ON THE ACTIVITY OF α-GLUCANPHOSPHORYLASE IN *PANICUM MILIACEUM* AND *SETARIA GLAUCA* DURING THE GERMINATION PERIOD

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Abstract: The present paper represents a comparative study on the activity of α-glucanphosphorylase in *Panicum miliaceum* (millet) and *Setaria glauca* (bristle grass) during 240 hours of germination. In the case of bristle grass, the minimum activity of α-glucanphosphorylase is to be recorded in the stage of impregnated seed while, in the case of millet, the minimum activity was registered in the last day of germination taken into study. In both species under study, the maximum value of the α-glucanphosphorylase activity has been evidenced in the 5th day of germination.

INTRODUCTION

As generally known, the main ways for starch degradation are hydrolysis and phosphorylis, the latter one being the metabolic means selected for correlating the activity of α-glucanphosphorylase and the mobilization ratio of starch, in a comparative study dedicated, on one hand, to a cultured plant (*Panicum miliaceum*) and, on the other, to a species of the spontaneous flora (*Setaria glauca*), both from the Poaceae family (STRATU *et al*., 2001; BURZO *et al*., 2005).

α-Glucanphosphorylase (α-1,4-D-glucan: orthophosphate-glucosyl transferase) is an enzyme catalyzing the depolymerization of glycogen and starch in its similar regions, thus inducing the phosphorylase of such α-glucans, which is accompanied by the formation of glucose-1-phosphate. The enzyme acts in a repetitive manner on the non-reducing terminal ends of glucan's chains, until meeting the α-1,6-branching points. The action of such an enzyme assures the introduction of glucose-1-phosphate in the glycolytic sequence (PELMONT, 1995; TANASE *et al*., 1997).

MATERIALS AND METHOD

The experiments have been developed on germinated caryopses of millet (*Panicum miliaceum*) and bristle grass (*Setaria glauca*) of the 2004 crop, from the Station for Agricultural Researches at Podu-Iloaiei, the district of Jassy.

First, the caryopses have been treated with 3% oxygenated water, for the removal of the possible pathogenic germs or of some substances that might have influenced the germination process, and then let to soak for 24 hours. Germination of caryopses was made at room temperature, in Petri boxes lined inside with filtering paper wetted with distilled water, samples' taking over being performed at intervals of 24 hours, for 10 days.

The method of α-glucanphosphorylase determination is based on phosphate's transformation into a phosphomolybdenic complex and its reduction by means of the ascorbic acid (ARTENIE *et al*., 1981).

For each sample subjected to analysis, 3 parallel determinations have been made, the obtained results, processed statistically, being expressed in μg P/g (VĂLEANU *et al*., 1990).

RESULTS AND DISSCUSION

A prioritary objective in the determination of α-glucanphosphorylase activity in the *Panicum miliaceum* and *Setaria glauca* species has been plotting of the standard curve, with a standard solution of monopotassium phosphate, the extinctions being read at a wavelength equal to 700 nm (Fig. 1).

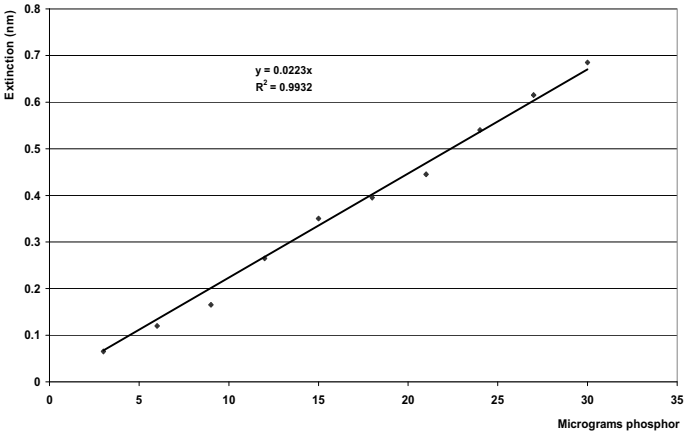

Fig.1. Standard curve for dosing of anorganic phosphor

The experimental results obtained have evidenced that, in *Panicum miliaceum*, the activity of α-glucanphosphorylase along the ten germination days varies between 1 500 - 29 000 μg P/g. Thus, in the beginning of germination, in the impregnated sample (the zero moment), the enzymatic activity had been minimum, the values recorded being, on the average, around a value of 1833.33 μg P/g, a possible explication being that, in the first hours, a series of biochemical and physiological processes occur at low speeds, as seeds react to the installation of optimum environmental conditions first by an increased membrane permeability, for the absorption of the environmental water and only secondly by the reactivation of the enzymatic equipment involved in the catabolism of the reserve substances, for the production of energy and of the precursors necessary to the various biosynthetic processes.

In the following hours of germination, a progressive increase of the enzymatic activity is to be noticed as early as the first 24 hours of germination (2 500 μg P/g), up to the 5th day (29 500 μg P/g), followed by a gradual decrease until the last germination day considered for the study (1 500 μg P/g) (Fig. 2).

