

ON THE AMYLASIC ACTIVITY OF *SORGHUM SUDANENSE* DURING GERMINATION

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Abstract: The results of the investigation devoted to the activity of total amylase, α - and β -amylase in caryopses of Sudan grass (*Sorghum sudanense*), performed along 10 days of germination under laboratory conditions, are discussed. The total amylolytic activity evidences a Gauss-type dynamics, minimum values being recorded in the beginning of the germination process and maximum values at 168 germination hours, after which a progressive decrease is registered, up to the attainment of a new minimum threshold, at 240 germination hours.

INTRODUCTION

The possibility of entering the world of plants, of understanding the processes developing inside it, of following their transformations during germination - when a simple seed gives rise up to a small plant that will become a delicate flower or an impressive tree - is a unique, extremely interesting and challenging experience, indeed, the more so that plants represent an indestructible part of the human universe, which explains man's permanent attempt of integrating them into his urban environment.

Germination assumes a certain capacity form the part of plants, which depends on several internal and external factors (soil humidity, soil aeration and temperature, permeability of the seminal tegument, seed's water content, chemical nature of the reserve substances, formation and maturation of the embryo, age and validity of seeds).

Mobilization of the reserve substances from seeds occurs immediately after their water impregnation, the most significant modifications appearing after the first 72 hours from the debut of germination.

It is generally agreed that the germination process goes on until the elaboration of chlorophyll, when the plant initiates photosynthesis reactions, thus becoming an autotrophic-photo synthesizing organism, trophically independent (TOMA, 1998; BURZO *et al.*, 1999; ZAMFIRACHE, 2001).

During germination, the reserve substances present in seeds are enzymatically degraded, a process during which the large molecular mass ones are transformed into low molecular mass water-soluble substances, to be further employed in the feeding and growing of the embryo (BOLDOR *et al.*, 1981; NEAMȚU, 1981).

MATERIALS AND METHOD

The experiments have been developed on germinated caryopses of Sudan grass (*Sorghum sudanense*) of the 2007 crop, from the Station for Agricultural Researches at Podu-Iloaiei, Iași.

Total soluble proteins were quantitatively dosed by the Bradford method 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 (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 DISCUSSIONS

The activity of total, α - and β -amylase was determined for all samples under study, taken over at intervals of 24 hours, along 10 germination days, in caryopses germinated under laboratory conditions.

During *Sorghum sudanense* seeds germination, the activity of total amylases is subjected to some modifications. Thus, during biological rest, *i.e.*, moment 0, the activity of total amylase attains its minimum threshold, recording values between 65.189 - 66.123 μM maltose/g, with a mean value of 65.557 μM maltose/g.

After 24 hours of germination, total amylase registers a mean value of 111.491 μM maltose/g, then it progressively increases, up to a maximum value of 1407.702 μM maltose/g in the seventh germination day, followed by a gradual decrease, up to 410.947 μM maltose/g (at 240 hours of germination) (Fig. 1).

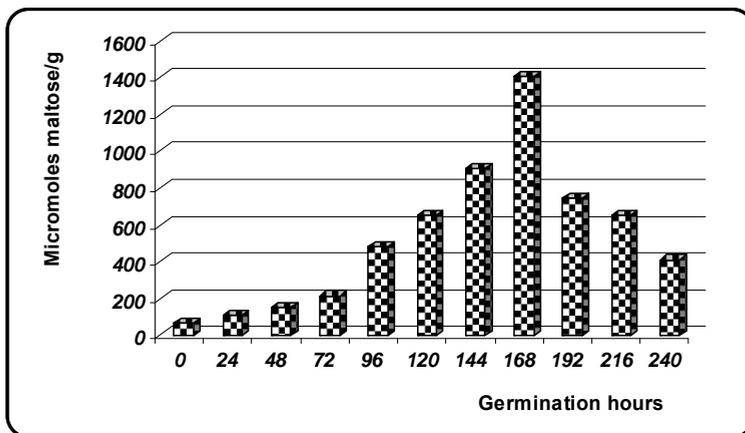


Fig.1. Total amylase activity (μM maltose/g) in germinated *Sorghum sudanense* caryopses

Starting from the mean values recorded and from the standard deviation, the superior and inferior limits of the confidence intervals were calculated - with a probability of 95% - for the activity of total amylase in germinated seeds of Sudan grass, along the germination period under investigation. Thus, as graphically illustrated, the values recorded are extremely low for all samples, with the only exception of the 7th day, when the interval is somehow larger (846.306 - 876.669 μM maltose/g) (Fig. 2).

