Effect of Jasmonic Acid and Wounding on Polyphenol Oxidase Activities during Leaf Senescence in *Lycopersicon esculentum*

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1. Introduction

Plant polyphenol oxidase(PPO) are widespread enzymes which oxidize plant phenolic compounds. The role of PPOs in the biology of plants remains unclear. Because of its conspicuous reaction products, PPO has frequently been assumed to play a role in plant defence(Thipyapong et al., 1995). PPOs have been studied in many different plant specis, and a recent survey found that leaf PPO is essentially ubiquitous in higher plants(Sherman et al., 1991). Although PPO is widely distributed, only in some species does it appear to be a component of the inducible anti-herbivore defense(Constabel and Ryan, 1998). Jasmonic acid and its methyl ester(MeJA) are closely related to components of the octadecanoid wound-signalling pathway and is a strong inducer of PPO and other anti-herbivore defense. JA and MeJA are widespread in the plant kingdom and exert various physiological activities, such as promotion of leaf senescence, inhibition of seedling growth, induction of rooting and tuberisation(Sembdner and Parthier, 1993). The senescence syndrome in detached leaves appears to be promising model system for studing the mode of action of the jasmonate group. Leaf senescence can be regarded as a genetically specified integral pattern of leaf development. Wounding plays in the syndrome of leaf senescence(Giridbar and Thimann, 1985). Here we describe the effects of wounding and JA on PPO in tomato seedlings and possible correlations between PPO and JA- and wound-induced resistance are examied.

2. Materials and Methods

Plant materials and treatments. Tomato(*Lycopersicon esculeutum*) were

grown in a growth chamber under 14 h of light(200 μEm ^{2}s ^{1}) at 26℃ and 10 h of dark at 18℃. Apical segments from the first true leaves were cut from the two-week-old seedlings and floated on distilled water or test solutions in the dark for up to 4 h.

Methods of wounding. Mechamical wounding of the leaves were done by subdivision into subsegments, scraping of adaxial surface or making punctures with a sharp needle.

PPO assays. PPO activity was assayed spectrophotometrically using 3,4-dihydroxyphenyl-alanine(DOPA) as described by Sherman et al.(1991).

Estimation of chlorophyll. Leaf segments were repeatedly extrated with 80 % ethanol at 80 ℃ and the total chlorophyll was estimated as absorbance at 665nm.

3. Results

In leaf segments floated in darkness, chlorophyll decreased continuously after abscission. The decrease was partially restored by benzyladenine. When the wounding was done, retention of chlorophyll was clearly evident and the extent of inhibition of chlorophyll loss was evidently roughly proportional to the area wounded, the punctures having the smallest effect. In darkness, the senescence-promoting effect of JA was significant. The activity of PPO in controls increased greatly during senescence. The degree of inducibility of PPO by wounding and JA varied widely. Tomato showed significant increase of PPO activity following the treatments. When the segments were wounded, the activities were higher than in their respective unwounded controls in all cases. JA caused a drastic increase of enzyme activity during the senescence. Simultaneous application of JA and BA led to a partial stabilization of the enzyme activity in light but not in darkness. JA and ABA, which have been reported to promote chlorophyll loss had the same effect when the segments were wounded, but in each case the PPO content was clearly increased by the wounding. However, the PPO values in wounded segments were higher than in their respective unwounded controls. The combination of wounding and JA resulted in stability of PPO for 4 d in the dark. The largest increase in the concentration of PPO occurred in wounded leaves of seedlings kept under bright lighting; a small but significant increase was also measured in seedlings kept in darkness after

wounding.

4. Conclusion

The senescence of tomato(*Lycopersicon esculeutum*) leaves after being detached and kept in the dark was studied in terms of loss of chlorophyll and accumulation of polyphenol oxidase(PPO). PPO activity increased greatly in detached primary leaves during senescence. The leaf segments responded to the application of putative plant growth regulator, jasmonic acid(JA) with accelerated senescence, as indicated by the loss of chlorophyll and the rapid increase in PPO activity. The senescence-promoting action of JA differed in light and in darkness. Wounding by scrapping the surface of segments or making punctures with needles considerably delayed the loss of chlorophyll and caused the concentration of PPO in leaf segments. The amounts were roughly proportional to the intensity of wounding. Severity of wounding, light intensity and temperature also affected wound-induced change in the concentration of PPO. The combination of wounding with JA resulted in the stability of chlorophyll and PPO in the dark. JA and ABA acted similarly on both unwounded and wounded leaves, but the amount of chlorophyll and PPO in the wounded segments was always more than in the respective controls.

References

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