The Mechanism of Dark-induced Senescence of Detached Maize Leaves

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The roles of ethylene, polyamines and peroxidase activity in regulating senescence of detached maize leaves in darkness are reviewed. This represents the summary of the work conductedin our labortory during the past three years. Increase in ethylene sensitivity and in putrescine level were observed to be responsible for senescence of detached maize leaves. However, peroxidase did not play a role in dark-induced senescence of detached maize leaves.

Key words: Ethylene, Leaf senescence, Maize, Peroxidase, Polyamines

Introduction

The most common definition of leaf senescesce refers to the deterioative changes which are natural causes of leaf death (Leopold, 1975). Leaf senescence is an intergal part of leaf development. It is, like other developmental processes, a well organized and regulated process rather than a passive decay of the leaf. Physiologists continue to make progress toward an understanding of senescence regulating mechanisms.

Detached leaves are often used for senescence experiments to facilitate samplings of leaves and uptake of test compounds, as well as to avoid the correlative complexity of the whole plant. When detached leaves are used to study senescence, wounding is always a problem. However, in the case of maize leaves, each long and narrow leaf is cut once transversely, the area of wounding is small.

In this review, we shall be concerned strictly with the senescence of detached maize leaves, and mostly with the work conducted in our laborotory during the past three years.

Role of Ethylene

In recent years ethylene has received inscreasing attention as a potential reglulator of senescence in leaf tissues. The question of whether ethylene is involved in the regulation of leaf senescence has been approached in a number of ways:measurement of ethylene production during senescence, treatment of leaf tissue with precursor or inhibitiors of ethylene

biosynthesis, and treatment of leaf tissue with inhibitiors of ethylene action. As each approach individually has its own limitations, we are especially interested in studies that have combined these approaches to answer the question.

Several different patterns of ethylene production have been reported in senescing leaf tissue. In some cases, ethylene production peaked prior to the phase of rapid chlorophyll degradation (Kao and Yang, 1983). In a second pattern, ethylene production peaked after the commencement of rapid chlorophyll degradation (Aharoni et al., 1979; Chen et al., 1991; Gepstein and Thimann, 1981). In a third patten, two peaks of ethylene productiona were observed in *Rumex* leaf discs during senescence (Hackett and Goldthwaite, 1983). We have observed a fouth pattern: maize leaf segments exhibited no peak of ethylene production during senescence in the dark (Kao, 1992).

Ethylene production is generally controlled by the level of 1-aminocyclopropane-1-caboxylic acid (ACC), the immediate precursor of ethylene biosynthesis (Yang and Hoffman, 1984). Application of ACC significanthy stimulated ethylene production of detached maize leaves (Kao, 1992). However, ACC application did not result in an enhancement of senescence (Kao, 1992). It seems that ethylene production does not play a role in regulating senescence of detached maize leaves. This conclusion is further supported by the observations that (a) benzyladenine retarded senescence but promoted ethylene production and (b) abscisic acid promoted senescence but inhibited ethylene production (Kao, 1992).

If a change of ethylene concentration is excluded as an explanation for the change in extent of senescence, a change in sensitivity is an alternate possibility. This possibility was tested by using ethylene action inhibitor, silver ion (Beyer, 1976). Our results indeed showed that silverion retarded dark-induced senescence of detached maize leaves (Kao, 1992). It is concluded that a change in sensitivity to ethylene might be involved in the regulation of senescence of detached maize leaves.

Role of Polyamines

Polyamines are ubiquitous nitrogen compounds and have been implicated in several aspects of plant development (Evans and Malmberg, 1989). Diaminopropane, an oxidation product of naturally occurriing polyamines, and exogenous polyamines are effective in retarding the senescence of excised leaves and protoplasts (Altman, 1982; Cheng and Kao, 1983 ; Cheng et al., 1984; Galston et al., 1978 ; Shih et al., 1982). Since polyamine levels have been observed to decrease in senescing leaves, it has been suggested that they play a role in the control of leaf senescence (Kaur-Sawhney et al., 1982; Srivastava et al., 1981). However, Birecka et al. (1984) reported that no significant decline of polyamines could be observed in oat (*Avena sativa* cv. Astro or Gary) and *Nicotiana alata* leaves, which exhibited typical dark-induced senescence syndrome.

