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Relationship between polyamines and paraquat toxicity in sunflower leaf discs

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Abstract

Polyamines have been reported as efficient antioxidant compounds in plants. Sunflower leaf discs, treated with 100 μM paraquat (PQ), a well known oxidative stress inducer, showed decreased levels of putrescine (Put), spermidine (Spd) and spermine (Spm) (between 33% and 80% with respect to the controls). Arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) activities decreased 42% and 33% respectively. Among the markers of oxidative stress measured after PQ treatment, chlorophyll and glutathione content were reduced (30% and 49% respectively) and thiobarbituric acid reactive substances (TBARS) content increased (60%). Superoxide dismutase (SOD) activity declined 60% with respect to the control and lipoxygenase (LOX) increased 25% when leaf-discs were treated with the herbicide. Pretreatment with exogenous polyamines (1 mM) reversed paraquat toxicity to different degrees according to the polyamine and/or the tested parameter. Spermidine was able to inhibit chlorophyll loss, while Spm reverted the effect of PQ on the level of TBARS almost completely and also restored SOD activity close to control values. Putrescine was the least effective as an oxidant protectant. These results provide support for the argument that polyamines are effective antioxidants through their ability to act as radical scavengers.

Abbreviations: ADC – arginine decarboxylase; ODC – ornithine decarboxylase; LOX – lipoxygenase; SOD – superoxide dismutase; PQ – paraquat; Put – putrescine; Spd – Spermidine; Spm – Spermine; TBARS – thiobarbituric acid reactive substances

1. Introduction

Polyamines (Pas) are ubiquitous nitrogen compounds which are present in plant cells at up to millimolar concentrations and are considered to participate in a variety of events related to plant growth and development [3, 18]. Despite extensive studies on polyamine metabolism, the exact role that these compounds play in plant physiology remains unclear [34]. The most important changes in Pas levels are related to plants under stress [16], e.g. osmotic stress [15, 2], ozone fumigation [24], UV radiation [22], atrazine treatments [37] and salt stress [15].

Paraquat (methyl viologen, 1,1′-dimethyl-4,4′-bipyrydinium dichloride, PQ) is a contact foliar herbicide, which causes rapid membrane damage by generating highly toxic superoxide anions [11]. It is well known that the herbicide exerts this effect by catalyzing the transfer of electrons from photosystem I of chloroplast membranes to molecular oxygen, producing free radicals that cause lipid peroxidation and membrane damage [11]. Protective mechanisms against the production of reactive oxygen species do exist in plants. They include low molecular weight antioxidants, such as ascorbate and glutathione, as well as enzymatic components, e.g. superoxide dis-

mutase, catalase, ascorbate peroxidase and glutathione reductase [17]. In the past years, a role for polyamines as antioxidants has been proposed. It has been reported that polyamines inhibit lipid peroxidation in rat liver microsomes and in phospholipid vesicles [21, 31]. Polyamines have also been reported as direct free radical scavengers [13], or to function as scavengers by interacting with other molecules [8]. More recently, paraquat oxidant resistance had been correlated with polyamine levels in *Conyza bonariensis* and *Triticum aestivum* resistant phenotypes [35]. In addition, the level of PQ-induced oxidant resistance can be further enhanced by exogenous application of Put [35]. Also, application of polyamines reduced the severity of ozone-induced leaf necrosis in tobacco [8].

In this study, our objectives were to examine, a) whether paraquat-induced oxidative stress could induce changes in polyamines levels in sunflower discs and b) whether exogenously added 1 mM Put, Spd or Spm could protect the plants against paraquat toxicity in sunflower leaf-discs.

2. Materials and methods

2.1 *Plant material and treatments*

Sunflower (*Helianthus annuus* L) seeds were surface sterilized with $HClO_4$ (8% active Cl_2) at 50% for 10 min and then thoroughly rinsed with distilled water. They were germinated in Petri dishes in the dark and then, transferred to plastic pots (1 L) filled with vermiculite and irrigated with Hoagland [20] solution for 4 weeks. Plants were grown at 26/20 °C (day and night), with a 16-h photoperiod under fluorescent white light (175 μmol/m^2 per s) in a controlled environmental growth chamber. Leaf discs (80 mm diameter) were cut with a cork borer from the third pair of leaves (counting from the bottom of the plant) and incubated in a rotatory shaker under continous illumination, in flasks containing 20 ml of the treatment solutions. After 20 h of treatment, leaf-discs were thoroughly washed with distilled water and extracted for analysis. Treatments were as follows: a) Controls in distilled water (C) b) Paraquat 100 μM (PQ) c) 3 h preincubation in putrescine (Put), spermidine (Spd) or spermine (Spm) and then in PQ 100 μM d) Controls with 1 mM polyamines instead of distilled water (Put C, Spd C or Spm C).

