

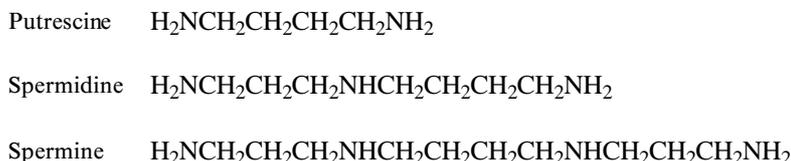
## STRESS AND POLYAMINE METABOLISM

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### Introduction

The **polyamines (PAs) putrescine (Put), spermidine (Spd) and spermine (Spm)** (Fig. 1) have been shown to be involved in a variety of plant growth and developmental processes, including cell division, vascular differentiation, root initiation, shoot formation, flower initiation and development, fruit ripening and senescence and embryoid formation in tissue cultures (Evans and Malmberg, 1989; Galston and Kaur-Sawhney, 1990; Galston and Kaur-Sawhney, 1995). Many of these functions are similar

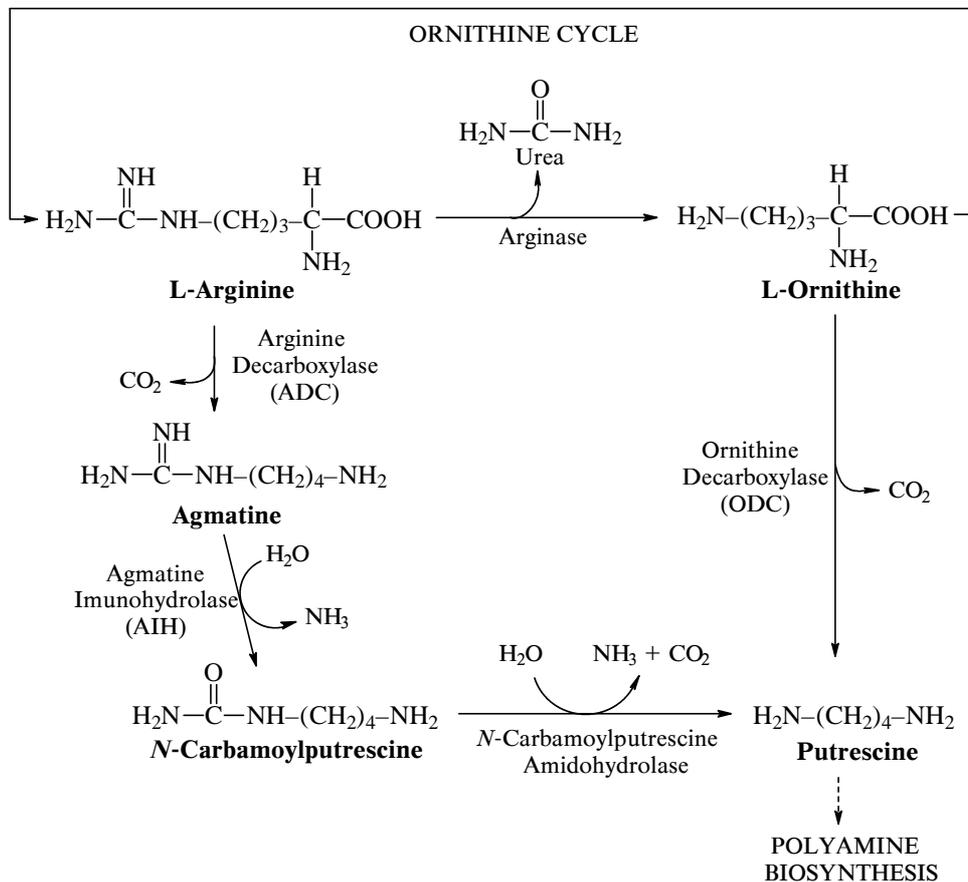


**Fig. 1.** Chemical structure of main polyamines

to those mediated by known plant hormones such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene. In addition, direct and putative interactions of plant hormones and PAs are also known. However, in spite of these facts many plant physiologists still have some doubts about recognizing PAs as another class of plant growth regulators.

