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Spermine and putrescine enhance oxidative stress tolerance in maize leaves

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Abstract

The protective effects of spermine (SPM) and putrescine (PUT) against paraquat (PQ), a herbicide in agriculture and oxidative stress inducer, were investigated in the leaves of maize. Maize leaves were pretreated to SPM and PUT at concentrations of 0.2 and 1 mM and treated with PQ afterwards. Pretreatment with 1 mM of SPM and PUT significantly prevented the losses in chlorophyll and carotenoid levels induced by PQ. Ascorbic acid content in the leaves pretreated with both polyamines was found to be higher than those of the leaves pretreated with water. Also, pretreatment with SPM and PUT was determined to have some effects on the activities of superoxide dismutase (SOD) and peroxidase (POD). 1 mM of SPM increased SOD activity, but PUT has no significant effect on SOD activity. On the other hand, POD activity was recorded to increase slightly in response to both concentrations of SPM and 1 mM of PUT. The results showed that such polyamine pretreated plants may become more tolerant to oxidative stress due to increases in the antioxidative enzymes and antioxidants.

List of abbrevations: EDTA: Ethylenediamine tetra acetic acid, NBT: Nitroblue tetrazolium, POD: Peroxidase, PQ: Paraquat, PUT: Putrescine, ROS: Reactive oxygen species, SOD: Superoxide dismutase, SPM: Spermine.

Introduction

Crop loss due to environmental stresses is the primary source of decrease in agricultural productivity. The reason is partly due to oxidative stress that is the overproduction of reactive oxygen species (ROS) in plant cells under these environmental conditions. Abiotic and biotic stresses (pollutants, herbicides, extremes of temperature and high light, high O_2 pressures, salinity and pathogen invasion) all cause increases in toxic ROS in plant cells (Sakaki *et al*. 1983, Kenylon and Duke 1985). The containment of ROS has proved to be important for problems as diverse as aging and cancer in human health and crop loss in agriculture. Therefore, understanding of oxidative stress and antioxidant defense mechanisms and alleviation of oxidative damage are important for plant productivity. It has been proposed that polyamines could take part in cellular defense mechanism against oxidative damage through the inhibition of lipid peroxidation (Tadolini 1988). In addition, polyamines are well known for their anti-senescence and anti-stress effects due to their acid neutralizing and antioxidant properties, as well as to their membrane and cell wall stabilizing abilities (Velikova *et al*. 2000). Free radical scavenging properties of polyamines have also been documented (Drolet *et al*. 1986). Despite extensive studies on polyamine metabo-

lism, the exact role that these compounds play in plant physiology remains unclear (Tiburcio *et al*. 1997).

Paraquat herbicidal activity in higher plants is thought to be the result of increased amount of superoxide radical. Paraquat accepts an electron from the primary electron acceptor of photosystem I to become a reduced free radical which rapidly reacts with oxygen to form the superoxide radical (Dodge 1994). Superoxide serves as a source of hydrogen peroxide and the highly active hydroxyl radical. Thus, toxicity of paraquat stems from the generation and activity of oxygen species that lead to oxidative stress in biological systems. Reactive oxygen species can react with numerous cell components causing inactivation of enzymes, pigment bleaching, lipid peroxidation, and proteolysis. Thus, they need to be scavenged for maintenance of normal plant growth. Plant cells contain substances such as glutathione, ascorbic acid and carotenoids, and also enzymes like superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), glutathione reductase (GR; EC 1.6.4.2) which participate in scavenging ROS (Halliwell 1982). The primary scavenger is the enzyme superoxide dismutase which converts superoxide to hydrogen peroxide (Asada and Kiso 1973). This toxic product of SOD is removed by POD. Enhanced production of oxygen free radicals is responsible for peroxidation of membrane lipids and the degree of peroxidative damage of cells was controlled by the potency of the antioxidative POD enzyme system (Sreenivasulu *et al*. 1999). In several plant species paraquat-tolerance was correlated with increased capacity of enzymes detoxifying activated oxygen species (Shaaltiel *et al*. 1988; Furusawa *et al*. 1984). Correspondingly, paraquat tolerance in *Conyza*, *Lolium* or *Nicotiana* was also accompanied by a cross-tolerance to other environmental factors involving oxidative stress, such as SO_2 or ozone (Shaaltiel *et al*. 1988; Tanaka *et al*. 1988).

