# DYNAMICS OF RESERVE POLYGLUCIDE MOBILIZATION UNDER THE ACTION OF α-AMYLASE IN VARIOUS GRAMINACEAE SPECIES FROM CULTIVATED AND SPONTANEOUS FLORA

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**Key words:** starch, α-amylase, germination, graminaceae

**Abstract:** The investigations followed the mobilization of reserve polyglucides under the action of α-amylase, during the germination process, in some species of graminaceae from both cultivated and spontaneous flora (*Panicum miliaceum*, *Sorghum vulgare* and *Bromus sterilis*). The experimental results obtained showed that - under the action of amylases - starch gets hydrolyzed at an amazingly high rate, hydrolysis beginning as early as the first hours of germination; at the same time, major differences have been observed from one species to another.

## INTRODUCTION

Natural lawns represent a well-established part of the ecosystems of the biosphere. Also, as part of the agricultural, anthropic or anthropizing ecosystems, the ecosystem represented by secondary, temporary lawns has a special importance of its own.

Study of lawns ecosystems is especially significant - from a scientific point of view - within the general, larger concept of biodiversity, while evidencing an equally considerable - economic, agricultural and zootechnical - practical importance.

The economic and ecologic value of permanent lawns is unanimously acknowledged nowadays, once they represent the main source of food for domestic animals and the habitat for the wild ones, to say nothing of their contribution to preventing soil erosion and to improving its structure and fertility.

That is why, the researches developed in this field have been constantly extended and permanently improved, in both conception and methodology, special stress being laid on the systematics, biology, plant reaction to fertilization, genetic aspects, melioration, plant resistance to un favorable environmental conditions, maladies and pests (MURARIU, 2003).

The present paper presents the specific dynamics of germination in graminaceae species belonging to cultivated and spontaneous flora, with botanical and technological value, but without any alimentary importance (with the exception of millet). Once known that such graminaceae may be equally viewed as contaminating species (weeds) for cereal cultures, the study of germination and of some potential inhibitors of germination is of special interest.

In this respect, knowledge on the mobilization pathways of starch - the main reserve substance - through the action of amylases, during germination, are extremely important.

## MATERIALS AND METHOD

The experiments have been developed on germinated caryopses of graminaceae from cultivated and spontaneous flora, namely *Panicum miliaceum*, *Sorghum vulgare* and *Bromus sterilis* of the 2007 crop. Germination was made in Petri boxes lined inside with filtering paper, under laboratory conditions, samples taking over being performed at intervals of 24 hours, for 10 days.

The quantitative determination of total soluble proteins was determined by Bradford method, of starch - by the polarimetric method, and the enzymatic activity by 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 calculated the mean, error and standard deviation, mean variation and precision coefficient, as well as limits of the confidence intervals (FOWLER *et al*., 2000).

## RESULTS AND DISCUSSIONS

The germination dynamics of the cultivated and spontaneous graminaceae taken into study evidences several differences among species, starch concentration being gradually reducing, as a result of the hydrolytic processes mediated by amylases.

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

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

A first objective of the present study involved determination of the amount of starch in caryopses during germination, once known that, in cereals, starch synthesis occurs - at the level of the endosperm - according to an unique mechanism, involving enzymatic isoforms which are not to be found in the tissues of other non-cereal plants (GUAN and PREISS, 1993; BLAUTH *et al*., 2002; GENSCHEL *et al*., 2002; DINGES *et al*., 2003; JAMES *et al*., 2003). On the other hand, starch represents the main reserve polysaccharide in cereals, which might mean that, during germination, a perfect correlation is manifested between the total amylolytic activity and the rate of starch degradation. Furthermore, intensification of the metabolic processes involves a dynamics characteristic to the concentration of total soluble proteins, at least due to the activity of the enzymes and to the acceleration of the biosynthesis of some new ones.

Table I. Values of the main statistical indices of α-amylase activity in germinated *Panicum miliaceum* caryopses

