ON THE AMYLOLYTIC ACTIVITY OF *SORGHUM VULGARE*

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Key words: amylase, proteins, *Sorghum vulgare*

Abstract: The present investigation follows the activity of some enzymes, involved in the mobilization of reserve substances, namely α- and β-amylase from *Sorghum vulgare* seeds germinated under laboratory conditions, determined by the Noelting - Brenfeld method, along with the quantitative dosage of the total soluble proteins from tissular homogenates - by the Bredford method, on using the Comassie Brilliant Blue G_{250} reactive.

INTRODUCTION

α-Amylase occurs in large amounts in vegetable organisms, especially in cereal grains, during their germination (while it is absent in non-germinated grains). It is assumed that the α-amylases pre-exist in the cereal grains as inactive precursors, which get activated during germination, by means of proteolytic enzymes.

According to STEUP *et al*., 1983, in spinach, α-amylase is the only enzyme isolated from chloroplasts which may possibly degrade starch. Determination of the sub-cellular localization of α-amylase is essential for putting into evidence the physiological role played by this enzyme in starch degradation.

By the application of techniques of sub-cellular fractionation and by a specific action upon the substrate, it has been demonstrated that α-amylase is present in the chloroplasts of the spinach leaves (OKITA *et al.*, 1979), pea (*Pisum sativum*), wheat (*Triticum aestivum*) (ZIEGLER and BECK, 1986) and Arabidopsis (LIN *et al.*, 1988).

Others authors (LEVI and PREISS, 1978; KAKEFUDA *et al*., 1986) report that, in barley (*Hordeum vulgare*), α-amylase is either absent or evidences a much reduced activity in chloroplasts.

According to BEERS and DUKE (1988), in most plant species, the activity of α-amylase at the level of the leaf tissues is manifested extrachloroplastically.

β-Amylase occurs only in superior plants, in both non-germinated and germinated cereals (rye, barley, wheat, rice, etc.). Starting from the above-mentioned literature data, one may assert that both α- and β-amylases may be present in plants as enzymes which transform the glucide reserve - occurring as starch - up to the stage of maltose, a form in which it may be employed for cellular requirements.

Relatively recent investigations, developed on *Calystegia sepium*, have shown that β-amylase occurs exclusively in the cytoplasm, evidencing high activity at the level of rhizomes (VAN DAMME *et al*., 2001).

MATERIALS AND METHOD

The experiments have been developed on germinated caryopses of *Sorghum vulgare* of the 2007 crop, from the Station for Agricultural Researches at Podu-Iloaiei, Iasi.

The quantitative determination of total soluble proteins was determined by Bradford method and the enzymatic activity 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 (BRADFORD, 1976; 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 (FOWLER *et al*., 2000).

RESULTS AND DISCUSSION

As generally known, in conditions of biological repose, the enzymatic activity is much reduced, up to its total absence while, with the absorption of environmental water, during germination, activation of the whole enzymatic equipment may be observed, together with the enzymatic degradation of the reserve substances from the seeds, for obtaining the necessary energy.

In germinated caryopses of *Sorghum vulgare*, the activity of total amylase follows an ascending curve in the first six germination days. The minimum value is recorded in the stage of impregnated seed (4.457 μM maltose/g), followed by an increase, up to 6^{th} day, when the maximum threshold is attained (91.565 μM maltose/g). Starting from 7^{th} germination day, the activity of total amylase progressively decreases, up to a mean value of 43.614 μM maltose/g, registered after 240 hours of germination (the tenth day).

Such a dynamics of total amylasic activity permits the assumption that the process of reserve starch mobilization for producing the energy necessary in metabolic processes starts as early as the first hours of the germination process, the *Sorghum vulgare* species evidencing quite a remarkable activity after only 24 germination hours. The gradual diminution of the amylolytic activity in the second stage of the germination interval under study might be explained by a gradual decrease of the starch amount as well as, probably, by the initiation of the photosynthetic process, known as assuring itself the precursors necessary for various metabolic processes.

The graphical representation of the percent values of total amylase activity in germinated caryopses of *Sorghum vulgare* shows that this enzyme records a slow increase of its activity in the first days of germination up to the sixth day, when the activity registered is about 21 times higher than that of the impregnated sample (moment zero) (Fig. 1).

