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ON THE ACTIVITY OF β-AMYLASE IN GERMINATED CARYOPSES OF *PANICUM MILIACEUM* AND *SETARIA PUMILA*

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INTRODUCTION

The metabolism of substances and the energy constitutes an astonishing characteristic of the living world, which differentiates in a clear - cut manner the living organisms from the inanimate ones.

The totality of reactions and transformations making up the two parts of metabolism (catabolism and anabolism) does not occur randomly, instead they manifest themselves in a permanent correlation with the environment, responding in a coordinate manner to cell's momentary necessities. This fact is possible as such transformations occur under the action of the enzymatic equipment of each cell, which means that a complex mechanism of self - regulation and control is in action each moment.

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 the biosynthetic process (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.), where they are accumulated (EVANS *et al*., 1997).

The amylases, enzymes belonging to the class of hydrolases, are involved in the hydrolysis of starch. β-Amylase (α-1, 4 - D- glucan-maltohydrolase) catalyzes the hydrolysis of the α - 1, 4, - glycosidic links from the polysaccharides, by successive removal of the maltose rests from the non-reductive ends (COJOCARU, 1997).

MATERIAL AND METHOD

The experiments have been developed on germinated caryopses of millet (*Panicum miliaceum*) and bristle grass (*Setaria pumila*) 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 μM maltose /g (VĂLEANU *et al*., 1990).

RESULTS AND DISCUSSIONS

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 wave length equal to 540 nm.

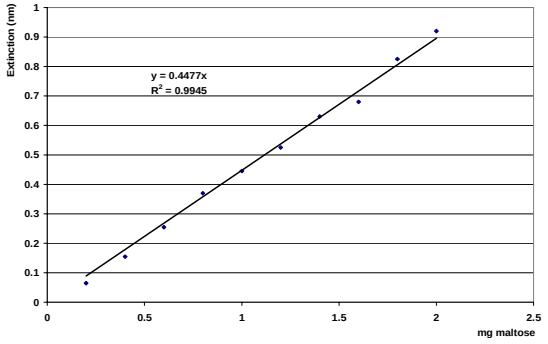

Fig. 1. Standard curve for dosing of maltose

On the basis of the graph, the regression straight line has been drawn and its regression equation has been calculated. According to the equation, the amounts of maltose corresponding to the samples subjected to analysis have been subsequently established, and the values obtained were referred to the amount of tissue employed (μM maltose /g).

Study of β-amylase in *Panicum miliaceum* permits the observation that the enzymatic activity oscillates considerably from one germination process to another, the variation recorded being between 40.206 - 326.871 μM maltose /g. The minimum value is registered in the beginning of germination, in the impregnated sample, 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.

The enzymatic activity increases progressively in the following days of germination, up to the sixth germination day, when the maximum value is recorded.

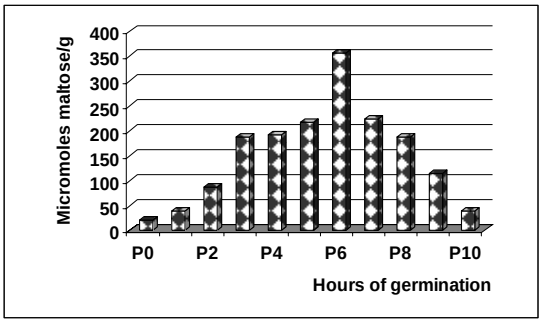

Fig. 2. Activity of β-amylase (μM maltose /g) in germinated *Panicum miliaceum* caryopses

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 α= 0.05 and n-1 degrees of freedom (Fig. 3).

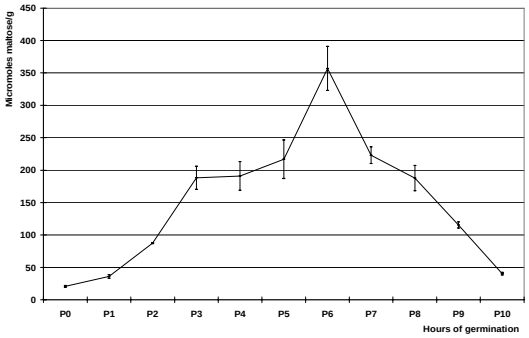

Fig. 3. Confidence intervals of β-amylase activity in *Panicum miliaceum*

Analysis of the experimental results on the amylolitic activity in *Setaria pumila* evidences that the enzyme records, in this case too, a minimum value in the zero moment of the germination process (8.963 μM maltose /g).

