ON THE MOBILIZATION OF RESERVE STARCH UNDER THE ACTION OF GLUCANPHOSPHORYLASE IN *PANICUM MILIACEUM* AND *BROMUS STERILIS*

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Key words: α-glucanphosphorylase, reserve polyglucides, soluble proteins, *Panicum miliaceum*, *Bromus sterilis*, germination

Abstract: The main objective of the study was the quantitative determination of the reserve starch and of the total soluble proteins, in correlation with the dynamics of the α-glucanphosphorylase activity from the caryopses, subjected to germination under laboratory conditions, belonging to two graminaceae species from the cultivated (*Panicum miliaceum*) and spontaneous (*Bromus sterilis*) flora. The experimental results obtained put into evidence a differentiated dynamics for both the enzymatic activity and protein and starch concentration, as a function of species - on one side - and germination time - on the other.

INTRODUCTION

α-Glucanphosphorylase, also known as phosphorylase, with the systemic denomination α-1,4-glucan: ortophosphate-glucozyltransferase, E.C. 2.4.1.1., is a transferase taking part in the transfer reactions of the glycozyl groups. It is largely occurring in superior plants (cereals, leguminous plants, potatoes), in animal organisms (muscles, heart, liver, brain) and in microorganisms (ARTENIE and TĂNASE, 1981).

The literature of the field provides more complex information on the structure and action mechanism of the α-glucanphosphorylase present in bacteria, fungi and animals, comparatively with other various plant species (FRANKOVA, 2003).

In many plants, such as: seeds and pea leaves (MU *et al*., 2001), bean (SUDA *et al*., 1987), barley (BAXTER and DUFFUS, 1973), rice seeds (RICHARDSON and MATHESON, 1977), spinach (STEUP and SCHACHTELE, 1986), sweet potato (CHANG *et al*., 1987; LU *et al*., 1995), banana (DA MOTA *et al*., 2002) and marine algae (FREDERICK, 1973; YU and PEDERSEN, 1991), the α-glucanphosphorylase has been identified both in its cytosolic and amyloplastic forms.

MATERIALS AND METHOD

The experimental investigations were developed on germinated caryopses of millet (*Panicum miliaceum*) and of brome grass (*Bromus sterilis*), from ecosystems with similar climatic and agrotechnical conditions.

Germination of caryopses was made at room temperature, in Petri boxes lined inside with filtering paper wetted with distilled water, sample taking over being performed at intervals of 24 hours, for 10 days.

The method of α-glucanphosphorylase determination is based on the transformation of the phosphate into a phosphomolybdenic complex and on its reduction by means of the ascorbic acid, the starch concentration being dosed by the polarimetric method, while soluble proteins - by the Bradford method (BRADFORD, 1976; ARTENIE and TĂNASE, 1981; ARTENIE *et al*., 2007).

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

RESULTS AND DISCUSSION

A comparative analysis was devoted to the activity of α-glucanphosphorylase, an enzyme involved in starch phosphorolytic scission, through a reversible reaction giving glucose - 1 - phosphate, on two graminaceae species from booth cultivated (*Panicum miliaceum*) and spontaneous (*Bromus sterilis*) flora, during their germination period.

As known, α-glucanphosphorylase contributes - at least to the same extent as α- and β-amylase - to the mobilization of reserve starch, during germination, determination of its activity being equally important for the fact that α-glucanphosphorylase is also involved in the biosynthesis of the polyssacharides from the α-glucan class. In the case of plants, the action of α-glucanphosphorylase is extremely important for both degradation and biosynthesis of starch (HACHEM *et al*., 2006). Immediately after the debut of the photosynthetic process, the glucose thus formed may act as a precursor in starch biosynthesis yet, simultaneously, it may be partially

degraded, which leads to the formation of acetyl - CoA, for subsequent biosynthesis of the other biomolecules involved in the growth of the future plantlet and plant.

Starting from all these considerations, in the present study, the activity of α-glucanphosphorylase during the germination period will be determined in the species under investigation, for elucidating the mechanism through which the reserve starch is mobilized during such processes.