The most common polyamines in higher plants include putrescine, spermidine and spermine. Putrescine and spermidine were present in detached maize leaves throughout senescence (Kao, 1994). Spermime, however, was undectable in detached maize leaves during dark-induced senescence (Kao, 1994). Two different patterns of putrescine level have been reported during dark-induced senescence of

detached leaves. In some cases, the putrescine level decreased during senescence (Chen and Kao, 1991; Kaur-Sawhney et al., 1982; Srivastava et al., 1981). In a second pattern the putrescine level remained unchanged during senescence (Birecka et al., 1984). We have obseved a third pattern: detached maize leave exhibited an increase in putrescine level during senescence (Kao, 1994). The increase in putrescine level in detached maize leaves was found to be prior to the commencement of senescence in darkness (Kao, 1994).

Several experiments have shown that putrescine has a deleterious effect when fed to plant tissues (Coleman and Richards, 1956; DiTomaso et al., 1989; Roberts et al., 1986; Shevyakova 1982). In fact, we observed that upon feeding putrescine to detached maize leaves a toxic symptom was observed (unpublished data). If the increase in level of endogenous putrescince in detached maize leaves in the dark is the factor responsible for the senescence, then senescence is expected to be retarded by inhibitors of putrescine biosynthesis. We indeed observed that D-arginine and -methtylornithine, which decreased the level of putrescine, retarded the senescence of detached maize leaves in darkness (Kao, 1994). Our results seem to indicate that an increase in putrescine level is likely to be the factor resposible for dark-induced senescence of detached maize leaves. This conclusion is further supported by the observations that benzyladenine, a synthetic cytokinin, which retarded dark-induced senescence of detached maize leaves, also decreased the level of putrescine (Kao, 1994).

Role of Peroxidase Activity

Parish (1968) first suggested 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 Miahra, 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 Biswa, 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 maize leaves in the dark. Peroxidase activity in detached maize leaves was found to decrease only in the later stage of senescence (Yeh and Kao, 1994a). Benzyladenine retarded senescence but had no influence on peroxidase activity (Yeh and Kao, 1994a). Abscisic acid and methyl jasmonate (MJ) promoted senescence but did not affect peroxidase activity (Yeh and Kao, 1994a). Gabaculine, an inhibitor of heme synthesis, and tunicamycin, a protein glycosylation inhibitor, decreased peroxidase activity but had no effect on senescence (Yeh and Kao, 1994a). Our available data seem to indicate that peroxidase did not play a role in dark induced senescence of detached maize leaves.

Concluding Remarks

In spite of recent progress, we are still far from understanding the mechanism of dark

induced senescence of detached maize leaves. However, it is clear that putrescine accumulation is a factor causing senescence of detached maize leaves under dark condition. To our knowledge, this is the first evidence that putrescine accumulation results in senescence syndrome.

It is generally thought that polyamines and ethylene have opposite effect on plant senescence (Evans and Malmberg, 1989). Since both polyamines and ethylene are responsible for dark induced senescence of detached maize leaves, it is possible that this general concept may not hold for senescence of detached maize leaves.

The fact that benzyladenine, abscisic acid, and MJ have no effect on peroxidase activity in detached maize leaves is peculiar. Peroxidase activity has been found to respond to cytokinins and MJ treatment in other plant leaf systems. For example, kinetin and benzyladenine increase peroxidase activity during leaf senescence of cucumber (Ford and Simon, 1972), rice (Kar and Mishra, 1976; Reddy et al., 1985), 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 our recent work, we also found peroxidase activity increased in MJ-treated detached rice leaves (Yeh and Kao, 1994b). Whether the lack of sensitivity of peroxidase activity to BA, ABA, and MJ treatment isspecific to maize or C_4 plants remainsto be seen.

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玉米切離葉片在暗中老化機制之研究

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本文係綜合本研究室過去三年就乙烯、多元胺及過氧化酵素活性與玉米切離葉片在暗中老化間關係的成果。乙烯的敏感度與 putrescine 含量的增加可能與玉米切離葉片老化有關。沒有證據顯示過氧化酵素活性與玉米切離葉片在暗中老化有因果關係。

關鍵詞:乙烯、葉片老化、玉米、過氧化酵素、多元胺。