THE DYNAMICS OF TOTAL AMYLASE'S ACTIVITY IN *PANICUM MILIACEUM* AND *SETARIA GLAUCA* DURING THE GERMINATION PERIOD

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Abstract: It was studied the dynamics of total amylase's activity in millet (*Panicum miliaceum*) and bristle grass (*Setaria glauca*) during the germination period. The enzymatic activity was determined by the Noelting - Brenfeld method, the results obtained being expressed in µM maltose / g. In both millet and bristle grass, it was evidenced that both parameters taken into study (the species and the germination time) do influence the enzymatic activity, although to a different extent.

INTRODUCTION

As generally known, the process of germination assumes enzymatic degradation of the reserve substances from seeds. Consequently, the reserve proteins are hydrolyzed by proteases and then decomposed in more and more simple substances, such as: peptides, amino acids, amides, ammonium, while the lipids get transformed - under the action of lipase - in glycerin and fatty acids; in a subsequent stage, glycerin is transformed into sugars, while the fatty acids enter the cycle of tricarboxylic acids, in embryo's breathing process (BURZO *et al*., 1999).

The richest reserve polyglucide is represents by the starch deposited in the endosperm. The starch is solubilized by the amylase released by the cells of the aleurone layer and degraded up to simple glucides which, once oxidized, produce the ATP.

Starting from the ever increasing, both theoretical and practical, interest raised, on one side, by the germination process in which the reserve substances are mobilized, at an astonishing rate, to assure to the embryo the energy and the metabolic precursors necessary in biosynthetic processes and, on the other side, by the special role played by the amylolytic enzymes in the degradation of starch, the authors of the present study have determined the activity of total amylase and established a possible correlation between the rate of starch mobilization and the enzymatic activity, in a comparative study developed on a cultured plant and a species of the spontaneous flora (ARTENIE, 1977; ZAMFIRACHE *et al.*, 2000).

Consequently, the study is devoted to the determination of total amylase in *Panicum miliaceum* and *Setaria glauca*, along 240 hours of germination, for putting into evidence the influence of the germination time and of the growing ratio upon the enzymatic activity (GIMBI et al., 2002).

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 µM 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.

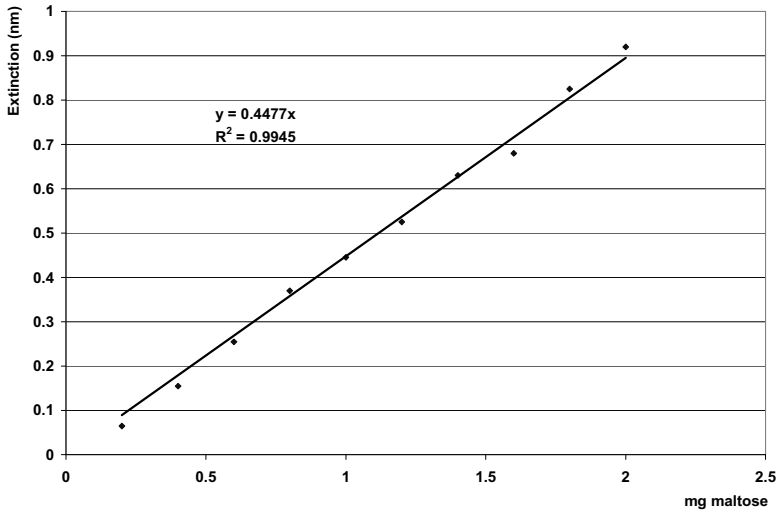

Fig.1. Standard curve for dosing of maltose

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).

The results of the experimental determinations have shown that the activity of total amylase attains its minimum threshold in the zero moment, in millet caryopses occurring in biological repose, the values recorded ranging between 58.873 - 60.561 μM maltose /g, at and an average value of 60.186 μM maltose /g.

After a 24 hour germination period, total amylase records an average activity of 115.307 μM maltose /g, which is followed by a progressive increase, the maximum value being noticed in the 5th germination day (812.707 μM maltose /g).

Starting with the 7th day of germination, the enzymatic activity is drastically decreasing, up to values of 508.592 μM maltose /g at 80.876 μM maltose /g (the minimum value of total amylase in the millet caryopses considered for analysis) (Fig. 2).