The enzymatic extracts utilized for dosing of the α-glucanphosphorylase have been obtained by extraction with bidistilled water, for 120 minutes, at a temperature of + 4°C, followed by centrifugation for 15 min at 3, 000 rpm.

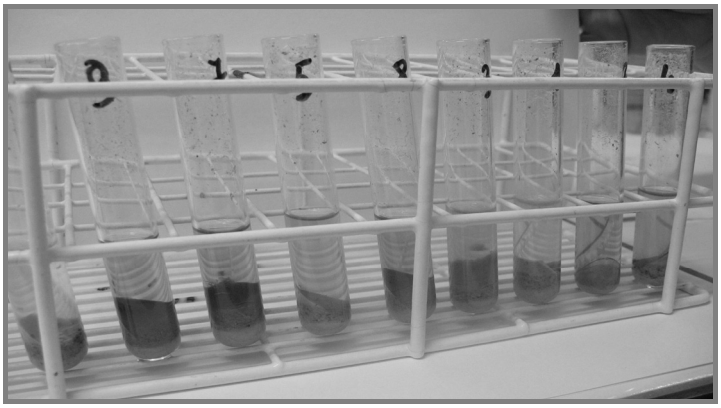

Fig.1. Enzymatic extracts for determining the α-glucanphosphorylase activity (original photo)

Determination of α-glucanphosphorylase activity involved dosing of the amount of anorganic phosphor, which assumed conversion of the extinction units into micrograms phosphor per calibration curve. A main objective in the determination of α-glucanphosphorylase activity has been plotting of the standard curve (Fig. 2), on using a standard solution of monopotassium phosphate, and various phosphorus concentrations from one sample to another (3 - 30 micrograms), the extinctions being read at a wavelength equal to 700 nm.

On the basis of the graph, the regression straight line has been drawn and its regression equation has been calculated, on establishing the amounts of phosphor corresponding to the samples subjected to analysis.

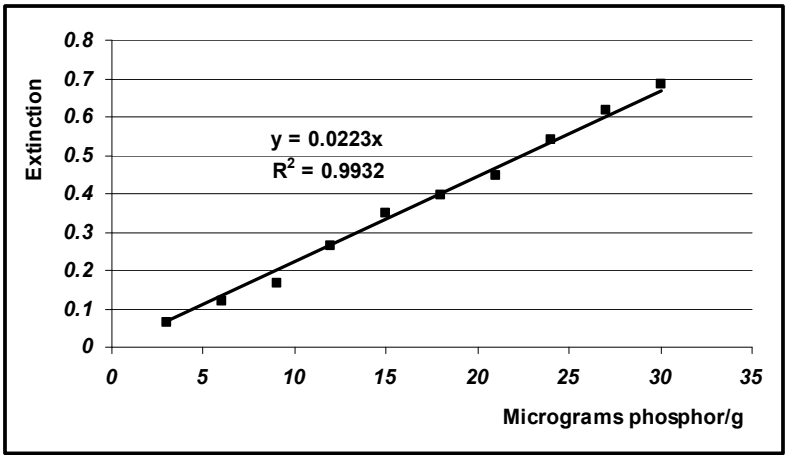

Fig.2. Etalon curve for dosing of phosphor (original)

In the beginning of germination, the enzymatic activity of *Panicum miliaceum* in the impregnated sample (moment zero) was minimum, an average value around 183.33 μg phosphor/g being registered, to be possibly explained by the fact that, in the first hours, certain biochemical and physiological phenomena occur, at low rates, once the first seed reaction *versus* the creation of optimum conditions refer to permeabilization of membranes and absorption of environmental water. Reactivation of the enzymes involved in the catabolism of reserve substances - for assuring the necessary energy and precursors for biosynthetic processes - occurs only in a subsequent step.

In the germination hours to follow - that is, starting with the first 24 hours of germination (250 μg phosphor/g), up to the fifth day (2950 μg phosphor/g) - a progressive increase of the enzymatic activity is recorded, followed by a gradual decrease up to the last germination day taken into study (150 μg phosphor/g). One may easily observe that, if, in the first four germination days, the increase of α-glucanphosphorylase may be considered as approximately linear, the values determined at 96 and 120 germination hours are wholly different, which supports the hypothesis that, at this age of the embryo, the process of starch mobilization is considerably intensified (CIORNEA *et al.*, 2006).

