

ON THE ACTIVITY OF AMYLASES *DACTYLIS GLOMERATA*

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Abstract: The paper discusses the results of the investigation on the enzymatic activity of total amylase and, respectively, of α -amylase, in the caryopses of *Dactylis glomerata*, along 10 germination days. The results obtained evidenced a maximum enzymatic activity, recorded 240 hours after the initiation of germination, both in the case of total and of α -amylase.

INTRODUCTION

Germination is an extremely complex process that involves a multitude of biochemical and physiological processes through which the glucides, lipids and reserve proteins are mobilized for assuring the precursors necessary to the biosynthetic processes occurring in the embryo and in the future plant, up to the initiation of the photo-synthetic transformations (BURZO *et al.*, 1999; BURZO and DOBRESCU, 2005).

As generally known, the plants contain both α - and β - amylases, the activity of which increases considerably during the germination of seeds containing high amounts of starch. The increase of amylasic activity during the germination of graminaceae seeds may be explained, in the case of α -amylase, by the “de novo” synthesis of the enzyme (CIORNEA *et al.*, 2006 a; 2006 b; 2006 c).

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 (COJOCARU, 1997; COJOCARU *et al.*, 2007).

The present study discusses the dynamics of the activity of both total and α -amylase in germinated *Dactylis glomerata* seeds.

MATERIALS AND METHOD

The experiments have been developed on germinated caryopses of *Dactylis glomerata* of the 2006 crop. 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 activity of both enzymes was determined by the Noelting - Brenfeld method, results being expressed in micromoles maltose/g (ARTENIE and TĂNASE, 1981).



Fig.1 . Impregnated seeds of *Dactylis glomerata* (original photo)

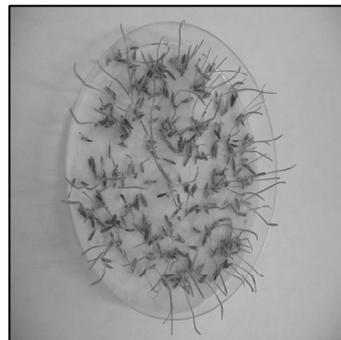


Fig.2 . Seeds of *Dactylis glomerata* at 96 hours of germination (original photo)

RESULTS AND DISCUSSION

The data listed in Table 1 show that, in *Dactylis glomerata* caryopses occurring in biological rest, at the zero moment, the activity of total amylase records its lowest value (58.873 - 60.561 μM maltose/g).

Table I. Total amylase activity of *Dactylis glomerata* as a function of the germination time

Hours of germination	Activity (μM maltose/g)	Average (μM maltose/g)	Standard error	Standard deviation
0 (P ₀)	58.873	60.186	0.505	0.875
	60.561			
	60.123			
24 (P ₁)	115.851	115.307	1.342	2.324
	112.759			
	117.312			
48 (P ₂)	299.435	302.232	1.631	2.825
	305.085			
	302.175			
72 (P ₃)	424.785	419.597	3.728	6.458
	412.364			
	421.643			
96 (P ₄)	647.910	652.487	2.781	4.817
	657.513			
	652.039			
120 (P ₅)	802.391	812.707	5.626	9.745
	821.759			
	813.972			
144 (P ₆)	798.325	791.837	3.373	5.843
	786.988			
	790.198			
168 (P ₇)	503.972	508.592	2.657	4.602
	513.176			
	508.627			
192 (P ₈)	470.513	470.613	0.987	1.71
	468.964			
	472.381			
216 (P ₉)	161.913	165.587	2.014	3.489
	168.856			
	165.991			
240 (P ₁₀)	78.113	80.876	1.494	2.588
	83.244			
	81.272			

Starting with the first 24 germination hours, the activity of total amylase increases considerably from one day to another. Therefore, the enzymatic activity takes values of 115.307 μM maltose/g in the first germination day, up to a maximum value of 812.707 μM maltose/g, recorded in the 5th day of germination. Further on, the activity of total amylases gradually decreases, from 791.837 μM maltose/g after 144 germination hours, up to 80.876 μM maltose/g, after 240 hours of germination (Fig.3).

