

*(Invited review paper)*

Polyamines and rice leaf senescence¹

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Introduction

Senescence can be defined as endogenously controlled deteriorative changes, which are the natural causes of death of cells, tissues, organs and organisms (Leopold, 1973). Thus, senescence is a natural developmental process, and it is completely endogenous. Senescence of leaves is a normal part of the growth and differentiation of the whole plant. We have seen that as

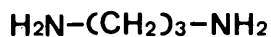
plants grow new leaves are continually formed, and the lowermost leaves gradually senesce. Leaf senescence is first evidenced by the degradation of the chlorophyll and later by the loss of leaf constituents. Browning or yellowing eventually takes place and the leaf wilts and shrinks or abscises. Polyamines have been studied in animals and bacteria for more than 50 years. It is only relatively recently, however, that their presence and significance have been recognized in higher plants. Among the physiological roles of polyamines, the association of polyamines with senescence has been extensively investigated. In this review, which is not intended to be comprehensive, we shall be concerned strictly with the senescence of rice leaves, and mostly with the work from our laboratory. Several articles will help provide a more comprehensive picture of the role of polyamines in senescence (Davies *et al.*, 1990; Evans and Malmberg, 1989; Flores, 1990; Galston and Sawhney, 1990; Kao, 1993).

Biosynthesis of Polyamines

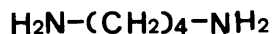
Polyamines are ubiquitous nitrogen compounds classified as plant growth substances (Evans and Mal-

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Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; ADC, arginine decarboxylase; DAO, diamine oxidase; DCH, dicyclohexylamine; DFMA, difluoromethylarginine; DFMO, difluoromethylornithine; dSAM, decarboxylated SAM; MTA, 5'-methylthioadenosine; MGBG, methylglyoxal bis(guanylhydrazone); ODC, ornithine decarboxylase; SAM, S-adenosylmethionine; SAMDC, SAM decarboxylase; PAO, polyamine oxidase.



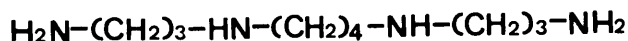
Diaminopropane



Putrescine



Spermidine



Spermine

Fig. 1. Structures of some polyamines found in higher plants.

mberg, 1989). The most common polyamines in higher plants include putrescine, spermidine, and spermine (Fig. 1). Figure 2 shows the biosynthetic pathways of polyamines. Putrescine is probably derived from ornithine in all plants, as it is in animals and microorganisms, utilizing the enzyme ornithine decarboxylase (ODC). Unlike that in animals, the putrescine in higher plants also derives from arginine, by the action of the enzyme arginine decarboxylase (ADC). Arginine can be converted to agmatine by ADC; agmatine then loses urea, forming putrescine. Both ADC and ODC are pyridoxal phosphate-dependent enzymes. It has been shown that ADC and ODC pathways have different tissue distributions (Smith, 1985) and different regulation (Hiatt *et al.*, 1986). ODC is primarily linked to rapid cell division (Cohen *et al.*, 1982; Heimer and Mizrahi, 1982), whereas ADC is usually linked to various stress responses (Flores, 1990).

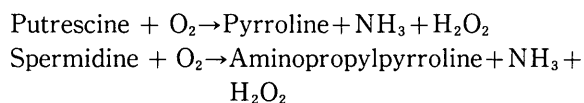
Putrescine is converted into spermidine by the action of an aminopropyltransferase called spermidine synthase. A second aminopropyltransferase, termed spermine synthase, adds an additional propylamine moiety to spermidine, forming spermine. The other product of the aminopropyltransferase reactions is 5'-methylthio-adenosine (MTA). The source of these propylamine moieties is decarboxylated S-adenosyl-

methionine (dSAM), which is formed by the action of S-adenosylmethionine decarboxylase (SAMDC). By the action of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ethylene-forming enzyme, SAM can also be converted into ethylene, an important hormone in higher plants.