| Germination hours | Mean activity (μM maltose/g) | S $\bar{x}$ | S (σ) | VC% | m% | SL | IL |
|---|---|---|---|---|---|---|---|
| M (0) | 39.476 | 0.398 | 0.691 | 1.75 | 1.01 | 41.193 | 37.76 |
| $P_1$ (24) | 76.298 | 0.785 | 1.361 | 1.783 | 1.029 | 79.679 | 72.917 |
| $P_2$ (48) | 214.993 | 1.669 | 2.891 | 1.344 | 0.776 | 222.175 | 207.81 |
| $P_3$ (72) | 228.618 | 2.131 | 3.692 | 1.615 | 0.932 | 237.79 | 219.445 |
| $P_4$ (96) | 466.394 | 6.055 | 10.487 | 2.248 | 1.298 | 492.447 | 440.34 |
| $P_5$ (120) | 595.870 | 4.459 | 7.724 | 1.296 | 0.748 | 615.060 | 576.681 |
| $P_6$ (144) | 435.020 | 4.835 | 8.374 | 1.925 | 1.111 | 455.824 | 414.215 |
| $P_7$ (168) | 321.192 | 2.280 | 3.949 | 1.229 | 0.71 | 331.004 | 311.379 |
| $P_8$ (192) | 247.546 | 2.382 | 4.127 | 1.667 | 0.962 | 257.798 | 237.294 |
| $P_9$ (216) | 50.256 | 0.938 | 1.626 | 3.235 | 1.868 | 54.295 | 46.216 |
| $P_{10}$ (240) | 37.182 | 2.675 | 4.634 | 12.465 | 7.196 | 48.696 | 25.669 |

S $\bar{x}$ = mean standard error, S (σ) = standard deviation, VC% = mean variation coefficient, m% = mean precision coefficient, SL = superior limit of the confidence interval, LI = inferior limit of the confidence interval

As to the activity of α-amylase, graphically plotted in Figure 1, it registers a minimum value in the impregnated seeds (39.476 μM maltose/g) after which, starting with the first day from the beginning of germination, a significantly higher activity may be observed, the maximum of which is attained in the 5$^{th}$ germination day (595.87 μM maltose/g); later on, after this maximum threshold is attained, there follows a gradual decrease in the activity of α-amylase, the value recorded at 144 germination days being of 435.02 μM maltose/g while, this time, the minimum occurs in the 10$^{th}$ germination day (37.182 μM maltose/g).

The experimental results obtained for all samples under investigation were processed statistically, on calculating the error and standard deviation, the variation and precision coefficient of the mean, as well as the (inferior and superior) limits of the confidence intervals (Table I).

The data thus collected support the idea that the limits of the confidence intervals are extremely narrow for each hour interval in part (37.76 - 41.193 µM maltose/g, 311.379 - 331.004 µM maltose/g).

The dynamics of amylasic activity permits the assumption that mobilization of reserve starch for assuring the energy necessary in metabolic processes initiates in the first hours of germination, even if, in the beginning, its catalytic activity is quite reduced.

The gradual decrease of the amylolytic activity in the second stage of the interval under investigation might be explained by a gradual reduction in the amount of starch, as well as by the initiation of the photosynthetic process, known as assuring the precursors of the metabolic processes.

Figure 1 illustrates the progressive decrease of starch concentration, the value of which was of 65.4 g% in the reference, which agrees with the literature data (ELIASSON and LARSSON, 1993, citated by KUKTAITÉ, 2004), the value attained after 10 days being of 10.6 g%.

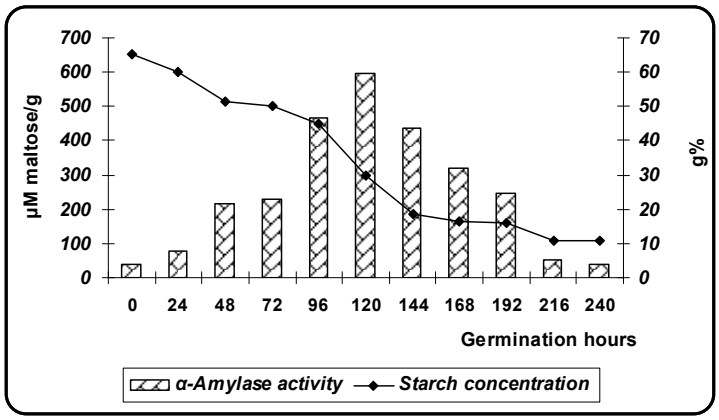


Fig.1. Correlation between starch concentration and absolute α-amylase activity in germinated *Panicum miliaceum* caryopses

To calculate the specific activity of α-amylase in millet caryopses subjected to germination under laboratory conditions, proteic concentration in the extracts obtained was calculated, the mean values being plotted in Figure 2. As observed, proteins vary over the 6.548 mg% (at 48 germination hours) and 14.235 mg% (at 120 germination hours) interval, the oscillations being significant from one sample to another.

