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Protective Effect of Exogenous Polyamines against Atrazine in Pea Plants

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Abstract. The effect of exogenously applied polyamines in reversing the effect of atrazine stress on pea seedlings (*Pisum sativum* L., cv. Koray) was investigated. The plants treated with combinations of atrazine (14 mM) + spermidine (1 mM) and atrazine (14 mM) + spermine (1 mM) possessed improved growth (30–35% increase of leaf area and 10–20% increase of fresh weight 10 days after treatment) and chlorophyll content (50–60% increase) in comparison with atrazine- (14 mM) treated plants. Spermine and spermidine also diminished the inhibitory effect of atrazine on gas exchange and photosystem II function. This fact supports the hypothesis of Yordanov and Goltsev (1990, Plant Physiol 4:42–51) that the interaction of polyamines with the thylakoid membrane surface led to their stacking, to separation of the photosystems, and to the association of light-harvesting complex II with the photosystem II core complex.

Aliphatic polyamines, a class of plant growth substances, are present in all plants so far examined for polyamines (Bagni 1986). The most common ones are putrescine, spermidine, and spermine. Polyamines are involved in the control of the cell cycle, cell division, and morphogenesis, in phytochrome and plant hormone-mediated processes, and the control of plant senescence, as well as in plant responses to various stress factors (Flores 1991, Galston and Kaur-Sawhney 1990).

Several effects of exogenous application of polyamines have been cited. A large number of reports refer to the capability of polyamines to restrain senescence-linked processes and certain responses to wounding. It has been demonstrated that polyamines may inhibit senescence-induced increase of protease activities (Kaur-Sawhney et al. 1982) and the loss of chlorophyll and synthesis of ethylene in oat leaves (Fuhrer et al. 1982; Shih et al. 1982). In detached radish leaves, exogenous polyamines inhibited the loss of chlorophyll and RNase activity (Altman 1982a). Polyamines effectively limited solute leakage and rise in RNase activity of potato tuber discs (Altman 1982b, Isola and Franzoni 1989). These and other related effects were called "antisenescence" effects of polyamines (Flores 1991).

In addition, Yordanov and Goltsev (1990) described a protective effect of putrescine and spermidine on the thylakoid membrane activity after high-temperature treatment. Applications of spermine, spermidine, or diaminopropane were effective in retarding the loss of D_1, D_2, and cytochrome *f* from the thylakoid membranes as well as Rubisco large subunits and chlorophyll from the osmotically stressed leaf tissue of oat seedlings (Besford et al. 1993). Polyamines were determined to be free radical scavengers and protectants against ozone damage (Bors et al. 1989). Srivastava and Smith (1982) showed that spermine reversed the growth inhibition induced by guanidines, of higher plants, and Preston et al. (1992) established that polyamines contributed to paraquat resistance in *Hordeum glaucum*.

Although it is difficult to ascribe a single mechanism of polyamine protective action, it is assumed that, due to their unique properties as biological polycations, polyamines bind to acidic sites of nucleic acids and cell membrane phospholipids, thereby stabilizing their structure and preventing the breakdown of macromolecules under stress conditions (Altman et al. 1982).

Atrazine is a widely used selective herbicide, which belongs to the group of triazines. Like other photosystem II (PS II) electron transport inhibitors,

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atrazine binds to the D_1 protein of the PS II reaction center, thus blocking electron transfer to plastoquinone. Inhibition of photosystem II electron transport prevents the conversion of absorbed light energy into electrochemical energy and results in the production of triplet chlorophyll and singlet oxygen, which induce the peroxidation of membrane lipids. Consequently, atrazine inhibits photosynthesis, growth, and other physiological and biochemical processes in treated plants (Iliev 1991).

Our previous investigations demonstrated that atrazine treatment resulted in the accumulation of putrescine, spermidine, and spermine (free and bound forms) in pea leaves, whereas the amount of conjugated polyamines decreased (Zheleva et al. 1993a). Atrazine-induced augmentation of free putrescine was accompanied by an increase of ornitinedecarboxylase (ODC) activity (Zheleva et al. 1993b). To elucidate the physiological significance of polyamine accumulation, we investigated the effects of exogenous polyamines on growth, leaf chlorophyll content, gas exchange, and de novo protein synthesis and function of PS II in atrazine-treated pea plants.

