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Influence of GA_3 and 4-PU-30 on leaf protein composition, photosynthetic activity, and growth of maize seedlings

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Abstract

The effects of gibberellic acid (GA_3) and N_1-(2-chloro-4-pyridyl)-N_2 phenylurea (4-PU-30) on maize seedling growth, photosynthetic parameters, and leaf protein composition were investigated. The agents used alone or in combination increased leaf growth and photosynthetic rate of the seedlings. Chlorophyll and total nitrogen contents in leaves as well as the quantity of individual protein fractions increased simultaneously. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of soluble proteins (albumins and globulins) revealed quantitative differences between 4-PU-30-treated plants and the other experimental variants. They differed in polypeptide composition associated with changes in soluble proteins and amino acids. However, GA_3 did not induce similar changes in polypeptide composition of soluble proteins.

Additional key words: amino acids, chlorophyll, cytokinins, electrophoresis, germination, shoot.

Introduction

In cereal grains the embryo controls the mobilization of food reserves through the production of gibberellic acid (GA_3, Bewly and Black 1994). GA_3 activates proteases in seeds during germination (Segundo *et al.* 1990, Pinthus and Abraham 1996) and m-RNA synthesis at some stages of plant differentiation (Koehler and Ho 1988, Jacobsen *et al.* 1994). GA_3 also influences plant growth, chlorophyll content and photosynthetic rate (*e.g.* Chaterjee *et al.* 1976, Sell *et al.* 1990). Phenylurea cytokinins stimulate cell division and differentiation, promote leaf and cotyledon expansion, increase fruit size, and retard leaf senescence (*e.g.* Okamoto *et al.* 1981, Mok *et al.*

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1982, Ogata *et al.* 1989). Phenylurea cytokinins regulate many biochemical processes (Nickell 1986, 1991). The phenylurea cytokinin 4-PU-30 provokes quantitative differences between some relevant polypeptides of soluble proteins isolated from control seedlings and treated maize seedlings (Stefanov *et al.* 1994). However, there are almost no data about the nature of these changes. Thus the aim of this investigation was to find the effects of 4-PU-30 and GA_3 applied alone and in combination on the content of the individual protein fractions of maize shoots and especially of soluble proteins as well as on the growth and photosynthetic parameters.

Materials and methods

Seeds of maize (*Zea mays* L.) cv. Knezha-530 were germinated in the dark on moist filter paper at 25 °C. On the 8th day, the seedlings were divided into 4 groups of 45 plants each. The first group was sprayed with 250 μM GA_3, the second group with 250 μM 4-PU-30, the third group with 250 μM GA_3 and 4-PU-30 and the control group was sprayed with distilled water. The suitable concentrations were determined in previous testing of effects of several concentrations of GA_3 and 4-PU-30 on seedling growth. After spraying, all seedlings were grown in the chamber under continuous ligth [irradiance 160 μmol(PAR) m^{-2} s^{-1} provided by "white" fluorescent lamps], temperature 25 °C and relative humidity of 60 % for 72 h. Photosynthetic rate in the seedlings was measured as the rate of $^{14}CO_2$ uptake as described by Stefanov *et al.* (1994) on leaf discs isolated from central part of the second leaf of 5 different seedlings (irradiance 200 μmol m^{-2} s^{-1}, temperature 25 °C, air humidity 80 %, CO_2 concentration 0.15 %). At the end of the growth period, shoots (stems and leaves) were excised, and their dry mass was determined after drying to constant mass at 110 °C. Each experiment was repeated 4 times with 6 replicates. For determination of chlorophyll content, all samples were weighed, ground with 80 % acetone, centrifuged at 6 000 × *g*, and supernatants were used for measuring absorbance at 663 nm (Arnon 1949). The determinations were repeated 3 times with 6 replicates. Protein fractions (albumins plus globulins, prolamins, and glutelins) were extracted from fresh shoots according to Landry and Moureaux (1970). Nitrogen content was measured by a micro-Kjeldahl method. Before electrophoresis, the protein extracts containing albumins plus globulins were dialyzed against 0.01 M Tris-glycine buffer (pH 8.5) with 0.2 % SDS, 5 % 2-mercaptoethanol, and 10 % sucrose. SDS-PAGE was performed according to Laemmli (1970). The SDS-PAGE mobilities (in 7.5 % homogenous gels) of all investigated proteins were compared with those of standard proteins of known molecular mass: α-lactalbumin 14 200, trypsinogen 24 000, ovalbumin 45 000 and bovine serum albumin 66 000. One hundred micrograms of protein were loaded in each tube for electrophoretic separation. Bands C from all investigated samples were cut with a razor blade from polyacrylamide gel. About 60 - 70 pieces containing band C were collected from each protein sample. Extraction of the protein-SDS-complexes from the gel was performed by the method of McGillivray and Rickwood (1974). The obtained supernatants were dialyzed against

0.01 M acetic acid, and lyophilized. These samples were resolved in 6 M HCl (2 mg cm^{-3} of a sample), and the liberated amino acids were quantitatively determined with an amino acid analyzer *339 M* (*Microtechna*, Prague, Czech Republic). The results were statistically analyzed using Fisher's criteria.

