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Метою роботи було вивчення активності ізоцитратліази за оксидативного стресу, викликаного впливом хлориду кобальту в насінні сої при пророщуванні

Ключові слова: хлорид кобальту, актиномицин Д, ізоцитратліаза, сім'ядоли сої

Целью работы было изучение активности изоцитратлиазы при оксидативном стрессе, вызванном влиянием хлорида кобальта в семенах сои при прорастании

Ключевые слова: хлорид кобальта, актиномицин Д, изоцитратлиаза, семядоли сои

The aim of the present paper is to research isocitrate lyase activity under the conditions of oxidative stress invoked by the influence of cobalt chloride in soybean seeds during germination

Keywords: cobalt chloride, actinomycin D, isocitrate lyase, soybean seeds

INFLUENCE OF ACTINOMYCIN D ON ISOCITRATE LYASE ACTIVITY DURING IN SOYBEAN SEEDS

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Introduction

Glyoxylate cycle is one of gluconeogenesis stages, i.e. transformation of the lipids stored in seeds into saccharum and functions in seeds of oily cultures in their germination. The key enzyme of glyoxylate cycle is enzyme isocitrate lyase (CF 4.1.3.1). This enzyme catalyzes reaction of isocitrate splitting into succinate and glyoxylate which serve as substrates for gluconeogenesis process and, thus for formation and sprout development [1]. Because of rapid industrial development a considerable accumulation of heavy metals in environment has been observed recently.

Cobalt is the metal playing an important role as a cofactor of many enzymatic reactions, for example, in such cobalt compounds as methylcobalamin and 5'-deoksiadenozilkobalamin, however cobalt excess can influence on growth of cultivated plants [2]. Because enzymes of glyoxylate cycle regulate transformation reactions of fatty acids in sprout formation, the study of key enzyme activity in glyoxylate cycle – ICL – in cultivated plants seeds germination under the influence of the essential heavy metal, i.e. cobalt chloride is of considerable interest for the purpose of forecasting of germinating capacity and productivity of plants in the conditions of soils contamination by metal salts.

Materials and Methods

The object of study were germinating seeds (s.Clark) soybean grown on the vivarium territory at V.N.Karazin national university. The seeds have been sterilised and germinated, as described in [3], cobalt chloride concentration is 10⁻⁴ M actinomycin D (Act D) was added in concentration of 100 mkg/ml into the medium of sprouting. Cotyledons of soybean seeds sprouting in one, three and five days have been used in the experiments.

A vegetative fabric shot has been homogenized in the cooled medium containing 0,1M tris-HCl buffer, pH 7,45,

0,5 M sucrose, 1mM EDTA, 5 mM DDT, 1 mM MgCl₂. Homogenate has been filtrated through four layers of bandage cloth and centrifuged at 1300 g. The received deposit containing incompletely destroyed cells, rejected. Fluid over sediments has been centrifuged for 20 minutes at 14000g. A sediment containing rough fraction of micro corpuscles, was destroyed by dipping of assays in liquid nitrogen and resuspended in 2 ml of initial tris - HCl buffers.

Then repeated centrifugation was carried out for 15 minutes. The sediment was solved in 0,4 % solution of digiton and repeatedly centrifuged in the specified mode [4]. To obtain the required size and cleared microcorpuscles («Sigma») a membranous filtration through membranous filters with a diameter 0,45 micrometers has been carried out. ICL activity has been found according to the Dixon and Kornberg method [5] at $\lambda = 324\text{nm}$ and expressed in nanomoles of phenylhydrazonglyoxylate per 1 mg of protein per 1 min. Statistical processing of the results has been carried out by method of variation statistics ANOVA with software package «Statistica 6,0».

Results and Discussion

Cobalt chloride raised ICL activity on the third day 1,65 times as to control indicators and 1,74 times as to ICL activity on the first day. On the fifth day the level of enzyme activity remained high, at the level of the third day though in relation to a control variant the activity of enzyme was 18 % higher.

It is known that activity increase of key enzymes of some metabolic ways, in this case ICL, not only AFO can activate, but can also occur due to induction of the enzyme. A significant progress in the research of heavy metals has been achieved due to development of molecular-biologic research which enables to identify some genes coding heavy metals transmitting agents. For example, representatives of CDF

family (cation diffusion facilitator) are found in yeasts, animals and plants and participate in transportation of heavy metals, namely, cobalt [6].

