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Lack of evidence for causal relationship between peroxidase activity and dark-induced senescence of detached corn leaves

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Abstract. We investigated the role of peroxidase activity in the regulation of dark-induced senescence of detached corn leaves. The optimum pH for peroxidase activity was 5.8. Peroxidase activity was found to decrease only in the later stage of senescence. Benzyladenine, a synthetic cytokinin, retarded senescence but had no influence on peroxidase activity. Abscisic acid and methyl jasmonate promoted senescence but did not influence peroxidase activity. Gabaculine, an inhibitor of heme synthesis, and tunicamycin, a protein glycosylation inhibitor, decreased peroxidase activity but had no effect on senescence. Our results suggest that peroxidase did not play a role in dark-induced senescence of detached corn leaves.

Keywords: Abscisic acid; Benzyladenine; Leaf senescence; Methyl jasmonate; Peroxidase; *Zea mays*.

Abbreviations: **ABA**, abscisic acid; **BA**, benzyladenine; **MJ**, methyl jasmonate.

Introduction

Chlorophyll loss and protein degradation are characteristic symptoms of leaf senescence. Parish (1968) suggested that the increase in the activity of peroxidase (EC 1.11.1.7) in tobacco leaves is one of the most reliable indicators of senescence, and plays an important role in it's regulation. Increased peroxidase activity during leaf senescence has been reported in several other plant species (Abeles and Dunn, 1989; Braber, 1980; De Jong, 1972; Hazell and Murray, 1982; Kar and Mishra, 1976; Kumar and Khan, 1983; Kuroda et al., 1990). Peroxidase activity of detached barley leaves, however, was found to decrease during dark-induced senescence (Sharma and Biswal, 1976). Birecka et al. (1979) reported that a decrease in peroxidase activity was observed only at the later stage of dark-induced senescence of oat leaves.

In view of this conflicting evidence, we set out to check whether total peroxidase activity contributes to the regulatory complex that controls the senescence of detached corn leaves in the dark.

Materials and Methods

Plant material and incubation conditions

Seedlings of corn (*Zea mays* cv. Tainung 1) were grown in vermiculite in a greenhouse with natural light at 30 °C day/25 °C night for 7 days, by which time the primary leaves were fully expanded. The apical 2.5-cm segments were excised from the primary leaves and were placed vertically in test tubes with the cut end submerged in 2 ml of distilled water or test solutions and incubated at 27 °C in the dark.

Determination of chlorophyll and protein

Chlorophyll was extracted and determined following the method of Wintermans and De Mots (1965) after extraction in 96% ethanol. For protein determination, leaf segments were homogenized in 50 mM sodium phosphate buffer (pH 7.5). The extracts were centrifuged at 17,000 x *g* for 20 min, and the supernatant liquids were used for determination of protein by the method of Bradford (1976). Chlorophyll and protein levels were expressed as mg g^{-1} fresh weight.

Peroxidase assay

Leaf segments were homogenized using a mortar and pestle in 50 mM sodium phosphate buffer (pH 6.8). The homogenate was centrifuged for 20 min at 12,000 x *g* and the supernatant liquid was used as crude extract. Peroxidase activity was assayed in a solution containing 7.2 mM guaiacol, 11.7 mM H_2O_2, 50 mM sodium phosphate buffer (pH 5.8), and 0.1 ml extract in a final volume of 3.0 ml. The reaction was initiated by the addition of H_2O_2 and the change in absorbance at 470 nm was measured. The activity was expressed as units g^{-1} fresh weight. One unit activity was defined as an increase of one A per min.

