ON THE DYNAMICS OF THE ACTIVITY OF SOME ENZYMES INVOLVED IN THE GLUCIDIC METABOLISM IN *SORGHUM SUDANENSE* AND *SORGHUM VULGARE* DURING THE GERMINATION PERIOD

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Key words: starch, amylase, glucanphosphorylase, *Sorghum sudanense*, *Sorghum vulgare*

Abstract: The main objective of the present investigation referred to the activity of amylase and α-glucanphoshorylase, two enzymes involved in the metabolism of reserve glucides in two graminaceae species (*Sorghum sudanense* and *Sorghum vulgare*), in correlation with the dynamics of starch concentration, followed along ten germination days. The results obtained evidenced that the enzymatic activity follows a Gauss-type curve, with a minimum in the beginning of the germination process and a maximum recorded over the 144 - 192 germination hour interval, unlike the starch concentration, characterized by a decreasing dynamics along the whole time interval under analysis.

INTRODUCTION

As a first product of photosynthesis, starch occurs in leaves in the form of primary granules. In a subsequent stage, under the action of enzymes, starch is hydrolyzed into the monosaccharide D-glucose-compound, which is carried, either free or as phosphoric acids, towards the other organs of the plant, where re-synthesis of starch - deposited as secondary granules - occurs.

In heterotrophic tissues, such as seeds and tubercles, starch is deposited for months or even years, being therefore defined as reserve starch, unlike the chloroplastic starch from leaves, which is a transitory element (GEIGENBERGER and RITTE, 2000; GEIGENBERGER *et al*., 2001).

Several studies have evidenced the high amounts of starch present in the plants frequently employed in food industry, such as: rice (GOPALDAS *et al.*, 1986; WAHED *et al*., 1994), barley (HANSEN *et al*., 1989), sorghum (LORRI and SVANBERG, 1993; MOSHA and SVANBERG, 1983), corn (GOPALDAS *et al*., 1988) and maize (HELLAND *et al*., 2001).

Amylase and α-glucanphosphorylase are quite frequently occurring in nature, both in the vegetal and animal reign. As a general rule, amylases are present where starch is also present, for example in potatoes, cereal flour, beans or soy bean. Equally, the amylases occur in tissues and liquids of animal origin (DUMITRU and IORDĂCHESCU, 1974). α-Glucanphosphorylase has been identified - in both its cytosolic and amyloplastic form - in pea seeds and leaves (MU *et al*., 2001), beans (SUDA *et al*., 1987), potatoes tubers, rice and barley seeds (RICHARDSON and MATHESON, 1977), spinach (STEUP and SCHACHTELE, 1986), sweet potato (CHANG *et al*., 1987; LU *et al*., 1995), bananas (DA MOTA *et al*., 2002) and marine algae (YU and PEDERSEN, 1991).

MATERIALS AND METHOD

The experiments were developed on germinated caryopses of *Sorghum sudanense* (Sudan grass) and *Sorghum vulgare* (sorghum). Seed germination was made in Petri plates, lined inside with filter paper wet with distilled water, at room temperature, following a first treatment with oxygenated water for the removal of the pathogenic germs, samples taking over being performed at intervals of 24 hours, for ten days.

The amylasic activity was determined by the Noelthing - Brenfled method, the α-glucanphosphorylase - by decreasing the amount of anorganic phosphorous in the reaction medium, as a result of starch phosphorolysis, while the starch concentration - by the polarimetric method, 3 repetitions being made for each sample in part (ARTENIE and TĂNASE, 1981; ARTENIE *et al*., 2007).

RESULTS AND DISSCUSION

As the amylolytic activity depends strictly on both the germination degree and temperature and pH of the incubation medium, a first stage of the present study was oriented towards the determination of the germination capacity of the seeds from the species under investigation and, on the other hand, of the temperature and pH of the incubation medium for each enzyme taken into consideration (Table I).

Our results agree with literature information, according to which, in the case of millet, the optimum action pH of α-amylase is of 5.4 (GIMBI and KITABATAKE, 2002), in bristle grass and hair grass is of 5.5 - 6.5 (CIORNEA and VASILE, 2008), in the lentil cotyledons (*Lens esculenta* L.) is of 6.1 (SHAHA *et al*., 2004), in the coffee beans is between 4.5 - 5.2 (VALENCIA

et al., 2000) while, in the case of rice, the optimum action temperature ranges between 45 - 50°C, in lentil cotyledons it is of 40°C (SHAHA *et al.*, 2004) and in germinated millet seeds the optimum temperature would be around 45°C (GIMBI and KITABATAKE, 2002).