In this study we investigated if prior exposure to SPM and PUT may protect plants against exposure to paraquat and if this protection may be related to antioxidative enzyme activities and antioxidant levels.

Materials and Methods

Plant material and treatments

Maize (*Zea mays* L. cv RX 947) seeds which were obtained from Agriculture Research Center in Trabzon were sown in plastic pots (11 cm high, 23 cm top and 13 cm bottom diameter) filled with soil and sand (5:1). They were maintained in a growth chamber under a 16-h light/8 h dark regime with a light intensity of 350 $\mu E \cdot m^{-2} \cdot s^{-1}$, 75 % relative humidity, and day/night temperatures of 25/22 °C. Ten day-old maize plants were sprayed until run off with SPM and PUT at 0.2 and 1 mM concentrations, each containing 0.05 % Tween 20 as a surfactant once daily for 4 days. Then, for paraquat treatment, leaves of 14-d-old plants were exposed in a surface application to 10^{-4} M paraquat (methyl viologen) in a 0.05 % solution of the Tween 20 for 24 h into the light period. Control plants were sprayed with 0.05 % Tween 20 in distilled water. Foliar samples were collected for analyses at 8, 12 and 24 h following PQ application, immediately frozen in liquid nitrogen and stored at –20 °C for determination of enzyme activities. All treatments were repeated at least three times on different days.

Determination of chlorophyll and carotenoids

For chlorophyll and carotenoid determinations, the leaves were homogenized in 5 ml of 80 % acetone and centrifuged at 3000 rpm for 5 min. The optical density of the supernatant was read at 450, 645 and 663 nm with a spectrophotometer. The amounts of total chlorophyll and carotenoids were estimated according to Arnon (1949) and Jaspars (1965), respectively.

Determination of ascorbic acid

The determination of ascorbic acid was performed using the procedure of Shieh and Sweet (1979) with pure ascorbic acid as the standard. Two g samples were homogenized with 0.01 M phosphate-citric acid buffer, pH 3.0, filtered and centrifuged at 5000 rpm, for 5 min at 25 °C. The supernatant was used to determine the ascorbic acid content. The assay mixture consisted of 0.5 ml of 0.01 M phosphate--citric acid, pH 3.0, 2.4 ml of 2,2′-Cu-biquinoline solution (1.0 mM 2,2′-biquinoline and 0.38 mM

$CuCl_2 \cdot 2H_2O$) and 0.1 ml of the plant extract. Ascorbic acid content was determined spectrophotometrically at 540 nm.

Determination of SOD activity

Leaf tissue (0.5 g) was ground to a powder in liquid nitrogen and then homogenized in 5 ml of cold 50 mM phosphate buffer (pH 7.0), containing 1 mM EDTA, 0.05 % triton, 2 % polyvinylpolypyrrolidone, and 1 mM ascorbic acid. The homogenate was filtered through two layers of cheesecloth and centrifuged at 20000 g for 20 min at 4 °C. The supernatant was used for enzyme analyses.

SOD activity assay was based on the method of Beauchamp and Fridovich (1971) as modified by Dhindsa and Matowe (1981), which measures the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT). In the spectrophotometric assay the 1 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionin, 75 μM NBT, 2 μM riboflavin and 50 μl of the plant extract. Riboflavin was added last and the reaction was initiated by placing the tubes under fluorescent white light. The reaction was terminated after 10 min by removal from the light source. Reaction product was measured at 560 nm. The volume of supernatant corresponding to 50 % inhibition of the reaction was assigned a value of 1 enzyme unit.

Determination of POD activity

0.5 g leaf tissue was ground to a powder in liquid nitrogen and then homogenized in 5 ml of cold 0.2 M sodium phosphate buffer (pH 7.0). The homogenate was filtered through two layers of cheesecloth and then centrifuged at 20000 g for 20 min at 4 °C (Canal *et al*. 1988). The supernatant was assayed for the enzymatic activity. Peroxidase activity was measured using a modification of the procedure described by Rodriguez and Sanchez (1982). The assay mixture contained 1.4 ml of 0.05 M phosphate citrate buffer (pH 4.6), 1 ml of 40 mM guaiacol and 0.5 ml of 26 mM H_2O_2. The mixture was incubated for 15 min at 25 °C. The assays were initiated by the addition of the enzyme extract and the formation of the oxidized tetraguaiacol polymer was monitored at 420 nm for 3 min. Peroxidase activity was expressed as A_{420}/min/g fresh weight.

