ON THE ACTIVITY OF α-GLUCANPHOSPHORYLASE IN *SETARIA PUMILA* AND *FESTUCA PRATENSIS*

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Key words: α-glucanphosphorylase, starch, proteins, bristle grass, hair grass

Abstract: The main objective of the present study involves determination of the α-glucanphosphorylase in germinated caryopses of *Setaria pumila* and *Festuca pratensis*, through dosing of the anorganic phosphorous, seed germination being performed in Petri plates, at room temperature, for 10 days, while taking over of the samples occurred at intervals of 24 hours. In the enzymatic extracts thus obtained, starch concentration was also determined, by the polarimetric method, while the concentration of total soluble proteins - necessary for the calculation of the specific phosphorolytic activity - was established by the Bradford method.

In both graminaceae species, the enzymatic activity was highly reduced in the impregnated seed stage, after which it progressively increases, in parallels with the intensification of starch hydrolysis, for producing the energy and precursors necessary for various metabolic processes.

INTRODUCTION

α-Glucanphosphorylase is an enzyme catalyzing the depolymerization of glycogen and starch in its similar areas, thus inducing the phosphorolysis of these α-glucans, accompanied by the formation of glucozo-1-phosphate. The enzyme acts upon the non-reducing terminal ends of the glucan chains in a repetitive manner, until reducing the α-1,6 ramification points. The action of this enzyme assures the introduction of glucozo-1-phosphate into the glycolytic sequence (COJOCARU, 1997; COJOCARU *et al*., 2007).

Considering the especially high intensity of the biochemical and physiological processes occurring during germination, it is hardly probable that the maltose and free glucose resulted from the amylase action will act as a quantitatively important precursor in the first moments of the biosynthesis of the new polysaccharides. Instead, it is highly probable that, in such moments, the determining role in the glucidic metabolism will be played by the phosphorolytic action of the α-glucanphosphorylase, an enzyme which catalyzes the reversible reaction of starch degradation, accompanied by the formation of phosphorylated glucose. In this way, a more rapid scission of starch into (phosphorylated) glucose occurs, concomitantly with the direct involvement of the newly-formed product in subsequent processes of polysaccharide biosynthesis, while the stages of maltose hydrolysis and glucose phosphorylation are left aside.

MATERIALS AND METHOD

The experiments were developed on germinated caryopses of bristle grass (*Setaria pumila*) and hair grass (*Festuca pratensis*) harvested in 2006.

The method of α-glucanphosphorylase determination is based on the transformation of the phosphate into a phosphomolibdenic complex, and on its reduction in the presence of ascorbic acid, the starch concentration being dosed by the polarimetric method, while the 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 being statistically processed (VĂLEANU and HÂNCU, 1990; DRAGOMIRESCU, 1998; GOMOIU and SKOLKA, 2001).

RESULTS AND DISSCUSION

In the *Setaria pumila* caryopses subjected to germination, the starch concentration attains its maximum threshold, after which it progressively decreases, recording values between 89.5% from the maximum value at 24 hours of germination, 65.4% at 120 germination hours while, at 240 hours from the beginning of germination, the minimum concentration of 25.4% is attained.

Protein concentration during the germination of bristle grass seeds is relatively constant, with the exception of the 5th and, respectively, 6th germination day, when the maximum values (of 18.589 and, respectively, 17.561 mg%) are recorded, which actually agrees with a high specific activity of α-glucanphosphorylase over this interval (Fig. 1).

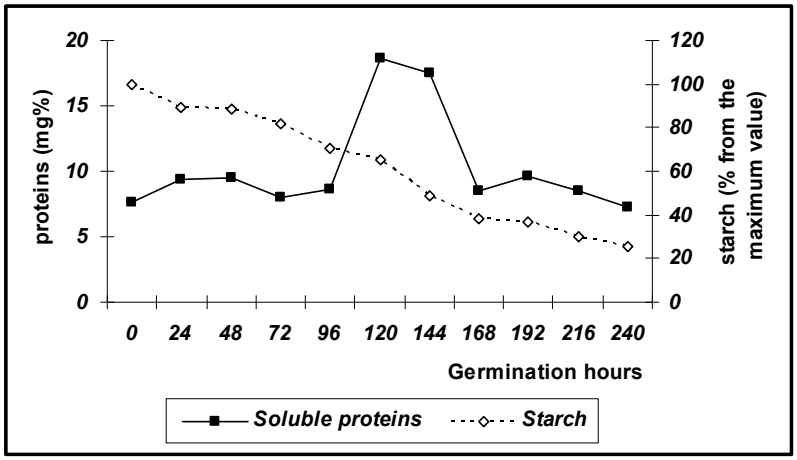

Fig.1. Comparative representation of the starch and total soluble proteins concentration in *Setaria pumila* germinated caryopses