Materials and Methods

Plant Material and Treatment

Seeds of pea plants (*Pisum sativum* L., var. Koray) were soaked for 3–4 h in tap water and then placed on moistened filter paper for germination at 25°C for 48 h. Pea seedlings were grown as water cultures on half-strength Hoagland solution in a growth chamber (12-h photo period, photon flux density about 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and temperature 25 ± 1°C). Aerial parts of 10-day-old pea plants were sprayed with a water solution of 14 mM atrazine, 1 mM spermine, 1 mM spermidine, 1 mM putrescine (purchased from Aldrich), or combinations of them (approximately 0.3 ml to each plant). Tween 80 (0.5%, v/v) was used as surfactant. The control plants were sprayed with a 0.5% (v/v) water solution of Tween 80.

CO_2 Exchange Measurements

Intensities of photosynthesis, dark respiration, transpiration, and stomatal resistance were measured by a Portable System for Photosynthesis Measurements LI-6000 (Li-Cor, USA). Whole plants were placed in a 1/4l chamber. Quantum flux density was 970 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. Leaf temperature was 34 ± 2°C. The initial CO_2 concentration in the leaf chamber was about 800 $\mu mol \cdot l^{-1}$. Dark respiration was measured after 90 min of darkness.

Investigation of De Novo Protein Synthesis

De novo polypeptide synthesis was studied 10 days after treatment. It was performed by incubating disks from the third leaf of pea plants for 4 h in 5 mCi of ^{14}C-amino acid mixture (^{14}C-AAM, Amersham) at 27°C and at a light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Labeling and extraction of proteins were carried out according to Nivison and Stocking (1983). Soluble protein content was measured according to Bradford (1976), using bovine serum albumin as standard.

Chlorophyll Fluorescence Measurements

Fluorescence was measured on leaf disks at room temperature with an impulse modulated fluorimeter PAM (Heinz Walz, Germany). Fluorescence with open traps (F_0) was measured after 3 min dark adaptation. Maximal fluorescence (F_m) was obtained by 5 s light impulse with intensity 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from a halogen lamp. A microcomputer program FIP of Tyysjarvi and Karunen (1990), analysing fluorescence data was used.

Preparation of Thylakoids and Determination of Hill Reaction Activity

Fresh thylakoid membranes were prepared by the method of Percival et al. (1984). 2,6-Dichlorophenolindophenol (DCPIP, Loba Chemie, Wien-Fischamend, Austria) was used as artificial electron acceptor. The photoreduction of DCPIP was measured by the decrease in optical density at 590 nm following irradiation with a saturating red light. The reaction mixture had a final volume of 2.0 ml and contained 50 mM Hepes buffer (pH 7.6), 300 mM sorbitol, 5 mM $MgCl_2$, 10 mM NaCl, 30 μM DCPIP, and chloroplasts equivalent to 15 μg Chl/ml. 1,5-Diphenilcarbazide (DPC, Loba Chemie, Wien-Fischamend) was used as artificial electron donor at a concentration 0.5 mM.

The chlorophyll content was measured according to Arnon (1949). The leaf area was determined by a computerized system for area measurements (Tsonev and Sergiev 1993).

The length, fresh weight of shoots and roots, the leaf area, gas-exchange parameters, and chlorophyll content were measured 2, 6, and 10 days after treatment. The chlorophyll fluorescence and Hill reaction activity were determined after 10 days of the treatment.

All experiments were repeated three times with two replications for each. The data were analyzed statistically, and the least significant deviation (LSD) was used to evaluate differences between the variants.

Results

Influence of Spermine, Spermidine, and Putrescine on the Growth of Atrazine-Treated Pea Plants

Shoot length, fresh and dry weights, and leaf area were determined to characterize the growth of pea plants treated with atrazine, spermine, spermidine, putrescine, and their combinations. Our data clearly showed that atrazine inhibited growth of treated plants, which was determined by measured