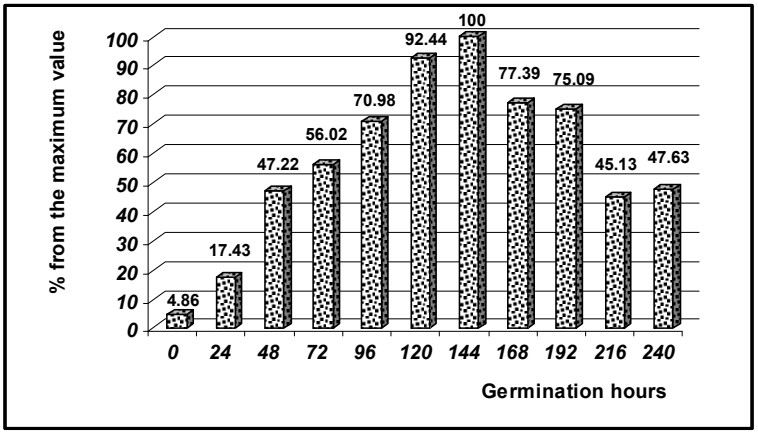

Fig.1. Relative activity (%) of total amylase in *Sorghum vulgare* germinated caryopses

As to the dynamics of soluble protein concentration in germinated *Sorghum vulgare* caryopses, extremely fluctuating values may be observed from one day to another. Thus, proteic concentration increases in the first four germination days then, after 120 hours of germination, it suddenly decreases, to be followed by another considerable leap, up to values higher than in the reference, which might be indicative of a possible acceleration of the biosynthetic processes, starting - probably - with the biosynthesis of all enzymes necessary to the metabolic processes developed within the vegetal cell, which assure the embryo and plantlet development (Fig. 2).

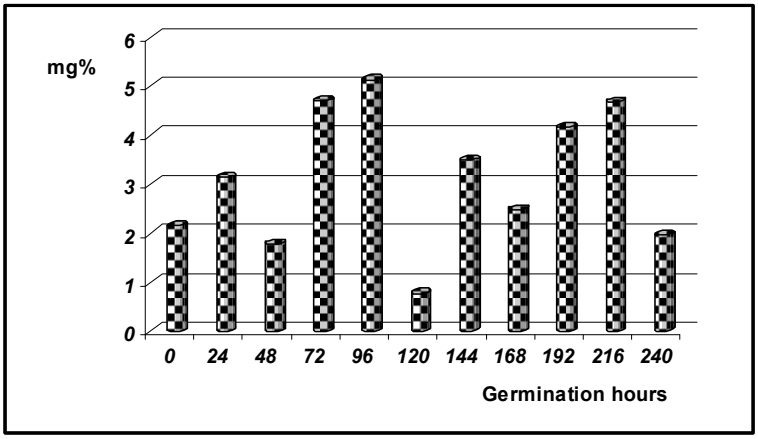

Fig.2. Protein concentration in *Sorghum vulgare* germinated caryopses

As the specific enzymatic activity reflects in a most faithful manner the real catalytic capacity of these enzymes, on also eliminating the possible errors, induced by the different conditions of homogenization and extraction, in the following, the specific activity of total amylase will be determined in *Sorghum vulgare*.

Consequently, as actually illustrated in Figure 3, the graph of the amylolytic activity gets modified, that is the maximum specific activity is evidenced at 120 hours from the beginning of germination.

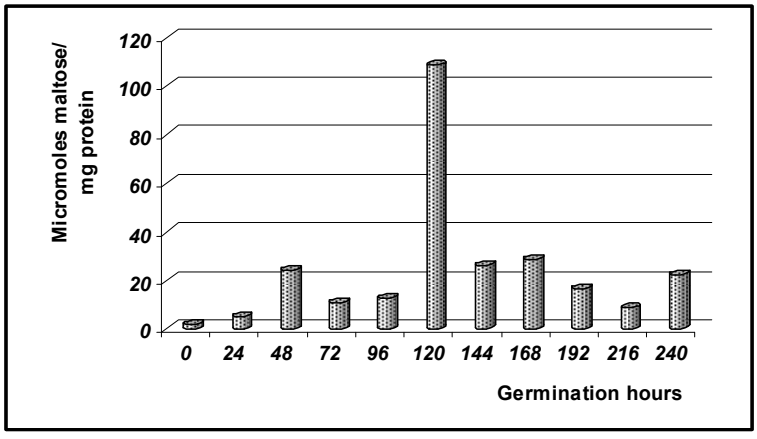

Fig.3. Specific activity dynamics of total amylase in *Sorghum vulgare* germinated caryopses

