

ON THE ACTIVITY OF α -AMYLASE IN SOME CULTURE AND SPONTANEOUS GRAMINACEAE, DURING THEIR GERMINATION PERIOD

ELENA CIORNEA ^{1*}, ARTENIE VLAD ¹, DUMITRU COJOCARU ¹,
GABRIELA VASILE ¹

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Abstract: Our study presents the results obtained on the activity of α -amylase in *Panicum miliaceum* (millet) and *Setaria glauca* (bristle grass) during ten days of germination. In both species under study, the minimum value of α -amylase activity has been recorded in the stage of impregnated seed. In the case of millet, the maximum activity of α -amylase (595.871 μ M maltose / g) has been evidenced 12 hours after the beginning of germination, while, in the case of bristle grass, α -amylase attains the maximum level of its activity after 144 hours of germination (429.824 μ M maltose / g).

INTRODUCTION

Seeds' germination represents an extremely complex biochemical and physiological process, in which the reserve substances are mobilized, at incredible speed, for assuring to the embryo the energy and the metabolic precursors necessary for biosynthetic processes (BURZO *et al.*, 1999).

To a certain extent, the biochemical transformations occurring during germination represent the reverse of the processes developed during seeds' ripening (and, respectively, bulbs' and tubercles' formation, etc.). Consequently, in the maturation stages, the main biochemical processes developed inside the vegetal organisms are actually biosynthetic processes, leading to the formation of more or less significant amounts - as depending on the species - of glucides, lipids and reserve proteins. Further on, these amounts are transported to the reproduction organs (seeds, bulbs, tubercles, etc.), in which where they are accumulated (EVANS *et al.*, 1997).

α -Amylase (systematically known as α -1-D glucan-glucohydrolase), also denominated diastasis, ptyaline, glycogenase, is an enzyme belonging to the class of hydrolases, which catalyzes the hydrolysis reaction of the α -1-4-glycosidic links from starch and glycogen, resulting in the formation of smaller polyglucidic fragments - the dextrans - and of a determined amount of maltose. The enzyme is totally inactive towards the α -1-6 glycosidic links from the branching points of substrate's molecule.

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 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 *et al.*, 1981).

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

RESULTS AND DISCUSSION

A first objective considered in the determination of amylases' activity in the species under study was plotting of the standard curve for converting the extinction units (Fig. 1). To this end, a series of reference samples - in which the concentration in maltose varied between 0.2 -

1.8 mg - has been employed. The values of extinction have been read at a wavelength equal to 540 nm.

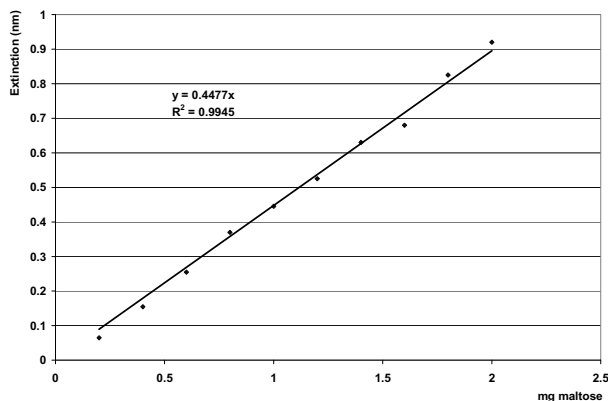


Fig.1. Standard curve for dosing of maltose

The results of the determination of α -amylase activity in *Panicum miliaceum* have evidenced that the enzymatic activity records significant variations along the whole germination period taken into study.

Thus, in the case of millet, the activity of α -amylase records a minimum value in the impregnated seeds (39.477 μM maltose / g) while, starting with the 1st day after the beginning of germination, a significant increase of the activity is to be recorded, the maximum being attained in the 5th germination day (595.871 μM maltose / g).

After the attainment of the maximum value, a gradual decrease in the activity of α -amylase is to be observed, the value recorded at 144 germination hours being of 435.02 μM maltose / g, while the minimum value is again to be recorded in the 10th germination day (37.183 μM maltose / g) (Fig. 2).