Results

Treatment with GA_3 resulted in a decrease of shoot dry mass in comparison with the control shoots (Table 1). GA_3 increased the length of shoots by 6 %. When 4-PU-30 was used, the shoots dry mass increased. The combination of GA_3 and 4-PU-30 increased the both dry mass and length of shoots. Chlorophyll (Chl) content and photosynthetic rate of leaves of plants treated with GA_3, 4-PU-30, and their combination were enhanced compared with control leaves (Table 1), but after treatment with sole GA_3 the differences in Chl content were statistically not significant. The total nitrogen content of shoots treated with GA_3 and 4-PU-30 added alone or in combination was higher than in the control (Table 2). Similar changes were found in individual protein fractions. Treatment with GA_3 and 4-PU-30 applied alone elevated the quantities of all investigated proteins, especially of prolamins and glutelins. When GA_3 and 4-PU-30 were used in combination, very little effect was found in the glutelin content. The 4-PU-30 provoked highest (20.4 %) elevation of total nitrogen in shoots. As shown by SDS-PAGE, each of the albumin and globulin fractions contained four polypeptides with similar molecular masses. Soluble proteins from 4-PU-30 treated shoots showed some differences in their mobilities as well as in the intensity of the protein bands C and D. In control, the band C prevailed in amount against the band D. This prompted us to extract bands C from all investigated samples and to compare the amino acid composition of these parallel bands (Fig. 1): all bands C

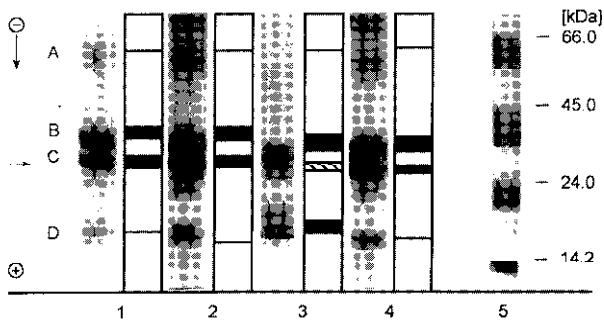

Fig. 1. SDS-PAGE of albumin plus globulin protein fractions extracted from control and treated maize seedlings. Control seedlings (*1*), and seedlings treated with 250 μM GA_3 (*2*), 250 μM 4-PU-30 (*3*), and 250 μM GA_3 plus 250 μM 4-PU-30 (*4*); *5* are protein standards. Band "C" isolated for amino acid composition determination from all 4 samples investigated.

Table 1. Effect of GA_3 and 4-PU-30 cytokinin on shoot dry mass, shoot length, chlorophyll content and photosynthetic rate of 11-d-old maize seedlings.

Concentration	Shoot dry mass [g per seedling]	[%]	Length of shoot [cm]	[%]	Chlorophyll *a* [mg m^{-2}]	[%]	Chlorophyll *b* [mg m^{-2}]	[%]	Chlorophyll *a+b* [mg m^{-2}]	[%]	Photosynthetic activity [mg ($^{14}CO_2$) $m^{-2}s^{-1}$]	[%]
Control	0.088	100.0	16.8	100.0	198	100.0	132	100.0	330	100.0	0.180	100.0
250 μM GA_3	0.082	93.2	17.8	105.9	205	103.5	140	106.1	346	104.8	0.227	126.1
250 μM 4-PU-30	0.098	111.4	15.3	91.1	255	128.8	175	132.6	430	130.3	0.216	120.1
250 μM GA_3 + 250 μM 4-PU-30	0.097	110.2	18.3	108.9	255	128.8	177	134.1	432	130.9	0.233	129.2
LSD 5 %	0.002		0.6		5		5		7		0.005	
LSD 1 %	0.003		0.9		7		6		10		0.008	

Table 2. Nitrogen content of 11-d-old maize shoots and individual extracted protein fractions.