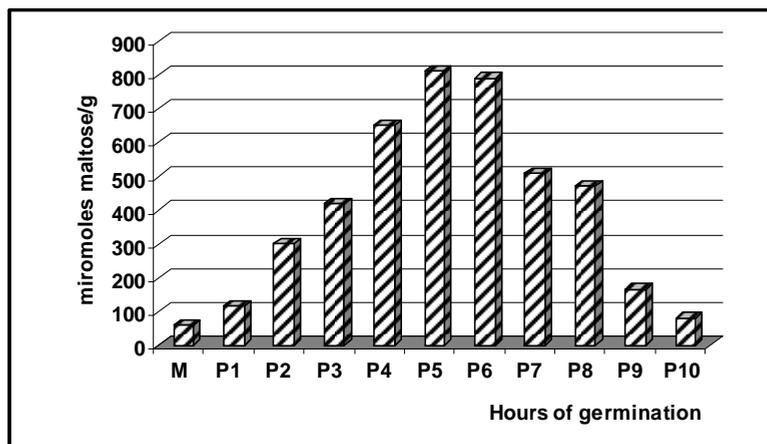


Fig.3. Dynamics of the absolute activity of total amylase in the germination of *Dactylis glomerata* seeds

Calculation of the percent value of the activity of total amylase in *Dactylis glomerata* permits the observation that, comparatively with the impregnated sample (the reference), the activity of total amylase increases gradually, its maximum (100%) being recorded after 120 germination hours, which is followed by a gradual decrease, up to 9.951%, in the last germination day (Fig.4).

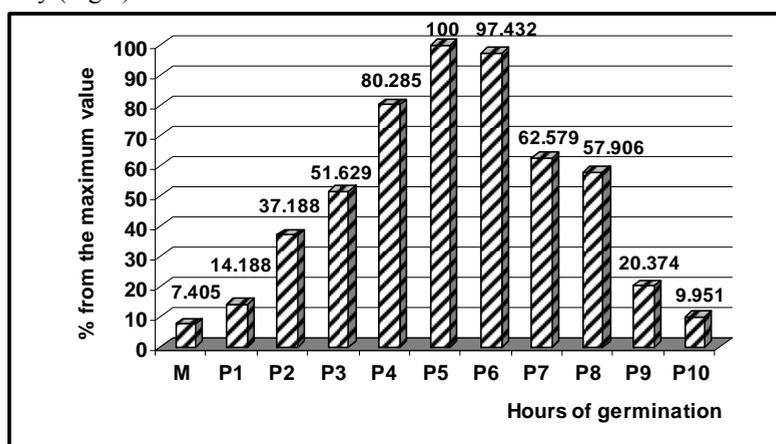


Fig.4. Dynamics of the relative activity (%) of total amylase in the germination of *Dactylis glomerata* seeds

The activity of α -amylase has been recorded by the same method for all samples taken into study, while the results obtained, expressed as μM maltose/g, have been listed in Table 2. The same variation of the enzymatic activity with the germination time is here recorded, as well, that is a progressive increase in the first days, up to a minimum value, recorded towards the end of the germination period.

Table II. α -Amylase activity of *Dactylis glomerata* as a function of the germination time

Hours of germination	Activity (μM maltose/g)	Average (μM maltose/g)	Standard error	Standard deviation
0 (P ₀)	39.538	39.477	0.398	0.691
	40.135			
	38.757			
24 (P ₁)	76.185	76.299	0.785	1.361
	74.998			
	77.713			
48 (P ₂)	211.988	214.993	1.669	2.891
	217.755			
	215.236			
72 (P ₃)	224.484	228.618	2.131	3.692
	229.783			
	231.588			
96 (P ₄)	457.613	466.394	6.055	10.487
	463.562			
	478.007			
120 (P ₅)	598.783	595.871	4.459	7.724
	601.716			
	587.113			
144 (P ₆)	425.781	435.02	4.835	8.374
	437.167			
	442.113			
168 (P ₇)	325.724	321.192	2.28	3.949
	319.371			
	318.481			
192 (P ₈)	251.681	247.546	2.382	4.127
	247.531			
	243.427			
216 (P ₉)	48.486	50.256	0.938	1.626
	51.684			
	50.598			
240 (P ₁₀)	38.184	37.183	2.675	4.634
	32.129			
	41.235			

As shown in Table 2, the activity of α -amylase records a minimum value in the impregnated seeds (39.477 μ M maltose/g) after which, starting with the first day of germination, a significant increase is recorded, the maximum being attained in the 5th germination day (595.871 μ M maltose/g).