2.2 *Analysis of polyamines*

Plant material (400 mg FW) was homogeinized with 5% perchloric acid, kept 30 min on ice and centrifuged at 5000 rpm for 10 min. The supernatants were derivatized using the dansylation method described by Smith and Meeuse [30] and 1,6 hexanediamine was used as an internal standard. Standards of Put, Spd and Spm were dansylated simultaneously. The dansylated derivatives were extracted with 1 ml ethylacetate. Polyamines were separated and identified by TLC, performed on high resolution silica gel plates (JT Baker, silica gel plates IB 2-F) using n-hexane:ethyl acetate (1:1) solvent system. Dansylated polyamines were identified by comparing the Rf values of dansylated standards. Silica plates were observed under UV light and bands corresponding to the polyamines in the samples and standards were scraped off the plates and eluted with 1 ml ethylacetate. Their fluorescence was measured at 365 nm excitation and 510 emission in an spectrofluorometer.

2.3 *Determination of ADC and ODC activities*

All assays were performed on fresh extracts according to the method described by Flores and Galston [15]. Samples (400 mg FW) were homogenized in a chilled mortar with 2 ml of 50 mM phosphate buffer (pH 7.8) containing 0.5 mM EDTA, 5 mM dithiothreitol, 1 mM PMSF and 1 mM pyridoxalphosphate. They were centrifuged at 20000 *g* for 20 min and the supernatants were immediately used for enzyme assays.

The incubation mixture for arginine decarboxylase (ADC) consisted of 100 μl of the crude extract, 70 μl of buffer, 10 mM pyridoxal phosphate, 25 mM dithiothreitol, and 1 mM concentration of the substrate [1-^{14}C] arginine (325 mCi/mmol, New England Nuclear) diluted with cold arginine to give a final concentration of 20 mM, in a final reaction volume of 200 μl. Ornithine decarboxylase (ODC) was assayed in a similar way using [1-^{14}C] ornithine (54.3 mCi/mmol, New England Nuclear) diluted with cold ornithine to give a final concentration of 20 mM. Specific activities of the enzymes are expressed as nmol $^{14}CO_2$ h^{-1} g^{-1} FW. Reaction mixtures were incubated for 60 min at 37 °C with continuous shaking. The reaction was stopped by adding 100 μl of TCA 20% and the incubation continued for 45 min. For blanks, TCA was added at zero time. The $^{14}CO_2$ released in the reaction was trapped in Whatmann filter papers moistened with Protosol (New England Nuclear) and placed above the

reaction mixture in glass tubes similar to the plastic wells of Kontes. When the reaction was finished, the filter papers were put in a scintillation solution in glass vials and the radioactivity was measured using a Beckmann LS 1801 scintillator counter.

2.4 *Chlorophyll content*

Chlorophyll was extracted by homogenizing and boiling 1 g fresh weight of leaves in 35 ml of 96% ethanol. After centrifugation for 10 min at 5000 rpm, the chlorophyll content in the ethanolic supernatant was measured spectrophotometrically at 654 nm, as described by Wintermans and De Mots [36].

2.5 *Thiobarbituric acid reactive substances (TBARS) determination*

Lipid peroxidation was measured as the amount of TBARS determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer [19]. Fresh leaves (0.5 g) were homogenized in 5 ml of 20% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 $\times g$ for 20 min. To 1 ml of the aliquot of the supernatant, 1 ml of 20% TCA containing 0.5% (w/v) TBA and 100 μl 4% BHT in ethanol were added. The mixture was heated at 95 °C for 30 min. and then quickly cooled on ice. The contents were centrifuged at 10000 $\times g$ for 15 min and the absorbance was measured at 532 nm. The concentration of TBARS was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.6 *Glutathione determination*

Non-protein thiols were extracted by homogenizing 1 g of tissues in 5 ml of 0.1 N HCl (pH 2), containing 1 g polyvinylpyrrolidone (PVP) [27]. After centrifugation at 10000 *g* for 20 min at 4 °C, the supernatants were used for the analysis. Total glutathione (GSH plus GSSG) was determined in homogenates by spectrophotometry at 412 nm, using glutathione reductase, DTNB and NADPH [1].

2.7 *Superoxide dismutase and lipoxygenase determination*

Extracts for determination of SOD activity were prepared from 0.3 g of leaves homogenized under ice-cold conditions in 3 ml of extraction buffer, containing 50 mM phosphate buffer (pH 7.4), 1 mM EDTA, 1 g PVP, and 0.5% (v/v) Triton X-100 at 4 °C. The homogenates were centrifuged at 10000 $\times$g for 20 min and the supernatant fraction was used for the assays. Total SOD (EC 1.15.1.1) activity was assayed by the inhibition of the photochemical reduction of NBT, as described by Becana et al. [4]. The reaction mixture contained 50–150 μl of the plant extract and 3.5 ml O_2^- generating solution which contained 14.3 mM methionine, 82.5 μM NBT, and 2.2 μM riboflavin. Extracts were brought to a final volume of 0.3 ml with 50 mM K-phosphate (pH 7.8) and 0.1 mM Na_2EDTA. Test tubes were shaken and placed 30 cm from a light bank consisting of six 15-W fluorescent lamps. The reaction was allowed to run for 10 min and stopped by switching the light off. The reduction in NBT was followed by obtaining the A_{560} values. Blanks and controls were run the same way but without illumination and enzyme, respectively. One unit of SOD was defined as amount of enzyme which produced a 50% inhibition of NBT reduction under the assay condition.