As generally known, determination of the specific enzymatic activity expresses most faithfully the real catalytic capacity of the enzymes, eliminating the errors induced by the different conditions of homogenization and extraction. That is why, the specific activity of α-amylase was calculated in germinated *Panicum miliaceum* seeds, which permitted to follow its dynamics along the whole germination period. The data obtained (Figure 2) show that they follow the same ascending curve in the first germination days under study, after which the curve gets a descending aspect, which is also the case of the absolute activity, with the only difference that the maximum value (58.379 µM maltose/mg protein) is attained at 96 hours of germination.

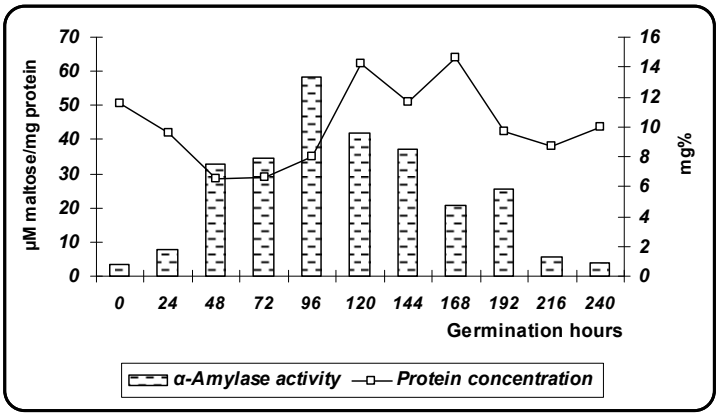


Fig.2. Correlation between soluble protein concentration and specific α-amylase activity in germinated *Panicum miliaceum* caryopses

As to the dynamics of starch concentration over the germination period considered for the study, it decreases over the whole experimental time, although only moderately, from 57.865 g% in the impregnated sample to 21.7 g% - in the 10th day of the study.

Table II. Values of the main statistical indices of α-amylase activity in germinated *Sorghum vulgare* caryopses

| Germination hours | Mean activity (µM maltose/g) | S $\bar{x}$ | S (σ) | VC% | m% | SL | IL |
|---|---|---|---|---|---|---|---|
| M (0) | 2.85 | 0.062 | 0.107 | 3.773 | 2.178 | 3.117 | 2.582 |
| $P_1$ (24) | 13.561 | 0.253 | 0.438 | 3.234 | 1.862 | 14.65 | 12.472 |
| $P_2$ (48) | 41.504 | 0.65 | 1.126 | 2.715 | 1.567 | 44.303 | 38.704 |
| $P_3$ (72) | 47.213 | 0.11 | 0.192 | 0.406 | 0.234 | 47.690 | 46.736 |
| $P_4$ (96) | 53.619 | 0.265 | 0.459 | 0.857 | 0.495 | 54.761 | 52.477 |
| $P_5$ (120) | 64.553 | 0.325 | 0.563 | 0.872 | 0.503 | 65.951 | 63.154 |
| $P_6$ (144) | 52.345 | 0.206 | 0.356 | 0.681 | 0.393 | 53.232 | 51.458 |
| $P_7$ (168) | 65.41 | 0.305 | 0.529 | 0.809 | 0.467 | 66.725 | 64.096 |
| $P_8$ (192) | 51.158 | 0.11 | 0.19 | 0.372 | 0.215 | 51.632 | 50.684 |
| $P_9$ (216) | 28.978 | 0.237 | 0.41 | 1.418 | 0.818 | 29.999 | 27.957 |
| $P_{10}$ (240) | 24.038 | 0.112 | 0.194 | 0.807 | 0.465 | 24.519 | 23.556 |

S $\bar{x}$ = mean standard error, S (σ) = standard deviation, VC% = mean variation coefficient, m% = mean precision coefficient, SL = superior limit of the confidence interval, LI = inferior limit of the confidence interval

Statistical analysis of the experimental results evidences that the lowest variation coefficient of the mean value (0.681%) is recorded in the 6th germination day, while the maximum value (3.773%) is attained in the impregnated seed stage; here again, the confidence intervals vary between quite narrow limits for all samples under analysis (Table II).

In *Sorghum vulgare*, the activity of α-amylase shows low values, ranging between 2.85 µM maltose/g in the impregnated seed stage and 65.41 µM maltose/g in the 7th germination day, after which it slightly decreases, up to a value of 24.038 µM maltose/g, recorded at 240 germination hours, which might be explained by the extremely low germination capacity of the seeds, as well as by a somehow belated germination (Fig. 3).

