Bot. Bull. Acad. Sin. (1997) **38:** 141-144

(*Invited review paper*)

Physiological significance of stress-induced changes in polyamines in plants

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Abstract. Polyamines (putrescine, spermidine, and spermine) are present in all living organisms. The possible role of polyamines under stress conditions is reviewed. Recent experiments indicate that polyamine levels are not associated with growth inhibition caused by nitrogen deficiency, sucrose starvation, and NaCl stress. Putrescine accumulation seems to be a factor causing growth inhibition under potassium deficiency and phosphate deprivation. Spermidine and spermine seem to be involved in protection against paraquat toxicity in rice leaves.

Keywords: Growth; Paraquat; Plant stress; Polyamines.

Contents

Introduction

A number of nitrogen-containing compounds accumulate in plants in response to environmental stress conditions (Rabe, 1990). The nitrogen-containing compounds which normally accumulate during stress conditions include amino acids (arginine, proline), amides (glutamine, asparagine), ammonium, quaternary ammonium (glycinebetaine) and polyamines (putrescine, spermidine, spermine).

Polyamines are low-molecular weight polycations, and are present in all living organisms. Putrescine is the obligate precursor for spermidine and spermine in all systems studied so far. In higher plants, putrescine is made from ornithine, utilizing the enzyme ornithine decarboxylase (ODC). An alternative pathway in higher plants for the synthesis of putrescine involves arginine decarboxylase. The product of this reaction, agmatine, is then converted to putrescine in two additional enzymatic steps. In animal cells, putrescine is generally thought to be synthesized solely via ODC. The recent discovery of a substantial amount of tissue agmatine in rats and of arginine decarboxylase activity in rat brains raises the possibility that there is an alternative pathway for putrescine biosynthesis in mammals via arginine decarboxylase (Li et al., 1994; Raasch et al., 1995). Experimental evidence now indicates that plant polyamines may be involved in growth, differentiation or morphogenesis, stress, and senescence (Evans and Malmberg, 1989). This mini-review will be limited to a discussion of the possible function of stress-induced changes in polyamines in plants.

Are Polyamines a Growth Factor Under Stress Conditions?

The involvement of polyamines in the growth of prokaryotic and eukaryotic cells was established through using a series of mutants in *Escherichia coli* and *Saccharomyces cerevisiae* (Tabor and Tabor, 1984). For example, in *Saccharomyces*, several mutants at the ODC locus are unable to grow without the addition of polyamines. Mammalian cells in tissue culture have also been demonstrated to require polyamines for normal growth (Bachrach, 1973; Cohen, 1971). Bertosi et al. (1965) were the first to report that polyamines stimulated the growth *in vitro* of explants from dormant tubers of *Helianthus tuberosus*. Subsequently, polyamine levels and plant growth rates were positively correlated in a wide variety of conditions when high levels of polyamines were associated with rapidly growing tissues (Evans and Malmberg, 1989).

Bagni et al. (1978) demonstrated that putrescine had the ability to substitute for inorganic nitrogen in the growth of *in vitro* explants from dormant tubers of *Helianthus tuberosus*. Bayley et al. (1972) tested putrescine for its ability to alleviate the reduced nitrogen requirement of soybean cells, grown in suspension culture on nitrate medium. They found that putrescine supported growth, although less effectively than NH_4^+ and glutamine. Nitrogen deficiency markedly inhibited rice cell growth and resulted in lower levels of putrescine, spermidine, and spermine than in the control culture supplied with nitrogen (Sung et al., 1995a). If polyamines can substitute for inorganic nitrogen in the growth of rice cells, then nitrogen deficient rice cells would be expected to recover the growth by the addition of polyamines. However, the growth inhibition of rice cells induced by nitrogen deficiency could not be recovered by the addition of polyamines, but could be recovered by the addition of a mixture of amino acids (glycine, aspartic acid, glutamic acid and arginine) (Sung et al., 1995a). Sucrose starvation also resulted in growth inhibition and lower polyamine levels in rice suspension cells than in the control cells (Sung et al., 1995b). As in the case of nitrogen deficiency, the growth inhibition of sucrose-starved rice cells did not recover after the addition of polyamines (Sung et al., 1995b). Clearly, in the case of rice cells, polyamines do not seem to regulate nitrogen deficiency- and sucrose starvation-inhibited growth.

NaCl is known to inhibit rice growth (Flowers and Yeo, 1981; Prakash and Prathapasenan, 1988a; Prakash et al., 1988). Several reports have shown that exogenous application of putrescine can overcome the harmful effects of NaCl stress on rice seedlings (Krishnamurthy, 1991; Prakash and Prathapasenan, 1988a; Prakash and Prathapasenan, 1988b). It has been shown that endogenous levels of putrescine decreased in rice seedlings under NaCl stress (Lin and Kao, 1995; Prakash et al., 1988). The addition of precursors of putrescine biosynthesis (L-arginine and L-ornithine) resulted in an increase in putrescine levels in NaCl-treated shoots and roots, but did not allow recovery of growth inhibition of rice seedlings induced by NaCl (Lin and Kao, 1995). Lin and Kao (1995) concluded that endogenous putrescine may not play a significant role in the control of the NaCl-inhibited growth of rice seedlings. Furthermore, putrescine accumulation in rice seedlings in response to NaCl has also been reported (Basu et al., 1988; Krishnamurthy and Bhagwat, 1989).