Study of α-glucanphosphorylase in germinated caryopses of bristle grass evidences that - as a function of the germination period - the enzyme has a fluctuating activity; consequently, in the beginning of germination (in the impregnated sample, representing the reference for the determinations made and at 24 hours of germination), the enzymatic activity is imperceptible, being plotted only beginning with the second germination day when it reaches a value of 12.25 µg phosphorous/mg protein, which permits therefore the conclusion that, probably, starch is mobilized, in view of hydrolysis, in the first stage of the germination process, under the action of amylases, while glucanphosphorylase becomes active only in a subsequent step. Starting with the 4th germination day, the activity of glucanphosphorylase significantly increases (124.4 µg phosphorous/mg protein), the maximum value being attained at 144 germination hours (231.54 µg phosphorous/mg protein), to be followed by a progressive decrease up to the last germination day under analysis (10.39 µg phosphorous/mg protein) (Fig. 2).

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 $t(\alpha, n-1)$, for $\alpha = 0.05$ and n-1 degrees of freedom, which means $t(0.05, 10)$.

As to the confidence intervals of α-glucanphosphorylase, one may observe that, in the case of bristle grass, they are quite narrow for all germination days taken into study, somehow larger limits being evidenced in the 2nd (149.04 - 149.416 µg phosphorous/g) and, respectively, 6th day (753.927 - 818.53 µg phosphorous/g) (Fig. 3).

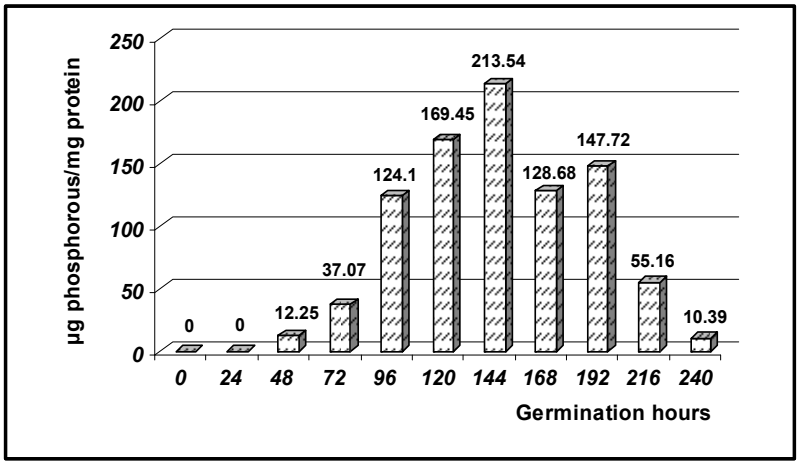

Fig.2. Specific activity dynamics of α-glucanphosphorylase in *Setaria pumila* germinated caryopses

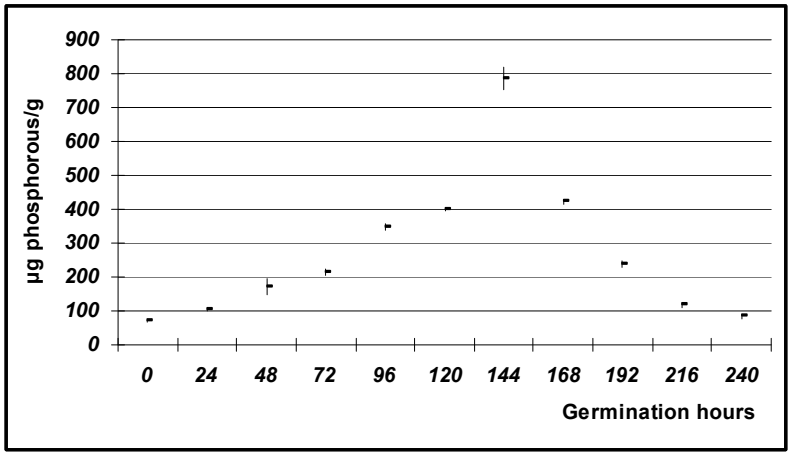

Fig.3. Confidence intervals limits of α-glucanphosphorylase activity in *Setaria pumila* germinated caryopses