Statistical analysis

Analysis of variance of data was evaluated by the Statistical Package for Social Sciences (SPSS for Windows 9.0). DUNCAN's Multiple Range Test was employed to determine the statistical significance of differences among the means.

Results

Bipyridyl herbicides such as paraquat are redox--active compounds that become reduced within the cell and subsequently transfer their electrons to oxygen forming the superoxide anion. Their main activity is exhibited in the light, where photosystem I is responsible for their reduction. In this study the plants were illuminated for 8, 12 and 24 h after they were sprayed with PQ. Physiological injuries included bleached and necrotic spots on the adaxial surface of the leaves were observed. Visible effects were dependent on exposure time. Until 8 h of continuous illumination plants treated with 100 μM PQ still looked healthy, but after that time the leaves began to tilt and, bleached and necrotic spots became evident. Visible leaf injury developed on all leaves within 24 h after treatment with PQ. A lower degree of leaf injury appeared on leaves pretreated with SPM and PUT at concentration of 1 mM.

Effects of SPM and PUT on photosynthetic pigment contents

Changes in photosynthetic pigment levels in maize leaves were determined for 8, 12 and 24 h after they were sprayed with PQ. A decrease in chlorophyll level induced by PQ was observed after 12 h of treatment (Table 1). Also, following of the treatment with 100 μM PQ, carotenoid contents of the leaves were significantly decreased (Table 2). After 24 h of treatment, total chlorophyll and carotenoid contents decreased by 41.6 % and 39.7 % in PQ-treated leaves compared to the control, respectively. In order to test if polyamines protect the leaves against photosynthetic pigment losses, leaves were pretreatment with SPM and PUT at concentrations of 0.2 and 1 mM, and then they were sprayed with PQ. Chlorophyll loss was significantly prevented

Table 1. Total chlorophyll content in the leaves pretreated with polyamines and treated with PQ afterwards.

Treatments	Total chlorophyll content (mg/ g dry weight) Hours after PQ treatment 8	12	24
Control	26.49 ± 0.48 ab	27.25 ± 0.4* b	25.95 ± 3.64 c
Paraquat (PQ)	22.11 ± 0.54 a	20.45 ± 0.26 a	15.15 ± 1.28 a
SPM (0,2 mM) +PQ	26.81 ± 2.03 ab	24.09 ± 3.06 ab	20.48 ± 0.27 b
SPM (1 mM) +PQ	27.80 ± 0.84 b	26.56 ± 0.59 b	21.56 ± 0.96 b
PUT (0,2 mM) +PQ	28.23 ± 3.71 b	22.28 ± 3.95 ab	18.17 ± 0.91 ab
PUT (1 mM) +PQ	28.00 ± 2.29 b	27.49 ± 4.16 b	20.01 ± 2.42 b

*Standard deviation of average of four replications. Values followed by different letters are significantly different from each other (P=0.05) according to Duncan's test.

Table 2. Carotenoid contents in the leaves pretreated with polyamines and treated with PQ afterwards.

Treatments	Carotenoid contents (mg/ g dry weight) Hours after PQ treatment 8	12	24
Control	6.18 ± 0.26*a	6.07 ± 0.02 b	5.79 ± 0.47 d
Paraquat (PQ)	5.01 ± 0.58 a	4.23 ± 0.36 a	3.49 ± 0.17 a
SPM (0,2 mM) +PQ	5.26 ± 0.09 a	4.71 ± 0.02 ab	4.01 ± 0.40 ab
SPM (1 mM) +PQ	6.10 ± 0.42 a	5.66 ± 1.14 ab	4.31 ± 0.23 b
PUT (0,2 mM) +PQ	6.07 ± 0.83 a	5.06 ± 0.08 ab	4.50 ± 0.07 bc
PUT (1 mM) +PQ	6.12 ± 0.09 a	5.69 ± 0.97 ab	4.94 ± 0.07 c

*Standard deviation of average of four replications. Values followed by different letters are significantly different from each other (P=0.05) according to Duncan's test.