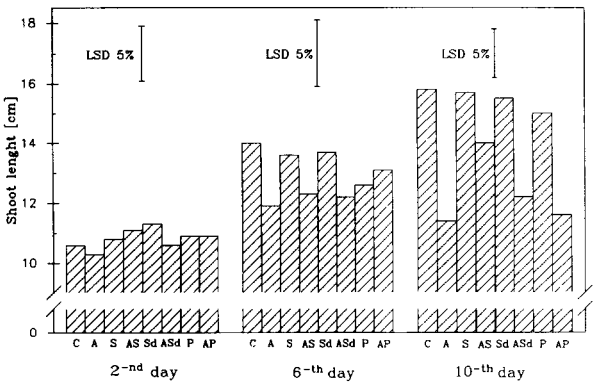

Fig. 1. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on shoot length of pea plants 2, 6, and 10 days after treatment. C, control; A, atrazine; S, spermine, AS, atrazine + spermine; Sd, spermidine; ASd, atrazine + spermidine; P, putrescine; AP, atrazine ± putrescine.

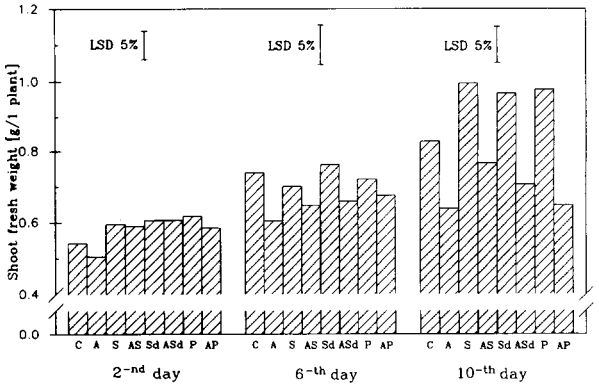

Fig. 2. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on shoot fresh weight of pea plants 2, 6, and 10 days after treatment. See Fig. 1 for legend.

length, fresh and dry weights of aerial parts, and leaf area (Figs. 1–4). On day 10 of the experiment, the herbicide-treated pea plants had shoots with 28% smaller length than the controls (Fig. 1). Spermine, applied in combination with atrazine, decreased this inhibitory effect; at the end of the experimental period, the shoots of pea plants, treated with atrazine + spermine were 11% shorter than the control. In relation to spermine, spermidine modified the atrazine action on shoot length to a lesser extent, and the effect of putrescine was negligible. As a consequence of atrazine application, the aerial parts of pea plants were lighter than those of untreated ones (Fig. 2). Spermine and spermidine, but not putrescine, decreased this inhibitory effect of the herbicide on shoot fresh weight. The same tendency was observed in relation to the dry weight of aerial parts of pea plants from the variants (Fig. 3). Atrazine-treated pea plants had smaller leaf area than controls (Fig. 4). Reduced leaf area (61.4% of the control value) was detected 6 days after atrazine treatment, and this inhibitory effect persisted to the end of the experimental period. Spermine and spermidine decreased this atrazine action; 10 days after treatment with atrazine + spermine and atrazine + spermidine, leaf areas of pea plants were, respectively, 83.2% and 78.6% of the control. Putrescine did not alter the herbicide effect on reducing leaf area. Polyamines, applied without atrazine, increased shoot fresh and dry weights and leaf area, but did not change the shoot length (Figs. 1–4). We did not observe any considerable inhibitory effect of the herbicide on length and fresh weight of the roots after plant spraying (data not shown).

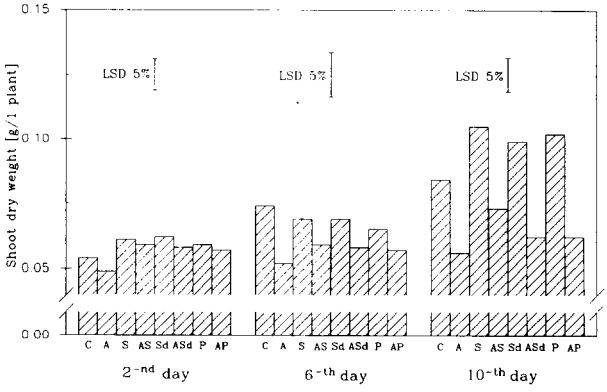

Fig. 3. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on shoot dry weight of pea plants 2, 6, and 10 days after treatment. See Fig. 1 for legend.