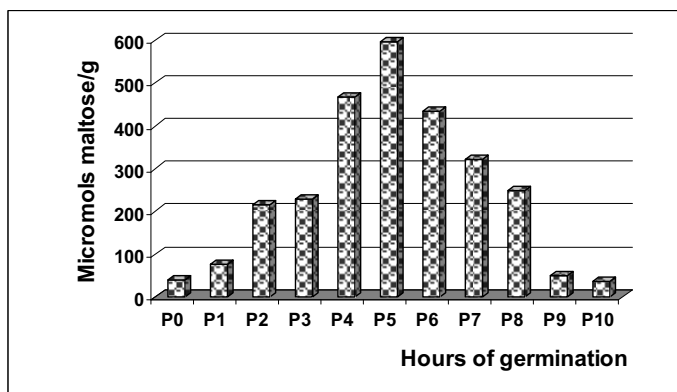


Fig.2. Activity of α -amylase (μM maltose /g) in germinated *Panicum miliaceum* caryopses

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 $\alpha=0.05$ and $n-1$ degrees of freedom (Fig. 3).

The narrowest confidence intervals have been recorded at 24, 48 and, respectively, 216 germination days, the largest confidence intervals being observed at 96, 120 and, respectively, 144 hours.

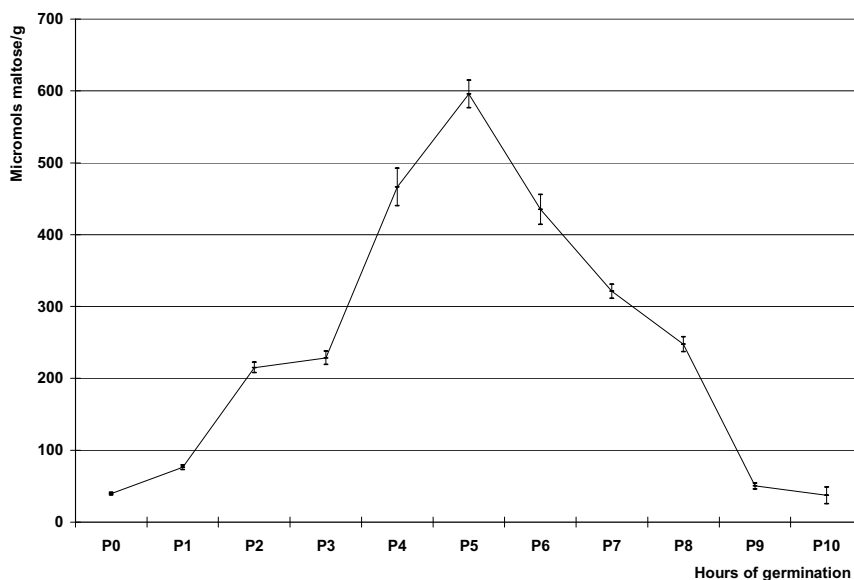


Fig.3. Confidence intervals of α -amylase activity in *Panicum miliaceum*

In the germinated caryopses of bristle grass, the activity of α -amylase follows the same ascending curve in the first days, which is followed by a drastic decrease up to the last day taken into study. The minimum value is recorded, in this case too, in the stage of impregnated seed ($61.645 \mu\text{M}$ maltose / g), which is followed by an increase until the 6th day, when the maximum threshold is finally attained ($429.824 \mu\text{M}$ maltose / g).

Starting with the 7th germination day, the activity of α -amylase decreases progressively, until reaching an average value of $61.387 \mu\text{M}$ maltose / g (after 240 hours of germination) (Fig. 4).