Table 3. Ascorbic acid content in the leaves pretreated with polyamines and treated with PQ afterwards

Treatments	Ascorbic acid content (mg/ g dry weight) Hours after PQ treatment 8	12	24
Control	5.73 ± 0.62*c	4.88 ± 1.76 b	3.99 ± 0.68 c
Paraquat (PQ)	3.01 ± 0.77 a	2.66 ± 0.62 a	1.20 ± 0.27 a
SPM (0,2 mM) +PQ	4.31 ± 0.84 b	3.82 ± 0.67 ab	2.76 ± 0.41 b
SPM (1 mM) +PQ	4.12 ± 0.26 b	4.03 ± 0.80 ab	3.05 ± 0.30 b
PUT (0,2 mM) +PQ	3.92 ± 0.09 ab	3.78 ± 0.06 ab	2.95 ± 0.27 b
PUT (1 mM) +PQ	4.11 ± 0.16 b	4.07 ± 0.12 ab	3.28 ± 0.50 b

*Standard deviation of average of four replications. Values followed by different letters are significantly different from each other (P=0.05) according to Duncan's test.

by SPM pretreatments. It was determined that especially 1 mM of SPM significantly decreased chlorophyll loss as compared with the leaves treated with PQ. At the end of the experiments, the loss of total chlorophyll in the leaves treated with PQ was 41.6 % whereas in the 1 mM concentration of SPM-pretreated leaves was 16.9 %. Also, chlorophyll level of the leaves pretreated with 1 mM of PUT was more than that of unpretreated leaves. Trends in carotenoid content were similar to those observed for chlorophyll. Leaves pretreated with 1 mM of PUT and 1 mM of SPM had significantly higher carotenoid contents than those pretreated with water after 24 h of PQ treatment. That is, 1 mM of PUT and SPM significantly prevented the loss in carotenoid levels induced by PQ. There was no statistical difference in carotenoid contents between the leaves pretreated with 0.2 mM of SPM and those pretreated with water.

Effects of SPM and PUT on ascorbic acid content

Ascorbic acid content was significantly reduced after PQ treatment. For example, it was found that ascorbic acid level decreased 69.9 % in PQ-treated plants after 24 h of PQ treatment. SPM and PUT significantly reversed PQ-induced ascorbic acid reduction by increasing its content with respect to that of PQ-treated leaves. Especially, 1 mM of SPM and PUT significantly prevented the decrease in ascorbic acid content. After 24 h of PQ treatment, leaves pretreated with 1 mM of SPM and PUT had 1.5 and 1.7-fold higher ascorbic acid level, respectively, than those pretreated with water (Table 3).

Effects of SPM and PUT on SOD activity

The results of investigations into the effects of SPM and PUT on SOD activity in maize leaves under oxidative stress are shown in Figure 1. SOD activity gradually increased after the plants were treated with PQ but statistically significant increases were recorded at 12 and 24 hrs. Pretreatment with SPM and PUT at concentrations of 0.2 and 1 mM increased SOD activity after 8 h of PQ treatment and it was recorded that 1 mM of SPM had a positive effect on the maintenance of SOD activity after 12 and 24 hrs of PQ treatment. Pretreatment with PUT was determined to have insignificant effect on SOD activity at 12 and 24 hrs.

Effects of SPM and PUT on POD activity

POD activity was increased slightly by the treatment of the leaves with PQ. After 24 h of PQ treatment, POD activity increased 15.7 %. Pretreatment with both concentrations of SPM increased POD activity. Also, the increase in POD activity in the leaves pretreated with 1 mM of PUT was found statistically significant. However, pretreatment with 0.2 mM of PUT had no significant effect on POD activity (Fig. 2).

