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DETERMINATION OF ACTIVITY OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE IN DIFFERENT SUNFLOWER (H.annuus L.) GENOTYPES

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INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase (RuBPCase; E.C. 4.1.1.39) is the enzyme which catalyses the initiation reaction in the pentose-phosphate reaction. It reperesents about 60% of soluble proteins in plant leaves (Miziorko, 1983). In the catalytic reaction it is transformed by carboxylation in two molecules of 3-phosphoglyceric acid (PGA):

$$CO_2 + RuBP \xrightarrow[(Mg^{2+})]{RuBPCase} 2\ PGA$$

This reaction is indispensable for organic matter synthesis since it represents a primary form CO_2 assimilation. Because of that RuBPCase can be used as the parameter in assessing the plant ability to bind CO_2 from air, i.e., for measuring the capacity for photosynthesis.

As to the present RuBPCase has not been investigated in sunflower grown in our country, the objective of the present work was the determination of activity of this enzyme in leaves of different genotypes of sunflower.

MATERIAL AND METHODS

The material for this investigation was leaves of three sunflower genotypes, taken at different stages of vegetation, namely: IM-8x7 ♀ in the beginning of flowering; OCMS-18x17 in the phase of buttonization; and H.tuberosus, F_1, also in the buttonization phase. All investigations were carried out on fresh plant material collected at the vegetation stages of 5, 10 and 15 leaves for the first two genotypes and 5, 10 and 33 leaves for the third genotype.

Determination of RuBPCase activity: Activity of RuBPCase was measured by the modified method of Lorimer et al. (1977). To the reaction medium (total volume: 0.45 ml) containing 50 mM HEPES-KOH, pH 8.2; 10 mM KCl; 20 mM $MgCl_2$; and 5 mM DDT was added 20 µl of the enzyme extract and 20 µl of 0.2 M $NaH^{14}CO_3$ of known specific activity. The test tube with reaction mixture was kept at 25°C for 1-2 min., after which the substrate (10 µl of 20 M RuBP) was added. Then, the reaction was stopped by adding 100 µl of 2 M HCl. After several minutes, the remaining amount of $^{14}CO_2$ was liberated and the tube content quantitatively transferred to the vessel containing scintillation liquid (4 g/l PPO; 0.25 g/l POPOP in toluene - triton X-100 in the ration 2:1) to measure activity on a scintillation counter. Total radioactivity of the reaction mixture was measured in the mixture prepared in the same way as that with the sample, but radioactivity was measured directly, with no addion of HCl.

Determination of soluble proteins: Soluble proteins content in the raw extract of sunflower leaf was determined by standard method according to Lowry et al. (1951).

Determination of chlorophyll: The dry plant material (0.05 g) was homogenized in a mortar with quartz sand and extracted with acetone. The extract was filtered and the filtrate collected in a 25 ml measuring flask. 1 ml of such stock solution was put into a 10 ml measuring flask and filled to the mark with acetone. Then, colour intentsity of the solution was measured (Brulnsma, 1961).

RESULTS AND DISCUSSION

The data about RuBPCase activity and contents of soluble proteins and chlorophyll are presented in Table 1.

Table 1. Activity of RuBPCase and contents of soluble proteins and chlorophyll in leaves of different sunflower genotypes at different stages of plant development (I - flowering; II,III - buttonization)

Genotype	Leaf number	RuBPCase ($\mu M\ CO_2 mg^{-1} chl.h^{-1}$)	Proteins (mg g^{-1} d.m.)	Chlorophyll (mg g^{-1} d.m.)
I) JM-8x7 (♀)	5	48	1.26	1.11
	10	105	5.23	2.76
	15	164	10.55	3.45
II) OCMS-18x17	5	64	2.27	1.65
	10	100	4.53	2.43
	15	187	12.46	2.12
III) H.tuberosus (F_1)	5	74	2.38	1.29
	10	92	3.36	0.99
	33	57	1.56	1.56

As seen from the table, the highest RuBPCase activity was found in two genotypes (JM-8x7 and OCMS-18x17) at the stage of 15 leaves, whereas for H.tuberosus (F_1) the highest activity was register at the stage of 10 leaves. This is in agreement to the dynamics of metabolic processes of organic matter synthesis in the sunflower genotypes under investigation.

The highest content of soluble proteins was found in the genotype OCMS-18x17 (buttonization phase) at the stage of 15 leaves (12.46 mg/g), while this quantity was the lowest in the fifth leaf (2.27 mg/g). Similar results were also obtained for the JM-8x7 (♀), while for H.tuberosus (F_1) the opposite held.

The presented results show a significant variability of RuBPCase activity and contents of soluble proteins and chlorophyll, depending on the genotype and vegetation stage. A systematic approach to the investigation of RuBPCase activity in different sunflower may be of great importance for selection.

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