Lipoxygenase (EC 1.13.1.12) was extracted according to the method of Lupu et al. [25]. Leaf tissue was homogenized in ice-cold 0.2 M sodium-phosphate buffer (pH 6.5, 1% Triton $\times$ 100) and the homogenates were centrifuged at 10000 $\times g$ for 20 min. Lipoxygenase activity was measured spectrophotometrically at 234 nm using linoleic acid (cis-9, cis-12 octadecadienoic acid) as the substrate. One ml of substrate solution containing 2.28 $\times$ 10^{-4} M linoleic acid and 0.25% Tween 20 in 0.2 M citrate-phosphate buffer (pH 6.5) was added to quartz cuvettes in a Beckman DU-65 spectrophotometer. The spectrophotometer was zeroed, and 5 to 15 μl leaf extract was added to the sample cuvette thorough mixing. The increase in absorbance at 234 nm (25 °C) was followed for 15 min and rates were calculated from the initial linear portion of at least two separated curves. Activity was expressed as the change in absorbance at 234 nm per min per mg protein.

2.8 *Protein determination*

Protein concentration was evaluated by the method of Bradford [9] using bovine serum albumin as a standard.

2.9 *Statistics*

All data presented are the mean values of two independent set of experiments. Each value was presented as means $\pm$ standard errors of the mean (SE), with a minimum of three replicates. Statistical assays were

carried out by one-way ANOVA using the Tukey test to evaluate whether the means were significantly different, taking $p < 0.05$ as significant.

3. Results

3.1 *Paraquat effect on putrescine biosynthetic enzymes*

The activity of arginine decarboxylase and ornithine decarboxylase, the two biosynthetic enzymes that lead to the production of putrescine in plants, was measured in paraquat-treated sunflower leaf discs. The herbicide negatively affected both enzymes activities, by reducing their values respect to the control by 42 and 33%, respectively. The addition of polyamines to the incubation medium for 3 h before PQ treatment did not change significantly the effect of the herbicide, except for the effect of spermine on ADC, where half of the decrease on the enzyme activity was reversed by the polyamine (Figure 1). Paraquat effect on ODC activity was not reversed by either of the exogenously added polyamines.

3.2 *Polyamine content*

Putrescine, Spd and Spm contents were considerably reduced after PQ treatment. Spermidine was the most abundant polyamine in control sunflower discs (3-fold Put and Spm levels) (Figure 2). It was also the one most affected after the treatment (decreased from 1795 nmol/g FW to 332 nmol/g FW). Spermine and Put levels were reduced by 33 and 69% respectively, in PQ-treated discs. Detached sunflower leaf-discs pretreated with Spm, Spd or Put showed considerably higher endogenous levels of the respective polyamine (Figure 2), than those in the control discs. This result indicated that exogenously added polyamines were effectively transported and accumulated in leaf discs after 3 h-pretreatment, an important fact to evaluate if polyamines could reduce paraquat toxicity by way of their increased amounts in the tissues.

3.3 *Chlorophyll, malondialdehyde and glutathione variation*

To examine PQ-induced oxidative damage, we selected some markers of oxidative stress and membrane injury. One of the parameters selected to monitor the damage on the photosynthetic apparatus was the chorophyll content. The pigment content decreased by 30% in PQ-treated discs after 20 h of treatment. In order to test if polyamines protect the discs against chlorophyll loss, leaf discs were preincubated with 1 mM Spm, Spd or Put for 3 h and then treated with PQ. Chlorophyll loss was completely prevented by Spd pretreatment. Spermine restored chlorophyll up, to 90% of the control value but putrescine was not effective (Figure 3). Chlorophyll was not affected by polyamines in control treatments.

Lipid peroxidation was measured in terms of TBARS content using the thiobarbituric acid reaction. Changes in TBARS levels in sunflower leaf discs after PQ treatment were determined 12 h and 20 h after treatment. After 12 h of incubation, a small increment (26%) was observed in TBARS levels in PQ-treated discs compared to control discs (Figure 4). Preincubation with Put or Spd for 3 h before PQ treatment did not modify significantly TBARS content with respect to the control, but Spm restored TBARS levels to control values. However, after 20 h of incubation, TBARS content increased 60% in PQ treated leaf discs. Incubation of discs with PQ after polyamine pretreatment resulted in a reduction of TBARS levels by different degrees according to the polyamine tested. Spermine completely prevented the rise in TBARS content, Spd reduced its increase by 38% but Put was not efficient as a protectant when compared to PQ-treated discs. Controls with polyamines did not affected TBARS content compared to control with water (Figure 4).