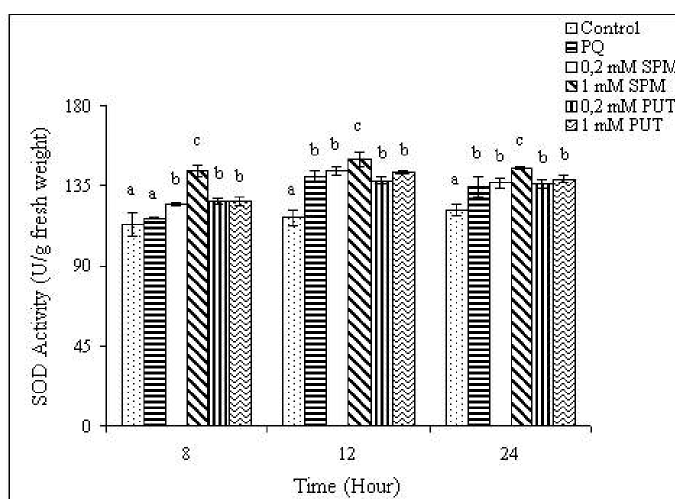

Fig. 1. Changes in SOD activity in maize leaves pretreated with polyamines and treated with PQ afterwards. (Vertical bars represent standard deviation of the means of four replicates. Within each hour, the data followed by the same letters are not significantly different at P=0.05).

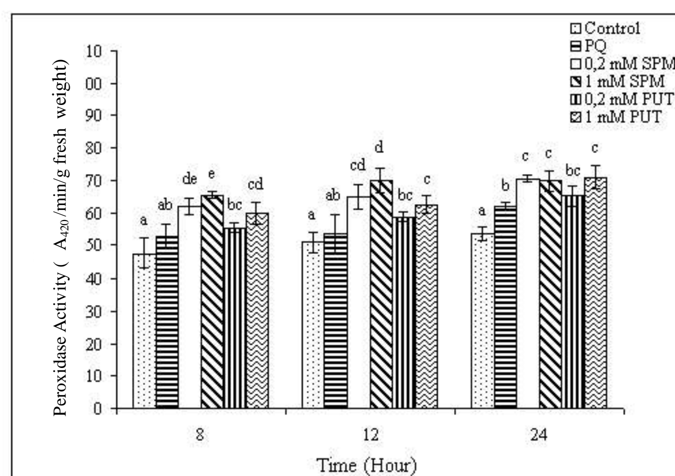

Fig. 2. Changes in POD activity in maize leaves pretreated with polyamines and treated with PQ afterwards. (Vertical bars represent standard deviation of the means of four replicates. Within each hour, the data followed by the same letters are not significantly different at P=0.05).

Discussion

Polyamines play an important role in a wide range of biological processes, including growth, development and stress responses of plants (Martin-Tanguy 2001). Although they appear to be involved in a wide range of plant processes, their exact role is not completely understood (Bais and Ravishankar 2002). In this study, the protective effects of polyamines against oxidative stress of maize leaves were investigated. Oxidative stress was induced by treating the plants with the herbicide PQ. In our study, 100 µM PQ induced oxidative damage in maize leaves incubated under continuous light for 24 h. PQ treatment caused stress symptoms including bleached and necrotic spots on the adaxial surface of maize leaves. These effects appeared with a lower degree of leaf injury on the leaves pretreated with SPM and PUT at concentration of 1 mM. Similarly, leaf necrosis caused by ozone in tomato and tobacco plants could be prevented by exogenously supplying putrescine, spermidine and spermine (Ormrod and Beckerson 1986, Langebartels *et al.* 1991). Also, data presented in our study showed that pretreatment of maize leaves with either SPM or PUT reduced the damage produced by PQ to different degrees, according to the studied parameter and the polyamine tested.

Chlorophyll loss, observed as a consequence of PQ treatment, was significantly prevented in maize leaves by the exogenous addition of SPM and PUT at concentration of 1 mM. It was also reported that PQ treatment caused significant losses of chlorophyll in other plants (Noctor *et al.* 1998, Cakmak and Marschner 1992). On the other hand, a major role of oxygen radicals in chlorophyll destruction by PQ in bean leaves was indicated (Cakmak and Marschner 1992). Also, oxidative stress mediated by superoxide and H_2O_2 causes disorganization of cellular and chloroplastic membrane (Thompson *et al.* 1987) and the breakdown of chlorophyll (Knox

and Dodge 1985). Polyamines have antisenescence properties and prevent the loss of chlorophyll by stabilizing the thylakoid membranes (Besford *et al.* 1993). In addition, polyamines might interact with membranes by inhibiting transbilayer movement of phospholipids (Bratton 1994).

