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GROWTH AND PHOTOSYNTHESIS RESPONSES OF YOUNG BEAN (*Phaseolus vulgaris* L.) PLANTS TO HERBICIDE STRESS. PROBABLE PROTECTIVE EFFECT OF POLYAMINE DIETHYLENETRIAMINE

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Abstract. Two-week-old *Phaseolus vulgaris* plants were subjected to herbicide stress. The possible protective effect of polyamine diethylenetriamine was studied. Biometric parameters, photosynthetic gas exchange and chlorophyll fluorescence were measured 10 days after the stress. The higher herbicide concentrations inhibited growth and photosynthesis in bean plants. The use of diethylenetriamine removed considerably the herbicide effect.

Keywords: leaf gas exchange, chlorophyll fluorescence, *Phaseolus vulgaris* L., Fusilade Super (FS), herbicide stress, diethylenetriamine (DETA).

AIMS AND BACKGROUND

Herbicides are widely applied in modern agriculture. Their misuse, however, could make them powerful stress factors inducing significant physiological disorders in plant organisms[1,2]. The latter could have a negative effect on yields and quality of agricultural produce.

To protect plants against herbicide stress, plant regulators have successfully been used[2-4]. In the last years, the group of regulators was enriched with polyamines that are natural cell components[5-7]. Endogenous concentrations of free polyamines in plant tissues are higher than those of plant hormones. All prokaryotic and eukaryotic organisms contain the diamine putrescine and the triamine spermidine, and the eukaryotic organisms – the tetraamine spermine, as well.

It was established that the stress, both physical and biological, increased the cell content of polyamines[8]. Putrescine accumulation and increased ornithine-decarboxylase were registered under conditions of osmotic stress, air pollutants and potassium deficiency[9]. The spraying of plants with difluoromethylornithine – an inhibitor of ornithine-decarboxylase, protected partially or completely against infection. The treatment with polyamines improved the status of plants grown under conditions of strong salinity, low temperatures, ozone damage, etc.[10] It was

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suggested that polyamine accumulation could play a specific protective role in plants, adapted to extreme conditions[9-13].

The physiological effect and the possibilities for polyamine application have been tested in different aspects[10,14,15]. A problem of topical interest is their use as antidotes against herbicide stress[3,16-18]. Benavides et al.[19] established that paraquat treatment of sunflower leaves led to decreased levels of polyamines and enzymes related to their metabolism. The exogenous application of polyamines (spermidine, 1 mM) reduced the negative effect of paraquat by recovering the enzyme activity. It was established that the polyamines spermidine and spermine (1 mM) reduced the damage of pea plants, induced by the application of the triazine herbicide atrazine. The positive effect of spermidines was monitored in terms of the restored growth rate, the increased chlorophyll content, the leaf gas exchange and the function of photosystem II[17,20].

To overcome the damage caused by herbicide misuse, it is necessary to know both the specific crop response to the herbicides applied and the methods for eliminating their negative effect. This motivated us to conduct the present research.

The objective of the investigation was to study the physiological response of young bean plants to increased dose rates of the herbicide Fusilade Super and the possibilities for overcoming their phytotoxic effect through the use of the synthetic polyamine DETA.

EXPERIMENTAL

Plant material and treatment. The subject of this study was young bean plants (*Phaseolus vulgaris* L.) cv. Plovdiv-11 M.

The applied herbicide Fusilade Super [Fluazifop-P-butyl] belongs to the group of aryloxy-propionic acids. It is a selective, leaf and systemic herbicide.

The polyamine diethylenetriamine (DETA) is a synthetic structural analogue of the polyamine spermidine. For the first time the plant growth regulating activity of DETA was determined in the laboratory for Chemical Phytoeffectors at the Institute of Plant Physiology of Bulgarian Academy of Sciences (BAS)[21,22].

The experiments were conducted in a climatic box at a light intensity of 150 μmol $m^{-2}s^{-1}$, a temperature of 24±2°C and a 12 h photoperiod. Plants were cultivated on sand with Knop's nutrient solution. After the emergence of the first compound leaf the following variants were formed: 1. Fusilade Super – 150 ml da^{-1} – recommended for practical use dose rate (0.5%) – control; 2. Fusilade Super – 300 ml da^{-1} – double dose rate (1.0%); 3. DETA – 0.1 mM; 4 combination of FS – 300 ml da^{-1} and DETA. Plants were foliarly treated with water solutions of the aforementioned compounds approximately 2.0 ml to each plant.

Analyses. Biometric parameters, photosynthetic gas exchange and chlorophyll

fluorescence were measured on the primary leaves of bean plants 10 days after treatment.

The leaf area was measured with a digital area meter NEO-2 (TU, Sofia, Bulgaria).