In germinated caryopses of *Festuca pratensis*, the starch concentration progressively decreases with the increase of enzymatic activity, from 58.2g% in the reference, up to 14.8 g% at 240 hours from the debut of the germination process, an idea agreeing with literature data, giving concentration values between 20 - 60 g% in *Festuca rubra*, *Sorghum sudanense* and *Sorghum vulgare* (MURARIU, 2003; CIORNEA and VASILE, 2008).

Another objective of the present study was the quantitative determination of the soluble proteins from the enzymatic extracts obtained for dosing of glucanphophorylase activity in the hair grass, the results attained evidencing a variation of theirs within large limits, ranging between 1.249mg% in the reference and 16.159 mg% at 240 hours from the beginning of the germination process. Such a situation might be explained by the fact that, after seed impregnation

and beginning of germination, reactivation of the enzymatic equipment occurs, along with intense processes of proteic biosynthesis (Fig. 4).

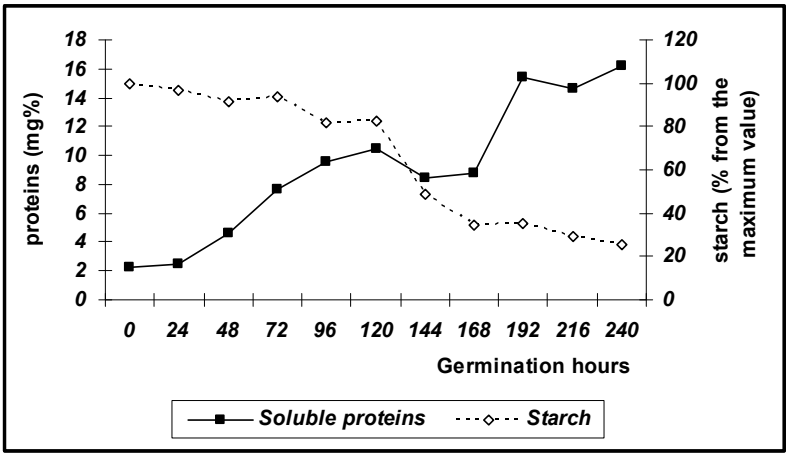

Fig.4. Comparative representation of the starch and total soluble proteins concentration in *Festuca pratensis* germinated caryopses

In *Festuca pratensis*, the activity of α-glucanphosphorylase progressively increases, towards phosphorylation along the whole germination period considered for analysis. Thus, in the case of hair grass, in the reference sample (impregnated seed stage), the activity of α-glucanphosphorylase is minimum (0.22 µg phosphorous/mg protein), following an ascending curve up to 168 hours of germination (126.63 µg phosphorous/mg protein), up to the last germination day under analysis, when it attains a value of 89.32 µg phosphorous/mg protein (Fig. 5).

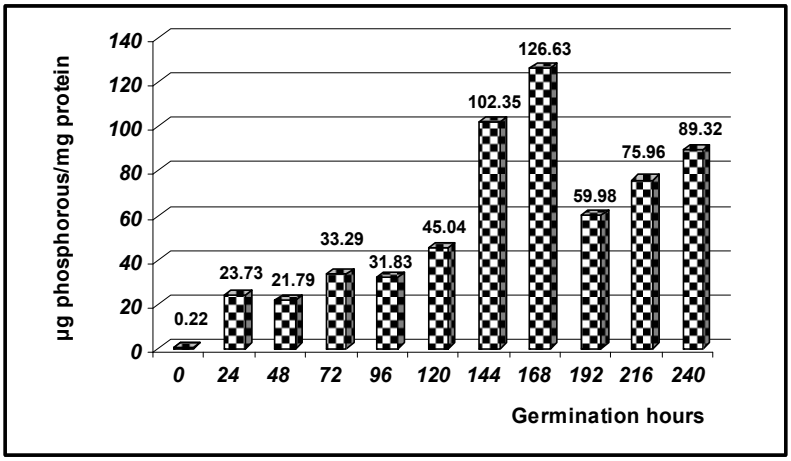

Fig.5. Specific activity dynamics of α-glucanphosphorylase in *Festuca pratensis* germinated caryopses

The limits of the confidence intervals of the α-glucanphosphorylase activity in germinated seeds of hair grass are somewhat larger, comparatively with those recorded in *Setaria pumila*, ranging between 0.024 - 0.541 µg phosphorous/g in the impregnated sample and 1293.597 - 1593.07 µg phosphorous/g, respectively, at 240 hours from the beginning of the germination process (Fig. 6).

