EXPERIMENTAL INVESTIGATIONS ON THE DYNAMICS OF β-AMYLASE IN SOME *GRAMINACEAE* SPECIES

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Key words: β-amylase, starch, bristle grass, hair grass, Sudan grass
Abstract: The present paper synthesizes the results of the investigations devoted to the dynamics of β-amylase activity in germinated caryopses belonging to some species of the *Poaceae* family, namely: *Setaria pumila* (bristle grass), *Festuca pratensis* (hair grass) and *Sorghum sudanense* (Sudan grass), as correlated with the dynamics of starch concentration, recorded along ten germination days. On one side, the obtained results evidence that, in the process of reserve polyglucides mobilization, this enzyme plays an at least as equally important role as the α-amylase and, on the other, the considerable differences observed among the three *Poaceae* species from cultivated and, respectively, spontaneous flora.

INTRODUCTION

β-Amylase - the systemic denomination of which is α-1,4-glucan maltohydrolase, E.C. 3.2.1.2., also known as saccharogenous amylase or glycogenase, catalyzes the hydrolysis of the α-1,4-glycosidic links from the polysaccharides, yet not chaotically but by successive removal of the maltose units from the non-reducing ends of the polysaccharide, the α-1,6 links acting as a final action point of this enzyme. It exercises its action on the starch, glycogen and related polysaccharides, as well as upon oligosaccharides, producing β-amylase, by a Walden type reversion (DUMITRU and IORDĂCHESCU, 1981).

According to CHEONG *et al*., 2004, the β-amylase present in sweet potato (crystallized in the absence of α-cyclodextrin) shows a tetrameric structure with identical subunits made of 498 aminoacids rests and a molecular mass of 55 800 Da, being therefore quite similar to bean subunits (complexed with cyclodextrin).

In cereals, the synthesis of starch, occurring at endosperm level, follows a unique mechanism which involves enzymatic isoforms not to be found in the tissues of other, non-cereal plants (GUAN and PREISS, 1993; BLAUTH *et al*., 2002; GENSCHEL *et al*., 2002; DINGES *et al*., 2003; JAMES *et al*., 2003). On the other hand, in cereals, starch represents the main reserve polysaccharide, which means that, during the germination period, a perfect correlation exists between the total amylolytic activity and the rate of starch degradation.

MATERIALS AND METHOD

The experiments have been developed on germinated caryopses of bristle grass (*Setaria pumila*), hair grass (*Festuca pratensis*) and Sudan grass (*Sorghum sudanense*) of the 2007 crop, from the Station for Agricultural Researches at Podu-Iloaiei, Iasi.

Starch was quantitatively dosed by the polarimetric method (ARTENIE *et al*., 2007) and the enzymatic activity was determined by the Noelting - Brenfeld method, based on the reduction of the free maltose resulting from the enzymatic hydrolysis of starch, with 3,5 - dinitrosalicylic acid, with formation of 3-amino-5-nitrosalicylic acid, orange in color, determined colorimetrically at 540 nm (ARTENIE and TĂNASE, 1981).

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

RESULTS AND DISCUSSION

A first objective of the experiment was dosing of the starch from the *Setaria pumila* seeds subjected to germination, under laboratory conditions. In this respect, mention should be made of the fact that, after one day from the initiation of the experiment, this represents only 93.3% of the maximum value, after which it slowly decreases up to the 5th day. In the sixth day, a sudden drop of starch concentration, up to 30.56%, is noticed while, towards the end of the germination period taken into study, it comes to represent about 17% of the initially determined value (Fig. 1).

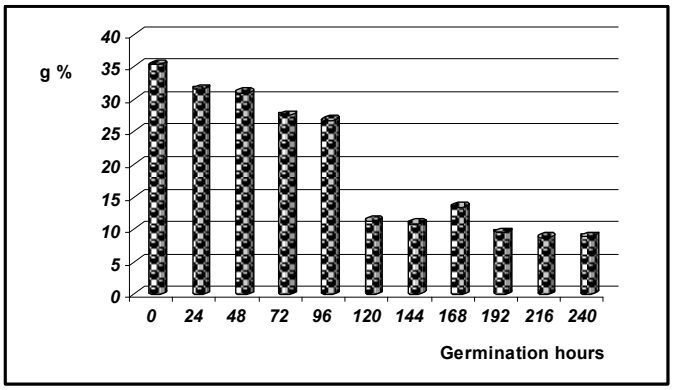

Fig.1. Starch concentration in *Setaria pumila* germinated caryopses

For better understanding the process of starch hydrolytic mobilization, the activity of β-amylase from bristle grass caryopses was determined along the 10 days of the germination process.