Glutathione is one of the major antioxidant components of the plant defense system. In PQ-treated discs without polyamine pretreatment, total glutathione decreased to almost half of the control values after 20 h of treatment (Figure 5). Putrescine, Spd or Spm partially, but significantly, reversed PQ-induced glutathione reduction by increasing its content with respect to that of PQ-treated discs. The three polyamines increased glutathione to similar values (up to 65%–71% of the control) (Figure 5).

3.4 *Lipoxygenase and superoxide dismutase activities*

To investigate whether or not lipoxygenase was involved in changes in lipid peroxidation, we measured the activity of this enzyme in the same experimental conditions previously detailed. Lipoxygenase activity increased 25% when discs were treated with 100 μM PQ. Pretreatment with polyamines produced varied effects. Putrescine did not modify LOX activity, Spd almost restored the enzyme activity to control

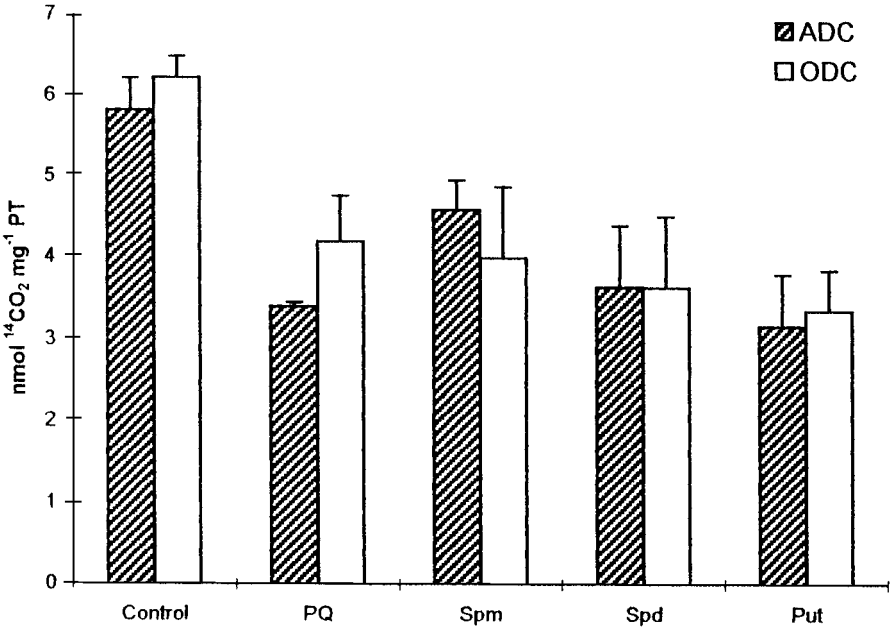

Figure 1. Arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) activities in 4 week-old 100 μM paraquat-treated sunflower discs, with or without polyamine pretreatments. Leaf discs were incubated for 20 h under continuous light. Polyamine (Put, Spd or Spm) pretreatments consisted of 3 h preincubation in 1 mM of the corresponding polyamine. Enzyme activities are expressed in nmol $^{14}CO_2$ h^{-1} mg-1 PT. Vertical lines in each point show ±SE (n = 3) ($p < 0.05$).