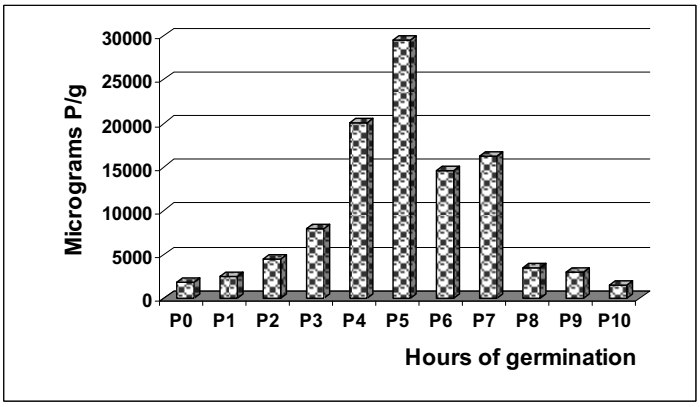

Fig.2. Activity of α-glucanphosphorylase (μg P/g) in germinated *Panicum miliaceum* caryopses

By means of the average values and of the standard deviation, there have been subsequently calculated the superior and inferior confidence limits, on the basis of the critical value *t* for α= 0.05 and n-1 degrees of freedom.

The graphical representation of the confidence intervals of the α-glucanphosphorylase activity evidences the fact that the largest interval is to be recorded in the 3rd germination day (8 000 - 9 000 μg P/g), larger intervals being also registered at 48, 120 and 192 hours of germination. In contrast to this, the narrowest confidence interval may be evidenced in the 4th day (20 000 - 20 200 μg P/g) (Fig. 3).

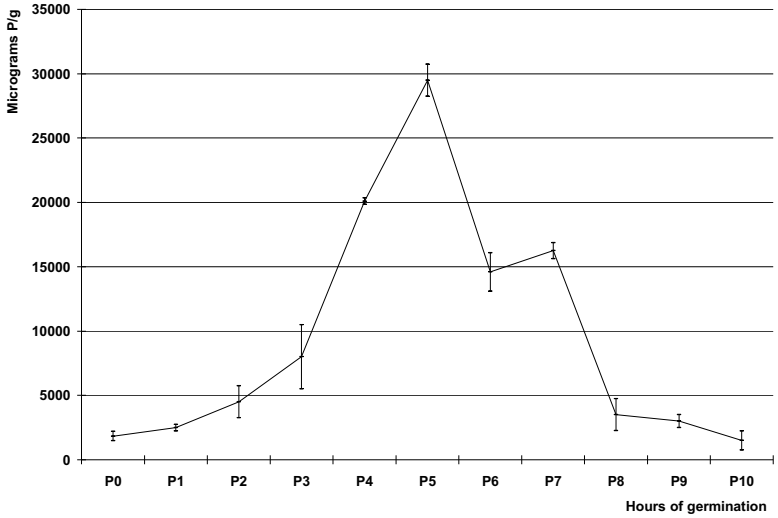

Fig.3. Confidence intervals of α-glucanphosphorylase activity in *Panicum miliaceum*

In *Setaria glauca*, the activity of α-glucanphosphorylase shows a similar dynamics, in the beginning of germination, the enzymatic activity in the impregnated sample (the zero moment) being minimum, occurring, on the average, around the value of 1 500 μg P/g.

There follows, in the subsequent germination days, a slight increase of the enzymatic activity while, starting with the 3rd germination day, the activity of α-glucanphosphorylase evidences a significant increase, very high values (29 500 - 31 500 μg P/g) being thus recorded.

Following the attainment of the maximum threshold, the activity of α-glucanphosphorylase decreases gradually up to the ninth germination day, when a value of 9666 μg P/g is recorded while, after about 240 hours, the enzymatic activity evidences an average value of 1 663 μg P/g, which is approximately equal with the one recorded in the stage of impregnated seed (Fig. 4).

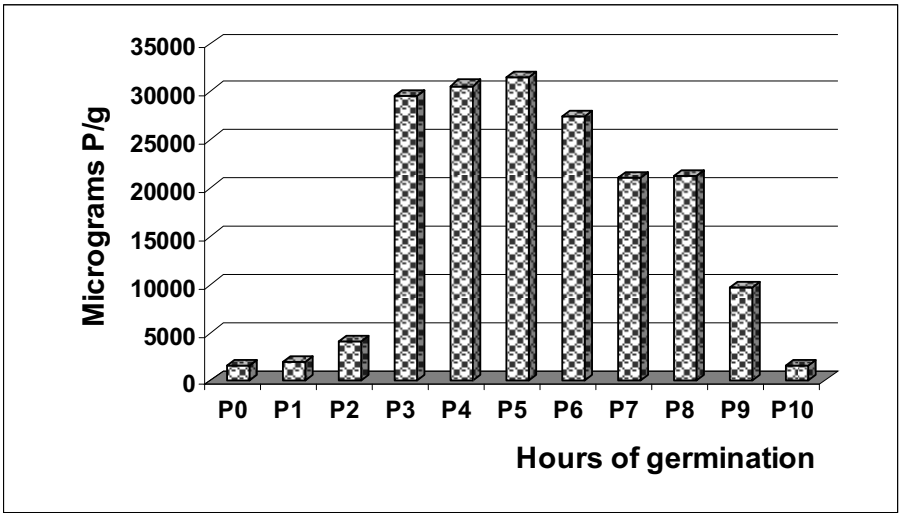

Fig.4. Activity of α-glucanphosphorylase (μg P/g) in germinated *Setaria glauca* caryopses

As to the intervals of confidence in bristle grass, they are very narrow for all germination days taken into study, which is a proof that the results obtained have a very low degree of error (Fig. 5).