Putrescine, Potassium Deficiency, and Phosphate Deprivation

The availability of potassium is known to have a large impact on plant growth. Richards and Coleman (1952) were the first to demonstrate that potassium deficiency induced an accumulation of polyamines, especially an accumulation of putrescine in barley leaves. Subsequently, the accumulation of putrescine under conditions of potassium deficiency has been observed in several plant species (Flores, 1991; Smith, 1984). P is one of the most important yet least available mineral nutrients for plant growth. Under conditions of phosphate deprivation, putrescine was found to accumulate only in tobacco and barley plants, and suspension-cultured rice cells (Shih and Kao, 1996; Sinclair, 1967; Takahashi and Yoshida, 1960).

Potassium deficiency- and phosphate deprivation-induced putrescine accumulation could represent (a) the cause of the injury syndrome, (b) the plant's defense against injury, or (c) a metabolic side-effect unrelated to stress. Using aseptic cultures of *Lemna paucicostala* 6746 and *Lemna gibba* G3, Tachimoto et al. (1992) were able to show that the growth under potassium deficiency recovered to some extent when putrescine was added, although the level of putrescine in these plants was considerably higher than that in the plants which did not receive putrescine. Murty et al. (1971) reported that putrescine compensated for potassium deficiency in black currant to the extent of 30% in terms of cations. These experiments suggest that putrescine plays a role in the replacement of potassium as an organic cation in potassium deficient plants (Murty et al., 1971; Tachimoto et al., 1992). However, this mechanism can not explain the role of putrescine in phosphate-deprived plants, since phosphate is an inorganic anion. Recently, we demonstrated that putrescine in excess of the level normally found in rice cells could be a factor causing growth inhibition of rice cells cultured under potassium deficiency and phosphate deprivation (Shih and Kao, 1996; Sung et al., 1994). This conclusion was mainly based on the observation that D-arginine, inhibitor of putrescine biosynthesis, caused a reduced level of putrescine in and a recovery of growth of rice cells cultured under potassium deficiency and phosphate deprivation, and growth recovery in potassium-deficient and phosphate-deprived rice cells by D-arginine was reversed by the addition of putrescine. Furthermore, it has also been shown that feeding putrescine to cut leaves of barley produced the same symptoms found in potassium deficient plants (Coleman and Richards, 1956).

Polyamines and Paraquat Toxicity

Paraquat is a herbicide widely used in agriculture. Very little is known about the effects of herbicides on polyamine levels in plants. It has been shown that nopropamide, a soil applied amide herbicide, increased putrescine level but had little effect on spermidine and spermine levels in pea roots (DiTomaso et al., 1988). In addition, atrazine, a widely used selective herbicide, increased putrescine, spermidine, and spermine levels in pea leaves (Zheleva et al., 1993). We recently observed that paraquat increased putrescine level but decreased spermidine and spermine levels in rice leaves (Chang and Kao, unpublished).

Minton et al. (1990) have shown that toxicity of paraquat for *Escherichia coli* is increased over tenfold in strains defective in the biosynthesis of spermidine compared to isogenic strains containing spermidine, and the increased sensitivity of these spermidine-deficient mutants to paraquat is eliminated by growing in medium containing

spermidine or by exogenous supplementation of spermidine by the use of a *spd* E^+D^+ plasmid. The protective effect of polyamines against paraquat has not been examined in higher plants. Recently, we investigated this effect in rice leaves (Chang and Kao, unpublished). Rice leaves treated with spermidine and spermine or with L-arginine and L-ornithine, precursors of polyamine biosynthesis, resulted in an increase in spermidine and spermine levels and also a reduction of paraquat toxicity.

In considering the possible mechanism for reduction of paraquat toxicity, inhibition of paraquat uptake by spermidine and spermine from the medium is one possibility. Since pretreatment with spermidine and spermine followed by treatment with paraquat also results in protection against paraquat toxicity, the effect of spermidine and spermine cannot be explained by direct competition between paraquat and spermidine or spermine.

Paraquat is known to increase lipid peroxidation in leaf tissue. It has been proposed that polyamines may take part in a cellular defence mechanism against oxidative damage through inhibition of lipid peroxidation (Kitada et al., 1979; Tadolini, 1988; Tadolini et al., 1984). However, spermidine and spermine had no effect on lipid peroxidation in rice leaves (Chang and Kao, unpublished), rendering the postulated property of reduction of lipid peroxidation of spermidine and spermine rather questionable.

It is believed that paraquat toxicity results from the production of hydrogen peroxide, superoxide radical and other highly toxic free radicals formed in the chloroplasts during photosynthesis (Calderbank, 1968). Thus the elevated activities of enzymes such as catalase, peroxidase, as well as the activities of enzymes in the Halliwell-Asada pathway in rice leaves, which would prevent excessive accumulation of such toxic products, might protect against paraquat toxicity. We observed that spermidine and spermine had no effect on the activities of superoxide dismutase, ascorbate peroxidase, or glutathione reductase, but increased the activities of catalase and peroxidase in rice leaves (Chang and Kao, unpublished). It appears that reduction of paraquat toxicity by spermidine and spermine is due to the increased activities of catalase and peroxidase.

Concluding Remarks

Although the involvement of polyamines in plant growth has been proposed for more than 20 years, recent experiments suggest that polyamines play no role in regulating growth inhibition by those stress conditions, under which the decline of polyamine levels usually occurs. On the contrary, evidence indicates that putrescine accumulation is a factor causing stress-induced growth inhibition of plants. We do not know the mechanism of growth inhibition caused by elevated putrescine levels. Further research is required. The finding that spermidine and spermine protect rice leaves against paraquat toxicity is intriguing. Whether spermidine and spermine would reduce paraquat toxicity in other plant systems is still to be determined. The possibility that rice leaves with high levels of spermidine and spermine can be used to select paraquat-tolerant rice plants should also be examined.

Acknowledgements. Over the years, our research on polyamines has received generous support from the National Science Council of the Republic of China.

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