A comparative view between the dynamics starch concentration (or, in other words, the degradation rate of this polysaccharide) and the activity of α-glucanphosphorylase shows that the two phenomena are not absolutely similar. On one side, the enzymatic activity progressively increases in the first period, attaining its maximum after 5 germination days, while - over the same period - starch concentration decreases - a phenomenon not perfectly superposable, however, with the activity of α-glucanphosphorylase. On the other side, along the second interval of the period over which the experiments were performed, the enzymatic activity decreases quite rapidly, while the starch degradation rate records no diminution.

Such phenomena confirm the hypothesis according to which the hydrolytic activity of both α- and β-amylases plays an essential part in the mobilization of reserve starch, the degradation of which continues, which explains the maintenance of the decreasing rhythm of starch concentration, even if the glucanphosphorylasic activity records a pronounced diminution (Fig. 3).

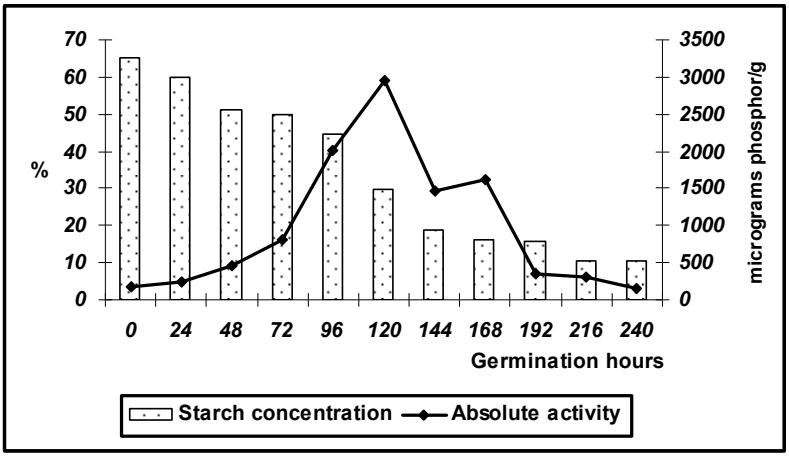

Fig.3. Graphical representation of the correlation between starch concentration and α-glucanphosphorylase in *Panicum miliaceum*

Another objective of the present study was the quantitative determination of the total soluble protein concentrations in the supernatants of the investigated samples. Figure 4 shows that the proteins act within a quite oscillating interval, higher values being registered towards the end of the germination period under investigation (the maximum value, of 18.892 mg%, being recorded at 240 germination hours). The results expressing the dynamics of the soluble protein concentration attest that - during germination - α-glucanphosphorylase plays a double part (of biosynthesis and degradation), the more so that, in the final period, proteic concentration remains at high values, its maximum being attained 10 days after the debut of germination, when the photosynthetic process is already initiated.

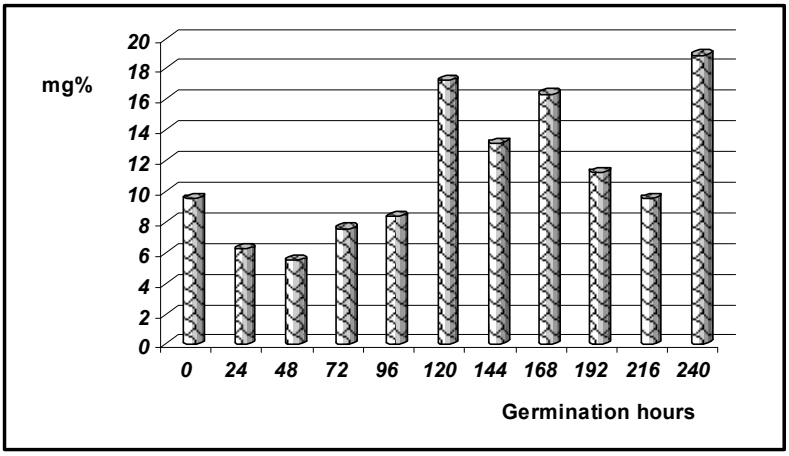

Fig.4. Soluble protein concentration in *Panicum miliaceum* germinated caryopses

The values of proteic concentration obtained permitted calculation and plotting of the specific glucanphosphorylase activity which, in the case of millet, reaches its maximum at 96 germination hours (240.545 μg phosphor/mg protein) while, at 240 hours from the debut of germination, it attains the minimum threshold of 7.939 μg phosphor/mg protein (Fig. 5).