The availability of specific, and potent, inhibitors for enzymes in the biosynthetic pathways of polyamines has provided an enormous stimulus to most biochemical and physiological studies of plant polyamines. Difluoromethylarginine (DFMA) and difluoroornithine (DFMO) have been developed to function as irreversible inhibitors for ADC and ODC, respectively (Kallio and McCann, 1981; Metcalf *et al.*, 1978). Methylglyoxal bis(guanyldrazone) (MGBG) is a potent inhibitor of SAMDC (Williams-Ashman and Schenone, 1972). Spermidine synthase is inhibited by dicyclohexylamine (DCH) (Hibasami *et al.*, 1980). These inhibitors are useful and have been widely used to change the levels of polyamines in plant tissues. However, not all inhibitors will cause the expected changes of polyamines levels. There is at least one report indicating that DCH is not an inhibitor of spermidine synthase (Batchelor *et al.*, 1986). Rice leaves treated with DCH showed a reduction in the level of spermine rather than that of spermidine (Chen and Kao, 1991b). Slocum and Galston (1987) discussed the application of these inhibitors in higher plants in considerable detail.

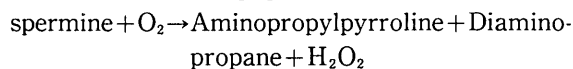
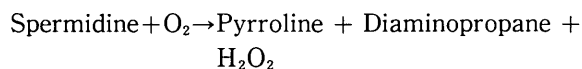
Metabolism of Polyamines

Polyamines can be oxidized by diamine oxidases (DAOs) and polyamines oxidases (PAOs). DAOs are widespread in higher plants (Rinaldi *et al.*, 1985; Smith, 1985) and are particularly active in leguminous plants. All the plant DAOs reported so far have been dimers, several of which have been shown to contain two copper atoms per molecule of enzyme (Smith, 1985). The following reactions are typical of those catalyzed by DAO:



In higher plants PAOs have been found only in the Gramineae family (Smith, 1985) and in water hyacinth (Yanagisawa *et al.*, 1987). PAOs from maize and barley

roots and from oat leaves have been purified and characterized (Smith, 1972, 1977; Suzuki and Yanagisawa, 1980). PAO, a flavoprotein, oxidizes spermidine and spermine to give, respectively, pyrroline and aminopropylpyrroline plus the coproducts diaminopropane and hydrogen peroxide:



Polyamines can be conjugated with several compounds in higher plants. Conjugates of putrescine, spermidine, and spermine, with cinnamic acid and its derivatives, are widespread in higher plants (Smith, 1981). Accumulated evidence suggests that hydroxycinnamoyl acid amides may play important role in flowering (Martin-Tanguy, 1985). Putrescine hydroxycinnamoyl transferase from tobacco callus has been isolated and purified (Meurer-Grimes *et al.*, 1989; Negrel, 1989). It has been shown that polyamines can be bound to RNA, DNAs, and proteins (Apelbaum *et al.*, 1988; Bagni *et al.*, 1981; Mizrahi *et al.*, 1989; Serafini-Fracassini and Mossetti, 1985; Serafini-Fracassini *et al.*, 1984). Serafini-Fracassini *et al.* (1988, 1989) provided the first evidence of the occurrence of transglutaminase, an enzyme capable of covalently binding polyamines to proteins in plants. Using thin-layer tobacco tissue culture, Apelbaum *et al.* (1988) demonstrated that the post-translational modification of a unique protein by attachment of spermidine may be causally connected to the appearance of flower buds.

Role of Polyamines in Rice Leaf Senescence

Influence of Exogenous Polyamines

Our interest in the affect of polyamines on rice leaf senescence arose mainly as a result of our earlier work, in which arginine was found to be effective in retarding senescence of detached rice leaves (Kao, 1980). Since arginine is known to be the precursor of polyamine biosynthesis (Evans and Malmberg, 1989), polyamines such as spermidine and spermine are expected to retard senescence of leaves. Polyamines have indeed been reported to retard leaf senescence and chlorophyll loss (Kaur-Sawhney and Galston, 1979). We demonstrated, however, that the retardation of chlorophyll loss by polyamines was localized to those areas along

the cut edges of detached rice leaves (Cheng and Kao, 1983). Kaur-Sawhney and Galston (1979) also reported that the retardation of chlorophyll loss by polyamines was localized to the peeled area of leaf blade. This localization of effect suggests that polyamines are poorly transported in leaf cells. This is not surprising if the polycationic nature of polyamines is taken into consideration.