Table 1

Cobalt chloride influence on isocitrate lyase activity in soybean cotyledon in germination (nmole phenylhydrazonglyoxylate/min per 1 mg of protein; $M \pm m$, $n=4$)

Factors of influence	Germination day		
	1-day	3-day	5-day
Control	19,62 ± 1,16	22,45 ± 1,43	31,80 ± 1,74#
CoCl ₂	21,31 ± 1,37	37,26 ± 1,70*#	37,38 ± 1,68 *#

* - $p \leq 0,05$ relative to control; # - $p \leq 0,05$ relative to first day

It is assumed that these proteins have six membrane domains, N-terminal sequence and C-terminal cation connecting area. To find out one of possible mechanisms of ICL activity change, we have investigated AmD influence (antibiotics blocking mRNA synthesis) on the enzyme activity under the influence of cobalt chloride (table 1). As one can see from table 1, AmD does not influence ICL activity during the first day of soybean seeds germination. We explain this by the fact that mRNA which codes necessary for information ICL synthesis, is available in dry seeds. It has been shown in the work with flax seeds [6].

Other authors have carried out molecular-biological research of biogenesis with glyoxysomes using methods of immunological analysis, separation of mRNA from areas responsible for glyoxysomal enzymes [7], in ricine endosperm, glyoxysom fraction was the object of the research. According to these experiments, the authors have made a conclusion that contain in malatsyntase and malatdehydrogenase available signal sequence.

Isocitraliase is not glycosylated, probably because this enzyme is localized in matrix, not in glyoxys membrane. In our opinion, it is possible to assume that Act D does not reach plants tissues on the first day [1]. In our experiments it has been convincingly proved that Act D influence on the activity of key enzyme in glyoxylate cycle ICL manifests in 66,7% inhibition of enzyme activity during soybean seeds germination, which makes 1,5 day. The inhibition of enzyme activity by antibiotics which can inhibit mRNA synthesis, goes in line with the idea of transcription-translation system functioning [9].

Table 2

Actinomycin D influence on isocitrate lyase activity influenced by cobalt chloride (nmole phenylhydrazonglyoxylate/min per 1 mg of protein; $M \pm m$, $n=4$)

Germination day	Control	CoCl ₂	CoCl ₂ + AmD	AmD
1-day	19,48 ± 1,25	21,77 ± 1,31	22,06 ± 1,30	19,14 ± 1,43
1,5-day	19,77 ± 1,19	24,91 ± 1,16	13,35 ± 0,92*	11,86 ± 1,01*
3-day	21,85 ± 1,34	36,62 ± 1,57*#	28,18 ± 1,66*#	20,43 ± 1,29

* - $p \leq 0,05$ relative to control; # - $p \leq 0,05$ relative to first day

Cobalt chloride does not influence on ICL activity on 1,5 day of germination and when cobalt chloride and Act D are simultaneously added into the incubation medium, we can see 1,48 times ICL activity inhibition as related to the control at the that time. In this case it is clear that ICL activity

inhibition because of combined action of heavy metal and antibiotics of mRNA synthesis on 1,5 seeds germination day occurs because of Act D influence on enzyme synthesis. In work [7] it has been shown that activity of glyoxysomal enzymes increase during seeds germination is connected with previous increase in mRNA level, carrying information for these enzymes, and such an increase precedes the increase in enzymes activity with one day difference.

Thus, the obtained data as to inhibition of ICL activity by antibiotics at the time when there was not true activity increase either in control, or under the influence of cobalt chloride as to the first day, agree with data of literature and form the integral picture of enzyme synthesis activity in glyoxysomes [7]. But on the third day of germination Act D did not influence on ICL activity which, most probably, is explained by short-time action of antibiotics.

Cobalt chloride increased ICL activity on third germination day in 1,52 times as to control indexes and in 1,68 times as to indexes of ICL activity on the first germination day. On the third day when Act D was added into seeds germination medium simultaneously with cobalt chloride, ICL activity increase in 1,52 times has been noticed relative control and 1,5 times relative to the first day.

Conclusions

The idea of this mechanism is in the fact that ICL activity increase is a result of de novo enzyme synthesis. Thus, according to the data obtained it is possible to conclude that cobalt chloride influences on ICL on transcriptional level.

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