A correlation between the β-amylasic activity and the starch consumption shows that the latter gets hydrolyzed in higher and higher amounts, proportionally to the amount of enzyme synthesized in the seeds under germination. Consequently, when the enzymatic activity is the lower, *i.e.*, in the first germination day, the amount of the hydrolyzed starch is also the lowest. Further on, with the increase of the amylasic activity, the amount of hydrolyzed starch also increases up to 7th day, when amylasic activity is maximum (CIORNEA *et al.*, 2006).

At 24 hours of germination, the enzymatic activity records an average value of 14.389 μM maltose/g, 51.657 μM maltose/g at 48 hours, the maximum activity being registered in the 6th germination day (223.886 μM maltose/g), to be followed by a drastic decrease, the average value recorded in the last germination day being of 20.404 μM maltose/g (Fig. 2).

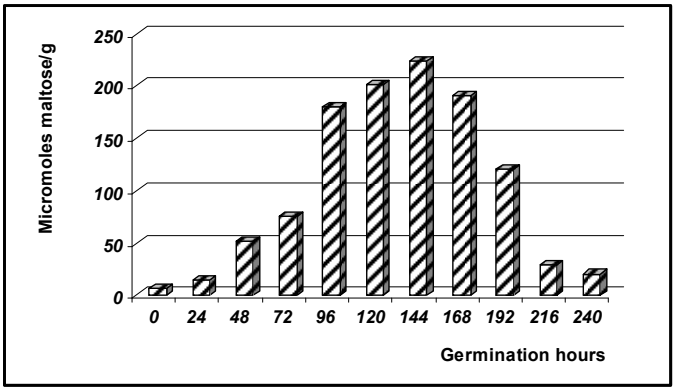

Fig.2. β-Amylase activity (μM maltose/g) in *Setaria pumila* germinated caryopses

One may observe therefore observe that, after 24, 48 and, respectively, 72 hours, the β-amylasic activity increases progressively, yet slowly, remaining nevertheless at high values in

the following five days, after which a relative sudden drop is noticed in the last part of the germination process, up to values close to those of the reference.

It is highly possible that - in the first day - the α-amylase hydrolytic action is predominant, followed by an increase in the β-amylase activity and by a new decrease of the amylasic activity towards the end of the interval, when the starch reserves get exhausted.

Graphical analysis of β-amylase percent activity in germinated *Setaria pumila* caryopses shows enzymatic values quite close to those of the maximum activity, over a somewhat larger time interval (80.56% at 96 germination hours, 89.66% at 120 germination hours and, respectively, 85.1% at 168 germination hours) (Fig. 3).

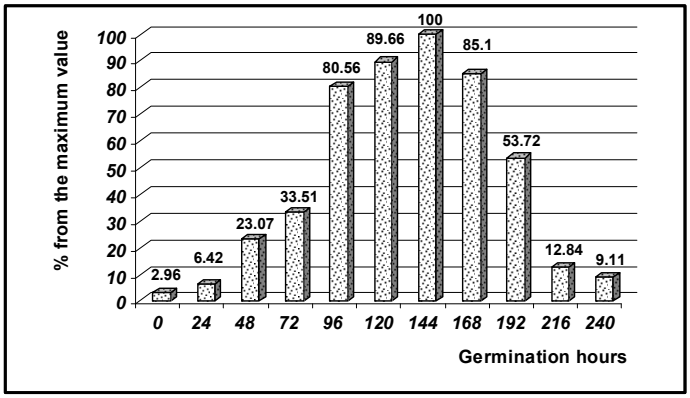

Fig. 3. Relative activity (%) of β-amylase in *Setaria pumila* germinated caryopses

Another species considered for the analysis was *Festuca pratensis*, in which the starch concentration decreases progressively with the increase of the enzymatic activity, from 58.2 g% in the reference up to 14.8 g% at 240 hours from the beginning of germination (Fig. 4), which agrees with literature data providing concentration values between 20 - 50 g% for *Festuca rubra* (MURARIU, 2003).