The biosynthesis of common PAs can be divided into two main steps: synthesis of the diamine Put and formation of the PAs, Spd and Spm resulting from propyl-amino addition to the Put precursor.

As can be seen in Fig. 2 Put is synthesized by one of the two main pathways in plants. It can be formed directly by decarboxylation of L-ornithine, in a reaction catalyzed by ornithine decarboxylase (ODC). This pathway is the only route to Put synthesis in animals and in most fungi (Tabor and Tabor, 1985).

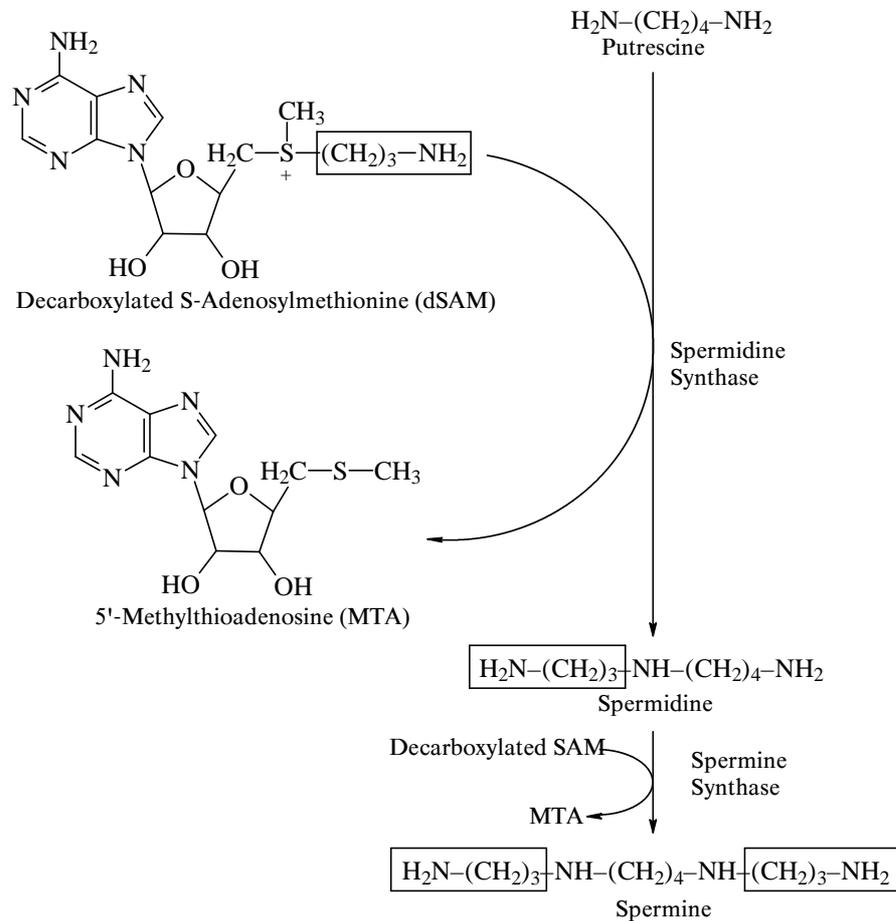


**Fig. 2.** Biosynthesis of putrescine (Slocum, 1991)

Alternatively the decarboxylation of L-arginine by arginine decarboxylase (ADC) leads to the formation of Put through agmatine and N-carbamoylputrescine intermediates.

Spd and Spm synthesis is carried out by addition of an aminopropyl group to one or both primary aminogroups of Put by Spd and Spm synthases respectively (Fig. 3). It is now well documented that decarboxylated S-adenosylmethionine (DSAM) is the aminopropyl donor in the synthesis of these amines. DSAM is derived from S-adenosylmethionine (SAM) in a reaction catalyzed by SAM decarboxylase (SAMDC). SAM is derived from L-methionine via SAM synthase.

