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Hydrogen peroxide-induced oxidative damage occurs on senescence green gram (*Vigna radiata* L.) leaves

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SUMMERY

In the present investigation, we evaluate the protective effect of ascorbic acid (AA) against the senescence of green gram leaves promoted by hydrogen peroxide (H_2O_2). Senescence of green gram leaves was determined by decreases in protein content. H_2O_2-treatment resulted in increases in leaf H_2O_2 content, induction of leaf senescence, increases in lipid peroxidation, and decrease catalase (CAT) activity observed with increase in the activities of superoxide dismutase (SOD) and glutathione reductase (GR). AA was effective in reducing H_2O_2-induced leaf senescence. AA prevented H_2O_2-increased H_2O_2 content, H_2O_2-induced lipid peroxidation, and H_2O_2-stimulated antioxidative enzyme activities. Reduction of H_2O_2-induced senescence by AA in green gram leaves is most likely mediated through its ability to scavenge H_2O_2.

Key words : Ascorbic acid, Hydrogen peroxide, Lipid peroxidation, Senescence, *Vigna radiata.*

Hydrogen peroxide (H_2O_2) is a constituent of oxidative plant metabolism and is itself reactive oxygen species (ROS). H_2O_2 can also react with superoxide (O_2ÿ$^{-}$) radicals to form more active hydroxyl (OHÿ) radicals in the presence of trace amounts of Fe or Cu. The OHÿ radicals initiate self-propagating reactions leading to destruction of proteins and peroxidation of membrane lipids (Halliwell, 1987; Thompson *et al*., 1987). H_2O_2 has been shown to promote leaf senescence and induction of senescence is accompanied by an increase in endogenous H_2O_2 content. Lipid peroxidation is considered an important mechanism of leaf senescence (Mondal and Choudhuri, 1981). The peroxidation of lipids can be initiated by ROS (Halliwell, 1987). Thus, H_2O_2-induced leaf senescence is mediated, at least in part, through lipid peroxidation. Ascorbic acid (AA) is seems to be a potent antioxidant, and its ability to directly scavenge ROS may be behind its action. More recently, we have shown that the promotion of leaf senescence in mustard by 2,4-dichlorophenoxyacetic acid (2,4-D, synthetic auxin), which induce lipid peroxidation and stimulate catalase (CAT) activity (Manoharan *et al*., 2005). In the present investigation, we examined the effect of AA on the H_2O_2-induced senescence of green gram leaves.

MATERIALS AND METHODS

Plant material and its germination condition:

Uniform green gram seeds (*Vigna radiata* L., obtained from TNAU, Coimbatore, India) were germinated in petridishes in the darkness containing Whatman No. 1 filter paper moistened with Hoagland nutrient solution (Hoagland and Arnon, 1950). After 48 hr of germination, seedlings were transferred to plastic glasses containing Hoagland nutrient solution at pH 5.8 and kept in growth chamber. The growth chamber was maintained at 25 ± 1°C with 16hL/8hD and 150 µmol $s^{-1}m^{-2}$ light intensity. RH was 35% in day and 60% by night. H_2O_2 and AA were supplemented along with the Hoagland nutrient solution. Seedlings treatment with water served as the control.

Assays of antioxidative enzymes:

For the extraction and assay of enzyme activities, 500 mg of leaves were macerated with 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% PVP in pre-cooled mortar and pestle. The homogenate was centrifuged at 15,000 rpm for 30 min at 4°C and the supernatant was used for analysis. CAT activity was determined according to the method of Aebi (1984). Absorbance was measured at 240 nm during the reaction. The CAT activity was expressed as U g FW^{-1}. Superoxide dismutase (SOD) activity was assayed according to the method of Beauchamp and Fridovich (1971). SOD activity was expressed as U g FW^{-1}. Glutathione reductase (GR) activity was determined according to the method of Schaedle and Bassham (1977). GR activity was expressed as U g FW^{-1}.

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