Leaf gas exchange parameters were measured by a portable infrared gas analyser LCA-4 (ADC Ltd., Hoddesdon, England) at 26±2 °C temperature, relative humidity – 70±2%, 800 μmol $m^{-2}s^{-1}$ photon flux density and 400 μmol mol^{-1} CO_2 concentration.

Parameters of chlorophyll fluorescence were determinated with a photosynthesis yield analyser MINI-PAM (H. Walz, Germany) after 30 min of dark adaptation.

All experiments were repeated three times. The data were statistically analysed. The differences between control and other variants were evaluated according to Student's *t*-criterion[23].

RESULTS AND DISCUSSION

The biometric parameters of bean plants showed (Fig. 1) an obvious negative effect of the double herbicide dose. On day 10, the herbicide-treated plants were by 38% shorter than those of the control. The linear growth inhibition was accompanied by phytotoxic symptoms, such as chlorosis and necrosis of leaf parts. These symptoms were typical for herbicide-damaged plants[3]. The polyamine DETA weakened this inhibitory effect and by the end of the study period the plants were by 16% shorter than those of the control. When applied alone, DETA did not affect significantly that parameter.

The fresh weight of plants subjected to herbicide stress was significantly

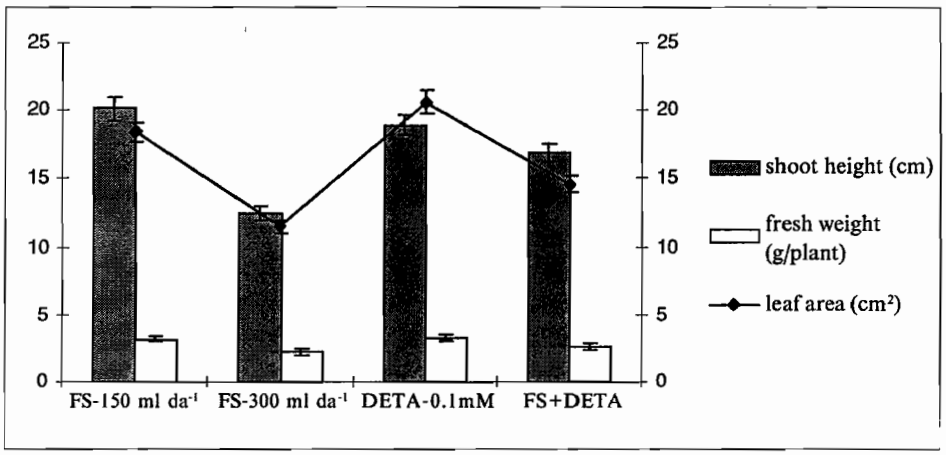

Fig. 1. Biometric parameters of young bean plants subjected to herbicide stress and diethylenetriamine treatment. Means of 3 separate experiments ± SE ($n = 9$). Bars indicate SE when larger than symbol

lower than that of the control. In terms of this parameter, it was again observed an obvious protective effect of the polyamine.

The treatment of plants with a double herbicide rate led to reduction in the photosynthetic potential. The formed leaf area was by 38% smaller than that of the control. The polyamine DETA modified partially the inhibiting effect of the herbicide.

It is known that all factors inducing stress response in plants affect directly or indirectly the function of their photosynthetic apparatus.

The results given in Fig. 2 showed that the treatment of bean plants with the herbicide Fusilade Super, applied at double dose rates, inhibited significantly their leaf gas exchange parameters. The photosynthetic rate decreased by 75%, and that of transpiration – by 32%.

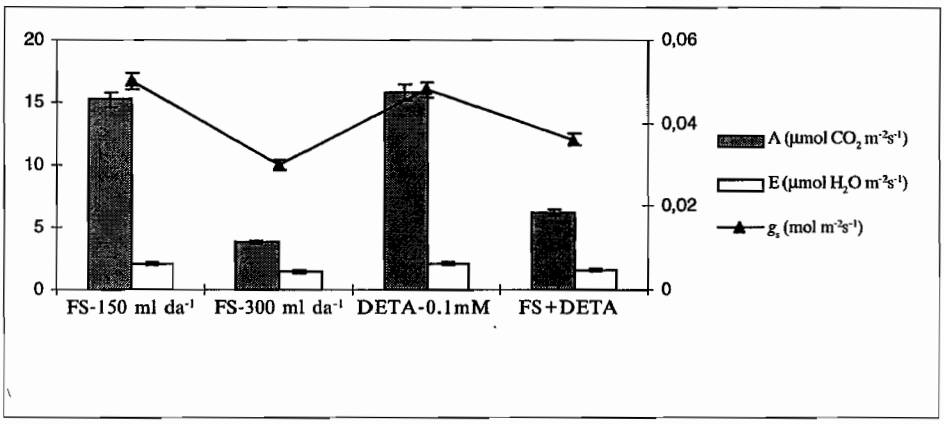

Fig. 2. Leaf gas exchange in young bean plants subjected to herbicide stress and diethylenetriamine treatment. A – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance. Means of 3 separate experiments ± SE ($n = 9$). Bars indicate SE when larger than symbol

The data showed that photosynthetic inhibition was related, to a great extent, to stomatal limitation. Probably, when penetrating the leaves, the herbicide damaged the functioning of stomatal apparatus. This led to a rapid loss of water in leaf cells and hydropassive stomatal closing.