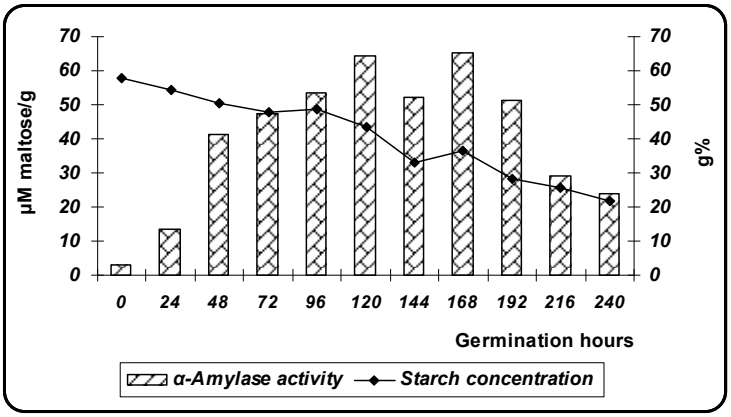


Fig.3. Correlation between starch concentration and absolute α-amylase activity in germinated *Sorghum vulgare* caryopses

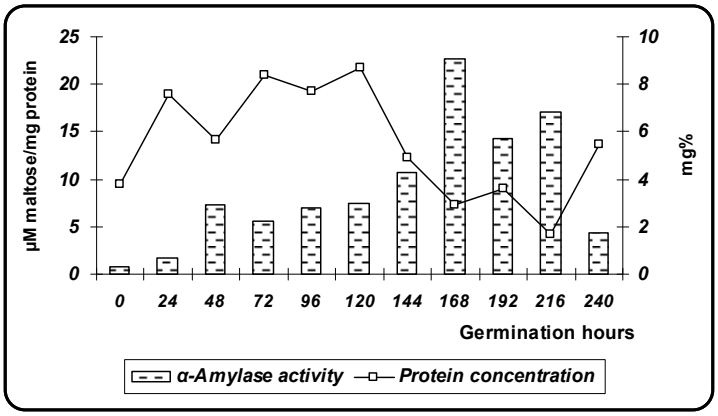


Fig.4. Correlation between soluble protein concentration and specific α-amylase activity in germinated *Sorghum vulgare* caryopses

Activation of the enzymatic equipment occurs immediately after caryopses impregnation, α-amylase intensifying its activity as early as the second germination day, when a value of 20.66% of the maximum value is registered, after which it gradually increases, remaining at maximum values for longer periods (72.18% at 72 germination hours, 81.97% at 96 germination hours, 98.68% at 120 germination hours and 78.21% at 192 germination hours, respectively).

The concentration of total soluble proteins in germinated hair grass seeds shows much more reduced values, comparatively with those of millet, representing about one-third of the values characterizing the previously mentioned species.

Determination of the specific activity of α-amylase from 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 (Fig. 4).

As known, starch occurs in leaves - as the first product of photosynthesis - in the form of primary granules; in a subsequent stage, under the action of enzymes, it is hydrolyzed in D-glucose monosaccharide, which is transported either freely or as phosphoric esters towards the other plant organs, where starch is re-synthesized and deposited as secondary granules.

The quantitative determination of starch concentration in the *Bromus sterilis* seeds subjected to germination evidences relatively constant values along the first six germination days under study, followed by a gradual decrease towards the end of the interval considered for analysis, according to the amylolytic activity put into evidence (CIORNEA *et al*., 2007).

Once again, the experimental results were processed statistically, the values obtained being listed in Table III.

Table III. Values of the main statistical indices of α-amylase activity in germinated *Bromus sterilis* caryopses

| Germination hours | Mean activity (μM maltose/g) | S $\bar{x}$ | S (σ) | VC% | m% | SL | IL |
|---|---|---|---|---|---|---|---|
| M (0) | 41.595 | 0.281 | 0.488 | 1.173 | 0.677 | 42.807 | 40.382 |
| $P_1$ (24) | 65.282 | 0.096 | 0.166 | 0.254 | 0.147 | 65.696 | 64.869 |
| $P_2$ (48) | 94.251 | 0.265 | 0.46 | 0.488 | 0.282 | 95.395 | 93.107 |
| $P_3$ (72) | 115.124 | 1.26 | 2.183 | 1.896 | 1.094 | 120.548 | 109.7 |
| $P_4$ (96) | 123.351 | 0.485 | 0.84 | 0.681 | 0.393 | 125.438 | 121.263 |
| $P_5$ (120) | 229.478 | 0.644 | 1.116 | 0.486 | 0.28 | 232.25 | 226.705 |
| $P_6$ (144) | 259.494 | 0.621 | 1.077 | 0.415 | 0.239 | 262.17 | 256.817 |
| $P_7$ (168) | 721.617 | 0.731 | 1.267 | 0.175 | 0.101 | 724.765 | 718.468 |
| $P_8$ (192) | 719.639 | 0.582 | 1.008 | 0.141 | 0.08 | 722.143 | 717.135 |
| $P_9$ (216) | 636.198 | 20.01 | 34.659 | 5.447 | 3.145 | 722.298 | 550.098 |
| $P_{10}$ (240) | 460.554 | 2.556 | 4.428 | 0.961 | 0.155 | 471.554 | 449.553 |