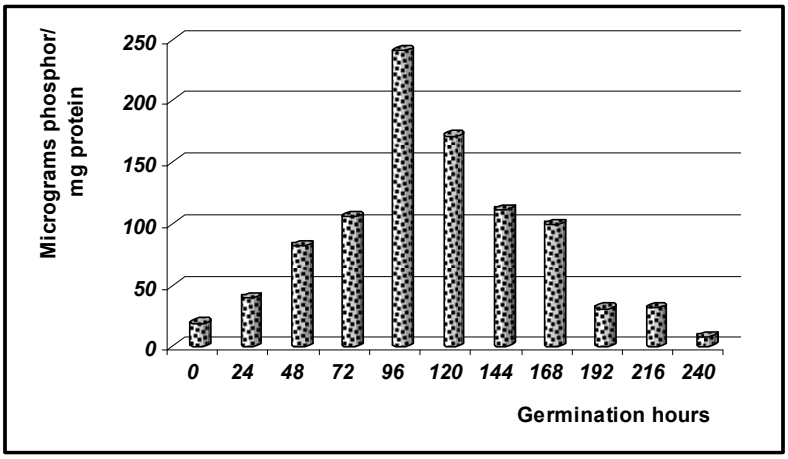

Fig.5. Specific activity dynamics of α-glucanphosphorylase in *Panicum miliaceum*germinated caryopses

In the case of *Bormus sterilis* species, the α-glucanphosphorylase from the germinated seeds evidences undistinguishable values in the first three analyzed samples, its action being probably oriented towards the synthesis - and not degradation - of starch, once known that α-glucanphosphorylase plays a crucial role in starch biosynthesis, as well.

In the first hours (moment zero), the enzymatic activity in the impregnated sample is indiscernible, a gradual intensification of the substrate's hydrolysis reaction under the action of the enzyme being observed at 72 germination hours, up to 216 hours (1601.667 μg phosphor/g), when the maximum activity is attained.

The graphical representation of the correlation between the α-glucanphosphorylase activity and the starch concentration of its substrate shows that, up to 168 germination hours, the two phenomena here discussed (*i.e.*, increase of the enzymatic activity and quantitative decrease of starch) are closely correlated by an inverse proportionality relation manifested by a progressive increase of the phosphorolytic activity, decrease in the substrate concentration occurring at the same rate while, starting with 192 hours from the debut of the germination process, the enzymatic activity is suddenly intensifying (Fig. 6).

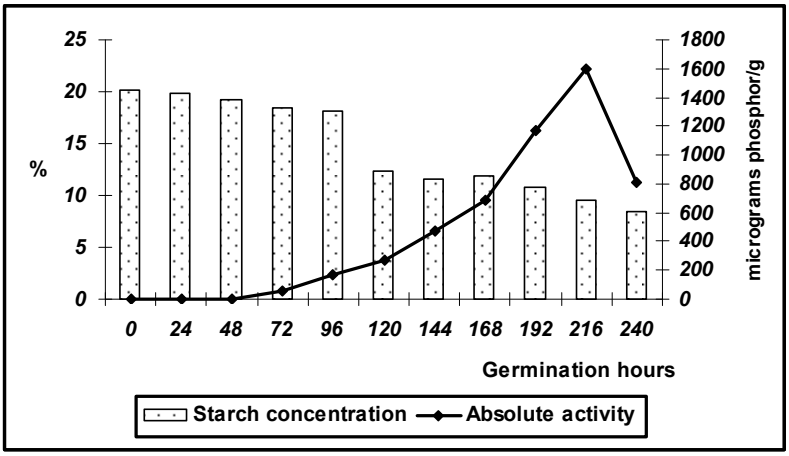

Fig.6. Graphical representation of the correlation between starch concentration and α-glucanphosphorylase in *Bromus sterilis*

Once again, proteic concentration was determined in the extracts obtained (the mean values oscillating between 0.111 - 12.589 mg%), the specific activity being graphically plotted (Figs. 7 - 8).