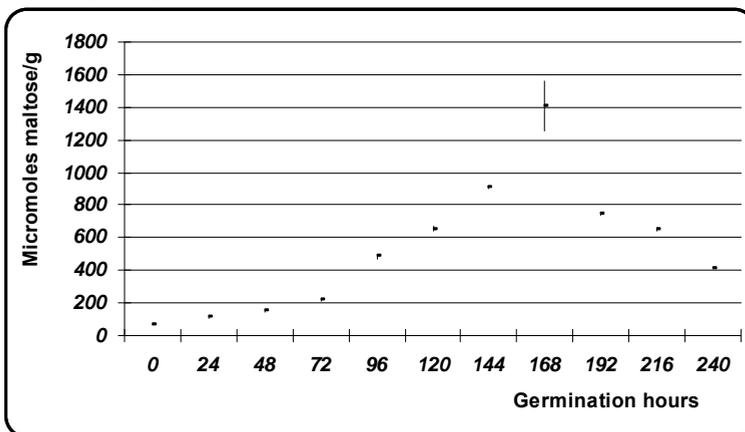


Fig.2. Confidence intervals of total amylase activity in germinated *Sorghum sudanense* caryopses

In the case of *Sorghum sudanense*, the concentration of proteins varies quite amply from one sample to another, between a minimum recorded after 48 germination hours (1.218 mg%) and a maximum (11.142 mg%) attained after 216 hours (Fig. 3).

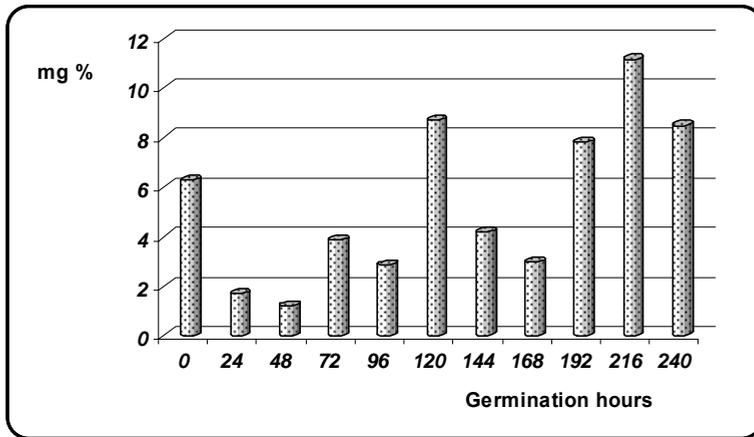


Fig.3. Protein concentration in germinated *Sorghum sudanense* caryopses

As to the specific activity of total amylase in *Sorghum sudanense*, one may observe that, in this case, the enzyme evidences fluctuating values, following an ascending line up to the 7th germination day, when the maximum threshold (473.973 μ M maltose/mg protein) is attained, followed by a sudden decrease up to 240 germination hours (48.477 μ M maltose/mg protein) (Fig. 4).

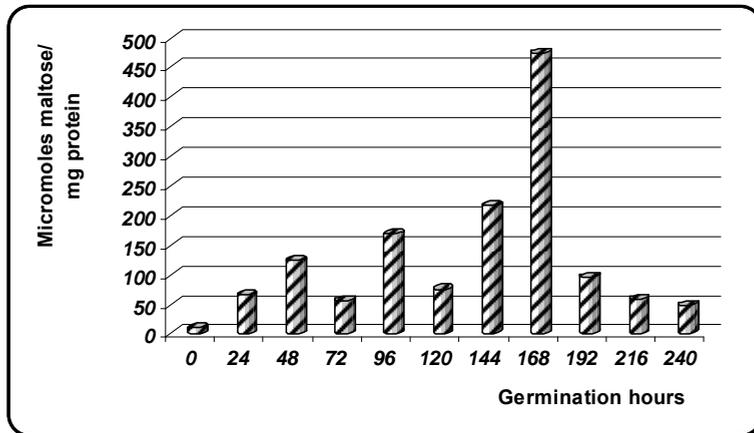


Fig.4. Dynamics of total amylase specific activity in germinated *Sorghum sudanense* caryopses

In the germinated caryopses of Sudan grass, the activity of α -amylase records, similarly with that of total amylase, a minimum value in the reference (45.588 μ M maltose/g), its maximum threshold occurring at 168 germination hours (861.488 μ M maltose/g) - which represents about two-thirds of total amyolytic activity (Fig. 5), the limits of the confidence intervals being extremely narrow, too (Fig. 6).