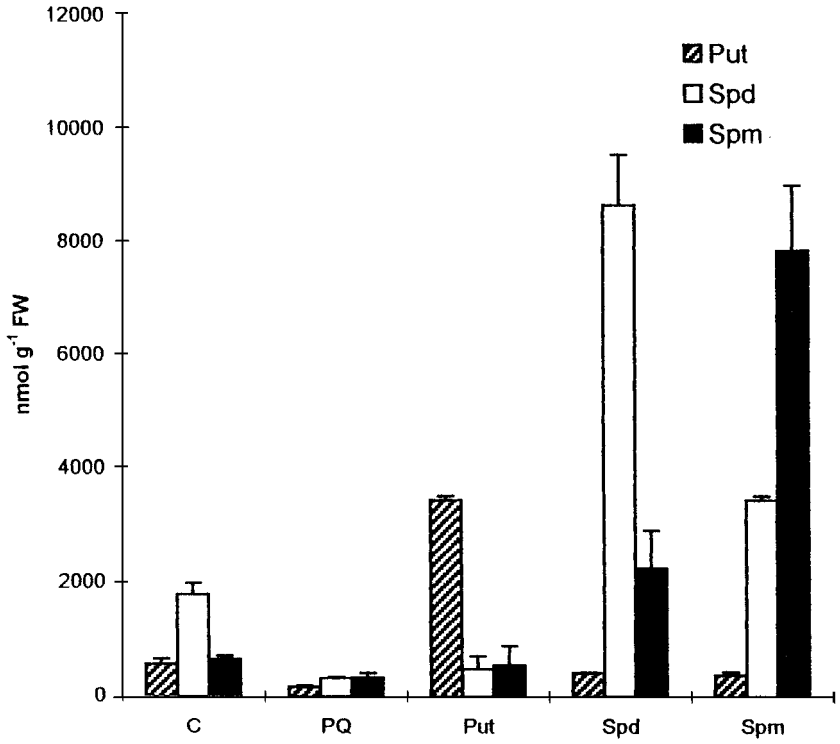

Figure 2. Polyamine changes in sunflower leaf discs after treatment with 100 μM paraquat. Leaf discs were incubated for 20 h under continuous light. Polyamine (Put, Spd or Spm) pretreatments consisted of 3 h preincubation in 1 mM of the corresponding polyamine. Polyamines are expressed in nmol g^{-1} FW. Vertical lines in each point show ±SE (n = 4) ($p < 0.05$).

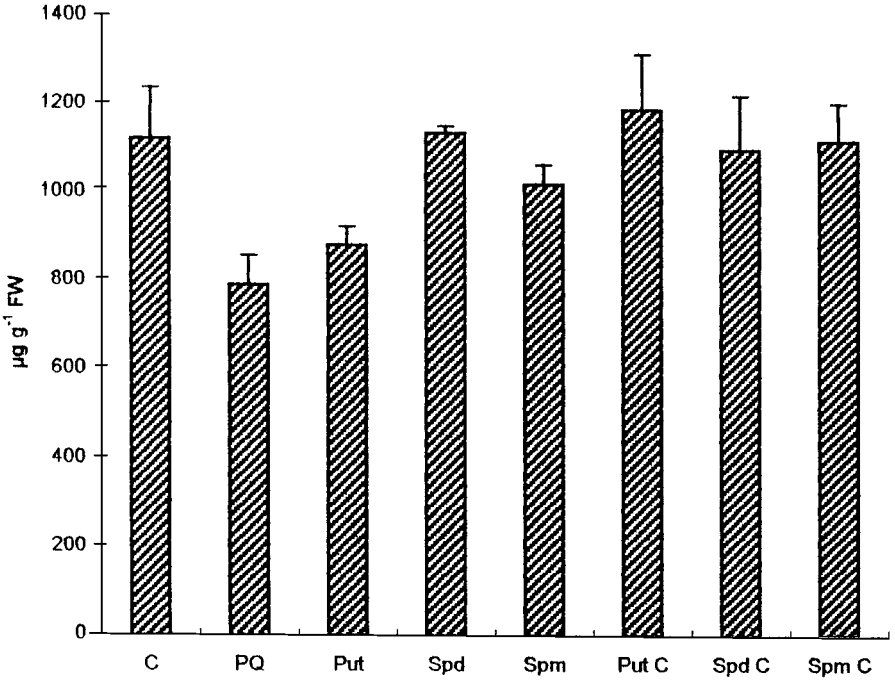

Figure 3. Chlorophyll changes in sunflower leaf discs after treatment with 100 μM paraquat. Leaf discs were incubated for 20 h under continuous light. Polyamine (Put, Spd or Spm) pretreatments consisted of 3 h preincubation in 1 mM of the corresponding polyamine. Chlorophyll is expressed in μg g^{-1} FW. Vertical lines in each point show $\pm$SE ($n = 4$) ($p < 0.05$). Put C, Spd C and Spm C are controls of the respective polyamine without significant differences respect to control in distilled water (C).