Influence of Spermine, Spermidine, and Putrescine on Chlorophyll Content of Atrazine-Treated Pea Leaves

The first visible consequence of atrazine action was chlorosis of the treated plants. The herbicide reduced chlorophyll a as well as chlorophyll b levels, as determined 2 days after treatment (Fig. 5a,b). At the end of the experimental period, chlorophyll a and chlorophyll b contents were, respectively, 47.6% and 49.3% of the control value. Our data clearly demonstrated the ability of exogenous polyamines to prevent chlorophyll loss following atrazine treatment. Ten days after spraying with atrazine + spermine, atrazine + spermidine and atrazine + putrescine leaf chlorophyll a levels were 72.4%, 82.9% and 69.5%, respectively, compared with the control (Fig. 5a). At the same time, chlorophyll b breakdown was also retarded as a result of

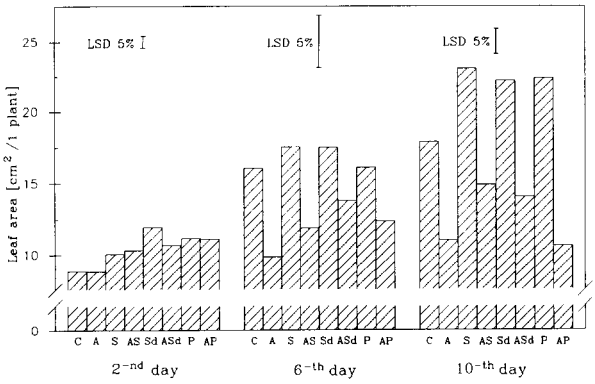

Fig. 4. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on leaf area of pea plants 2, 6, and 10 days after treatment. See Fig. 1 for legend.

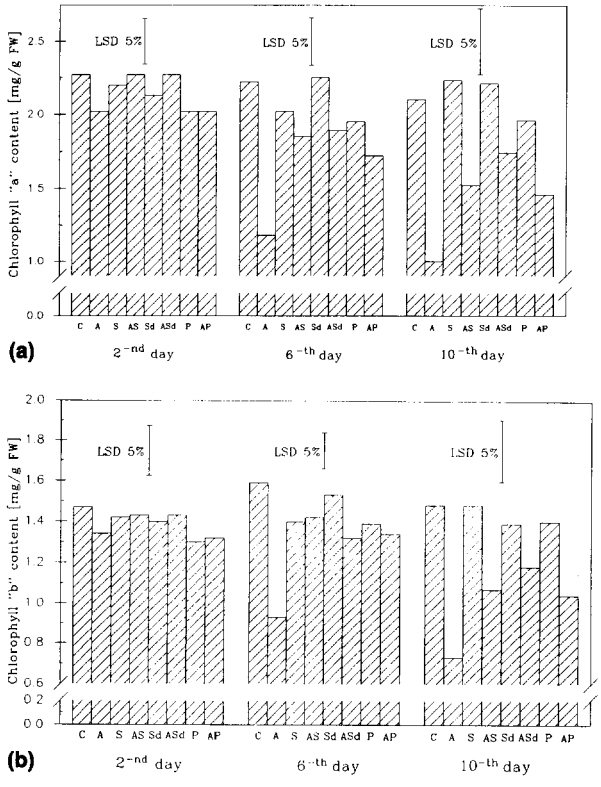

Fig. 5. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on chlorophyll content of pea plants 2, 6, and 10 days after treatment. (a) Chlorophyll a content; (b) chlorophyll b content. See Fig. 1 for legend.

the polyamine action, and its content was 72.3%, 79.7%, and 70.3% of the control value in atrazine + spermine, atrazine + spermidine, and atrazine + putrescine treated pea leaves, respectively (Fig. 5b). The chlorophyll a/b ratio was decreased as a consequence of atrazine spraying, and polyamines completely reversed this effect (data not shown). No significant changes in chlorophyll levels were found after spraying with spermine, spermidine, and putrescine (without atrazine).

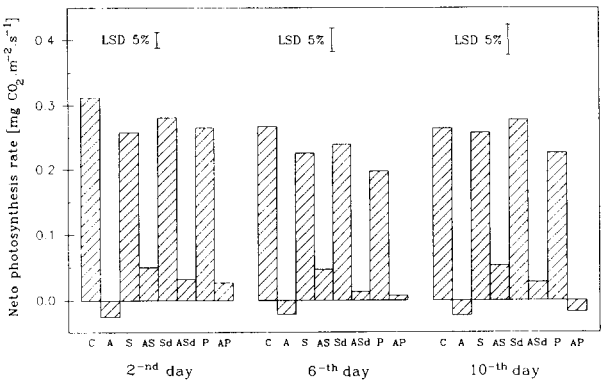

Fig. 6. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on net photosynthetic rate of pea plants 2, 6, and 10 days after treatment. See Fig. 1 for legend.