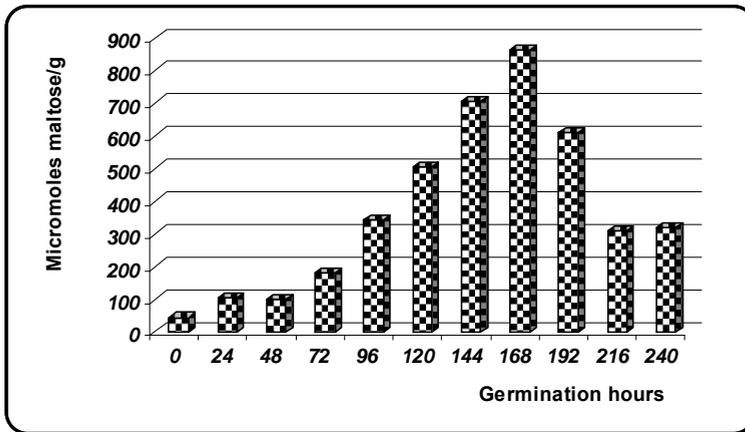


Fig.5. α -Amylase activity (μM maltose/g) in germinated *Sorghum sudanense* caryopses

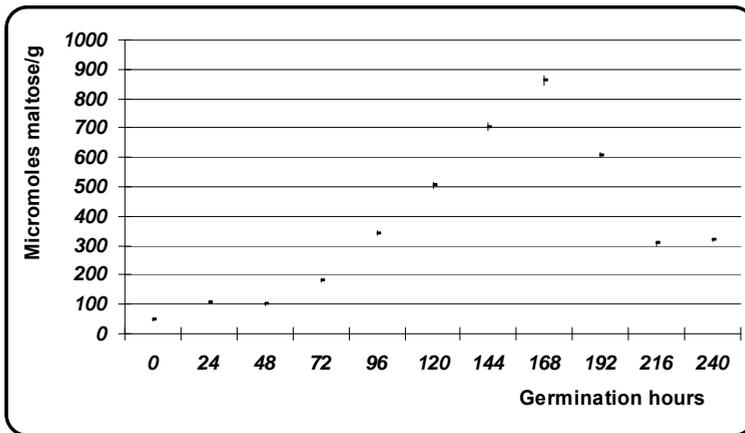


Fig.6. Confidence intervals of α -amylase activity in germinated *Sorghum sudanense* caryopses

When comparing the dynamics of proteins with the specific activity of α -amylase in *Sorghum sudanense*, a close interdependence of theirs may be observed, namely, in the interval between the 6th and 10th germination day proteic concentration decreases, concomitantly with a more intense specific activity (Figs. 7 - 8).