Attainment of the maximum value is followed by a gradual decrease of the α -amylase activity, the value recorded after 144 germination hours being of 435.02 μ M maltose/g, the minimum being recorded, here again, in the 10th germination day (37.183 μ M maltose/g).

For a more thorough representation of the α -amylase activity of *Dactylis glomerata* caryopses, the average values of the enzymatic activity along the 240 germination hours have been plotted graphically (Fig.5) while, for a correct estimation of the variation recorded by the α -amylase activity of *Dactylis glomerata* caryopses, the percent values have been represented graphically, as well. As shown in Figure 6, the α -amylase records its minimum value at 240 germination hours (6.24%), comparatively with the impregnated (reference) sample.

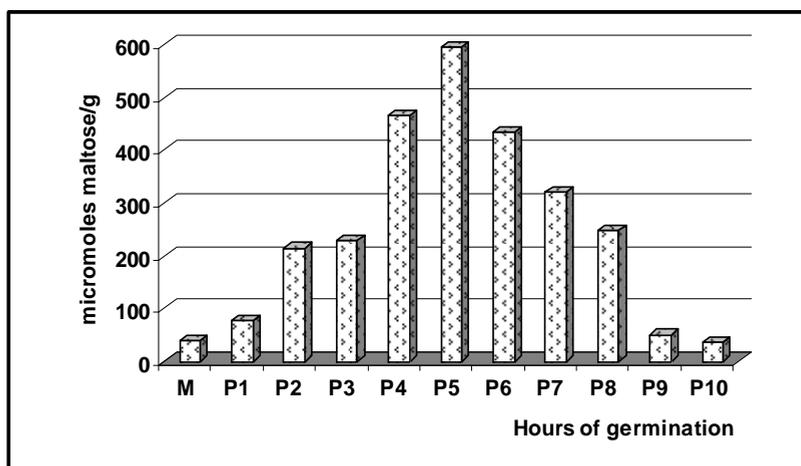


Fig.5. Dynamics of the absolute activity of α -amylase in the germination of *Dactylis glomerata* seeds

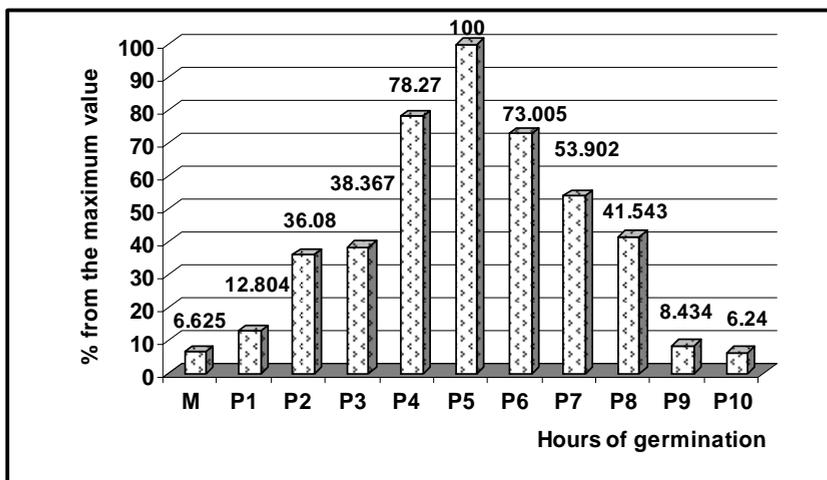


Fig.6. Dynamics of the relative activity (%) of α -amylase in the germination of *Dactylis glomerata* seeds

As a function of the average values and standard deviation for all samples under analysis, the upper and lower limits of the variability intervals have been subsequently calculated, on the basis of the critical value $t(\alpha, n-1)$, given by $\alpha = 0.05$ and $n-1$ degrees of freedom, that is $t(0.05, 10) = 2,228$ (VARVARA et al., 2001).

As evidenced by the graphical representations, too (Figs.7 - 8), the limits of the confidence intervals are extremely narrow, for both the activity of total and of α -amylase.