Since the plant hormone ethylene is also derived from a SAM precursor and because ethylene and PAs promote and inhibit senescence in plant tissues respectively, there has been considerable interest in the metabolic relation between the ethylene and PA biosynthetic pathways recently.

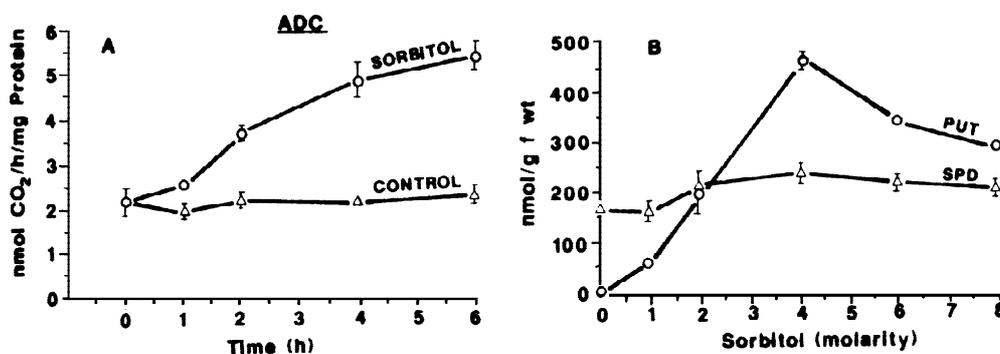


**Fig. 3.** Biosynthesis of spermidine and spermine (Slocum, 1991)

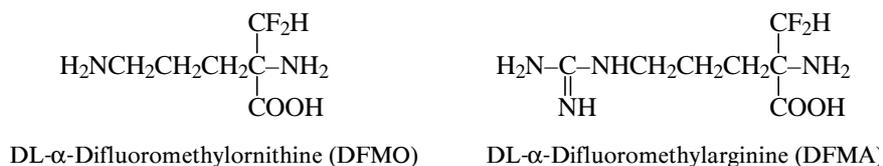
It has been known for the last 35 years that higher plants exposed to suboptimal or stress-inducing conditions of the environment may respond by accumulating high concentrations of the diamine Put. The original observation on this subject by Richards and Coleman (1952) dealt with barley plants grown in hydroponic culture and exposed to restricted levels of  $\text{K}^+$  ions. Since then the following stresses have also been shown to result in the accumulation of Put: water deprivation (Flores, 1983), high and external osmotic concentration (Flores and Galston, 1982, 1984), high concentration of  $\text{NH}_4^+$  (LeRudulier and Goas, 1971, 1975),  $\text{H}^+$  (Smith and Sinclair, 1967) and other monovalent cations (Smith, 1973) in the ambient solution, exposure to the atmospheric pollutant  $\text{SO}_2$  (Priebe et al., 1978),  $\text{Cd}^{2+}$  (Weinstein et al., 1986),  $\text{O}_3$  (Ormrod and Beckerson, 1986),  $\text{Pb}^{2+}$  (Aktan and Palavan-Ünsal, 1994), low temperature in subtropical crops (McDonald and Kushad, 1986) and high temperature (Kuehn et al., 1990).

## Osmotic Stress

Oat seedling leaves show a remarkably rapid response to osmotic stress (Flores, 1983; Flores and Galston, 1984). If such leaves are excised, peeled to remove the lower epidermis and floated in 1 mM pH 5.8 phosphate buffer in the light, then the Put level remains constant for at least 16 hours. However if 0.4 M sorbitol is added to the bathing medium, the Put concentration begins to rise within 1 hour and increases 40–50 fold within 6–10 hours. (Fig. 4). The accumulation of Put is also inhibited by the addition of  $\alpha$ -difluoromethylarginine (DFMA), a suicide inhibitor of ADC. The analogous inhibitor of ODC,  $\alpha$ -difluoromethylornithine (DFMO) (Fig. 5), produces no inhibition. Instead of this it produces 15 to 20% enhancement of Put level.