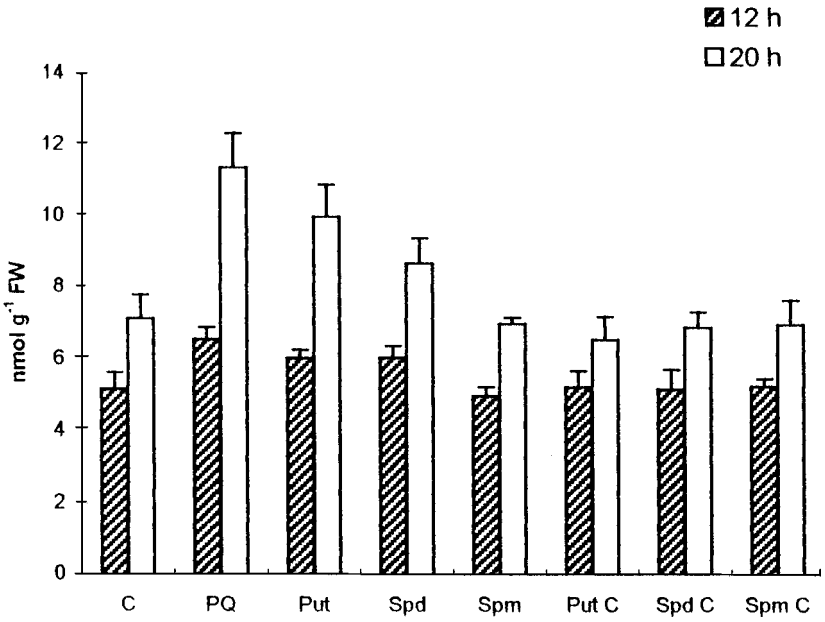

Figure 4. TBARS changes in sunflower leaf discs after treatment with 100 μM paraquat. Leaf discs were incubated for 20 h under continuous light. Polyamine (Put, Spd or Spm) pretreatments consisted of 3 h preincubation in 1 mM of the corresponding polyamine. TBARS are expressed in nmol g^{-1} FW. Vertical lines in each point show $\pm$SE ($n = 3$) ($p < 0.05$). Put C, Spd C and Spm C are controls of the respective polyamine without significant differences respect to control in distilled water (C).

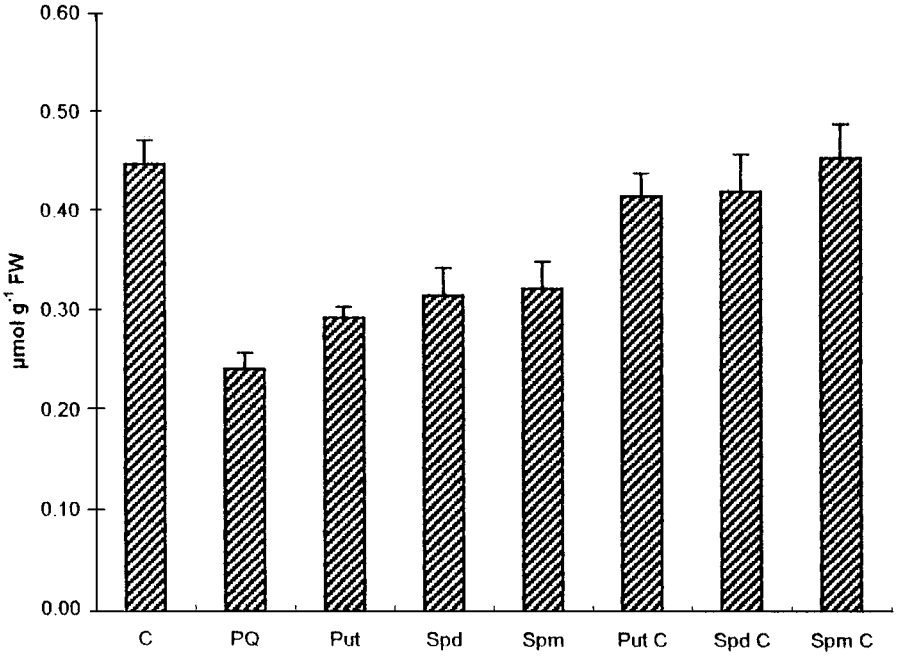

Figure 5. Total glutathione changes in sunflower leaf discs after treatment with 100 μM paraquat. Leaf discs were incubated for 20 h under continuous light. Polyamine (Put, Spd or Spm) pretreatments consistedof 3 h preincubation in 1 mM of the corresponding polyamine. Total glutahione is expressed in μmol g^{-1} FW. Vertical lines in each point show $\pm$SE (n = 3) ($p < 0.05$). Put C, Spd C and Spm C are controls of the respective polyamine, without significant differences with respect to controls in distilled water (C).

values (96% of the control), but preincubation of the discs with Spm reduced LOX activity 30% (Figure 6). Almost the same decrease was observed when discs were treated with Spm (instead of water) as a control, indicating that Spm directly inhibited LOX and that this effect was not related to PQ. Controls with Put or Spd did not affect LOX activity, compared to a water control.

To test the hypothesis that polyamines antioxidant capacity may be mediated through their scavenging properties, SOD activity was measured as another indicator of oxidative stress. It was observed that PQ decreased SOD activity to 60% of the control (Figure 7). Spermine was the most effective polyamine in inhibiting the decrease in enzyme activity, restoring SOD activity almost completely. Spermidine and Put partially reverted PQ-reduced SOD activity, to 87% and 76% of the control, respectively.