Table I. Germination degree, optimum pH and temperature in some cultivated and spontaneous graminaceae

Species	Germination degree	Optimum pH		Optimum temperature	
		Amylase	Glucanphosphorylase	Amylase	Glucanphosphorylase
Sorghum sudanense	91%	5,5	4	45ºC	40ºC
Sorghum vulgare	68%	5,8	4,5	40ºC	40ºC

In another series of experiments, the starch concentration was determined in the germinated caryopses of the species taken into study as - during germination - the enzymatic degradation of the reserve substances from seeds occurs.

It is 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, 2002; 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.

As graphically plotted, starch evidences a decreasing dynamics along the whole germination interval considered for analysis, with a maximum in the impregnated sample, moment zero while, at 240 hours from the beginning of the germination process, the maximum threshold was attained (15.04% from the maximum value) (Fig. 1).

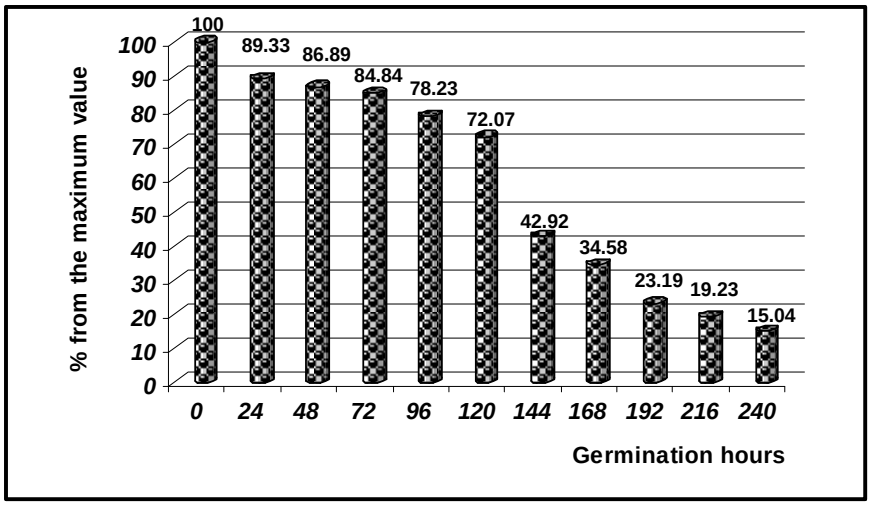

Fig.1. Starch concentration in *Sorghum sudanense* germinated caryopses

However, a comparison between the dynamics of starch concentration (or, in other words, the rate at which this polysaccharide gets degraded) and the total amylasic activity evidences no perfect similarities. On one hand, the total amylolytic activity increases progressively in the first period, being maximum after seven days of germination, while the starch concentration records a progressive decrease in the same period, although not entirely superposable with the amylasic activity. On the other hand, in the second period over which the experiments were performed, the amylasic activity decreased quite rapidly, yet without any diminution in the rate of starch degradation.

All these phenomena support the hypothesis put forward by the authors, according to which an at least equally important role, if not even more important, is played - in the mobilization of the reserve starch - by the phosphorolytic action of α-glucanphosphorylase. Under the catalytic action of this enzyme, the reserve starch continues its degradation, which explains the maintenance of the decreasing rhythm of starch concentration, even if the total amylolytic activity is drastically diminished (Figs. 2 - 3).

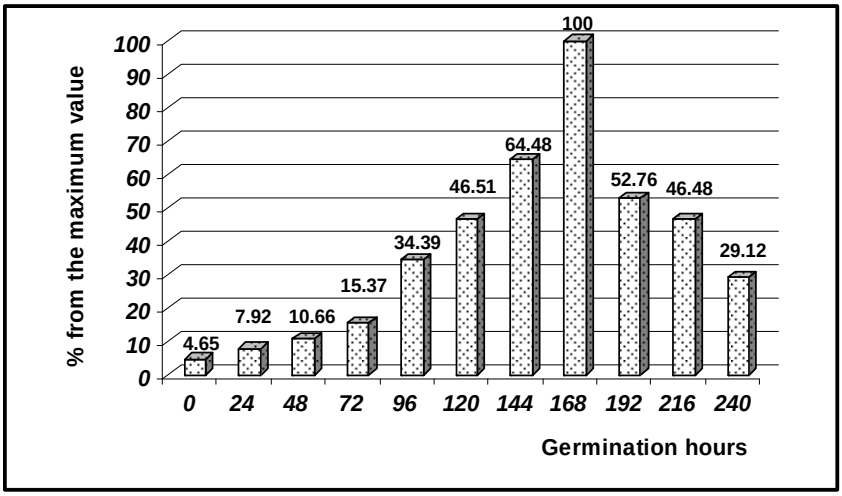

Fig.2. Relative activity dynamics of total amylase in *Sorghum sudanense* germinated caryopses

In the beginning of germination, the α-glucanphosphorylase evidences an imperceptible activity while, in the following hours, the substrate degradation starts, accompanied by a progressive increase of the enzymatic activity from 120 germination hours up to the 8th day, followed by a gradual decrease in the last germination day under analysis, up to the value of 40.353 µg phosphorous/g, *i.e.*, 39.48% from the maximum value (Fig. 3).