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Design of the experiment

For all measurements, each treatment was performed four times. All experiments described here were performed at least three times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

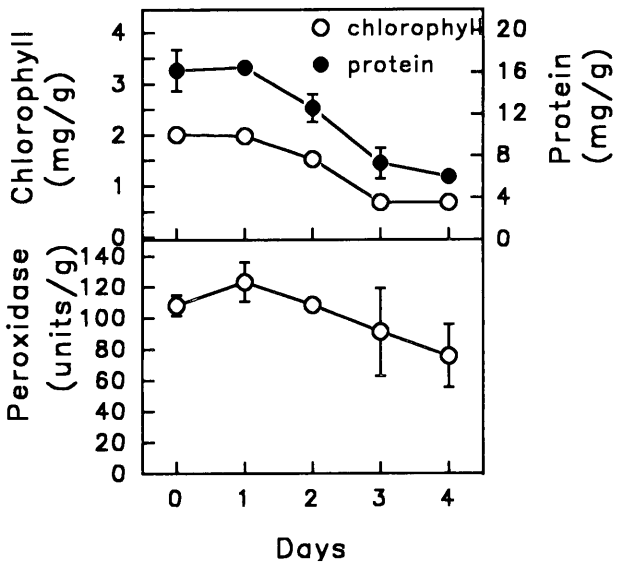

Figure 1. Chlorophyll and protein levels and peroxidase activity in detached corn leaves during dark-induced senescence. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.

Results

The optimum pH for peroxidase extracted from detached corn leaves was 5.8 (data not shown). The senescence of corn leaves was tracked by measuring the decrease of chlorophyll and protein. Figure 1 shows the time courses of chlorophyll and protein levels and peroxidase activity of detached corn leaves under dark conditions. A decrease of chlorophyll and protein was evident 2 days after leaf detachment. Peroxidase activity remained unchanged during the first 3 days of dark incubation and then decreased by day 4.

The influence of BA (a synthetic cytokinin) on senescence and peroxidase activity is shown in Figure 2. Benzyladenine retarded senescence of detached corn leaves but had no effect on peroxidase activity. Figure 2 shows the influence of ABA on senescence and peroxidase activity of detached corn leaves in the dark. ABA promoted senescence but had no influence on peroxidase activity. Jasmonates and ABA have a number of structural and functional similarities (Parthier, 1991). The influence of MJ on this system was investigated. Similar to ABA, MJ effectively promoted senescence but had no influence on peroxidase activity (Figure 3).

Peroxidase is a hemoglycoprotein and requires a heme moiety for its normal function (Chibbar et al., 1984a; 1984b). To further characterize the role of peroxidase on

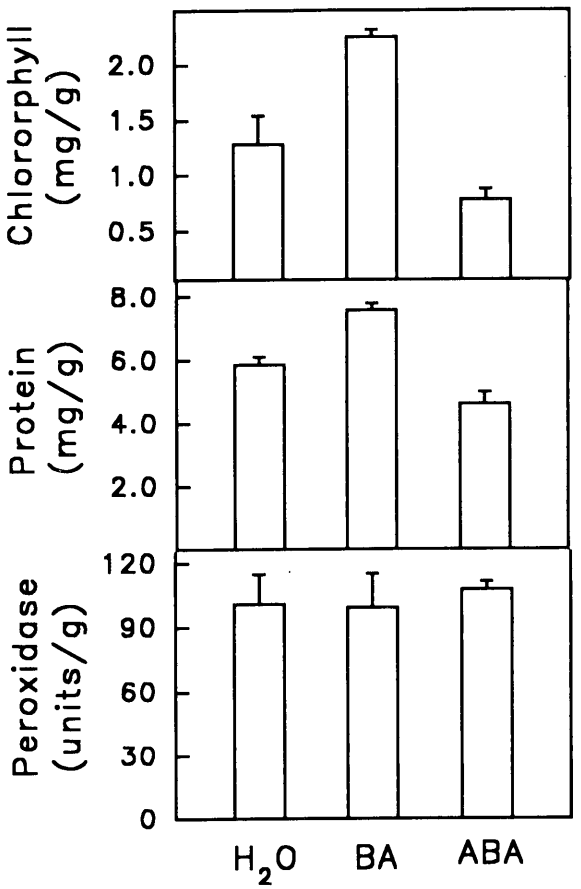

Figure 2. Influence of BA and ABA on chlorophyll and protein levels and peroxidase activity of detached corn leaves. The concentrations of BA and ABA are 10 μM and 45 μM, respectively. Chlorophyll and protein levels and peroxidase activity were determined at 4 days after treatment. Vertical bars represent standard errors.