Concentration	Total N [g g^{-1}(d.m.)]	[%]	Albumins and globulins [g g^{-1}(d.m.)]	[%]	Prolamins [g g^{-1}(d.m.)]	[%]	Glutelins [g g^{-1}(d.m.)]	[%]	Unextracted plus nonprotein [g g^{-1}(d.m.)]	[%]
Control	5.74	100.0	0.70	100.0	0.23	100.0	021	100.0	4.60	100.0
250 μM GA_3	6.58	114.6	0.72	102.8	0.27	117.4	028	133.3	5.31	115.4
250 μM 4-PU-30	6.91	120.4	0.87	124.3	0.29	126.1	032	152.4	5.43	118.0
250 μM GA_3 + 250 μM 4-PU-30	6.01	104.7	0.81	115.7	0.29	126.1	022	104.8	4.69	101.9
LSD 5 %	0.35		0.02		0.02		003		0.07	
LSD 1 %	0.37		0.03		0.03		004		0.10	

contained soluble proteins characteristic for maize, *i.e.* high amounts of glutamic acid, aspartic acid, glycine and alanine, and little amounts of methionine, histidine, and tyrosine (Table 3). Band C from maize shoots treated with 4-PU-30 contained high amounts of lysine, threonine, glutamic acid, valine and leucine. Bands C extracted from maize shoots treated with GA_3 and with GA_3 plus 4-PU-30 were closer in their amino acid composition to the control.

Table 3. Amino acid composition of albumin plus globulin protein fractions [mg g^{-1}(protein)] extracted from control and treated (250 μM GA_3, 250 μM 4-PU-30, or 250 μM GA_3 + 250 μM 4-PU-30) maize seedlings.

Amino acid	Control	GA_3	4-PU-30	GA_3 + 4-PU-30
Lysine	60	67	71	63
Histidine	24	22	24	23
Arginine	57	57	62	51
Aspartic acid	110	107	112	114
Threonine	50	51	61	52
Serine	62	66	68	74
Glutamic acid	132	130	145	136
Proline	68	69	76	71
Glycine	96	101	71	93
Alanine	101	104	97	98
Valine	69	71	61	72
Methionine	19	22	20	16
Isoleucine	43	47	47	46
Leucine	80	82	70	84
Tyrosine	29	32	34	37
Phenylalanine	47	48	43	39

Discussion

During seed germination GA_3 promotes elongation of plant shoots (Pressman *et al.* 1985, Pinthus and Abraham 1996). The increased length of maize shoots treated with GA_3 in comparison with control coincided with this assumption. The decrease in shoot length and increase in dry matter after the 4-PU-30 treatment may be in connection with cytokinin ability to enhance stomatal conductance and transpiration rate (Raschke 1975). When GA_3 and 4-PU-30 were added in combination, both these parameters were elevated, probably due to mutual influence of the two agents. Leaves treated with 4-PU-30 cytokinin as well as with the GA_3 + 4-PU-30 combination showed a 30 % elevation of total Chl content and a 20 - 30 % increase in photosynthetic activity. Nevertheless, GA_3 treatment induced a very little increase in Chl content but a significant enhancement of photosynthetic rate. A lack of relation between Chl content and photosynthetic activity has been reported by Ashour *et al.* (1973), Chaterjee *et al.* (1976), Baroncelli *et al.* (1995), and others. The fairly high increase of the quantities of total nitrogen and soluble proteins in seedlings treated

with 4-PU-30 was expected because exogenously applied cytokinins induce an increase in total nitrogen content in maize and other plants (Baruch *et al.* 1994, Stefanov *et al.* 1994). However, we expected a larger increase in these parameters after application of GA_3. This expectation was based on the functions of GA_3 in most cereals during germination, *i.e.* to synthesize hydrolytic enzymes *in situ* in endosperm (Torent *et al.* 1989, Bewly and Black 1994, Yoshida and Hirasawa 1996). These enzymes degrade storage proteins and supply the embryo with amino acids to support seedling growth (Moureaux 1979, Mohammad and Esen 1990). Protein quantities in leaves represent a balance between synthesis and breakdown. As GA_3 activates endopeptidases and other hydrolytic enzymes it might reduce a part of proteins in leaves. Moreover, in maize leaves protein and Chl concentration decrease in parallel with incresing endopeptidase activity (Feller *et al.* 1977). Of course, these processes are complicated and this is only one possibility. The effects of GA_3 upon the activity of leaf proteins may be different (Polard 1969, Mappelli and Ranieri 1978, Koehler and Ho 1988). In addition, the subcellular distribution and properties of proteins in leaves are different from those in the storage tissues of seeds (Fröhlisch and Feller 1987). The differences in intensities between band C from leaves treated with 4-PU-30 and the other variants could probably be due to the differences in amino acid composition, because even a small change in amino acid composition of polypeptides may significantly influence band intensity (Wilson 1981, 1986).

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