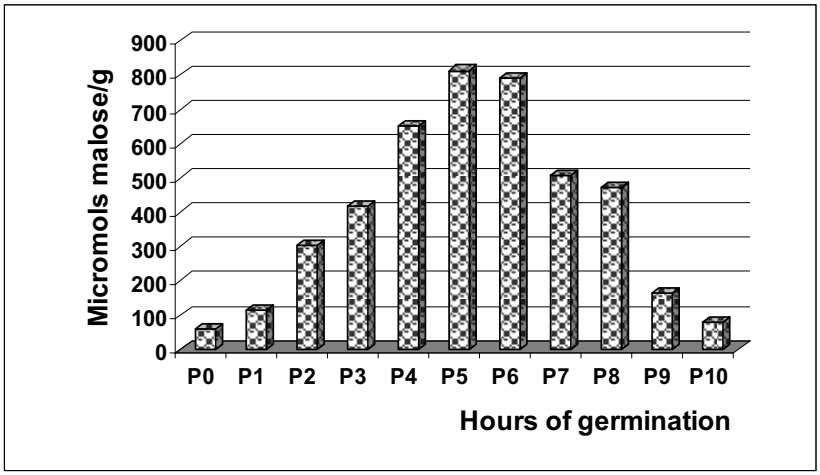

Fig.2. Activity of total amylase (μM maltose /g) in germinated *Panicum miliaceum* caryopses

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).

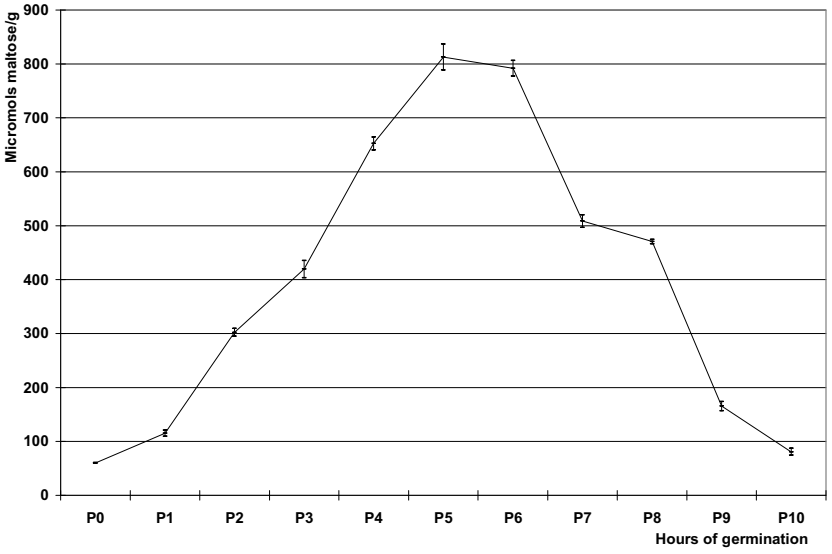

Fig.3. Confidence intervals of total amylase activity in *Panicum miliaceum*

Figure 3 evidences that the limits of the confidence intervals for the activity of total amylase are very narrow, for all germination hours taken into study. The largest confidence intervals are recorded at 120 germination hours, the limits ranging between 788.495 - 836.918 μM maltose / g.

In the case of bristle grass, one may notice that, in the caryopses found in biological repose, the activity of total amylase records the lowest value (69.986 - 71.512 μM maltose /g).

Starting with the first 24 germination hours, the activity of total amylase increases considerably from one day to another. Thus, if in the 1st germination day the enzymatic activity records values of 105.236 μM maltose /g, in the 6th germination day a maximum value of 786.229 μM maltose /g will be attained.

After this period, the activity of total amylase decreases progressively, (421.977 μM maltose /g at 192 germination hours, up to 83.457 μM maltose /g at 240 germination hours) (Fig. 4).

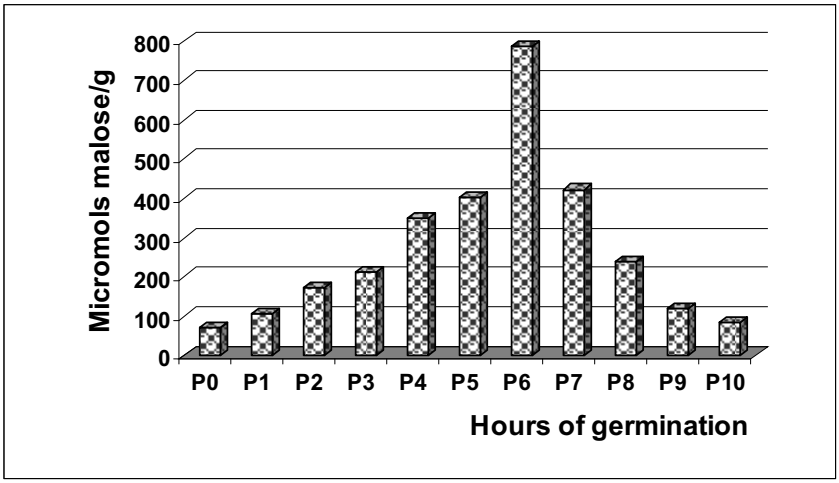

Fig.4. Total amylase activity (μM maltose /g) in the germinated *Setaria glauca* caryopses