Influence of Spermine, Spermidine, and Putrescine on Gas Exchange of Atrazine-Treated Pea Plants

Atrazine strongly influenced the gas exchange of treated plants, and the most sensitive process to this is photosynthesis (Ebert and Dumford 1976). The data concerning the net photosynthesis intensity are presented in Fig. 6. Even as early as 2 days after herbicide treatment photosynthesis was strongly inhibited, and the obtained negative value clearly showed that intensity of respiration in light exceeded the photosynthetic rate under the conditions employed. Spermine and spermidine partially reversed atrazine-induced inhibition of photosynthesis. This protective effect in relation to photosynthetic rates of atrazine-treated pea plants was observed on day 2 and did not change in following measurements. Ten days after treatment, photosynthetic intensities of plants treated with atrazine + spermine and atrazine + spermidine were 20.3% and 10.6% of the control value. It should be mentioned that a partially protective effect of the diamine, putrescine, was observed 2 days after spraying. However, after that, putrescine did not sufficiently reverse atrazine-induced inhibition of net photosynthesis. The polyamines, applied without atrazine, slightly decreased photosynthesis in comparison with the control. The inhibition was observed 2 and 6 days after treatment, but statistical significance of differences was registered only at day 6 and then the photosynthetic rates reached the control value.

Gas-exchange measurements showed a strong in-

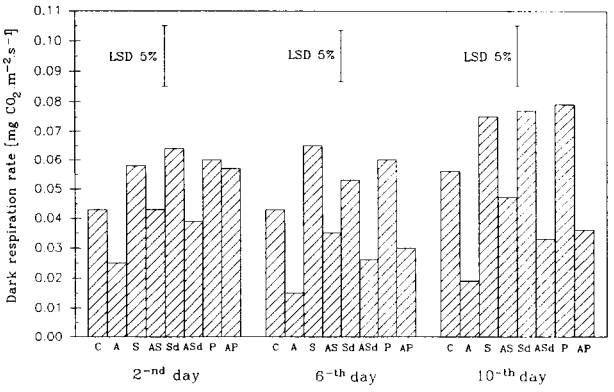

Fig. 7. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on dark-respiration rate of pea plants 2, 6, and 10 days after treatment. See Fig. 1 for legend.

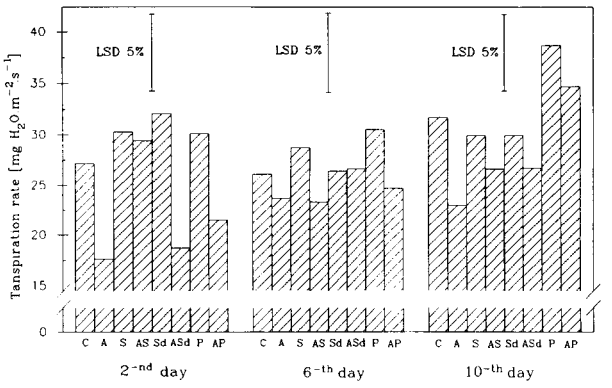

Fig. 8. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on the transpiration level of pea plants 2, 6, and 10 days after treatment. See Fig. 1 for legend.

fluence of atrazine and polyamines on dark-respiration rate (Fig. 7). The herbicide inhibited dark respiration, which was recognized 6 days after application (34.5% of the control) and did not alter to the end of the experimental period. Exogenous spermine, spermidine, and putrescine decreased atrazine-induced inhibition of dark respiration; 10 days after spraying with atrazine + spermine, atrazine + spermidine, and atrazine + putrescine, dark-respiration rates were, respectively, 83%, 59.7%, and 64.8% of the control value. Polyamines, applied alone, activated this process, the most effective being putrescine. The last measurement showed that the rates of dark respiration of spermine-, spermidine-, and putrescine-treated pea plants exceeded the control by 33.1%, 36.9%, and 41.1%.