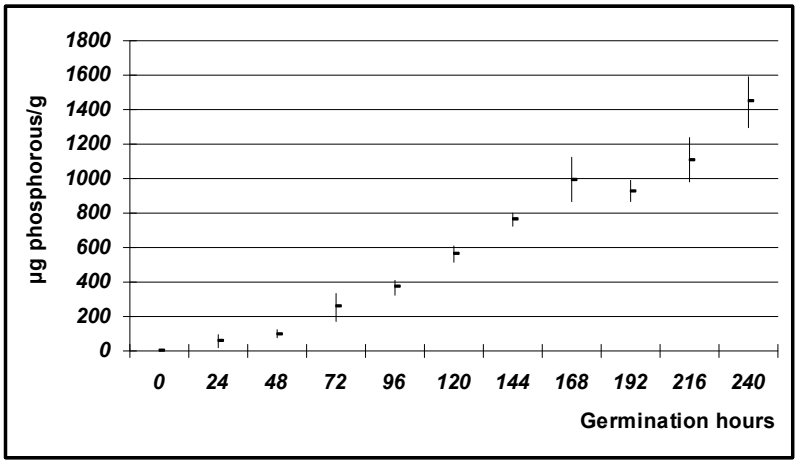

Fig.6. Confidence intervals limits of α-glucanphosphorylase activity in *Setaria pumila* germinated caryopses

CONCLUSIONS

The results of the investigations devoted to the activity of α-glucanphosphorylase activity in germinated caryopses of *Setaria pumila* and *Festuca pratensis* permitted drawing of the following conclusions:

- Starch concentration and α-glucanphosphorylase activity are reversely proportional, that is, in parallels with the increase of the enzymatic activity, a progressive decrease of the reserve starch may be noticed, in spite of the fact that the two phenomena are not perfectly superposable, hydrolytic degradation of the substrate occurring, too, under the action of amylases.
- The protein content in the germinated caryopses of the species under study oscillates within quite large limits, somehow higher values being observed towards the end of the germination period under consideration, when intense biosynthesis processes begin, for the formation and differentiation of both tissues and organs.

REFERENCES

Artenie, Vl., Tanase, Elvira, 1981. *Practicum de biochimie generală*, Ed. Univ. „Alexandru Ioan Cuza” Iaşi.
Artenie, Vl., Ungureanu, E., Negură, Anca Mihaela, 2007. *Metode de investigare a metabolismului glucidic şi lipidic*, Ed. Pim, Iaşi.
Bradford, M. M., 1976. *Anal. Biochem*., **72:** 248 - 254.
Ciornea, Elena, Vasile, Gabriela, 2008. *Lucrări Ştiinţifice, Seria Zootehnie*, U.S.A.M.V. Iaşi, **51:** 48 - 58.
Cojocaru, D. C., 1997. *Enzimologie*, Ed. Gama, Iaşi.
Cojocaru, D. C., Olteanu, Zenovia, Ciornea, Elena, Oprică, Lăcrămioara, Cojocaru, Sabina Ioana, 2007. *Enzimologie generală*, Ed. Tehnopress, Iaşi.
Dragomirescu, L., 1998. *Biostatistică pentru începători*, Ed. Constelaţii, Bucureşti.
Gomoiu, T. M., Skolka, M., 2001**.** *Ecologie. Metodologii pentru studii ecologice*, Ed. Univ. „Ovidius” Constanţa.
Murariu, Alexandrina, 2003. *Fiziologia plantelor din pajişti*, Ed. Junimea, Iaşi.

Văleanu, I., Hâncu, M., 1990. *Elemente de statistică generală*, Ed. Litera, Bucureşti.

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