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EFFECT OF CALCIUM IONS AND 4-PU-30 CYTOKININ ON THE PROTEIN QUANTITY AND THE ACTIVITIES OF PEROXIDASE, SUPEROXIDE DISMUTASE AND CATALASE IN ETIOLATED MAIZE COLEOPTILES

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Introduction. It is well known that calcium participates in cell regulation mechanisms influencing the conformation and functional activity of many proteins and enzymes [1]. Calcium ions have also influence on the amounts of the individual protein fractions isolated from germinating maize seedlings [2].

As plant growth regulator phenylurea cytokinin 4-PU-30 possesses a number of physiological effects. It stimulates cell division and differentiation, promotes leaf and cotyledon expansion and exerts influence on protein composition of maize seedlings [3, 4].

Storage protein hydrolysis has been studied for a long time in germinating seeds, the starting points being the establishment of protein degradation and the detection of proteolytic activity in seeds. However, very little is known about the influence of some important physiological agents like calcium and phenylurea cytokinins on the processes taking place in germinating seeds.

This work aims to characterize the influence of both calcium ions and phenylurea cytokinin 4-PU-30 on the growth, protein composition and activities of peroxidase (POD), superoxide dismutase (SOD) and catalase in an important initial period of development of maize seedlings. The investigated enzymes present the protective enzyme complex of the cells against activated oxygen forms.

Materials and methods. Seeds of "Knezha 530" maize hybrid were used in these experiments. The seeds were placed to germinate on filter paper in dark at 25°C. All seeds germinated in distilled water but before germination they were divided into four groups and soaked for three hours as follows - only in distilled water (control); in 10^{-5} M calcium sulphate solution (variant Ca^{2+}); in 10^{-5} M 4-PU-30 cytokinin solution (variant Cyt) and in solution containing together calcium ions and 4-PU-30 cytokinin (variant Ca^{2+}/Cyt). Coleoptiles were isolated manually at the 96th h from placing the seeds to germinate on filter paper. Fresh weights of the excised coleoptiles were determined and the results were statistically analysed using Fisher's criteria.

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Table 1

Fresh weight of 96 h old etiolated maize coleoptiles

Sample	Coleoptiles fresh weight	
	g/seedling	%
Control	0.185	100
Ca^{2+}	0.187	101.08
Cyt	0.198	107.03
Ca^{2+}/Cyt	0.208	112.61
LSD 5%	0.007	
1%	0.010	

Protein fractions containing both albumins and globulins were extracted from fresh coleoptiles according to LANDRY and MOUREAUX [5] with 0.5 M sodium chloride solution. The content of total nitrogen (TN), protein nitrogen (PN), non-protein nitrogen (NPN) and of soluble protein fractions was determined by Kjeldahl method and data were expressed in per cent to dry matter. Enzyme extracts were prepared from fresh material homogenized in 0.1 M Tris-HCl buffer containing 0.1 mM EDTA, pH 7.8. After 30 min extraction at 4°C, the homogenate was filtered through 6 layers of gauze and centrifuged 30 min at 15000 g. The supernatant was dialysed for 24 h against halfstrenght extraction buffer, centrifuged for 20 min at 15000 g and the supernatant was used for enzyme assays. POD activity was determined measuring the product of guaiacol oxidation in the presence of H_2O_2 at 420 nm. SOD activity was measured by the method of BEAUCHAMP and FRIDOVICH [6]. Catalase activity was determined by tracing H_2O_2 exhaustion in the reaction mixture as described by AEBI [7]. PAGE by DAVIS [8] was used for separation of POD and SOD isoforms. The isoperoxidase patterns were revealed by incubation in benzidine-guaiacol solution for 5 min and addition of H_2O_2 at final concentration 0.05%. SOD isoforms were visualized according to BEAUCHAMP and FRIDOVICH [6].

Results and discussion. The results demonstrate that seeds treatment with calcium ions and the phenylurea cytokinin 4-PU-30 insignificantly influenced growth of 96 h old etiolated maize coleoptiles (Table 1). However, a definite stimulation was observed when calcium and 4-PU-30 were used together.

The quantities of TN, PN, NPN and SP in coleoptiles of treated plants were higher in comparison with the control (Table 2). Table 3 shows the changes of enzyme

Table 2

Nitrogen content (in % of dry weight) of maize coleoptiles at the 96th h from the beginning of germination

Sample	Nitrogen content of coleoptiles							
	TN	%	PN	%	NPN	%	SP	%
Control	1.06	100	0.61	100	0.43	100	0.38	100
Ca^{2+}	1.17	110.89	0.69	113.11	0.48	112.91	0.42	110.52
Cyt	1.21	114.87	0.72	118.36	0.49	115.73	0.46	121.05
Ca^{2+}/Cyt	1.28	121.91	0.76	125.25	0.52	123.01	0.49	128.94

TN – total nitrogen, PN – protein nitrogen, NPN – non-protein nitrogen, SP – soluble protein