dark-induced senescence of detached corn leaves, experiments were carried out with tunicamycin, a protein glycosylation inhibitor (Handa, 1985), and gabaculine, an inhibitor of transaminase that has been used to inhibit synthesis of chlorophyll and phytochrome (Gardner and Gorton, 1985; May et al., 1987). Gabaculine and tunicamycin were applied to detached corn leaves in the dark. As shown in Figure 4, both gabaculine and tunicamycin had no influence on senescence but did cause a decrease of peroxidase activity. Our results are consistent with those obtained by Abeles and Dunn (1989), who reported that neither gabaculine or tunicamycin reduced chlorophyll breakdown, but they did inhibit both endogenous and ethylene-induced peroxidase synthesis.

Discussion

Several different patterns of peroxidase activity have been reported during dark-induced senescence of detached leaves. In most cases, peroxidase activity increased during senescence (Abeles and Dunn, 1989;

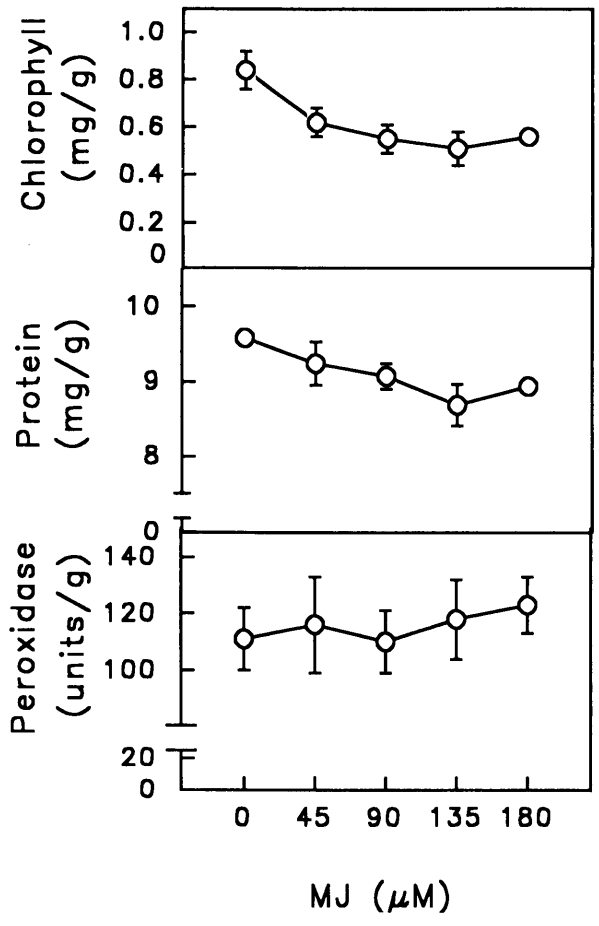

Figure 3. Influence of MJ on chlorophyll and protein levels and peroxidase activity of detached corn leaves. Chlorophyll and protein levels and peroxidase activity were determined at 4 days after treatment. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.

Braber, 1980; De Jong, 1972; Hazell and Murray, 1982; Kar and Mishra, 1976; Kumar and Khan, 1983; Kuroda et al., 1990; Parish, 1968). In a second pattern, peroxidase activity decreased during senescence (Sharma and Biswal, 1976). In a third pattern, peroxidase activity decreased only at the later stage of dark-induced senescence (Birecka et al., 1979). Our results are in agreement with the third pattern. These observations are not consistent with the hypothesis that peroxidase functions as a senescence-inducing enzyme (Parish, 1968).

If peroxidase were linked with senescence due to its ability to eliminate hydrogen peroxide, the production of which increases in leaf tissues with senescence (Mondal and Choudhuri, 1982), and if its activity was rate-limiting, then one would expect a decreased enzyme activity in ABA- and MJ-treated segments and an increase in BA-treated segments. Peroxidase activity, however, was not affected by ABA, MJ and BA treatments, though senescence was promoted by ABA and MJ and retarded by BA. These results further support our conclusion that there is no causal relation between peroxidase activity and dark-induced senescence of detached corn leaves. Additional evidence supporting our conclusion is the observation that both tunicamycin and gabaculine treatment decreased peroxidase activity but had no effect on senescence. We also examined the changes in the isoperoxidase pattern during senescence of corn leaves, but no difference could be found during senescence or treatment with BA, ABA, or MJ (data not shown).