**Fig. 4.** Effect of osmotic stress on arginine decarboxylase activity. Leaf segments were floated over buffer or buffer + 0.4 M sorbitol under light, and samples were taken at the indicated times for enzyme assay (Flores and Galston, 1984)

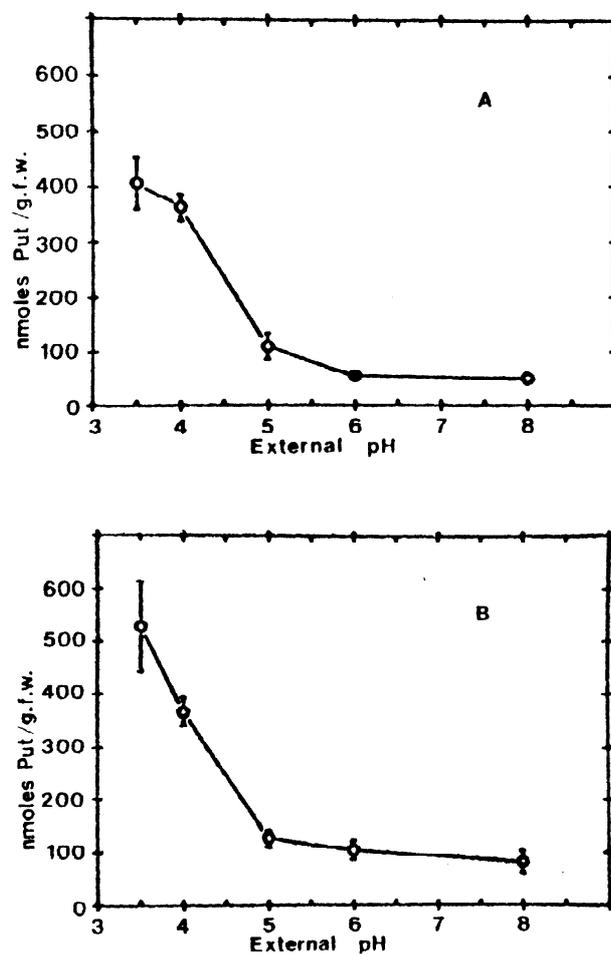


**Fig. 5.** The chemical structure of DL- $\alpha$ -difluoromethylornithine and  $\alpha$ -difluoromethylarginine

There is evidence also that the response of osmotic stress in excised oat leaves causes Put accumulation and this response occurs not only from ADC activation, but also from a decrease in Spd synthase activity (Tiburcio et al., 1993).

## pH Stress

The effect of high acidity on ADC activity was first discovered in microorganisms in 1940 by Gale. In the excised, peeled oat leaf segment system Put levels (Young, 1984) are low and stable at pH 8, 7 and 6 but increase rapidly and proportionately as the pH is progressively decreased at least down to pH 3.5 ( Young and Galston, 1983). The increase in Put is accompanied by a decrease in Spd, implying and acid-induced inhibition of Spd synthase (Fig. 6).

