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Effect of the Phenylurea Cytokinin 4-PU-30 on the Growth and Protein Composition of Maize Seedlings

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Abstract. The action of the phenylurea cytokinin 4-PU-30 on maize seedling growth, photosynthetic parameters, and leaf protein composition was investigated. The applied phenylurea cytokinin increased leaf growth and photosynthetic activity of the seedlings. It also elevated chlorophyll and total nitrogen content in leaves, as well as the quantity of individual protein fractions. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of soluble proteins (albumins plus globulins) revealed four protein polypeptides with molecular masses of 27,000, 24,000, 17,000, and 15,000. Comparison of the polypeptides from treated plants with relevant polypeptides from control plants showed some significant, quantitative differences. New proteins, of similar molecular mass, may be produced in treated plants.

Cytokinins have been implicated in the regulation of many physiological and biochemical processes, including protein synthesis, seed germination, dormancy and apical dominance, stomatal function, leaf senescence, fruit set, and fruit growth (Karanov et al. 1992, Nickell 1986, 1991). They also have been shown to affect the control of assimilate translocation in plants by altering sink strength and activity (Hayes and Patrick 1985, Thomas 1985).

Phenylurea and adenine cytokinins have a number of physiological properties in common. Both types of cytokinins stimulate cell division and differentiation, promote leaf and cotyledon expansion, and retard leaf senescence (Letham 1978, Mok et al. 1987, Okamoto et al. 1978). Some phenylurea derivatives, such as *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (4-PU-30) and *N*-(1,2,3-thiadiazol-5-yl)-*N'*-phenylurea (Thidiazuron, Dropp), exhibit cytokinin activity similar to or higher than that of adenine derivatives, for example, kinetin, N^6-benzylaminopurine, and zeatin (Kulaeva et al. 1992, Mok et al. 1982, Okamoto et al. 1981, Takahashi et al. 1978). However, neither type of cytokinin has found broad practical application in agriculture, especially in important crops such as wheat and maize (Thomas and Blakesley 1987).

The effect of the cytokinin 4-PU-30 on the growth of maize seedlings and on some photosynthetic parameters and the protein composition of maize leaves was investigated.

Materials and Methods

The experiments described here were carried out with maize seeds, "Kneja-530." The seeds were germinated in the dark on moist filter paper at 25°C. On the eighth day, the seedlings were divided into three groups of 45 plants each. Two groups of these seedlings were sprayed with solutions containing 4-PU-30 at two concentrations, 100 and 250 μM, respectively. After spraying, all seedlings were grown in continuous light for 72 h. At the end of this growth period, shoots (stems and leaves) were excised from all variants investigated, and both fresh and dry weights of the excised shoots were determined. The dry weights were measured after drying the samples to constant weight at 110°C. Each experiment was repeated five times with six replicates.

The intensity of photosynthesis in the seedlings was measured radiometrically, after Yordanov et al. (1969), on leaf disks (8 mm). The disks were isolated from the second leaf of all variants 72 h postspray. Photosynthetic pigments were extracted from leaf disks with 80% acetone (Arnon 1949). One sample contained five disks, isolated from the second leaf of five different seedlings. All samples were weighed, ground with 80% acetone, and centrifuged at 6000 *g*, and supernatants were used for measuring optical density. The optical density was measured at 663 and 645 nm (MacKinney 1941). All disks used for photosynthetic measurements were taken from the central part of the leaves. These experiments were repeated three times with six replicates.

Soluble protein fractions (albumins plus globulins, prolamins, and glutelins) were extracted from fresh shoots according to Landry and Moureaux (1970). Nitrogen content was measured

Table 1. Effect of 4-PU-30 cytokinin on 11-day-old maize seedlings.