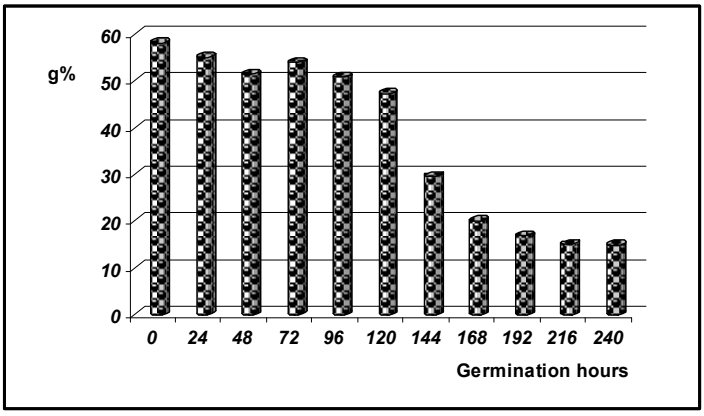

Fig.4. Starch concentration in *Festuca pratensis* germinated caryopses

The β-amylase from the germinated caryopses of *Festuca pratensis* evidences much higher values than in *Setaria pumila*. Thus, the maximum β-amylasic activity, recorded in the 6th day (1090.584 μM maltose/g) is obviously higher than that recorded in bristle grass (223.886 μM maltose/g) (Fig. 5).

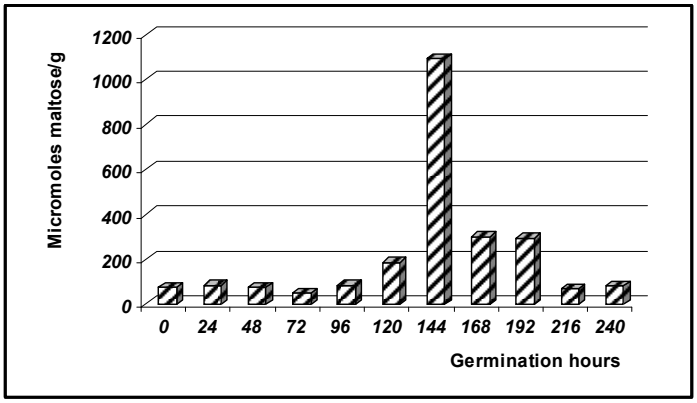

Fig.5. β-Amylase activity (μM maltose/g) in *Festuca pratensis* germinated caryopses

Figure 6 evidences a certain uniformity of the mean percent values of β-amylase from germinated hair grass seeds (between 4.61 - 7.9% of the maximum value) up to 96 hours from the beginning of the germination while, at 120 germination hours, they attain 17.16%, a sudden leap being recorded in the 6th germination day under analysis.

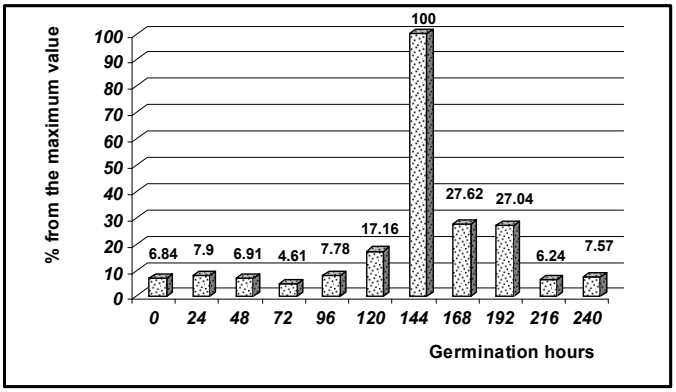

Fig. 6. Relative activity (%) of β-amylase in *Festuca pratensis* germinated caryopses

In *Sorghum sudanense*, one may observe that starch is hydrolyzed at a remarkable speed, its concentration being progressively diminishing from 68.6 g% to 13 g%, with increasing the enzymatic activity (Fig. 7).