Within 24 hours of germination, the enzymatic activity records an average value of 16.722 μM maltose /g, and 68.324 μM maltose /g after 48 hours, the maximum activity being recorded after 144 hours of germination (356.404 μM maltose /g), which is followed by a drastic decrease, the average value recorded in the last day of germination being of 22.071 μM maltose /g (Fig. 4).

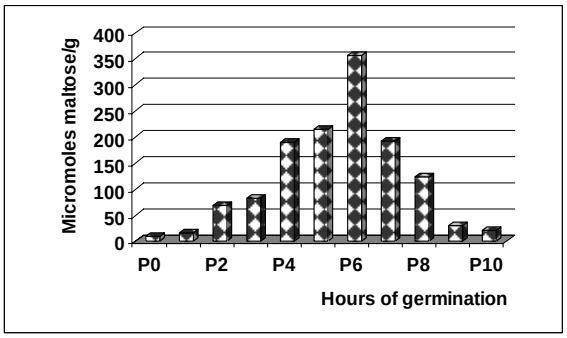

Fig. 4. β-Amylase activity (μM maltose /g) in the germinated *Setaria pumila* caryopses

Similarly with the millet, the confidence intervals are quite narrow for the whole germination period taken into study, as actually evidenced by the graphical representation, too (Fig. 5).

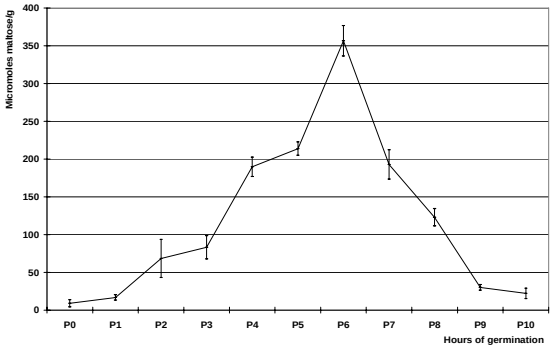

Fig. 5. Confidence intervals of the β-amylase activity in *Setaria pumila*

In order to check the possible difference or similarities occurring between the activity of β-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 (Fig.6) (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 resulted obtained from statistically analysis shows that both factors (the species and the germination time) influence the enzymatic activity, although to a different extent.

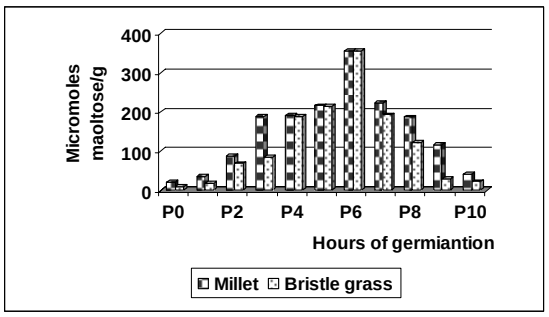

Fig. 6. β-Amylase activity (μM maltose /g) in *Panicum miliaceum* and *Setaria pumila*

CONCLUSIONS

The results obtained from the determination of β-amylase activity in germinated caryopses of millet and bristle grass permitted drawing of the following general conclusions:

- In both *Panicum miliaceum* and *Setaria pumila*, the minimum value of β-amylase activity is recorded in the stage of impregnated seed.
- The maximum activity, recorded after 144 hours of germination, is approximately equal in the species under study, taking values of 356.871 μM maltose /g in millet and 356.404 μM maltose /g, respectively in bristle grass.
- The confidence intervals of β-amylase activity, in millet and bristle grass, are relatively narrow, which means that the results obtained have a very low degree of error.
- Comparison of the calculated and critical values of the factors showed that the enzymatic activity is influenced both by the germination time and by the species, although to a different extent.

REZUMAT

Determinarea activității β-amilazei s-a realizat pe cariopse de mei (*Panicum miliaceum*) şi mohor (*Setaria pumila*) germinate la temperatura laboratorului, probele fiind recoltate din 24 în 24 de ore, timp de zece zile.

Activitatea enzimatică s-a determinat prin metoda Noelting - Brenfeld, rezultatele obținute fiind prelucrate statistic.

La ambele specii studiate valoarea minimă a activității β-amilazei s-a înregistrat în stadiul de sămânță îmbibată, activitatea maximă fiind evidențiată la 144 de ore de germinare. Analiza datelor experimentale relevă o influență semnificativă a timpului de germinare asupra activității amilolitice, enzima prezentând o dinamică asemănătoare la gramineele luate în studiu.

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