As the α- and β-amylases have different mechanisms of action and play different roles in the process of starch hydrolysis, their activities will be determined separately.

In the seeds of germinated plants, α-amylase degrades the starch accumulated in seeds, known as having two important functions: on one hand, the catalysis as such and, on the other,

the capacity of breaking the links from the starch molecule, the enzymes of various sources having the same mechanism of action (MORI, 2006; NEAGU *et al*., 2006).

Consequently, the activity of α-amylase in *Sorghum vulgare* shows low values, ranging between 2.850 μM maltose/g in the stage of impregnated seed and 65.410 μM maltose/g in the seventh germination day, followed by a slight decrease, up to reaching a value of 24.038 μM maltose/g at 240 hours of germination.

Activation of the enzymatic equipment occurs immediately after the impregnation of caryopses, α-amylase intensifying its activity as early as the second germination day, when it reaches a value of 20.66% from the maximum value, after which it gradually increases, being maintained at maximum values (72.18% at 72 germination hours, 81.97% at 96 germination hours, 96.68% at 120 germination hours and, respectively, 78.21% at 192 germination hours) (Fig. 4).

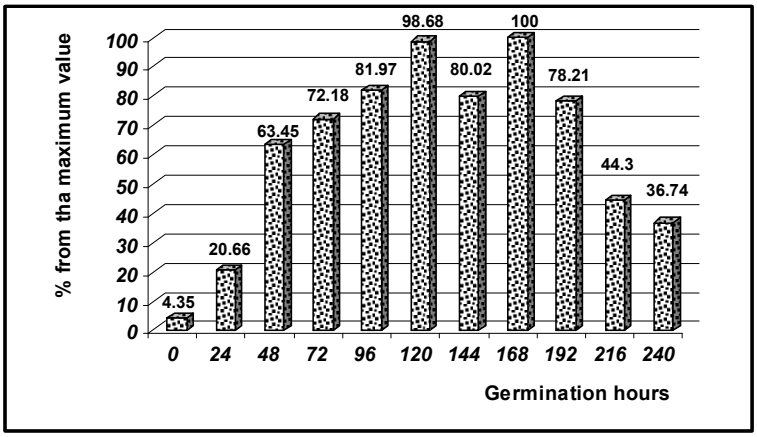

Fig.4. Relative activity (%) of α-amylase in *Sorghum vulgare* germinated caryopses

Determination of the specific activity of α-amylase in germinated *Sorghum vulgare* seeds evidences an ascending dynamics, as a function of the germination time, the maximum activity (22.625 μM maltose/mg protein) being evidenced at 168 germination hours (Figs. 5 - 6).