4. Discussion

Polyamines are rapidly affected by different types of environmental stress [14, 18]. However, very little is known about the effect of herbicides on polyamine metabolism [35, 23]. In contrast to other reports, a general decrease in the three main polyamines was observed in PQ-treated sunflower leaf discs. The decreased level of Put was directly attributed to the decrease in ADC and ODC activities. Spermidine and Spm also declined significatively, mainly because their biosynthesis is closely related to Put. Chang and Kao [12] reported that Put increased in rice leaves treated with PQ, while Spd and Spm levels decreased after 24 h of treatment. Sunflower leaf discs pretreated with Put, Spd or Spm for 3h had higher endogenous levels of Put, Spd and Spm respectively, than those incubated in water. However, polyamine pretreatment did not reverse the PQ effect on ADC or ODC activities, except for the slight increase in ADC activity induced by Spm (20% of reversion). This indicated that the increased polyamine content after preincubation could not be attributed to an increase in the biosynthetic enzymes, but only to the transport of polyamines inside the cells. Polyamines have specific transport systems to bring about a rapid cellular distribution [28] and in mammalian cells, the concentration of free intracellular Spd is considered to be the most important regulator of Pas uptake [29].

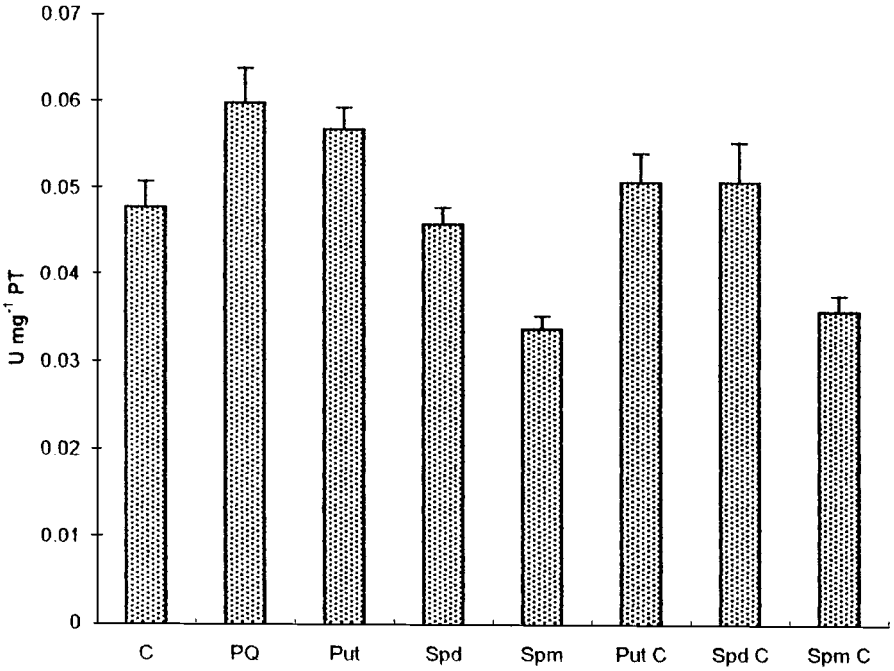

Figure 6. Lipoxygenase (LOX) activity as affected by paraquat treatment in sunflower leaf discs. Leaf discs were incubated for 20 h under continuous light. Polyamine (Put, Spd or Spm) pretreatments consisted of 3 h preincubation in 1 mM of the corresponding polyamine. LOX activity is expressed in U mg^{-1} PT. Vertical lines in each point show ±SE (n = 3) ($p < 0.05$). Put C, Spd C and Spm C are controls of the respective polyamine, without significant differences with respect to controls in distilled water (C).