As generally known, during the biological rest, the enzymatic activity is much, almost wholly reduced while, simultaneously with the absorption of the environmental water during the germination process, activation of the whole enzymatic equipment occurs, along with the enzymatic degradation of the reserve substances from seeds, for the production of energy.

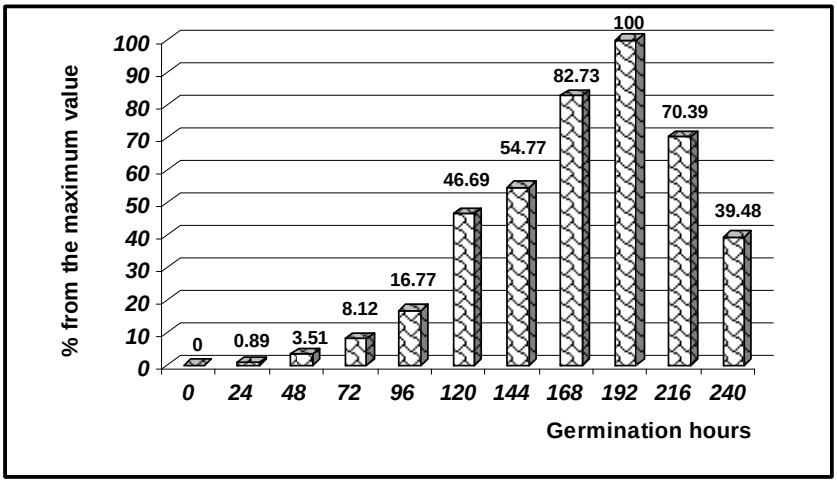

Fig.3. Relative activity dynamics of α-glucanphophorylase in *Sorghum sudanense* germinated caryopses

Starch, the richest reserve polyglucide, is deposited in the endosperm, then solubilized by means of the amylase secreted by the cells of the aleurone layer and degraded up to simple glucides, which are oxidized, thus forming the ATP, the main supplier of energy subsequently used by the cell as a function of its requirements (OLTEANU, 2003).

Therefore, in terms of the dynamics of starch concentration during the germination period of *Sorghum vulgare*, one should mention its rather moderate decrease along the whole duration of the experiment, up to 100% in the impregnated sample, to 36.64% in the 10th day of the experiment, which agrees with the total amylolytic activity (Fig. 4).

In the germinated caryopses of *Sorghum vulgare*, the activity of total amylase follows an ascending curve in the first 6 days of germination. The minimum value is recorded in the stage of impregnated seed (4.457 μM maltose/g), followed by an increase up to the 6th day, when the maximum threshold is attained (91.565 μM maltose/g). Beginning with the 7th germination day, the amylasic activity progressively decreases, up to an average value of 43.614 μM maltose/g, attained at 240 germination hours (10th day) (Fig. 5).

Such a dynamics of the total amylasic activity supports the assumption that the mobilization of reserve starch begins as early as the first hours of the germination process, although the enzymatic activity is much more reduced that that of the first species taken into study, which agrees with the less significant diminution of starch concentration, to be possibly explained, as well, by a very low germination degree.

The gradual decrease of the amylolytic activity in the second stage of the germination interval under analysis might be explained by the gradual decrease of the amount of starch, as well as, possibly, by the beginning of the photosynthetic process, which assures itself the precursors necessary in various metabolic process.