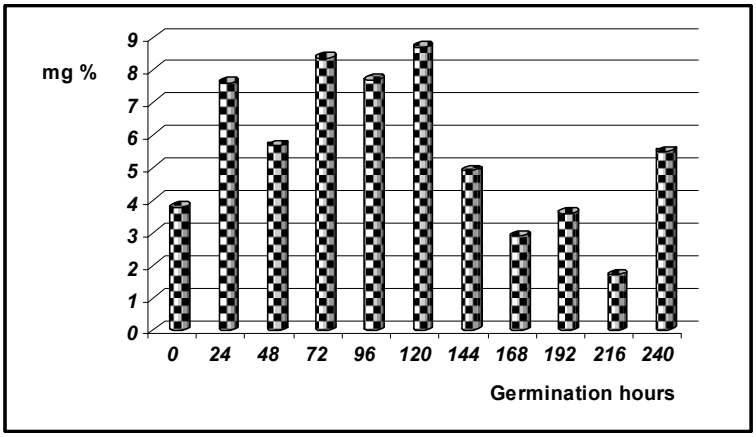

Fig. 5. Protein concentration in *Sorghum vulgare* germinated caryopses

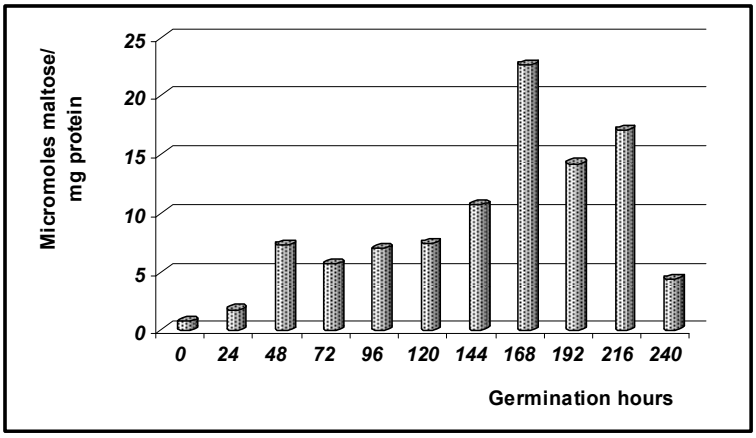

Fig.6. Specific activity dynamics of α-amylase in *Sorghum vulgare* germinated caryopses

Study of the substrate degradation by the amylolytic reaction in germinated caryopses of *Sorghum vulgare* should not be exclusively restricted to the determination of α-amylase activity, on considering plants capacity of synthesizing the α-amylase which, together with the β-amylase, contributes to the hydrolytic splitting of the macromolecules of reserve polyglucides.

In the four samples under analysis, β-amylase shows a hardly perceptible activity, ranging between 1.210 μM maltose/g at 48 germination hours and 2.897 μM maltose/g at 72 germination hours, after which it increases up to 28.988 μM maltose/g at 144 germination hours while, towards the end of the period taken into study, a new intensification of the substrate hydrolysis capacity is to be observed (Fig. 7).

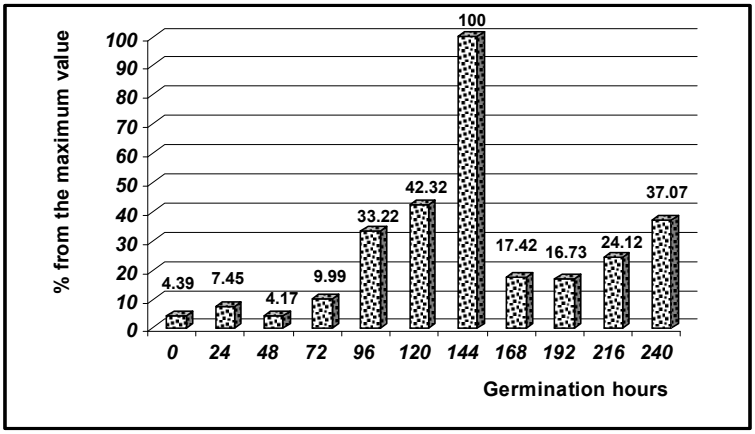

Fig.7. Relative activity (%) of β-amylase in *Sorghum vulgare* germinated caryopses

Quantitative dosage of proteins from the enzymatic extracts of *Sorghum vulgare* caryopses evidences higher concentrations up to 144 germination hours, followed by a drastic diminution up to the end of the germination interval under analysis, inversely proportional to the specific activity, which is minimum in the first half of the interval, after which it is obviously intensified in the last samples (Figs. 8 - 9).