Correlation with Endogenous Polyamines

If polyamines indeed play an important role in the regulation of rice leaf senescence, a decrease in the levels of polyamines and of the activity of polyamine biosynthetic enzymes would be expected. We found that levels of putrescine in detached rice leaves decreased with increasing duration of dark incubation (Chen and Kao, 1991b). To further characterize the role of endogenous putrescine in dark-induced rice leaf senescence, inhibitors of polyamine biosynthesis were used to change the level of intracellular putrescine in detached rice leaves (Chen and Kao, 1991b). DFMA and DFMO, inhibitors of putrescine biosynthesis, significantly decreased the level of putrescine without affecting senescence of detached rice leaves. By contrast, treatments with DCH and MGBG resulted in elevated levels of putrescine in detached rice leaves and promoted senescence. These observations suggest that a lowering of the level of putrescine is unlikely to be the factor responsible for the senescence of detached rice leaves in darkness. This conclusion is further supported by the observations that the affect of hormone (abscisic acid or benzyladenine) on senescence of detached rice leaves is separate from that on polyamine levels or polyamine biosynthesis (Chen and Kao, 1991b; Chen and Kao, 1992).

LT-8 is a chlorophyll-deficient mutant of rice derived from Noring no. 8. Chlorophyll level in the normal (Noring no. 8) leaves decreased with increasing age. However, chlorophyll level in the mutant leaves began to decrease only when more than 60% of the initial protein had been degraded (Chen *et al.*, 1991a). If chlorophyll is used as the prime indicator of senescence, then the leaves of the mutant would be regarded as nonsenescent, or slowly senescent. The levels of spermidine and spermine in the mutant leaves, however, decreased with increasing age at a rate similar to that in the normal leaves (Chen *et al.*, 1991a). The pattern of the putrescine level in the normal leaves behaved some-

what similarly to that in the mutant leaves.

Taking all data into account, we conclude that endogenous polyamine levels do not seem to be part of the chain of events leading to the initiation of rice leaf senescence. Several other examples of systems where polyamine levels did not decrease during senescence have also been reported (Birecka *et al.*, 1984; Birecka *et al.*, 1990; Smith and Davies, 1985). In our work with corn leaves, there was marked increase in levels of putrescine (Kao, unpublished). The increase in putrescine level preceded the commencement of dark-induced senescence. D-Arginine and α -methylornithine caused a reduction in levels of putrescine and a retardation of senescence. It seems that in corn leaves, an increase rather than a decrease in the endogenous putrescine level is likely to be the factor responsible for senescence. LA 5 is a low-alkaloid line of tobacco, whereas tobacco cultivar Speight G-70 contains high levels of nicotine. Putrescine is an intermediate in the synthesis of nicotine in tobacco (Tiburcio and Galston, 1986). It is also known that spermidine and spermine

require putrescine during synthesis (Fig. 2). We found that leaves from LA 5 plants contained 12-, 13-, 2-, and 6-fold less nicotine, putrescine, spermidine, and spermine, respectively, than those from Speight G-70, at 7 days after topping (Hurng and Kao, unpublished). However, senescence syndrome in LA 5 leaves was not more pronounced than that in Speight G-70.

How Exogenous Polyamines Retard Senescence of Detached Rice Leaves

Cheng *et al.* (1984) studied senescence of detached rice leaves and found that exogenous diaminopropane, spermidine, and spermine all appeared to retard senescence in the dark, and all promoted chlorophyll degradation in the light. The presence of β -hydroxyethylhydrazine, an inhibitor of conversion of polyamines to diaminopropane by polyamine oxidase, reversed the effects of spermidine and spermine in the light, indicating the possible requirement to convert to diaminopropane. However, neither intact nor detached rice leaves contained detectable diaminopropane throughout senescence (Chen and Kao, 1991a; Chen and Kao, 1991b; Chen *et al.*, 1991a).

Since diaminopropane and polyamines are positively charged at cellular pH, they could possibly affect rice leaf senescence by virtue of this cation property. Calcium was shown to competitively inhibit the observed effects of exogenous polyamines and diaminopropane on rice leaf senescence (Cheng *et al.*, 1984), suggesting that initial attachment of polyamines or diaminopropane to a membrane site may be required. Thus, the hypothesis that stabilization of cell membranes by polyamines must be involved in the control of senescence was postulated. Roberts *et al.* (1986) presented evidence to show that exogenously applied polyamines simply reflect membrane rigidification rather than a true physiological response. A recent experiment conducted by DiTomaso *et al.* (1989) showed that putrescine did not replace calcium in maintaining membrane stability. Thus, the postulated role of polyamines in stabilizing cell membranes is far from clear.