Analysis of proteinemy indicated a decrease in the concentration of soluble proteins in the first three germination days, higher than in those of the reference, which demonstrates - in an indirect manner - the global intensity of the metabolic processes.

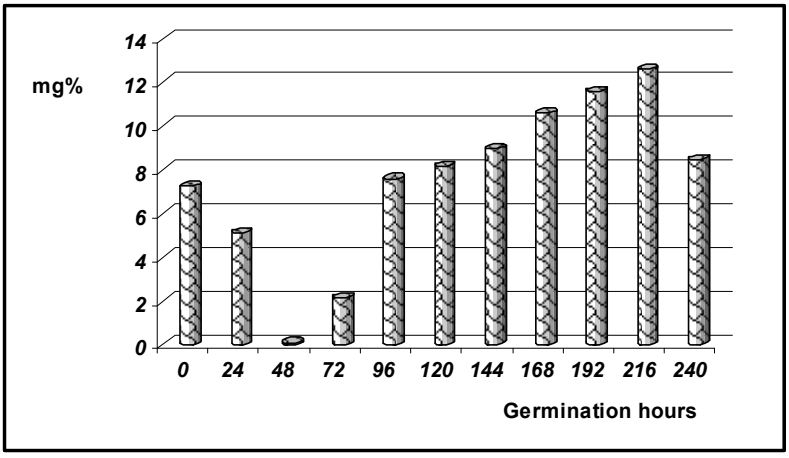

Fig.7. Soluble protein concentration in *Bromus sterilis* germinated caryopses

In *Bromus sterilis*, the specific activity shows a similar dynamics to that recorded in *Panicum miliaceum*, with the only difference that the maximum value, slightly lower than in the

previously mentioned case (127.227 µg phosphor/mg protein *versus* 240.545 µg phosphor/mg protein), is recorded only in the 9th day (Fig. 8).

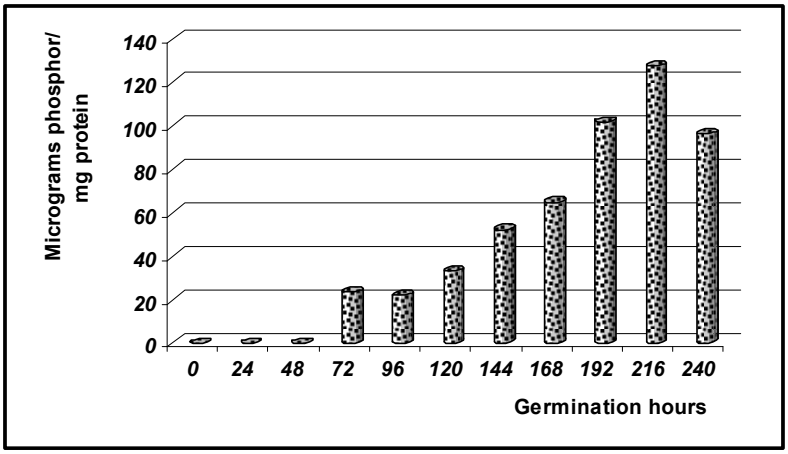

Fig.8. Specific activity dynamics of α-glucanphosphorylase in *Bromus sterilis* germinated caryopses

CONCLUSIONS

Determination of the starch content in the caryopses of the species under analysis evidences its gradual, progressive diminution, along the whole duration of the experiments, which confirms that the reserve starch is phosphorolytically mobilized, under the action of α-glucanphosphorylase.

As to the dynamics of the total soluble protein concentration, it differs from one species to another, showing fluctuating values which exceed the ones of the reference, followed by a stabilization around the same level, a possible explanation being that, after the first days, the biosynthetic processes are highly accelerated, probably starting with the biosynthesis of all enzymes necessary in the metabolic processes which assure the embryo and plantlet development.

The data obtained on the specific activity of α-glucanphosphorylase during germination in some cultivated and spontaneous graminaceae evidence a dynamics differentiated, on one hand, as a function of species and, on the other, of the germination degree.

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