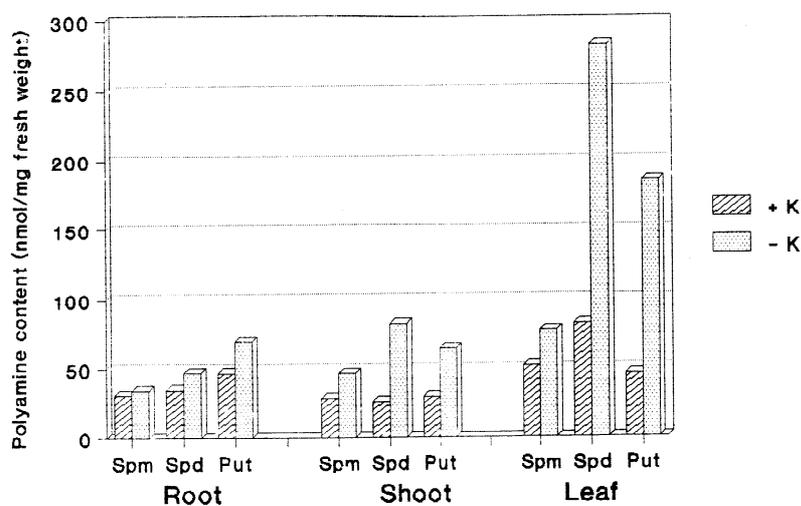


**Fig. 6.** Response of endogenous Put concentration to a range of external pH in excised oat leaf segments (A) and pea leaf discs (B) incubated for 8 h+ (Young and Galston, 1983)

## Nutritional Stress

As mentioned before,  $K^+$  starvation results in an increase of Put titer (Richards and Coleman, 1952). Deficiencies of  $Mg^{2+}$  and  $Ca^{2+}$  also induce the rise in Put, but these increases are smaller and take longer to develop. Put increase following  $K^+$  deprivation is accompanied by a rise in the activities of ADC. All these effects are reversed within 48 h after addition of  $K^+$  to the medium.  $Rb^+$  and  $Na^+$  can substitute  $K^+$  to some extent, while  $Li^+$  is completely ineffective. There also appears to be some organ specificity, since Galston in 1989 indicated that Put accumulation in  $K^+$  deficient barley occurs in the aerial organs and he also expressed that it is not known whether roots are capable of demonstrating the effect.

In our lab we demonstrated the Put accumulation in the roots of 15-day-old *Heliotropium europeum* plants in  $K^+$  deficiency conditions. We obtained maximum increase in the leaves, but the free and bound PA contents were also higher in the leaves than in the roots and shoots of these plants (Fig. 7) (Delibasoglu and Palavan-Ünsal, in preparation). On the other hand, the shoot and root lengths were decreased in  $K^+$  deficient conditions (Fig. 8 and 9).



**Fig. 7.** The effect of potassium deficiency on polyamine content in different organs of 15-day-old *Heliotropium europeum* plants (Delibasoglu and Palavan-Ünsal, in preparation)

Salinity is also known to cause an increase in Put (Stroganov et al., 1960). This appears to be an ion effect rather than an osmotic effect since addition of 50mM NaCl to the nutrient medium results in a sharp rise of Put and Cad titers. Later Shevyakova (1981) established a considerable accumulation of Put and cadaverine (Cad) in pea and bean plants grown in NaCl above 50mM. PA accumulation and activation of both

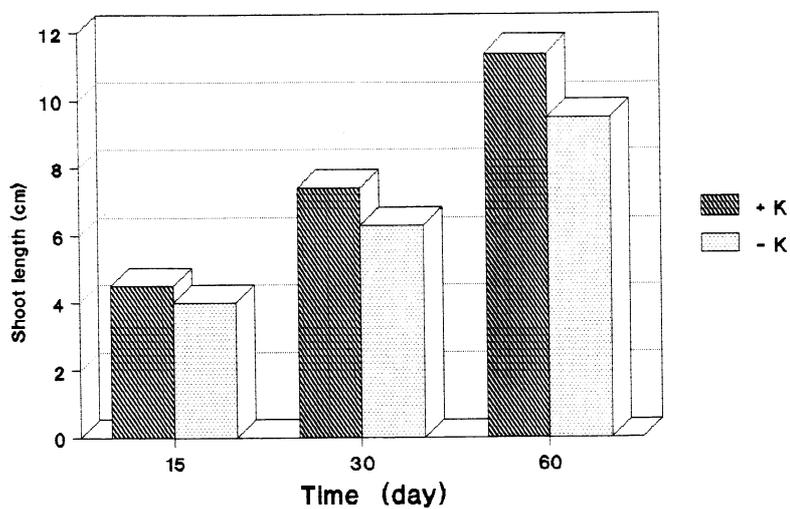


Fig. 8. The effect of potassium deficiency on shoot length of *Heliotropium europeum* plants (Delibasoglu and Palavan-Ünsal, in preparation)