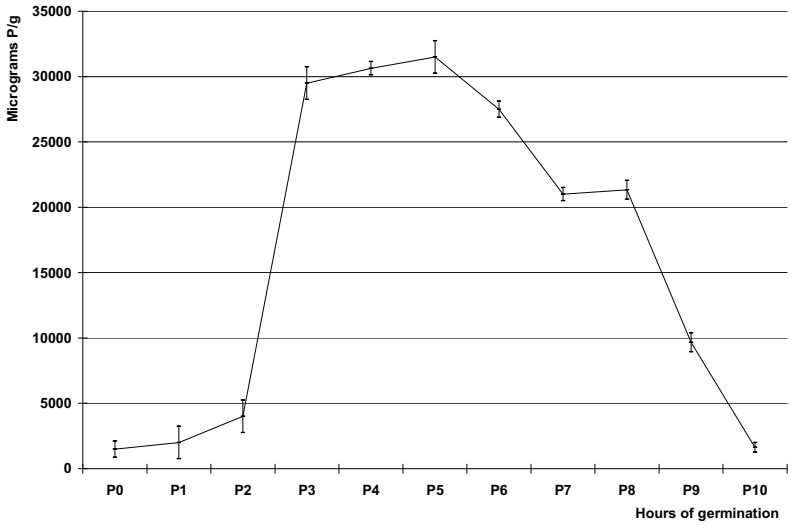

Fig.5. Confidence intervals of α-glucanphosphorylase activity in *Setaria glauca*

In order to check the possible differences or similarities occurring between the activity of α- glucanphosphorylase in the two species under study, the Anova test - the bifactorial

model, with an equal number of observations in the cell, has been applied, which permitted calculation of the square sums - on the basis of the (external, internal and total) variability sources, of the factor value, as well as of its critical value (FOWLER *et al*., 2000).

Starting from the experimental results obtained, the null (H_0) and the alternative (H_1) hypothesis of the test have been formulated.

The results obtained statistically show that both factors (the species and the germination time) influence the enzymatic activity, although to a different extent.

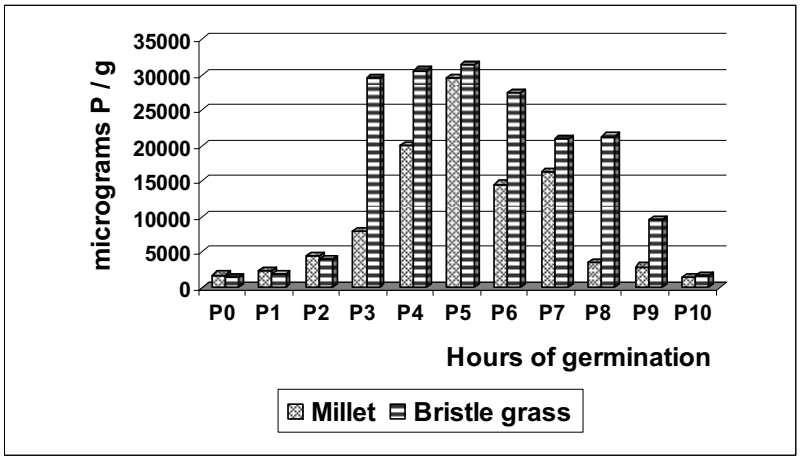

Fig.6. Average values of the α-glucanphosphorylase activity in *Panicum miliaceum* and *Setaria glauca*

CONCLUSIONS

The experimental results obtained from the determination of the α-glucanphosphorylase activity in the germinated caryopses of *Panicum miliaceum* and *Setaria glauca* permitted drawing of the following conclusions:

- In the case of bristle grass, the minimum activity of α-glucanphosphorylase is to be recorded in the stage of impregnated seed (1500 µg P/g) while, in the case of millet, the minimum activity was registered in the last day of germination taken into study (1500 µg P/g) - which is probably the result of the starch reserve consumption.
- In both *Panicum miliaceum* and *Setaria glauca*, the maximum value of the α-glucanphosphorylase activity has been evidenced in the 5th day of germination (29500 µg P/g for millet and 31500 µg P/g, respectively, for bristle grass).
- With both species, after the attainment of the maximum value, a progressive decrease of the α-glucanphosphorylase activity, is to be noticed until the last germination day.
- A comparative study on the α-glucanphosphorylase activity in both species considered for the study has evidenced a higher enzymatic activity in the spontaneous species, *versus* the cultivated one.

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