The fact that BA, ABA, and MJ had no effect on peroxidase activity in detached corn leaves is peculiar. Peroxidase activity has been found to respond to cytokinins and MJ treatment in other plant leaf systems. For example, kinetin increases peroxidase activity during leaf senescence of cucumber (Ford and Simon, 1972), rice (Kar and Mishra, 1976; Reddy et al., 1985), barley (Kuroda et al., 1990), oats (Birecka et al., 1979), and *Eleusine coracana* (Kumar and Khan, 1983). MJ has been shown to increase peroxidase activity in barley leaf segments during senescence (Weidhase et al., 1987). In

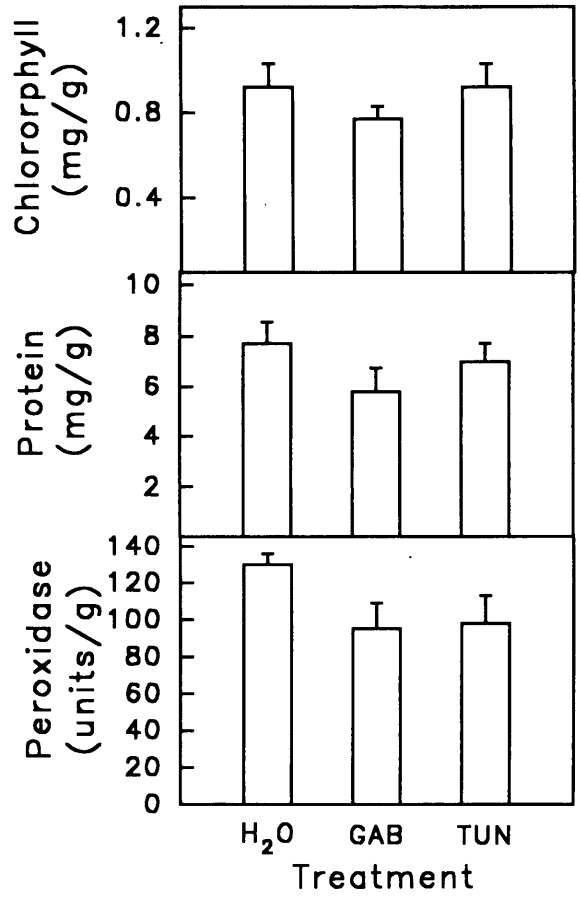

Figure 4. Influence of gabaculine (GAB) and tunicamycin (TUN) on chlorophyll and protein levels and peroxidase activity of detached corn leaves. The concentrations for GAB and TUN are 1 mM and 0.1 μg ml^{-1}, respectively. Chlorophyll and protein levels and peroxidase activity were determined at 4 days after treatment.

our recent work, we also found peroxidase activity increased in MJ-treated detached rice leaves (Yeh and Kao, 1994). Whether the lack of sensitivity of peroxidase activity to BA, ABA, and MJ treatment is specific to corn or C_4 plants remains to be seen.

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過氧化酵素活性與玉米切離葉片在暗中老化無因果關係

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本研究主要探討台農一號玉米切離葉片老化過程中，過氧化酵素活性所扮演之角色。由玉米葉片所抽取出之過氧化酵素，其最適 pH值爲 5.8 。過氧化酵素活性在老化初期活性不變，僅在老化後期活性下降。benzyl-adenine (BA) 處理，可延緩葉片老化；methyl jasmonate (MJ) 與 abscisic acid (ABA) 處理，可加速老化。然而，BA、MJ 與 ABA 處理均不影響過氧化酵素之活性。gabaculine (heme合成的抑制劑) 與 tunicamycin (蛋白質醣化作用之抑制劑) 處理，可降低過氧化酵素之活性，但不影響老化。因此，過氧化酵素活性似乎與玉米葉片老化無因果關係。

關鍵詞：離層酸；Benzyladenine；葉片老化；Methyl jasmonate；過氧化酵素；玉米。