Ethylene is known to promote senescence of rice leaf segments (Kao and Yang, 1983). Ethylene and polyamines are known to have opposite effects on rice leaf senescence. Both ethylene and polyamines have S-adenosylmethionine as a common precursor (Fig. 2). Thus, the mechanism by which exogenous polyamines

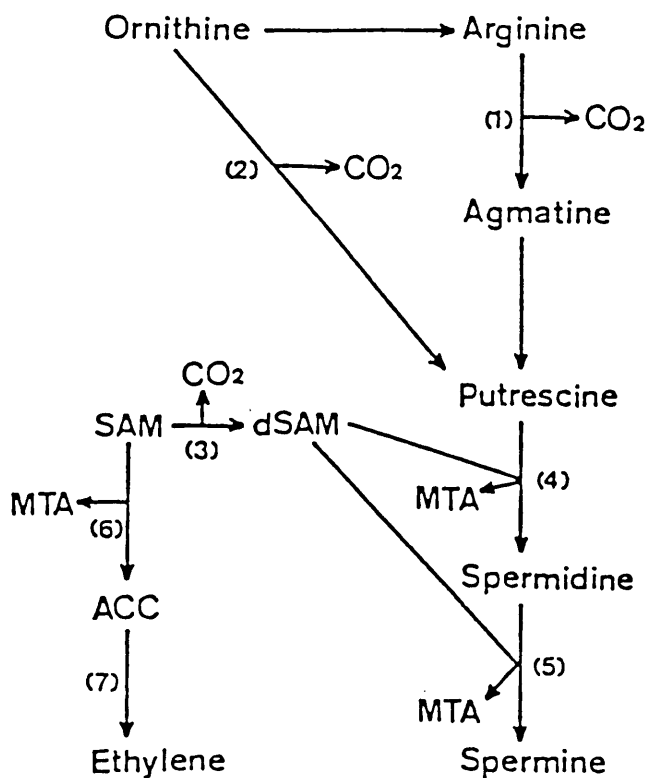


Fig. 2. Polyamine and ethylene biosynthetic pathways. The enzymes involved are: (1) ADC, (2) ODC, (3) SAMDC, (4) spermidine synthase, (5) spermine synthase, (6) ACC synthase, (7) ethylene-forming enzyme.

retard rice leaf senescence may be related to the possibility that they inhibit the biosynthesis of ethylene. Indeed, polyamines have been reported to inhibit ethylene production in a number of plant tissues, including fruits, leaves, petals, and hypocotyls (Apelbaum, 1981; Fuhrer *et al.*, 1982; Ke and Romani, 1988; Suttle, 1981). In order to understand whether the mechanism of the retardation of senescence of detached rice leaves by polyamines is related to the inhibition of the biosynthesis of ethylene by these compounds, we studied the effects of polyamines, specifically putrescine, on the biosynthesis of ethylene (Chen *et al.*, 1991b). Unexpectedly, we found that polyamines effectively promoted the production of ethylene in detached rice leaves, under both light and dark conditions. Putrescine stimulated ethylene production via enhancement of the synthesis of ACC and the conversion of ACC to ethylene. Exogenous polyamines also stimulated ethylene biosynthesis by detached tobacco and soybean leaf tissues (Pennazio and Roggero, 1989; Pennazio and Roggero, 1990). In some instances, polyamines have been shown to have no detectable effect on the production of ethylene (Downs and Lovell, 1986; Kramer and Wang, 1990). Thus, the mechanism by which polyamines may retard rice leaf senescence by inhibiting ethylene production is not clear.

Concluding Remarks

There is no doubt that exogenous polyamines retard rice leaf senescence. However, we do not know the exact mechanism by which polyamines bring about retardation, nor do we know the subcellular sites where polyamines act. In the past few years, much attention has been paid to the changes in free polyamine levels during rice leaf senescence. It would be of great interest to know whether polyamines bound with specific regulatory proteins are causally related to rice leaf senescence. To determine the functional role of polyamines, it is important to understand the metabolic fate of these compounds during senescence. We also need to know whether the effects of polyamines on senescence are artifacts or nonspecifically toxic, as suggested by some workers. More work is required to clarify the involvement of polyamines in rice leaf senescence. There is potential for breakthroughs. The prospects are promising.

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