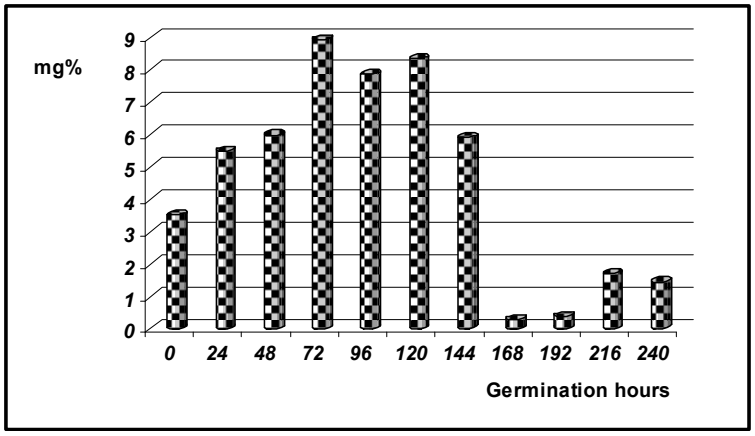

Fig. 8. Protein concentration in *Sorghum vulgare* germinated caryopses

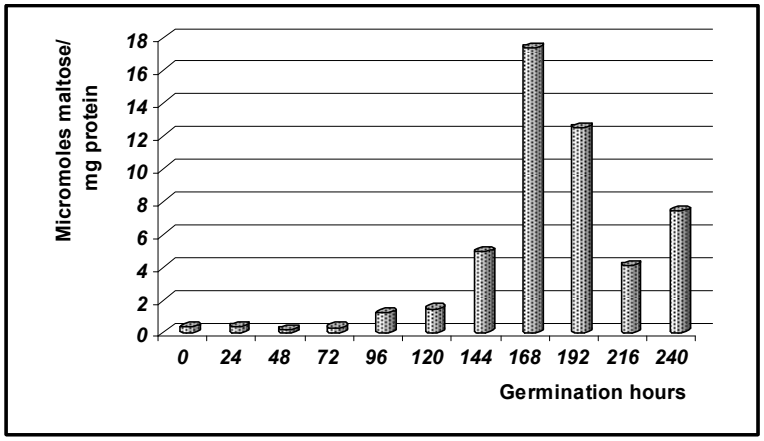

Fig.9. Specific activity dynamics of β-amylase in *Sorghum vulgare* germinated caryopses

There followed the statistical processing of the obtained results, by the **Anova bifactorial model test** with an equal number of observations in the cell, for evidencing the influence of the germination time on the amylasic activity.

As evidenced by the hypotheses of the test, as well as by the comparative graphical representation of the individual values characterizing the activity of the three enzymes, considerable differences appear both between the activity of the enzymes under study and as a function of the germination time (Fig. 10).

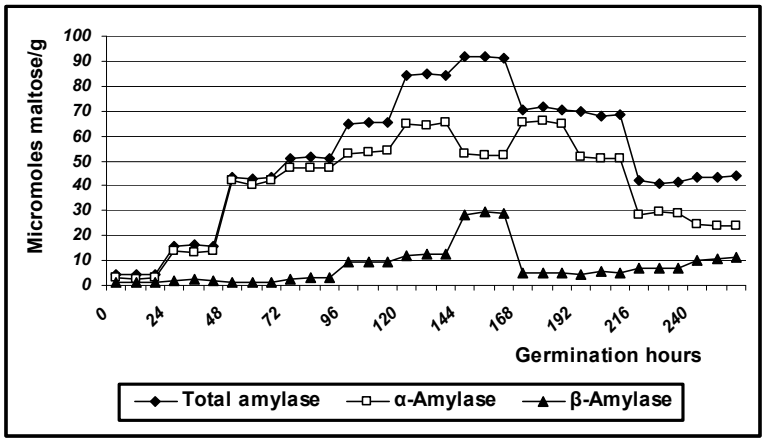

Fig.10. Individual values of amylases activity in *Sorghum vulgare* germinated caryopses

CONCLUSIONS

Analysis of the experimental results shows that the amylolytic activity in germinated caryopses of *Sorghum vulgare* does not always represent the arithmetic mean of the activities of α- and β-amylase, which might suggest that the values obtained for the two enzymes of the tissular homogenates do not fully reflect the entire catalytic potential of such enzymes.

Study of the specific activity, known as most faithfully reflecting the real catalytic capacity of such enzymes, along with eliminating the errors induced by the various homogenization and extraction conditions, evidences significant differences among the activities of total, α- and β-amylases, and also the decisive influence of the germination time on the enzymatic activity.

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