Concentration	Shoot fresh weight		Shoot dry weight	
	g/seedling	%	g/seedling	%
Control	0.38	100	0.087	100
100 μM 4-PU-30	0.35	92.0	0.092	105.9
250 μM 4-PU-30	0.34	89.5	0.097	112.3
LSD 5%	0.04		0.003	
1%	0.06		0.004	

by a micro Kjeldahl method. Before electrophoresis, the protein extracts of albumins plus globulins were dialyzed against 0.01 M Tris-glycine buffer (pH 8.5) containing 0.2% sodium dodecyl sulphate (SDS), 5% 2-mercaptoethanol (ME), and 10% sucrose. SDS-PAGE was performed according to Laemmli (1970). The SDS-PAGE mobilities (in 7.5% of homogeneous 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. Proteins for molecular mass standards were obtained from Sigma. Each reported molecular mass value is an average of five separate SDS-PAGE runs on the same protein sample.

The results were statistically analyzed using Fisher's criteria.

Results and Discussion

The fresh weight of the excised shoots from maize seedlings treated with 4-PU-30 showed a decrease in comparison with the fresh weights of the shoots of control plants (Table 1). Thidiazuron (Dropp) has been reported to have similar effects on fresh weights of leaves and stems of cereals (Devlin et al. 1989). It seems likely that this phenomenon may be related to the known ability of cytokinins to cause reduction in stomatal resistance (opening of stomata) and enhancement of transpiration (Raschke 1975). In contrast to the data for fresh weights, we found that the absolute dry weights of shoots of treated plants were increased, and the differences were statistically significant. An elevation of the absolute dry weight of some cereals occurred following the application of 4-PU-30, and Dropp has been reported by other investigators (Hodgson and Snyder 1988, Karanov et al. 1992). The increase in dry matter may be related to a stimulation of biosynthetic processes as a result of 4-PU-30 treatment. This assumption is supported by data for the chlorophyll content and photosynthetic activity of leaves of treated plants (Table 2). Treated leaves showed 25–35% elevation of both parameters in comparison with control leaves. These results confirm other studies with maize seedlings treated with 4-PU-30, Dropp, N^6-benzylaminopurine, and kinetin (Dimitrova and Vassileva 1987, Tsenova and Vassileva 1990). Similar results obtained when wheat flag leaves were treated with 4-PU-30 were correlated with slower chlorophyll degradation and higher photosynthetic activity in treated plants (Alexieva and Karanov 1992).

The total nitrogen content of shoots of treated plants was higher than that of control plant organs (Table 3). This effect was most pronounced in plants exposed to the higher cytokinin concentration. The nitrogen content of individual protein fractions isolated from the indicated organs of treated maize seedlings also increased compared with control plants, and the increase again correlated with the applied 4-PU-30 concentration. These quantitative changes were observed in all extracted protein fractions, but the highest increase in nitrogen content was detected in glutelins (61%), whereas the lowest increase in the nitrogen content was detected in prolamins (only 15%; Table 3). These results indicate that 4-PU-30 selectively affected the relative quantities of these protein fractions. This is very interesting because maize prolamins are deficient in the essential amino acids lysin and tryptophan.

No data have been reported on the influence of 4-PU-30 on protein fractions in maize. However, Karanov et al. (1992) reported that 4-PU-30 caused an elevation in the quantity of soluble proteins in soybean plants.

We suspect that 4-PU-30 stimulates protein synthesis. The observations of Klyachko et al. (1987) and Ananiev et al. (1987) that thidiazuron, 4-PU-30, and N^6-benzylaminopurine accelerate polysome formation and stimulate RNA polymerase activity in pumpkin cotyledons supports this assumption. Moreover, other data suggest that cytokinins increase the total protein quantity in maize and other plants (Martin and Sabater 1989, Ohya and Suzuki 1990, Romanov 1990, Tsenova and Vassileva 1990). However, the activities of proteolytic leaf enzymes undoubtedly affect the concentrations of leaf protein and chlorophyll (Feller et al. 1977, Huffaker and Peterson 1974, Martin and Thimann 1972). Consequently, the observed differences in protein content could be due to an effect of cytokinins on leaf proteolytic activities.

SDS-PAGE bands of the albumin-plus-globulin fractions from control and treated plants are shown in Fig. 1. All of these fractions contained the same four polypeptides of molecular mass: 27,000, 24,000, 17,000, and 15,000 daltons. However, control and treated plants exhibited significant, quantitative differences in the relative abundance of these polypeptides. The differences detected in protein

Table 2. Influence of 4-PU-30 on the chlorophyll content and photosynthetic activity on maize leaves from 11-day-old seedlings.