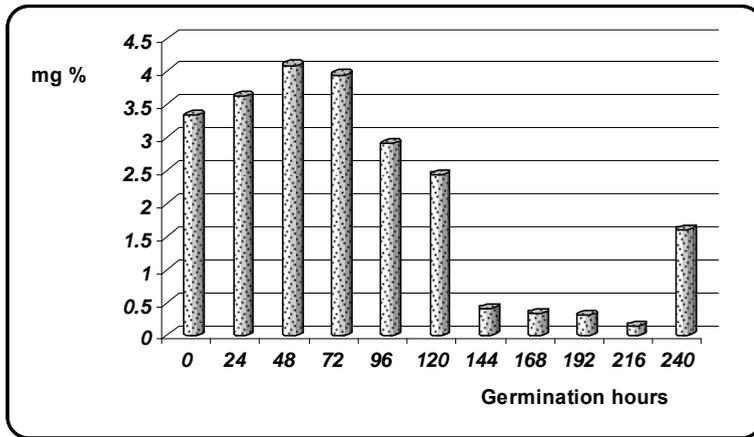


Fig.7. Protein concentration in germinated *Sorghum sudanense* caryopses

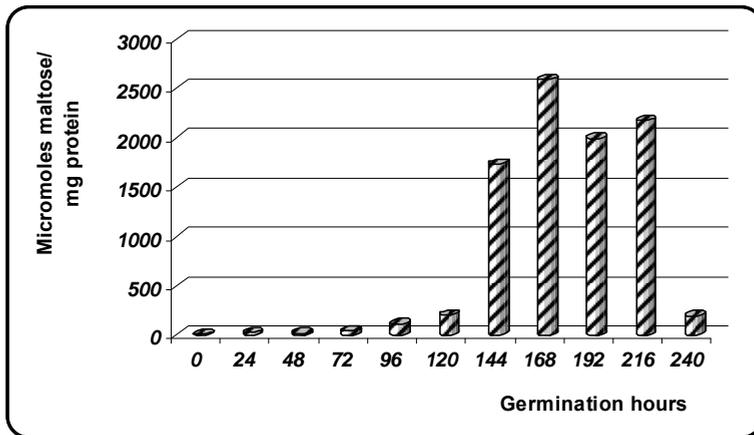


Fig.8. Dynamics of α -amylase specific activity in germinated *Sorghum sudanense* caryopses

Figure 9 shows that, in caryopses of Sudan grass occurring in biological rest, moment 0, the activity of β -amylase records a value of 18.574 μM maltose/g, followed by a decrease in the enzymatic activity up to 6.164 μM maltose/g (the minimum value) in the first 24 germination hours, and by a significant increase from one germination day to another. Consequently, the enzymatic activity records values of 128.355 μM maltose/g at 96 germination hours, up to a maximum value of 145.526 μM maltose/g, attained in the 7th germination day, after which the activity of β -amylase decreases progressively up to the last germination day taken into study (79.225 μM maltose/g).

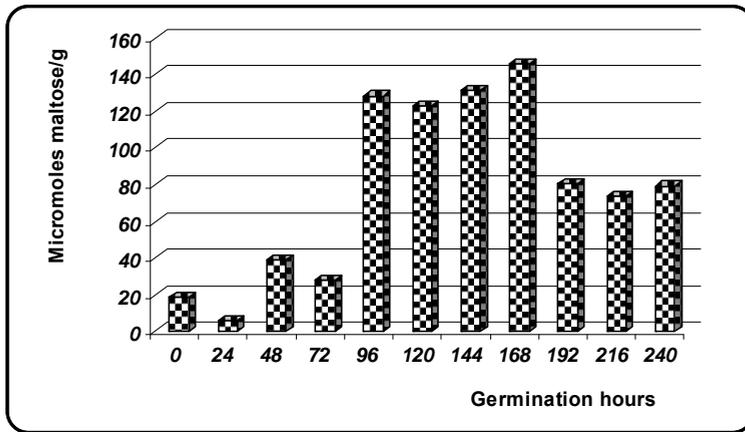


Fig.9. β-Amylase activity (µM maltose/g) in germinated *Sorghum sudanense* caryopses

Generally, the limits of the confidence intervals of β-amylase activity in germinated caryopses of Sudan grass are quite reduced, larger intervals being evidenced in the third (20.792 - 35.661 µM maltose/g), fourth (121.174 - 135.535 µM maltose/g) and ninth germination day (66.411 - 80.410 µM maltose/g), respectively (Fig. 10).

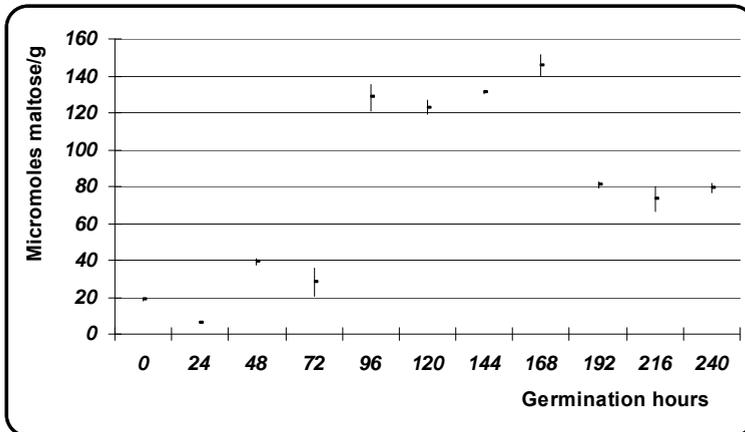


Fig.10. Confidence intervals of β-amylase activity in germinated *Sorghum sudanense* caryopses

To calculate the specific activity of β-amylase in the germinated caryopses of Sudan grass, the concentration of proteins, recorded along the 240 hours of germination, has been also determined and graphically plotted, which permitted the observation that, over the first half of the interval, proteic concentration is higher and, concomitantly, the activity is significantly lower, while it drastically decreases along the second half of the interval, the specific activity recording, too, a considerable increase (Figs. 11 - 12).