S $\bar{x}$ = mean standard error, S (σ) = standard deviation, VC% = mean variation coefficient, m% = mean precision coefficient, SL = superior limit of the confidence interval, LI = inferior limit of the confidence interval

The graphical representation of the correlation between the α-amylase activity and the concentration of its specific substrate shows that, up to 144 germination hours, the two phenomena *i.e*., increase of enzymatic activity and quantitative decrease of starch, are closely connected by a reversely proportional reaction, namely a progressive increase of the amylolytic activity is recorded, while decrease in substrate concentration occurs at the same rate.

In *Bromus sterilis*, the α-amylase activity is also minimum (41.595 μM maltose/g) in the impregnated seed stage, followed by a slow increase (259.494 μM maltose/g) up to the 6$^{th}$ day,

the maximum threshold (721.617 µM maltose/g) being attained in the last 96 hours of the interval under analysis (Fig. 5).

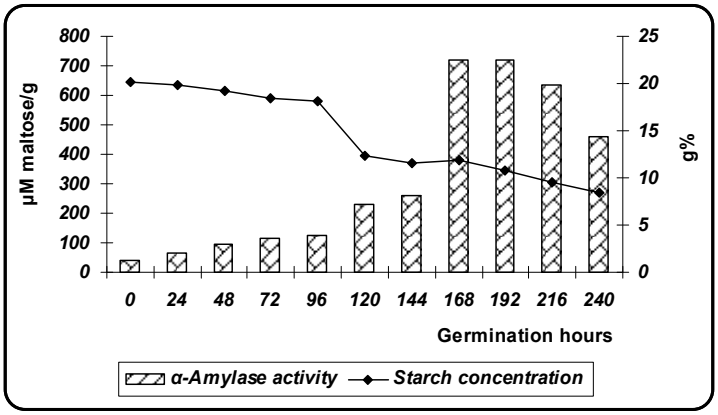


Fig.5. Correlation between starch concentration and absolute α-amylase activity in germinated *Bromus sterilis* caryopses

Protein concentration in the extracts employed for determining the α-amylase activity show fluctuating values, ranging between 0.789 mg% (in the reference), 1.563 mg% in the second germination day and 7.264 mg%, respectively, in the last day under study. The dynamics of the specific activity of α-amylase in *Bromus sterilis* varies within quite large limits, the maximum (29.661 µM maltose/mg protein) being attained at 192 germination hours (Fig. 6).

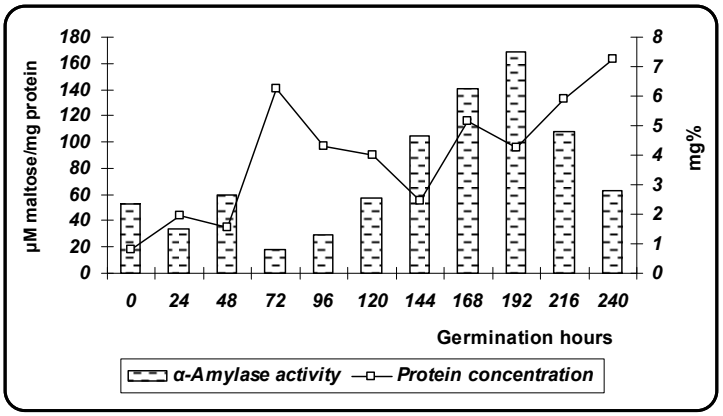


Fig.6. Correlation between soluble protein concentration and specific α-amylase activity in germinated *Bromus sterilis* caryopses

## CONCLUSIONS

Analysis of the experimental results obtained led to the following general conclusions:

During germination, the process of reserve starch mobilization under the action of amylase is extremely intense, in close correlation with the enzymatic activity characterized by an increasing dynamics, the two phenomena being connected by a reversely proportional relation.

As to the specific enzymatic activity expresses most faithfully the entire catalytic capacity of the enzymes, the total soluble proteins were determined, evidencing an intense variation, induced, on one side, by the germination time and, on the other, by the species.

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