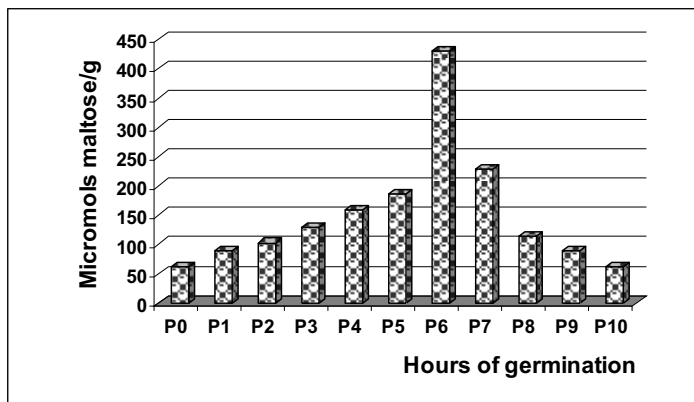


Fig.4. α -Amylase activity (μ M maltose /g) in the germinated *Setaria glauca* caryopses

As to the limits of the confidence intervals of α -amylase activity, one may observe that the largest interval is recorded during the 6th germination day (411.243 - 452.117 μ M maltose / g) (Fig. 5).

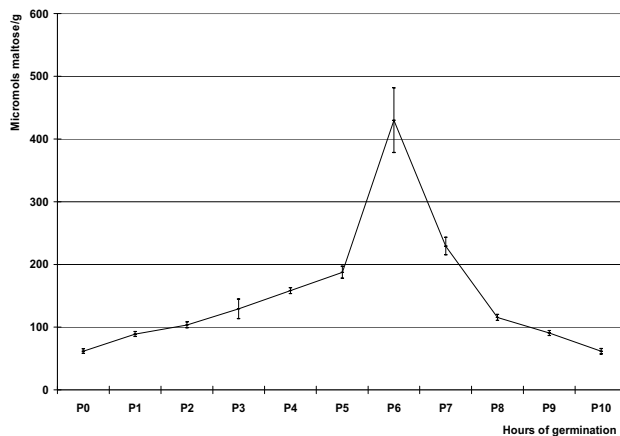


Fig.5. Confidence intervals of α -amylase activity in *Setaria glauca*

In order to check the possible difference or similarities occurring between the activity of α -amylase 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.

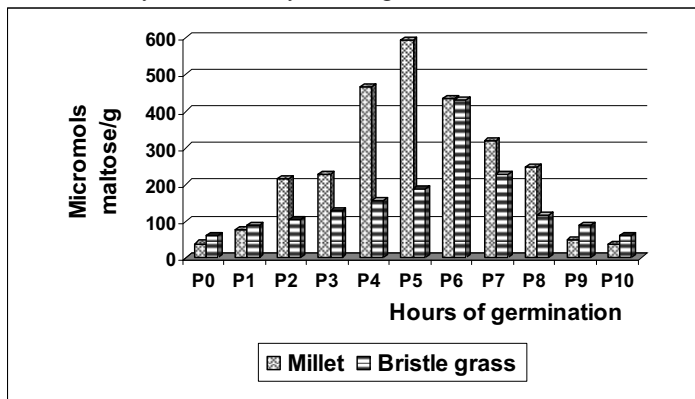


Fig.6. α -Amylase activity (μM maltose / g) in *Panicum miliaceum* and *Setaria glauca*

CONCLUSIONS

The experimental results obtained in the determination of α -amylase activity in the germinated caryopses of *Panicum miliaceum* and *Setaria glauca* permitted the formulation of the following general conclusions:

- In both *Panicum miliaceum* and *Setaria glauca*, the minimum value of α -amylase activity has been recorded in the stage of impregnated seed.
- In the case of millet, the maximum activity of α -amylase ($595.871 \mu\text{M}$ maltose / g) has been evidenced 12 hours after the beginning of germination, while, in the case of bristle grass, α -amylase attains the maximum level of its activity after 144 hours of germination ($429.824 \mu\text{M}$ maltose / g).
- In both species, after the attainment of the maximum value, a progressive decrease of amylases' activity is to be noticed until the last germination day considered in the study.

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1) "Alexandru Ioan Cuza" University of Jassy, Faculty of Biology

*) ciornea@uaic.ro