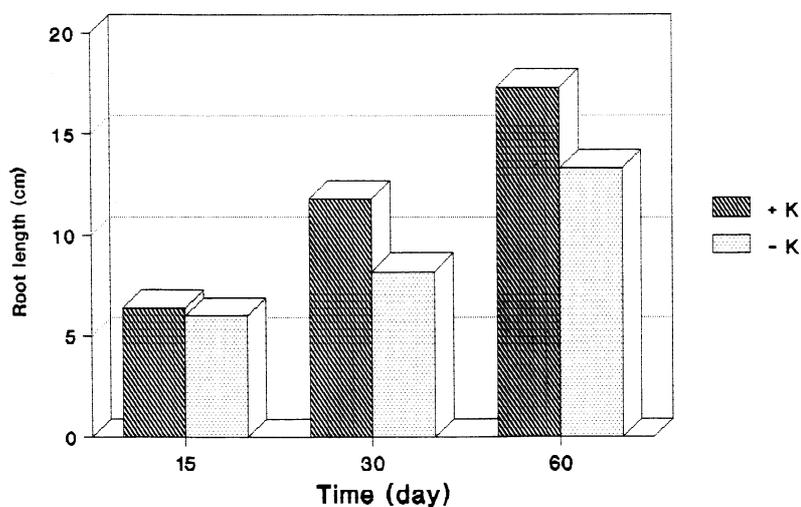


Fig. 9. The effect of potassium deficiency on root length of *Heliotropium europeum* plants (Delibasoglu and Palavan-Ünsal, in preparation)

ADC and ODC were found also in mung bean plant in response to NaCl stress (Friedman et al., 1989). On the other hand, Tattini et al. (1993) determined the dramatic decrease in root Put content in one-year-old *Olea europaea* plants supplied with increasing NaCl concentrations (50–150 mM). It is clear that the nutritional state of the plant is frequently reflected in its Put titer.

## Temperature Stress

Most studies relating PAs to stress responses have been done with the common widespread compounds Put, Spd and Spm. The usual response to stress is an increase in one or more of the PAs already present at the start of the stress. Recent findings suggests that at least under heat stress, plant cells can be induced to synthesize new PAs. Thermophilic bacteria usually contain Spd when grown under normal conditions. When the temperature is increased above 50°C, a whole new series of PAs appears, mostly symmetrical analogs of Spd and Spm (Oshima, 1983). The most common of these **thermopolyamines** are **norspermidine (caldine)** and **norspermine (thermine)**. Until recently, the presence of thermopolyamines was assumed to be restricted to thermophilic bacteria. Studies with heat tolerant cultivars of cotton have given surprising results. Pollen and cell cultures of heat tolerant cotton genotypes also accumulate the uncommon PA, **caldopentamine**, when exposed to high temperature (Kuehn et al., 1990).

Injury caused by chilling also results in significant increases in Put levels in a variety of fruits and vegetables (McDonald and Kushad, 1986; Wang and Ji, 1989). According to the results PAs protect plants from chilling injury.

## Atmospheric Pollutant

It was shown that SO<sub>2</sub>, a common atmospheric pollutant causes an increase in free and bound Put in peas (Priebe et al., 1978). This effect may also be attributed to the release of H<sup>+</sup> ions, because SO<sub>2</sub> absorption into cells results in the formation of acids.

On the other hand, treatment of bean and oat leaf discs with CdCl<sub>2</sub> causes an increase in Put content. As little as 0.001 mM Cd<sup>2+</sup> produces some effect and maximum effects are produced by 0.10 mM (Weinstein et al., 1986).