The results presented also show that 1 mM of SPM and both concentrations of PUT significantly decreased the loss in carotenoid levels induced by PQ after 24 h of treatment. Carotenoids are known to act as efficient quenchers of triplet chlorophyll (Noguchi *et al.* 1990) and singlet oxygen (Demming-Adams 1990), and elevated amounts of these accessory pigments in polyamines-pretreated leaves should enhance the capacity to limit the damage caused by reactive oxygen species. However, higher chlorophyll and carotenoid contents in tolerant genotypes have also been reported in earlier studies (Pastori and Trippi 1992).

In the detoxification of reactive oxygen species, ascorbic acid is a key antioxidant such as carotenoids. It was determined that PQ treatment significantly decreased ascorbic acid content in maize leaves. Pretreatment with SPM and PUT significantly prevented the decrease in ascorbic acid level induced by PQ. This effect may be due to the radical scavenging ability of polyamines. Polyamines can act directly as free radical scavengers (Drolet *et al.* 1986), or function as scavengers by interacting with other molecules such as free ferulic and caffeic acids (Bors *et al.* 1989). The increment in ascorbic acid content can increase oxidant tolerance in plants. Because, as an antioxidant, ascorbic acid has an important role in protection against oxidative stress. It eliminates ROS through multiple mechanisms. Ascorbic acid has the capacity to directly eliminate several different ROS including singlet oxygen, superoxide and hydroxyl radicals (Padh 1990). It also maintains the membrane--bound antioxidant -tocopherol in the reduced state (Liebler *et al.* 1986), and indirectly eliminates H_2O_2 through the activity of ascorbate peroxidase. Thus, the increment of ascorbic acid content in polyamines-pretreated maize leaves indicates an enhanced capacity to scavenge reactive oxygen species.

In addition to non-enzymatic oxygen radical scavengers such as carotenoids and ascorbic acid, antioxidative enzymes could also play an important role in protection against oxidative stress. Thus, in this study SOD and POD activities were measured in PQ-treated leaves and it was determined that these enzyme activities gradually increased after PQ treatment. These results are consistent with those obtained by Pastori and Trippi (1993) who found increases in SOD and ascorbate peroxidase (AP) activities in maize leaves incubated in paraquat. However, Kirtikara and Talbot (1996) found that AP activity in PQ-treated plants was similar to the control and SOD level did not significantly change in the PQ-treated tomato plants. Recent evidences suggest that induction of resistance to light- and heat-induced oxidative damage is dependent on concurrent elevation in both SOD and POD activities (Gupta *et al.* 1993). We found that the activities of both enzymes were higher in 1 mM of SPM pretreated leaves than in the unpretreated leaves. SOD activity in both concentrations of PUT-pretreated leaves did not significantly change but pretreatment with 1 mM of PUT increased POD activity. The increased activities of SOD and POD by SPM or PUT may contribute to the reduction of PQ toxicity. The importance of SOD in decreasing PQ toxicity was demonstrated in *E. coli* which showed extreme sensitivity to PQ after deletion of the SOD gene (Carlioz and Toutai 1986). However, peroxidases may be at least as important for protecting cells against oxidative stress as is already established for SOD. The efficient removal of hydroperoxides is important for cells because hydroperoxides may give rise to the formation of highly reactive singlet oxygen molecules and hydroxyl radicals which are both known to induce lipid peroxidation. Also, Mehlhorn (1990) recorded that ethylene production in plants exposed to oxidative stress is induced in order to enhance ascorbate peroxidase activity and thereby, plant susceptibility to oxidative stress is reduced. So, the increases in the activity of SOD or POD induced by SPM or PUT can contribute to the mechanism of tolerance in plants against oxidative stress. On the other hand, Ye *et al.* (1997) reported that the high levels of PUT and high levels of antioxidant enzyme activities in *Conyza bonariensis* could together confer the highest levels of oxidant resistance.