As a result of herbicide action, the intensity of transpiration decreased (Fig. 8). Atrazine reduced transpiration to 64.7% of the control by day 2 of the treatment; this inhibitory effect was slightly reduced by day 6 and at the end of the experimental period its value was 72.3% of the control. Spermine, spermidine, and putrescine, applied in combination with atrazine, partially reversed the inhibition of transpiration. However, it should be noted that the differences obtained between transpiration rates of pea plants treated with atrazine (with or without polyamines) were not significant (except for atrazine and atrazine + putrescine on day 10). Exogenous spermine and spermidine (without the herbicide) did not alter transpiration. A tendency toward an increase of transpiration as a result of treatment with putrescine was observed; at the end of the experimental period, the transpiration rate of diamine-treated plants exceeded the control value by 22.1%.

Atrazine-induced inhibition of transpiration was accompanied by an augmentation of stomatal resistance (Fig. 9). Ten days after spraying, stomatal resistance of pea plants treated with the herbicide was two times that of the control value. The data, presented in Figs. 8 and 9, showed that the polyamine protective activity, with regard to the transpiration rate, correlated with a decrease of atrazine-induced rise of stomatal resistance. Polyamines (without atrazine) did not influence this gas-exchange parameter of pea plants.

Influence of Spermine, Spermidine, and Putrescine on De Novo Protein Synthesis in Atrazine-Treated Pea Leaves

Our data (Table 1) showed that both the uptake and incorporation into de novo synthesized polypeptides of ^{14}C-AAM was reduced as a result of atrazine action (45.8% and 18.7% of the control, respectively). Polyamines spermine and spermidine (applied simultaneously with atrazine) weakened herbicide effects in relation to the uptake, as well as to the rate of protein labeling. ^{14}C-AAM uptake in leaf disks from plants treated with atrazine + spermine and atrazine + spermidine were, respectively, 56.5% and 56.7%, and its incorporation into polypeptides was 31.9% and 33.9% of the control. Compared with the polyamines, spermine and spermidine, the diamine putrescine had a slight effect on the herbicide-induced inhibition of protein synthesis.

Treatments with spermine, spermidine, or putrescine (without atrazine) led to a considerable increase of label uptake, and this effect may be due to the capability of these organic polycations to attach to the cell membranes and thus change the membrane permeability. We noticed augmentation of the

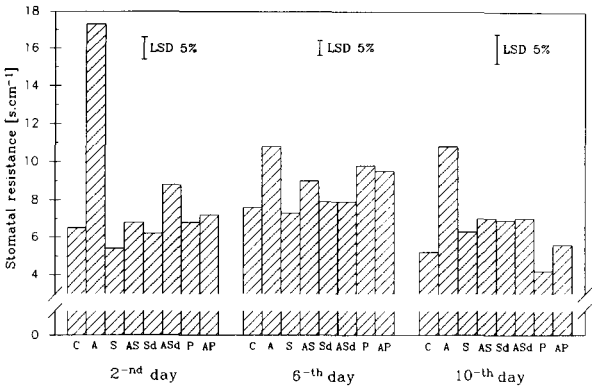

Fig. 9. Influence of atrazine, spermine, spermidine, putrescine, and their combinations on stomatal resistance of pea plants 2, 6, and 10 days after treatment. See Fig. 1 for legend.

polypeptide synthesis rate after spraying with spermine or spermidine (but this increase was smaller than those observed in relation to ^{14}C-AAM uptake); the effect of putrescine was not significant. The changes in soluble protein contents were similar to these established for the rates of de novo polypeptide synthesis (Table 1).

Influence of Spermine on Chlorophyll a Fluorescence of Atrazine-Treated Pea Leaves

Ten days after spraying of pea plants with atrazine, spermine, and a combination of them, we determined the components of fluorescence in leaf disks with a PAM fluorimeter, namely, initial (F_0), maximal (F_m), and variable (F_v) as well as ratios F_v/F_0 and F_v/F_m (Table 2). F_0 was recorded after a 3-min dark adaptation when all reaction centers were "opened." Our data showed that after atrazine treatment (with or without spermine) F_0 value increased 2.5-fold in comparison with the control. This was partly due to the reduced plastoquinone acceptor, Q_A^-, being unable to be oxidized completely due to an interruption of the electron flow through PS II. DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], a photosynthetic inhibitor binding to the Q_B site of the D_1 protein of the PS II reaction center, changed F_0 in the same manner (Krause and Weis 1991). F_m was altered in a different way, depending on the treatment. F_m slightly decreased as a result of atrazine application (84% of the control value) but increased when pea plants were sprayed simultaneously with atrazine and spermine (134% of the control).