Ozone (O<sub>3</sub>) is a major component of air pollution and is considered to have serious effects on vegetation, even when applied at low levels. When exogenous PAs were fed to tomato and tobacco plants there was a significant suppression of O<sub>3</sub>-induced leaf injury (Ormrod and Beckerson, 1986; Bors et al., 1989). In O<sub>3</sub>-treated barley leaves ADC activity increased before the injury was apparent (Rowland and Bamford, 1989). These results suggests that PAs may have a protective role against O<sub>3</sub> damage.

Lead is an another important environmental contaminant like Cd<sup>2+</sup>, not only because of the phytotoxicity but also because its uptake and accumulation in plants may introduce it into the food chain. We sprayed the half leaves that were removed from the main vein of 5-week-old sunflower plants, with different lead concentrations (Pb(NO<sub>3</sub>)<sub>2</sub>, 0.05, 1.25 and 5.00 mM) on both sides everyday for a week (Aktan and Palavan-Ünsal, 1994). 0.05, 1.25 and 5.00 mM Pb<sup>2+</sup> treatments increased Put con-

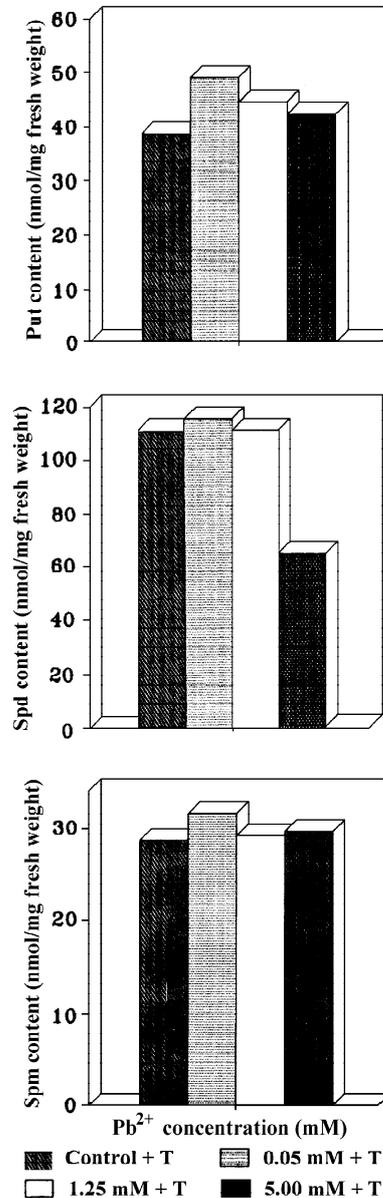
tent by 27, 15 and 9% respectively (Fig. 10). The effect of lead on Spd and Spm content were not significant.