They suggest that both the antioxidant enzymes and PUT might be constitutively linked at the peak period of oxidant tolerance. However, the reason for the increase in antioxidant enzymes, effected by polyamines is unknown. It is possible that polyamines-induced increases in the antioxidant enzyme activities may be due to an up-regulation of the genes controlling the synthesis of these enzymes or an increased activation of constitutive enzyme pools. Besides, it is reported that polyamines may have effects at the gene level (Panagiotidis *et al*. 1995).

In conclusion, this study shows that PQ treatment leads to major changes in the amounts of total chlorophyll, carotenoids and ascorbic acid, and the activities of some enzymes associated with cellular defence mechanisms against oxidative stress in maize leaves. Pretreatment with SPM and PUT may be reduced PQ toxicity because of increased antioxidant enzyme activities and antioxidant levels. It can be said that SPM and PUT increased the tolerance to the oxidative stress caused by PQ in maize leaves.

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References

Arnon D.I. 1949. Copper enzymes in chloroplasts, polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24**:** 1-15.

Asada K., Kiso K.. 1973. Initiation of aerobic oxidation of sulfite by illuminated spinach chloroplast. Eur. J. Biochem. 33: 253-257.

Bais H.P., Ravishankar G.S. 2002. Role of polyamines in the ontogeny of plants and their biotechnological applications. Plant Cell, Tissue and Organ Culture 69: 1-34.

Beauchamp C., Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 44: 276-287.

Besford R.T., Richardson C.M., Campos J.L., Tiburcio A.F. 1993. Effect of polyamines on stabilization of molecular complexes of thylakoid membranes of osmotically stressed oat leaves. Planta 189: 201-206.

Bors W., Langebartels C., Michel C., Sandermann J.H. 1989. Polyamines as radical scavengers and protectants against ozone damage. Phytochem. 28: 1589-1595.

Bratton D.L. 1994. Polyamine inhibition of transbilayer movement of plasma membrane phospholipids in the erythrocyte ghost. J. Biol. Chem. 269: 22517--22523.

Cakmak I., Marschner H. 1992. Magnesium deficiency enhances resistance to paraquat toxicity in bean leaves. Plant, Cell and Environment 15: 955-960.

Canal M.J., Tames R.S., Fernandez B. 1988. Peroxidase and polyphenol oxidase activities in *Cyperus esculentus* leaves following glyphosate applications. Physiol. Plant. 74: 125-130.

Carlioz A., Toutai D. 1986. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? The EMBO Journal 5: 623-630.

Demming-Adams B. 1990. Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. Biochem. Biophys. Acta 1020: 1-24.

Dhindsa R.S., Matowe W. 1981. Drought tolerance in two mosses: Correlated with enzymatic defense against lipid peroxidation. J. Exp. Bot. 32: 79-91.

Dodge A. 1994. Herbicide action and effects on detoxification processes. In: Foyer C.H. and Mullineaux P.M. (eds), Causes of photooxidative stress and amelioration of defense systems in plants. CRC Press, Boca Raton, FL, pp. 219-236.

Drolet G., Dumbroff E.B., Legge R.L., Thompson J.E. 1986. Radical scavenging properties of polyamines. Phytochem. 25: 367-371.

Furusawa I., Tanaka K., Thanutong P., Mizuguchi M., Yazaki M., Asada K. 1984. Paraquat resistant tobacco calluses with enhanced superoxide dismutase activity. Plant and Cell Physiol. 25: 1247-1254.

Gupta A.S., Webb R.P., Holaday S.A., Allen R.D. 1993. Overexpression of superoxide dismutase protects plants from oxidative stress. Plant Physiol. 103: 1067-1073.

Halliwell B. 1982. The toxic effects of oxygen on plant tissues. In: Oberley L.W. (ed), Superoxide dismutase. CRC Press, Boca Raton, Florida, pp. 89-124.

Jaspars E.M.J. 1965. Pigmentation of tobacco crown--gall tissues cultured in vitro in dependence of the composition of the medium. Physiol. Plant. 18: 933-940.

Kenylon W.H., Duke S.D. 1985. Effects of acifluorfen on endogenous antioxidants and protective enzymes in cucumber. Plant Phsiol. 79: 216-220.

Kirtikara K., Talbot D. 1996. Alteration in protein accumulation, gene expression and ascorbate- glutathione pathway in tomato (*Lycopersicon esculentum*) under paraquat and ozone stress. J. Plant Physiol. 148: 752-760.