F_v describes the change in fluorescence emission between F_0 and F_m. The F_v value of atrazine-treated leaves was reduced by a considerable extent, and spermine completely reversed the herbicide effect.

Table 1. ^{14}C-AMM uptake and inclusion in soluble protein fraction (cpm 10^6/30 leaf disks) and soluble protein content (mg/g fresh weight) of third leaves of pea plants 10 days after treatment with atrazine, spermine, spermidine, and putrescine (applied alone and in combination).

Variants	^{14}C-AMM uptake	Protein labeling	Soluble protein content
Control	9.56	3.48	18.07
Atrazine	4.38	0.65	12.96
Spermine	15.14	3.79	20.90
Atrazine + spermine	5.40	1.11	15.43
Spermidine	14.94	3.59	20.81
Atrazine + spermidine	5.42	1.18	15.94
Putrescine	15.40	3.43	19.61
Atrazine + putrescine	3.50	0.82	12.50
LSD 5%	0.40	0.13	2.40
LSD 1%	0.61	0.20	3.56

Table 2. Components of fast chlorophyll flourescence (relative units) of pea leaf disks 10 days after treatment with atrazine, spermine, and combination of them.

Variants	F_0	F_m	F_v	F_v/F_0	F_v/F_m
Control	0.087	0.413	0.326	3.76	0.79
Atrazine	0.215	0.361	0.147	0.69	0.40
Spermine	0.093	0.417	0.324	3.43	0.78
Atrazine + spermine	0.220	0.555	0.336	1.65	0.60
LSD 5%	0.045	0.091	0.082	0.53	0.05
LSD 1%	0.061	0.123	0.111	0.71	0.07

F_0, initial fluorescence; F_m, maximal fluorescence; F_v, variable fluorescence.

Because atrazine alters chlorophyll content, the ratios F_v/F_0 and F_v/F_m are more representative of stress-induced inhibition of photosynthetic function than the absolute heights of F_0 and F_m, which depends on the chlorophyll content per leaf area unit (Lichtenthaler 1988). As a result of atrazine spraying, the F_v/F_0 and F_v/F_m ratios decreased to 18.4% and 50.4%, respectively, of the control. These ratios were higher (F_v/F_0: 43.9%, and F_v/F_m: 75.7% of the control value) when atrazine was applied in combination with polyamine, which showed spermine protective action on photosynthetic activity of atrazine-treated pea plants. Treatment with spermine (without atrazine) did not lead to significant changes in chlorophyll fluorescence components.

Influence of Spermine on Hill Reaction Activity in Thylakoid Membranes, Isolated from Atrazine-Treated Pea Leaves

Spermine protective effect on PS II function of atrazine-treated pea leaves was also manifested from Hill reaction activity measurements (Table 3). Pho-

Table 3. Photochemical reduction of DCPIP in thylakoid membranes, isolated from pea leaves 10 days after treatment with atrazine, spermine, and combinations.

Variants	mmol DCPIP/mg chl · h	
	−DPC	+DPC
Control	91.1	96.7
Atrazine	22.7	57.0
Spermine	97.4	105.2
Atrazine + spermine	64.1	61.5
LSD 5%	18.9	21.0
LSD 1%	25.8	32.3

tochemical reduction of an artificial electron acceptor, DCPIP, in thylakoid membranes isolated from atrazine-treated pea leaves decreased four-fold compared with the control. Spermine considerably reversed the herbicide effect on Hill reaction activity. The addition of DPC as an artificial donor led to a decrease of atrazine-induced inhibition of the photochemical reduction of DCPIP. The same results were obtained by Holt et al. (1983), who explained this fact as a result of injury to the acceptor, as well as the donor, part of PS II (including the water oxidizing system). DPC did not change the Hill reaction activity in thylakoid membranes, which were isolated from atrazine + spermine-treated pea leaves. This leads us to suggest that the polyamine protective effect is localized in the donor part of PS II. Our data did not show altered DCPIP reduction in thylakoid membranes of pea leaves sprayed with spermine (without atrazine).