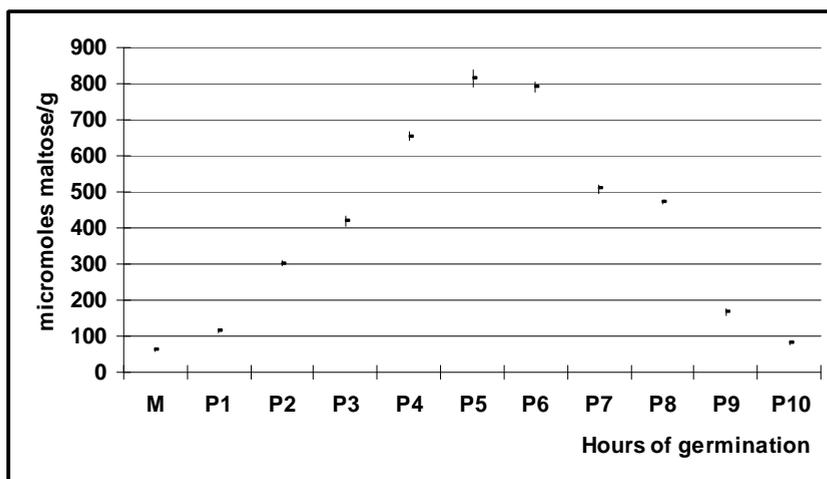


Fig.7. Confidence intervals of total amylase activity in *Dactylis glomerata*

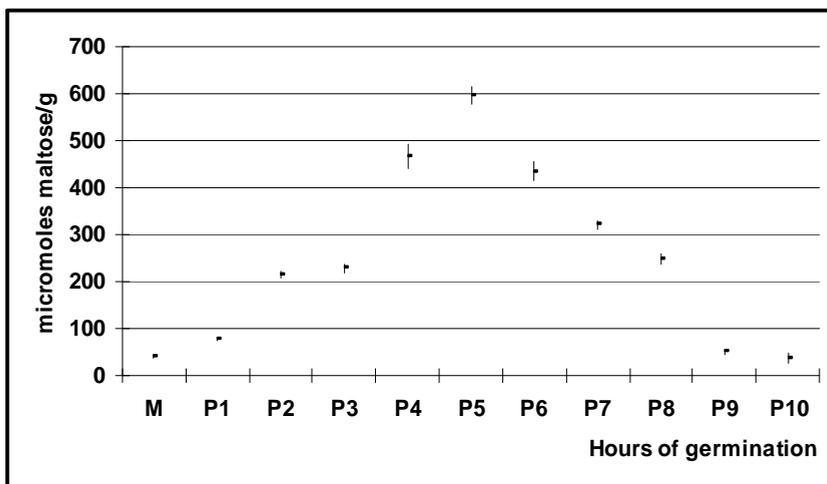


Fig.8. Confidence intervals of α -amylase activity in *Dactylis glomerata*

Figure 9 plots comparatively the activity of total and α -amylase. As evidenced, the activity of both enzymes follows the same curve, both attaining the maximum in the 5th germination day.

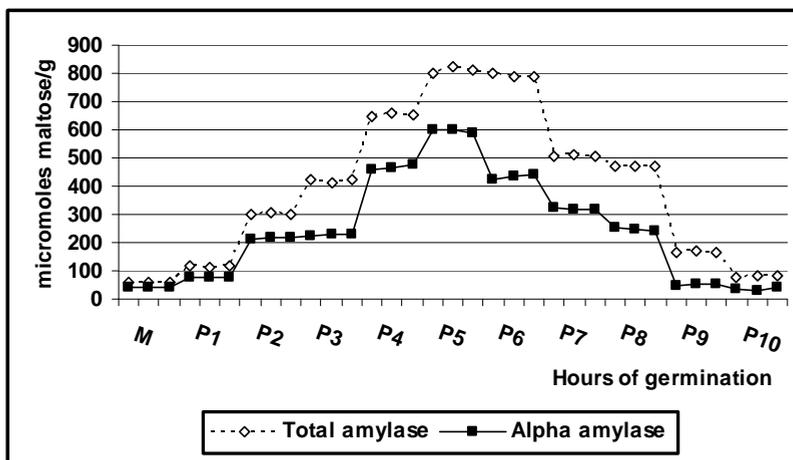


Fig.9. Comparative representation of individual values of the amylases activity in *Dactylis glomerata*

CONCLUSIONS

The results obtained in determining the activity of total and α -amylase in germinated *Dactylis glomerata* caryopses led to the following conclusions:

In the case of both total and α -amylase, the maximum value of the enzymatic activity has been recorded in the impregnated seed stage.

The maximum threshold of total (812.707 μM maltose/g) and α -amylase (595.871 μM maltose/g) was recorded in the 5th day of germination.

The limits of the confidence intervals of the activity manifested by the two enzymes under study are extremely narrow.

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