### Biotic Stress

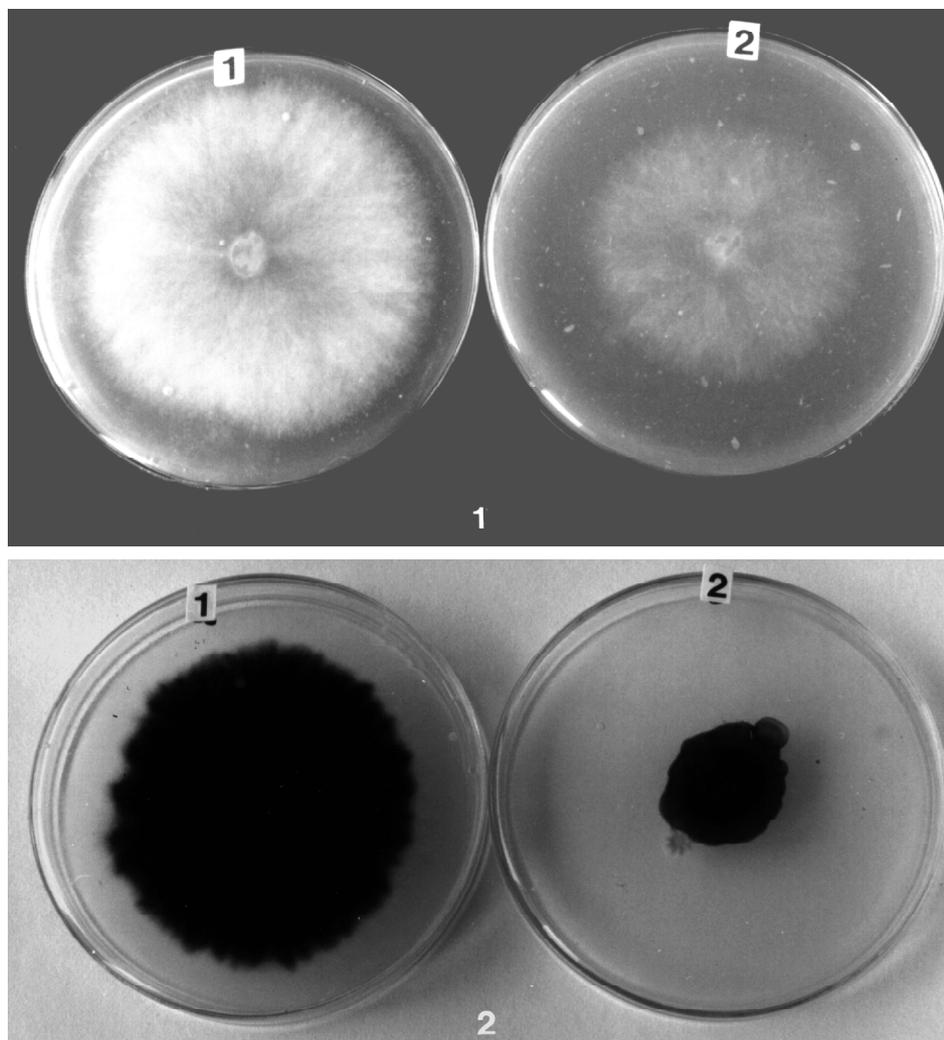
At least some fungi lack ADC and produce the Put they need for higher PA biosynthesis exclusively through decarboxylation of ornithine by ODC. If PAs are indeed required for growth, then it would appear that infection of higher plants by fungal pathogens might be prevented by blocking ODC activity. Rajam and Galston (1985) showed that 0.5 mM DFMO supplied to cultures of phytopathogenic fungi *Botrytis* on synthetic medium resulted in a marked inhibition of growth and that such inhibition is reversible by 0.1–1.0 mM Put or Spd. Later, Birecka et al. (1986) confirmed this observation in *Helminthosporium maydis*. Again Rajam and Galston (1985) determined that DFMO sprays were effective in growth inhibition of *Uromyces phaseol* also. The effective DFMO concentrations (0.5 mM) produced no abnormal symptoms on the host plants and were also able to protect unsprayed parts of the same or other leaves.

We also tried to search the DFMO effect on growth of 10 different phytopathogenic fungi which are important for Turkish agriculture. As a result we obtained the inhibition of growth in *Botrytis cinerea*, *Macrophomina phaseolina* and *Drechslera sorokiniana* (Palavan-Ünsal et al., 1993) (Fig. 11).

As a conclusion, the occurrence of a single biochemical response to such a diverse array of stress-inducing conditions suggests that Put accumulation may be taken as an indicator of stress metabolism in the plants.



**Fig. 10.** The effect of different concentrations of lead on polyamine content in 5-week-old *Helianthus annuus* half leaves. Values are average of 5 experiments and each experiment has duplication, C: Distilled water (Aktan and Palavan-Ünsal, 1994)



**Fig. 11.** 1: The effect of DFMO on growth of *Botrytis cinerea* on 72nd hour. Left: Control, Right: 2 mM DFMO. 2: The effect of DFMO (2 mM) on growth of *Drechslera sorokiniana* on 7th day. Left: Control, Right: 2 mM DFMO (Palavan-Ünsal et. al. 1993)

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