Discussion

As a result of treating pea plants with 14 mM atrazine, we observed the following changes: retardation of growth; reduced content of chlorophyll and soluble proteins; inhibition of photosynthesis, dark respiration and transpiration, and simultaneously increased stomatal resistance; the uptake and incorporation of ^{14}C-AMM into de novo synthesized polypeptides was decreased; and the PS II activity was strongly inhibited.

Our results showed that the simultaneous application of the herbicide and polyamines led to retarded chlorophyll loss. Similar data were obtained by Popovic et al. (1979) and Kaur-Sawhney and Galston (1979) who established polyamine-induced delaying of chlorophyll degradation in senescing barley and oat leaf discs. Polyamines partially reversed the inhibitory effect of atrazine on plant gas exchange. Their protective activity might be due to a stabilization of cellular membranes. However, the products of polyamine oxidative catabolism are involved in the Krebs cycle (Flores and Filner 1985) and that treatment with polyamines increased in vitro NADH-dehydrogenase oxidative activity in mitochondria isolated from *Helianthus tuberosus* tubers (Ruggolo et al. 1991). This suggested a regulatory role in vivo of these compounds on respiratory metabolism of the plants, which could explain the stimulatory effects of spermine, spermidine, and putrescine on the dark-respiration rate.

It was suggested that the atrazine inhibitory effect on protein synthesis was not direct and might be due to the herbicide-induced changes in the energy, water, and hormonal balance of plant cells (Ebert and Dumford 1976). However, the involvement of polyamines in metabolic regulation of protein synthesis in eukaryotic organisms is well known (Barbiroli et al. 1989). In addition, polyamine protective activity in relation to de novo polypeptide synthesis could be explained by the ability of these organic polycations to replace Mg^{2+} as activators of protein synthesizing systems (Cohen and Zalic 1978). It was determined that, as a result of atrazine action, Mg^{2+} uptake and concentration in plants were strongly reduced, and that this was one of the factors negatively influencing de novo protein synthesis (Brenchley and Appleby 1971). We observed that putrescine had the least effect on atrazine-induced inhibition of polypeptide synthesis (Table 1). Similarly, in the model system of Cohen and Zalic (1978), putrescine had the most weakly compensatory effect compared with spermine and spermidine.

Spermine improved photosynthetic activity of atrazine-treated pea plants in accordance with the chlorophyll fluorescence and Hill reaction activity measurements (Tables 2 and 3). Similar protective effects of exogenous polyamines against heat shock in bean plants were observed by Yordanov and Goltsev (1990) who suggested that the interaction of polyamines with the thylakoid membrane surface led to their stacking, to separation of the photosystems, and to the association of light-harvesting complex II with the PS II core complex.

It could be concluded that the polyamines, spermine and spermidine, partially reversed atrazine-induced inhibition of pea plants; spermine treatment was more effective; and plants, treated with atrazine + spermine, possessed improved growth, gas exchange, chlorophyll content, and intensity of de novo protein synthesis than atrazine-treated plants.

The precise mode of polyamine protective action is unknown and could be explained by the following possibilities: 1) because of their polycationic nature, polyamines might bind with photosynthetic membranes, resulting in membrane conformational

changes and alteration of their atrazine-binding properties; and 2) polyamines could prevent the destructive consequences of atrazine interruption of photosynthetic electron transport.

The target site for atrazine is the reaction center of PS II. One of the most deleterious consequences is the production of free radicals that induce lipid peroxidation and membrane damage (Fuerst and Norman 1991, Renger et al. 1988). Polyamines (especially when conjugated with trichloroacetic acid soluble molecules) act as free radical scavengers and protect ozone-treated tobacco plants (Langerbartels et al. 1991). In our model, polyamines could act as free radical scavengers and prevent atrazine damage, but knowledge of the precise mode of polyamine protective action is needed for further elucidation. The observed differences between protective activity of exogenous spermine, spermidine, and putrescine might be due to distinctions in their chemical structure, uptake, transport, and metabolism, as well as influence on endogenous polyamine concentrations. Using labeled polyamines could shed light on this problem in the future.

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