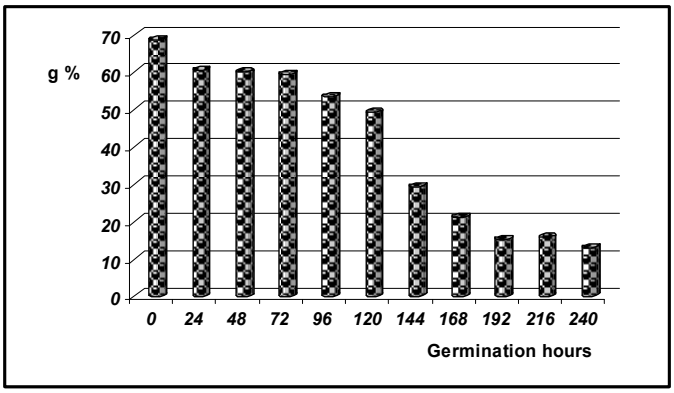

Fig.7. Starch concentration in *Sorghum sudanense* germinated caryopses

In Sudan grass caryopses in biological repose, moment zero, the activity of β-amylase records a value of 18.574 μM maltose/g while, in the first 24 hours of germination, the enzymatic activity decreases up to 6.164 μM maltose/g (the minimum value), after which it considerably increases from one germination day to another. Therefore, the enzymatic activity takes values of 145.526 μM maltose/g in the 7th germination day, then progressively decreases until the last germination day considered in the study (79.225 μM maltose/g) (Fig. 8).

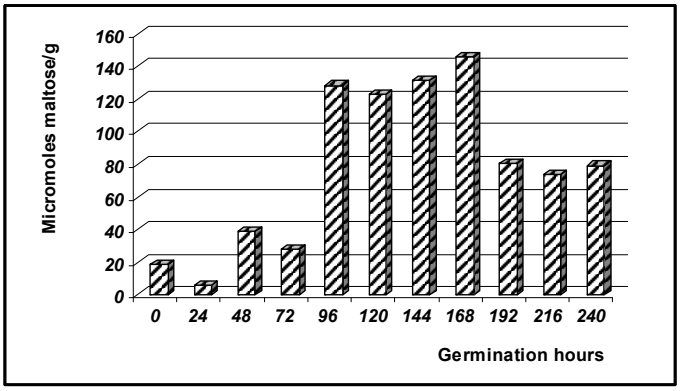

Fig.8. β-Amylase activity (μM maltose/g) in *Sorghum sudanense* germinated caryopses

Once again, percent concentration has been calculated and graphically plotted, analysis of the obtained results evidencing that the relative activity of β-amylase in germinated *Sorghum sudanense* seeds remains quite close to the maximum value for four consecutive germination days, after which it decreases up to 54.44 % of the maximum value (Fig. 9).

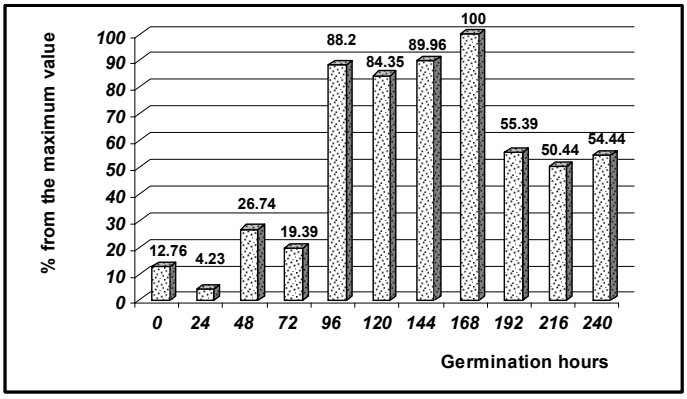

Fig. 9. Relative activity (%) of β-amylase in *Sorghum sudanense* germinated caryopses

CONCLUSIONS

Determination of the starch content in the caryopses of the species under investigation evidenced a gradual, progressive diminution along the whole experimental period, which confirms that mobilization of the reserve starch occurs hydrolytically, under the action of amylases.

Analysis of the experimental results on the dynamics of β-amylase activity in germinated caryopses evidences, on one side, that, in the process of reserve polyglucides mobilization, this enzyme plays an at least as equally important role as the α-amylase and, on the other, some considerable differences among the three *Poaceae* species from the cultivated and, respectively, spontaneous flora.

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