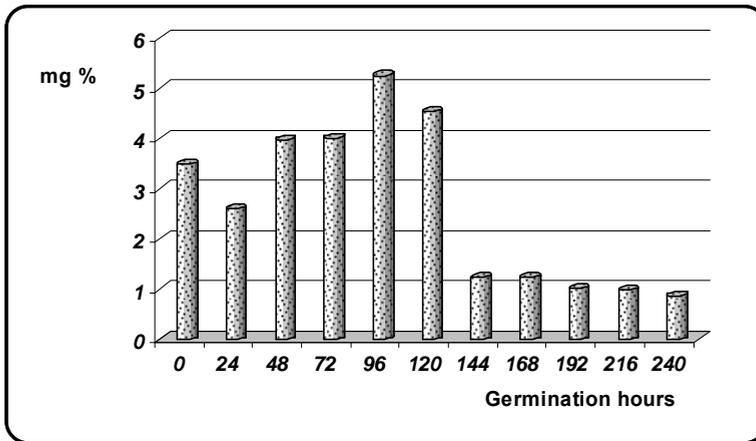


Fig.11. Protein concentration in germinated *Sorghum sudanense* caryopses

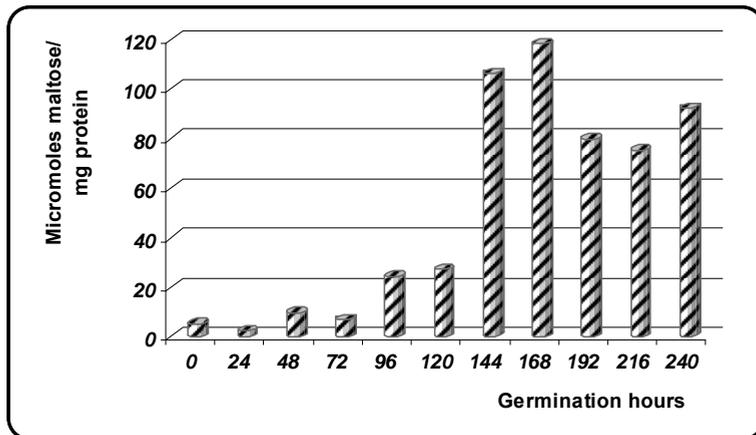


Fig.12. Dynamics of β -amylase specific activity in germinated *Sorghum sudanense* caryopses

The comparative graphical illustration of the individual values of the activity of total, α - and β -amylase in germinated caryopses of *Sorghum sudanense* evidences a much more reduced β -amylase activity, while α - and total amylase record significantly higher values, the maximum enzymatic activity being attained at 168 hours from the beginning of the germination process (Fig. 13).

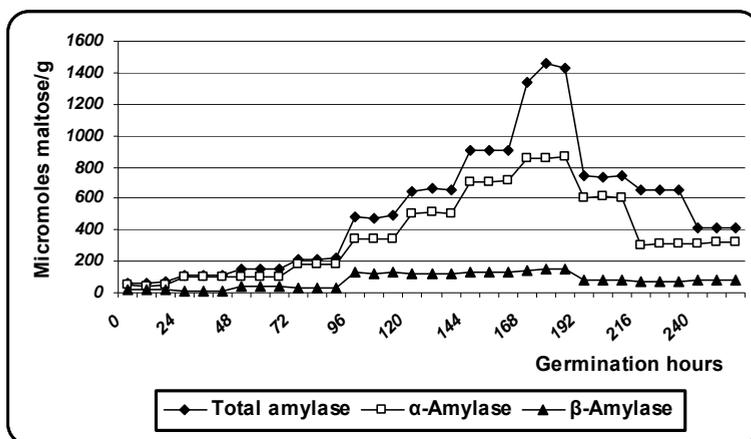


Fig.13. Individual values of amylases activity in germinated *Sorghum sudanense* caryopses

CONCLUSIONS

Along the first ten days of the germination process, amylolytic activity shows a Gauss-type dynamics, with a minimum recorded at 138 germination hours.

The concentration of total soluble proteins is somehow different and fluctuating from one enzyme to another, attaining values higher than the reference ones, after which they get stabilized at approximately the same level - meaning, possibly, a considerable acceleration of the biosynthetic process, starting with the biosynthesis of all enzymes involved in the metabolic process assuring the development of both embryo and plant.

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