Similarly to the procedure applied in the case of millet, the limits of the confidence intervals of the activity of total amylase have been plotted graphically. They are seen as taking quite narrow values, the largest interval being recorded at 144 germination hours. In the other germination periods taken into study, the limits of the confidence intervals are extremely narrow, which means a very reduced degree of error (Fig. 5).

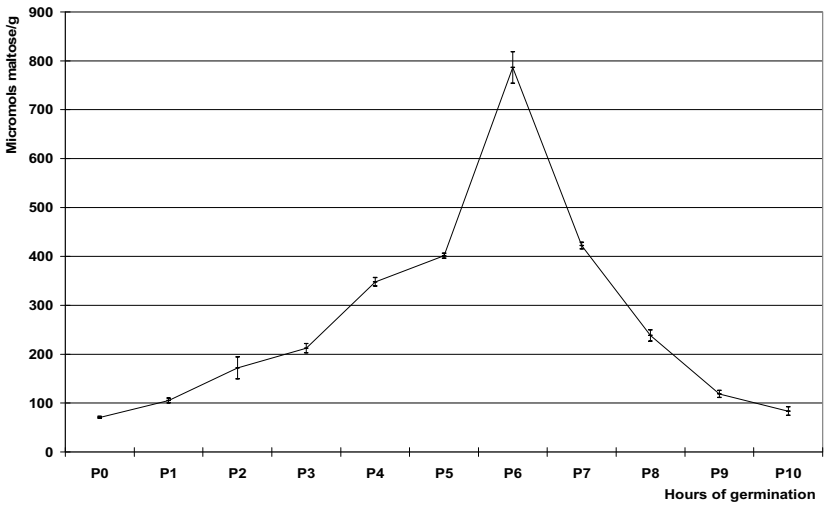

Fig.5. Confidence intervals of total amylase activity in *Setaria glauca*

In order to check the possible differences or similarities occurring between the activity of 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 from statistically analysis show that both factors (the species and the germination time) influence the enzymatic activity, although to a different extent.

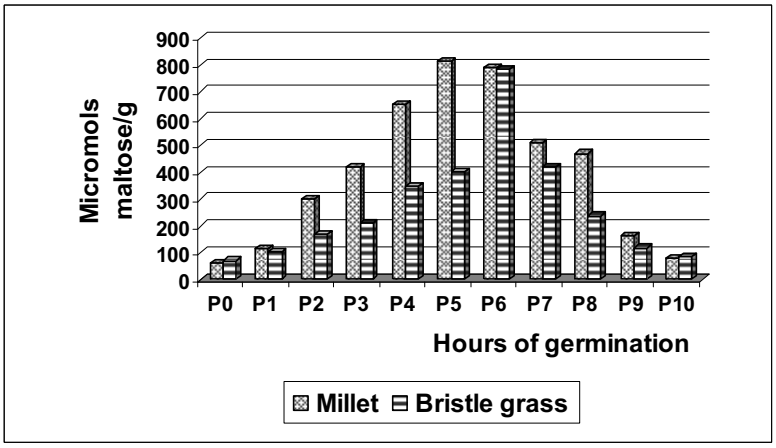

Fig.6. Total amylase activity (µM maltose /g) in *Panicum miliaceum* and *Setaria glauca*

CONCLUSIONS

- In the case of both millet and bristle grass, the minimum value of the activity of total amylase has been recorded in the stage of impregnated seed.
- In *Panicum miliaceum*, the maximum value of the activity of total amylase (812.707 μM maltose /g) has been evidenced in the 5th germination day while, in *Setaria glauca*, the maximum has been attained in the 6th germination day (786.229 μM maltose /g).
- In both species, a progressive decrease in the activity of total amylase may be noticed up to the last germination day considered in the study.
- The limits of the confidence intervals are very narrow in both species and, respectively, in each experimental moment in part.
- A comparative study of the activity of total amylase, in both millet and bristle grass, evidences that both parameters taken into study (the species and the germination time) do influence the enzymatic activity, although to a different extent.

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