The stronger inhibition of the photosynthetic rate, as compared to that of the transpiration intensity, showed the presence of mesophilic disturbances also. The latter could involve both photochemical and biochemical processes of the Calvin cycle.

In the treatment with DETA, a tendency to photosynthetic improvement was observed. That tendency was significantly less expressed in terms of transpiration. The DETA effect was probably related to the antidote action on mesophilic processes.

No significant difference was established between the photosynthetic and transpiration intensities in the control plants and those treated with DETA alone.

The parameters of chlorophyll fluorescence (Table 1) reflected the activity of the photosynthetic apparatus[24,25]. In dark-adapted leaves, F_0, F_m, F_v, F_v/F_0 and F_v/F_m characterised the potential activity of photosystem II (PSII). The data showed that in result of the treatment with an increased dose of the herbicide Fusilade Super the functional activity of PSII decreased. Marked trends to increase of F_0 and decrease of F_m, F_v/F_0 and F_v/F_m were outlined. The F_0 parameter reflected the fluorescence emission of the excited antenna chlorophyll before the energy migration to the reaction centers. The strong increase (about 2 times) of F_0 in the plants treated with the increased herbicide dose showed the presence of structure disturbances in the photosynthetic apparatus[25]. The same tendency was observed in the combined treatment of plants with herbicide and DETA. According to Briantais et al.[26] the rise of F_0 could be interpreted as a reflection of reduced energy transport effectiveness from antenna chlorophyll *a* to the reaction center of PSII and/or to a disturbed function of the latter.

Table 1. Chlorophyll fluorescence parameters in bean plants subjected to herbicide stress and treatment with the polyamine DETA

Parameters	Treatments			
	FS-150 ml da^{-1}	FS-300 ml da^{-1}	DETA-0.1 mM	FS-300 ml da^{-1} + DETA
F_0	425 ± 8.2	840 ± 21.6**	438 ± 9.7	852 ± 30.8**
F_m	1720 ± 63.5	1445 ± 57.1*	1725 ± 78.2	1790 ± 75.0
F_v	1295 ± 55.2	605 ± 38.1**	1287 ± 68.5*	938 ± 54.1*
F_v/F_0	3.05 ± 0.03	0.72 ± 0.03**	2.94 ± 0.05	1.10 ± 0.02**
F_v/F_m	0.75 ± 0.005	0.42 ± 0.005**	0.75 ± 0.003	0.52 ± 0.006*

Note: F_0, F_m, F_v, F_v/F_0, F_v/F_m – initial, maximum, variable and Chl fluorescence ratios in dark-adapted leaves. Means of 3 separate experiments ±SE ($n = 9$) – * $p < 0.05$; ** $p < 0.01$.

F_m represented the reduction degree of the PSII acceptor side. F_m decreased in result of the herbicide action and slightly increased after the combined herbicide + polyamine application. The herbicide induced a significant decrease in the variable fluorescence. The polyamine DETA eliminated partially this herbicidal effect. A decrease of F_m and F_v at a double herbicide rate treatment was due to a lower electron transport rate as compared with control plants.

The F_v/F_m ratio is a parameter for the potential PSII efficiency in the photochemical reactions. It is known that in healthy leaves this ratio is in the range of 0.75-0.85 (Ref. 24). The significant F_v/F_m decrease in the plants subjected to herbicide stress (by about 40%) was indicative of serious PSII disturbances.

The plants subjected to combined treatment with herbicide and polyamine maintained that ratio at a higher level. This demonstrated the higher tolerance

of their photosynthetic apparatus to herbicide stress. When applied alone, the polyamine DETA did not affect significantly the parameters of chlorophyll fluorescence.

CONCLUSIONS

On the basis of the parameters studied the following conclusions can be made:

1. The herbicide Fusilade Super, applied at a double dose rate, exerts a phytotoxic effect on bean plants. It is expressed in growth suppression and inhibition of leaf gas exchange and chlorophyll fluorescence parameters. The most sensitive parameters were F_v and the ratio F_v/F_0.

2. The synthetic polyamine DETA, applied at a concentration of 0.1 mM, has a positive antidote effect on the function of the photosynthetic apparatus and the growth of Fusilade Super-damaged bean plants.

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