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Lipid Peroxidation and Antioxidative Defense Systems in Early Leaf Growth[1]

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Abstract. Changes in lipid peroxidation activities—superoxide dismutase (SOD), peroxidase (POD), and antioxidation—have been studied in leaves of *Cucumis sativus* during early growth until full expansion in situ. The content of malonyldialdehyde (MDA) rapidly decreased and then increased gradually. The changes in SOD, POD, and antioxidation activities were similar to each other and inverse to the changes in MDA contents. In the first phase, decrease in MDA is accompanied by increase in the activities of antioxidative defense systems, and in the second, exponential growth phase, increase in MDA content is accompanied by the decrease in defense systems.

Changes in lipid peroxidation (induced by reactive oxygen species) and in the defense system during leaf senescence have been reported (Dhindsa et al. 1981, Lin et al. 1984, Zhang et al. 1990). However, we have not yet found studies on changes during early leaf growth. Both the rates of photosynthesis and respiration increase gradually during early leaf growth. Photosynthetic electron transport is often the source of the superoxide anion, which is further reduced to other reactive oxygen species (Halliwell 1982). Several other potential sources of reactive oxygen species exist within organelles, such as mitochondria, microsomes, and peroxisomes, for respiration processes and autoxidation of phospholipids (Fantone and Ward 1985). Therefore, we suggest that oxygen radical production and its defense systems in early leaf growth should be more active than those in leaf senescence.

Reactive oxygen species can initiate lipid peroxidation reactions. Quantitations of diene conjugates or of malonyldialdehyde (MDA) content in tissues are frequently used to indicate lipid peroxidation and indirectly to indicate the formation of reactive oxygen species. Plants possess enzymatic antioxidative defense systems, including superoxide dismutase (SOD) to clear away the superoxide anion, and peroxidase (POD) to remove hydrogen peroxide and organic peroxides. Plants also possess nonenzymatic systems, including water-soluble and fat-soluble scavengers. We have, therefore, examined changes in the MDA content and the activities of SOD and POD, as well as the antioxidative ability in cucumber during early leaf growth.

Materials and Methods

Cucumis sativus seedlings were grown at temperatures of 26°C during the day and 19°C at night with 16 h/day under white fluorescent lights (3.5×10^3 lux). Leaves were collected during early growth when three young leaves were present up to the period when leaves were fully expanded. Under the above environmental conditions, leaf area (A) was found to be in a linear correlation with fresh weight (W): $A = 72.7\ W$ ($n = 5$, $r = 0.975$). Thus, fresh weight was determined and the leaf area was calculated. The area of a fully expanded leaf was 14.54 cm^2.

MDA content was determined by the thiobarbituric acid reaction with minor modifications (Heath and Packer 1969). The absorbance at 535 nm of the butanol phase was measured. The concentration of MDA was calculated using its extinction coefficient of 155 $mM^{-1}\ cm^{-1}$.

The activity of SOD was assayed by measuring its ability to inhibit the reduction of nitroblue tetrazolium (Droillard et al. 1987). The absorbance at 560 nm of the reaction mixture was measured. From the resultant graph the volume of enzyme extract corresponding to 50% inhibition of the reaction was read and was considered as 1 enzyme unit.

POD was assayed by the method of Evans and Alldridge (1965) with minor modification. Optical density readings at 470 nm were taken at 60-s intervals. POD activitiy was expressed as mmol of tetraguaiacol produced (mg protein)$^{-1}$ min^{-1}. The amount of tetraguaiacol produced was calculated using an extinction coefficient of 26.6 $mM^{-1}\ cm^{-1}$ (Chance and Maehly 1955).

An aliquot of the enzyme extract or the MDA supernatant was

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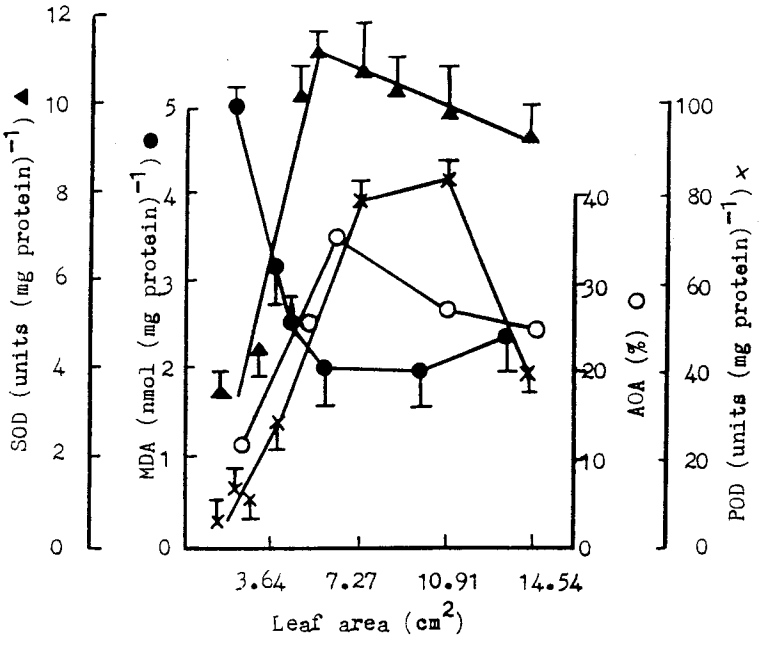

Fig. 1. Changes in MDA (●), SOD (▲), POD (×), and AOA (○) in leaves of *Cucumis sativus* during early growth until full expansion in situ (N = 3).

used to determine its protein content by the method of Lowry et al. (1951).

Fat-soluble antioxidants were extracted from the leaf (McKersie et al. 1982). The relative quantity of antioxidants in the tissue extract was determined by monitoring the inhibition of linoleate oxidation in an emulsion consisting of 3 ml of 0.02 M phosphate buffer solution, pH 7.0, 0.33 ml of 0.2 M linoleic acid in ethanol, and 1.5 ml of 0.5 mM Fe^{2+}-EDTA at 37°C. The diene conjugates (the oxidative product of linoleic acid) was monitored by measuring the absorbance at 232 nm. The relative antioxidative activity (AOA) was calculated by slops (S) of A_{232}-t straight lines:

$$AOA = (S_{control} - S_{extract})S_{Control}^{-1} \times 100$$

Results

The observed decrease in MDA content during the first growth phase was accompanied by the increase in SOD, POD, and AOA, whereas in the second, exponential growth phase MDA content increased and defense system activity decreased. Turning of all four curves occurs within a short period of leaf growth characterized by leaf areas of 5.45–7.27 cm^2. This stage is reached after about 5 days growth (Fig. 1). Wareing and Phillips (1978) found the first turning point of sigmoid leaf growth of *Cucumus sativus* to occur at about the same time.

The results described are similar to those published by Dhindsa et al. (1981) concerning tobacco leaf growth. Until full expansion, MDA content decreased at first and then increased, and changes in catalase in tobacco leaf were the same as those of SOD and POD observed in cucumber leaf. However, in tobacco leaf the SOD activity slowly declined until full leaf expansion and then sharply declined.

Decrease of lipid peroxidation and increase of antioxidative defense systems in leaves during early growth stages are inverse to those occurring during leaf senescence: increase of lipid peroxidation and decrease of antioxidative defense systems (Dhindsa et al. 1981, Lin et al. 1984, Zhang et al. 1990). It is suggested that the decline of MDA content in early stages of leaf growth may be a consequence of enhancements of antioxidative defense systems.

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