Knox J.P., Dodge A.D. 1985. Singlet oxygen and plants. Phytochem. 24: 889-896.

Langebartels C., Kerner K., Leonardi S., Schraudner M., Trost M., Heller W., Sandermann H. 1991. Biochemical plant responses to ozone. Differential induction of polyamine and ethylene biosynthesis in tobacco, Plant Physiol. 95: 882-889.

Liebler D.C., Kling D.S., Reed D.J. 1986. Antioxidant protection of phospholipid bilayers by á- tocopherol. Control of a-tocopherol status by ascorbic acid and glutathione, J.Biol. Chem. 261:12114-12119.

Martin-Tanguy J. 2001. Metabolism and function of polyamines in plants: recent development (new approaches). Plant Growth Regul. 34: 135-148.

Mehlhorn H. 1990. Ethylene- promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone and paraquat. Plant, Cell and Environ. 13: 971-976.

Noctor G., Arisi A.M., Jouanin L., Kunert K.J., Rennenberg H., Foyer C. 1998. Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. J. Exp. Bot. 49: 623-647.

Noguchi T., Hayashi H., Tasumi H. 1990. Factors controlling the efficiency of energy transfer from carotenoids to bacteriochlorophyll in purple photosynthetic bacteria. Biochem. Biophys. Acta. 1017: 280-290.

Ormrod D.P., Beckerson D.W. 1986. Polyamines as antiozonants for tomato. Hort. Scien. 21: 1070-1071.

Padh H. 1990. Cellular functions of ascorbic acid. Biochem. and Cell Biol. 68: 1166-1173.

Panagiotidis C.A., Artandi S., Calame K., Silverstein S.J. 1995. Polyamines alter sequence-specific DNA protein interactions. Nucleic Acids Res. 23:1800-1809.

Pastori G.M., Trippi V.S. 1992. Oxidative stress induces high rate of glutathione reductase synthesis in a drought-resistant maize strain. Plant Cell Physiol. 33: 957-961.

Rodriguez R., Sanchez T.R. 1982. Peroxidase and IAA oxidase in germinating seeds of *Cicer arietinum* L. Rev. Esp. Fisiol. 38: 183-188.

Sakaki T., Kondo N., Sugahara K. 1983. Breakdown of photosynthetic pigments and lipids in spinach leaves with ozone fumigation: role of active oxygens. Phsiol. Plant. 50: 28-34.

Sreenivasulu N., Ramanjulu S., Ramachandra-Kini K., Prakash H.S., Shekar-Shetty H., Savithri H.S. 1999. Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of foxtail millet with differential salt tolerance. Plant Science 141:1-9.

Shaaltiel Y., Glazer A., Bocion P.F., Gressel J. 1988. Cross tolerance to herbicidal and environmental oxidants of plant biotypes tolerant to paraquat, sulfur dioxide and ozone. Pest. Biochem. and Physiol. 31: 13-23.

Shieh H.H., Sweet T.R. 1979. Spectrophotometric determination of ascorbic acid. Anal. Biochem. 96: 1-5.

Tadolini B. 1988. Polyamine inhibition of lipoperoxidation: The influence of polyamines on iron oxidation in the presence of compounds mimicking phospholipid polar heads. Biochem J. 249: 33-36.

Tanaka K., Furusawa I., Kondo N., Tanaka K. 1988. SO_2 tolerance of tobacco plants regenerated from paraquat-tolerant callus. Plant Cell Physiol. 29: 743-746.

Thompson J.E., Ledge R.L., Barber R.F. 1987. The role of free radicals in senescence and wounding. New Phytol. 105: 317-344.

Tiburcio A.F., Altabella T., Borrel T., Masgrau C. 1997. Polyamines metabolism and its regulation. Physiol. Plant. 100: 664-674.

Velikova V., Yordanov I., Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. Plant Science 151: 59-66.

Ye B., Müller H.H., Zhang J., Gressel J. 1997. Constitutively elevated levels of putrescine and putrescine--generating enzymes correlated with oxidant stress resistance in *Conyza bonariensis* and wheat. Plant Physiol. 115: 1443-1451

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