	Chlorophyll *a*		Chlorophyll *b*		Chlorophyll *a* + *b*		Photosynthetic activity	
Concentration	mg/dm^2	%	mg/dm^2	%	mg/dm^2	%	mg CO_2/dm^2/h	%
Control	2.54	100	1.39	100	3.93	100	8.80	100
250 μM 4-PU-30	3.38	133.1	1.96	141.0	5.34	135.9	10.87	123.5
LSD 5%	0.44		0.42		0.85		1.29	
1%	0.69		0.65		1.34		2.02	

Table 3. Nitrogen content of 11-day-old maize shoots and individual extracted protein fractions.

	Total N		Albumins and globulins		Prolamins		Glutelins		Unextracted plus nonprotein N	
Concentrations	mg/g d.w.*	%	mg/g d.w.	%	mg/g d.w.	%	mg/g d.w.	%	mg/g d.w.	%
Control	4.78	100.0	0.71	100.0	0.34	100.0	0.27	100.0	3.45	100.0
100 μM 4-PU-30	5.38	112.5	0.76	107.2	0.34	100.0	0.34	125.0	3.94	114.0
250 μM 4-PU-30	5.76	120.6	0.88	122.9	0.39	115.0	0.44	161.0	4.05	117.4
LSD 5%	0.03		0.06		0.03		0.04		0.08	
1%	0.05		0.08		0.04		0.05		0.12	

* d.w., dry weight.

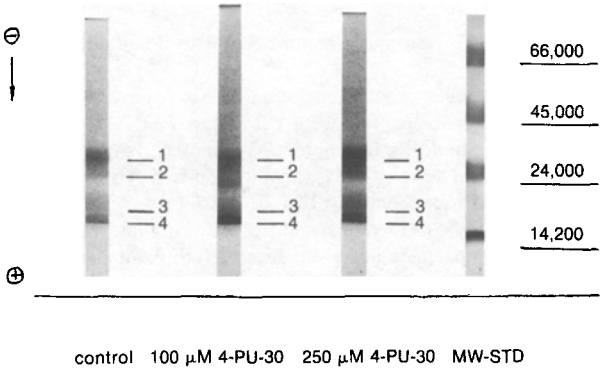

Fig. 1. SDS-PAGE of albumin-plus-globulin protein fractions extracted from control and treated maize seedlings. Numbers 1, 2, 3, and 4 indicate protein bands. MW-STD, molecular-weight protein standards.

samples extracted from treated plants are especially apparent for bands 2 and 3 with molecular masses of 24,000 and 17,000 daltons, respectively. How can these differences be explained when equal amounts of protein were loaded on each sample? Coomassie Brilliant Blue R-250 tends to stain more strongly polypeptides that have higher proportions of the amino acids lysine and arginine (Weber and Osborn 1969, Wilson 1979). In addition, proteins with the same molecular mass often exhibit microheterogeneity in their primary structure (Landry et al. 1987, Wilson 1986). Usually, microheterogeneity is expressed in substitutions of one or more amino acids on a polypeptide chain. But substitutions of only one amino acid may affect the electrophoretic mobility of SDS-protein complexes (De Jong et al. 1978). We suppose that 4-PU-30 treatment may affect the biosynthesis of polypeptides with small differences in primary structure. However, these small differences may significantly influence the band intensity (Wilson 1981). Therefore, we assume the observed differences to be quantitative, but it is possible that a new protein or protein class of very similar molecular mass may have been produced in treated plants.

Our results suggest that there is probably a stimulation of biosynthetic processes and a higher carbon-assimilative activity in treated maize leaves as a result of 4-PU-30 treatment. An enhancement of the total nitrogen content, photosynthetic activity, chlorophyll content, the quantity of individual protein fractions, and absolute dry weights of plant organs was observed in treated maize seedlings. These results also indicate that 4-PU-30 may selectively affect the quantities of particular albumin and globulin proteins.

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