOS via O_2^- in chloroplast under illumination. It should be noted that, among potential targets of MV-induced oxidative stress, a decrease in the total AsA and inactivation of chloroplastic APX isoenzymes occurred, especially in older leaves. On the other hand, the levels of MDAR, DHAR, GR, and GSH are relatively stable under photooxidative stress conditions. These results suggest that the chloroplastic APX isoenzymes are the earliest targets among the AOS scavenging system. Under the conditions where the concentration of AsA is lower than 20 μM, chloroplastic APX isoenzymes are inactivated with a half-life of only 15-20 s (Asada 1999). As about 30% to 40% of cellular AsA content is localized in the chloroplasts of higher plants (Gillham and Dodge 1986), it may be proposed that the AsA level in the chloroplasts in older leaves is drastically depleted under photooxidative stress conditions. In older leaves, the activities of chloroplastic APXs remained relatively high under normal conditions. The levels of AsA, DHAR, MDAR and GR in older leaves were significantly lower than those in younger leaves. Accordingly, the level of AsA is insufficient to scavenge H_2O_2 by chloroplastic APXs in older leaves under photooxidative conditions, which may accelerate the drastic degradation of chlorophyll.

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