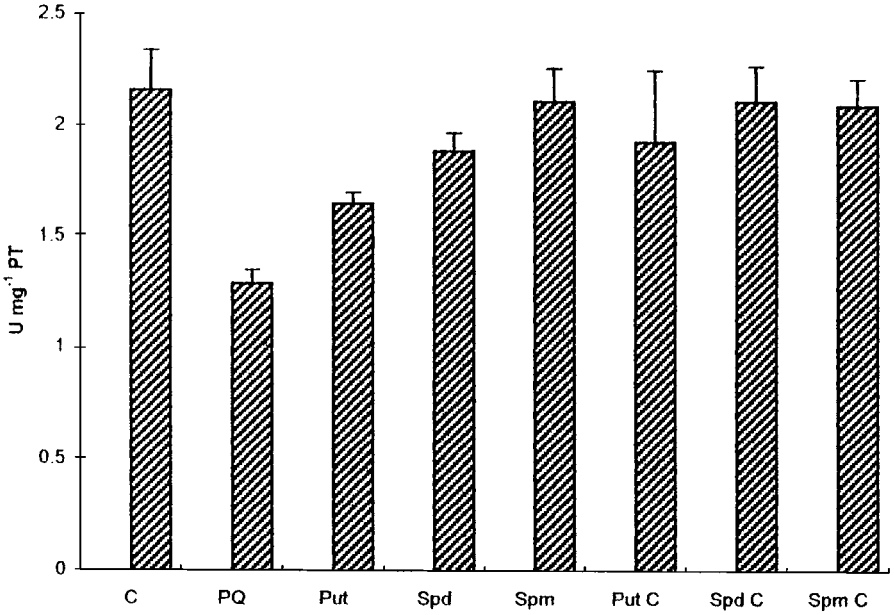

Figure 7. Superoxide dismutase (SOD) activity as affected by paraquat treatment in sunflower leaf discs. Polyamine (Put, Spd or Spm) pretreatments consisted of 3 h preincubation in 1 mM of the corresponding polyamine. SOD activity is expressed in U mg^{-1} PT. Vertical lines in each point show ±SE (n = 3)($p < 0.05$). Put C, Spd C and Spm C are controls of the respective polyamine, without significant differences with respect to controls in distilled water (C).

It is well known that paraquat induces oxidative stress through photosynthetically generated superoxide radicals [11]. Some markers of oxidative stress were selected in order to test whether polyamines could modulate paraquat toxicity in sunflower leaf discs. In our study, 100 μM paraquat induced oxidative damage in sunflower discs incubated under continous light for 20 h. Although the exact role that polyamines play during stress remains to be determined, the deleterious effect of some stresses can be alleviated by exogenous addition of polyamines. The exogenous application of polyamines protected pea plants against atrazine [37] and against ozone-induced leaf necrosis in tobacco [24]. Data presented in our study showed that pretreatment of sunflower leaf-discs with either Put, Spd or Spm reduced the damage produced by 100 μM PQ to different degrees, according to the studied parameter and the polyamine tested.

Lipid peroxidation measured through the TBARS content, was almost completely prevented by Spm, while Put and Spd were less effective. Kitada et al. [21] reported that spermine was the most effective in inhibiting lipid peroxidation in rat liver microsomes due to direct binding to microsomal phospholipids, and Tadolini [31] attributed the inhibition of lipid peroxidation by spermine and spermidine to the formation of a polycation/phospholipid vesicle complex. This is related to the ability of Spm to form a tertiary complex with iron, changing the suceptibility of Fe^{2+} to autooxidation [32]. Moreover, the results obtained in relation to LOX activity were related to the fact that Spm was the most effective in prevention of lipid peroxidation. Spermine inhibited LOX activity and taking into account that LOX is closely related to TBARS formation, this could explain why Spm was the most effective in inhibiting peroxidative damage. Spermidine and spermine also inhibited lipid peroxidation in oat leaves induced to senesce by an osmotic treatment [7].

Chlorophyll loss, observed as a consequence of PQ treatment, was completely reversed in sunflower leaf discs by the exogenous addition of Spd and Spm, which showed a similar efficacy in inhibiting chlorophyll decay. Noctor et al. [26] also reported a significant loss of chlorophyll after 7 h of PQ treatment under illumination. Polyamines have antisenescence properties and prevent the loss of chlorophyll by stabilizing the thylakoid membranes [6]. Although Spm has been reported as the polyamine which best preserved thylakoid integrity in oat leaves [33], Spd was the most effective in PQ-treated sunflower leaf discs.

The deterioration of cellular organelles that leads to senescence could be attributed, in part, to the free radical-mediated lipid peroxidation, and the ability of polyamines to delay senescence may be due to their action as radical scavengers [13]. Paraquat toxicity is mediated by the generation of superoxide radicals ($O_2^{\bullet -}$) produced in chloroplasts membranes and SOD is a key enzyme for detoxification of superoxide anions. In sunflower leaf-discs treated with PQ, SOD activity decreased significantly and Spm was the most effective polyamine in reversing this effect, followed by Spd and Put. Drolet et al. [13] reported that polyamines were more effective than diamines in scavenging superoxide radicals produced by senescing bean cotyledons.

All polyamines had a similar effect in protecting sunflower discs against glutathione decline and this effect may also be due to the radical scavenging ability of polyamines, considering that glutathione, a major antioxidant compound in plant tissues which directly reduces most active oxygen species [26], usually decreased under oxidative stress.

The mechanisms by which polyamines conferred protection against paraquat toxicity remain to be determined. Our results concerning chlorophyll loss may be related to the membrane stabilizing properties of polyamines [6], or through the inhibition of the transbilayer movements of phospholipids [10], while the radical scavenging ability could be the explanation for the protective effect of exogenously added polyamines on SOD activity and GSH content and the inhibition of lipid peroxidation. All the proposed mechanisms may not be mutually exclusive and/or could be additive. Contrary to other reports [35], Put was the least effective as an antioxidant against PQ induced damage in sunflower. Considering that the antioxidant capacity is related to the scavenger ability, Spd and Spm could be more effective only because of the size of the molecules.

Our data show an evident relationship between polyamines and antioxidant protection against paraquat-mediated damage and strongly support the assumption that polyamines are efficient protectors against oxidative damage in plant cells.

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