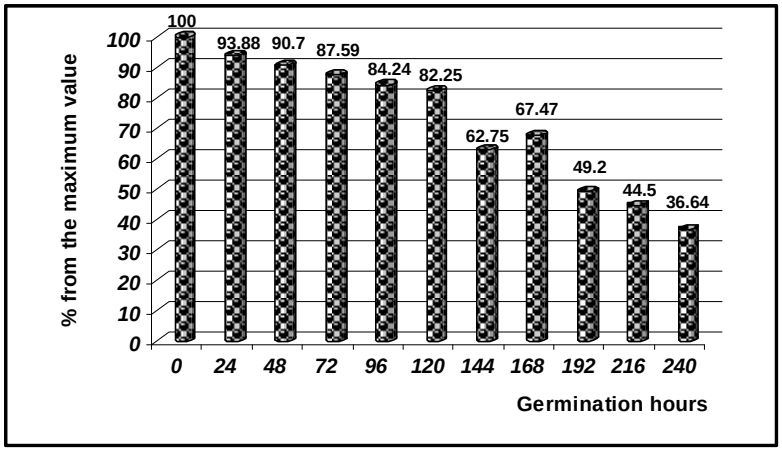

Fig.4. Starch concentration in *Sorghum vulgare* germinated caryopses

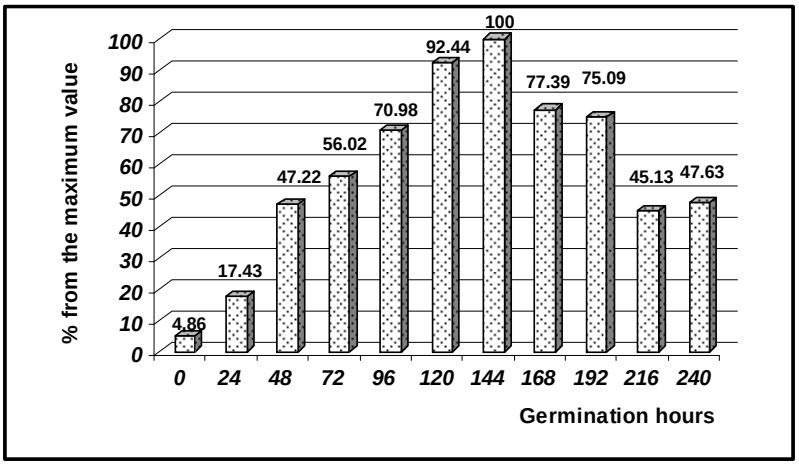

Fig.5. Relative activity dynamics of total amylase in *Sorghum vulgare* germinated caryopses

If considering the special intensity of the biochemical and physiological processes developed during germination, it is hardly probable that the maltose and free glucose resulted from the action of amylases will represent an important quantitative precursor in the first moments of biosynthesis of the new polysaccharides. Quite possibly, in such moments, the main role in the glucidic metabolism is played by the phosphorolytic action of α-glucanphosphorylase, an enzyme catalyzing the reversible reaction of starch degradation, accompanied by the formation of phosphorylated glucose. In this way, a more rapid scission of starch into (phosphorylated) glucose takes place while, at the same time, the product thus formed enters directly into future biosynthesis processes of the polysaccharides, the stages of maltose hydrolysis and glucose phosphorylation being thus omitted.

In *Sorghum vulgare*, α-glucanphosphorylase contributes to substrate degradation, a progressive increase of the enzymatic activity being observed beginning with the 96 hours of germination (31.666 μg phosphorous/g) up to the 7th day (865 μg phosphorous/g), followed by a decrease up to the last germination day, when a value of 268.333 μg phosphorous/g is attained.

The relative activity of α-glucanphosphorylase is reduced in the first hours, immediately following the absorption of environmental water, representing only 3.66% from the maximum activity at 72 germination hours, to be followed by a progressive increase up to 80.92%, and immediately, beginning with 192 germination hours, by a progressive decrease, so that, in the last day of the analysis, a value of 48.31% from the maximum activity is recorded (Fig. 6).

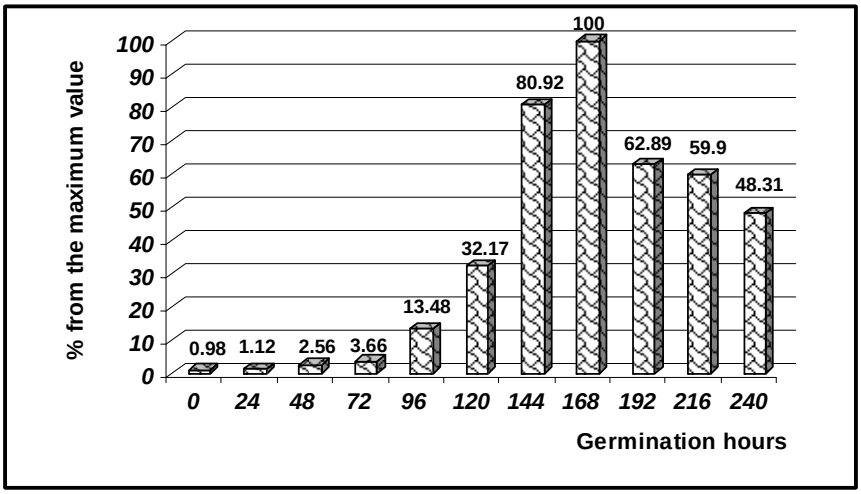

Fig.6. Relative activity dynamics of α-glucanphophorylase in *Sorghum vulgare* germinated caryopses

To evidence the possible differences or similarities observable, on one side, between species and, on the other, between the two enzymes involved in the glucidic metabolism, the enzymatic activity has been graphically represented and compared. Mention should be made, on one hand, of the fact that, in the two graminaceae species taken into study, the maximum enzymatic activity is recorded over the same germination interval while, on the other, no significant differences are to be noticed between species from the view point of the enzymatic activity, the only element modifying the activity being the germination time (Fig. 7).

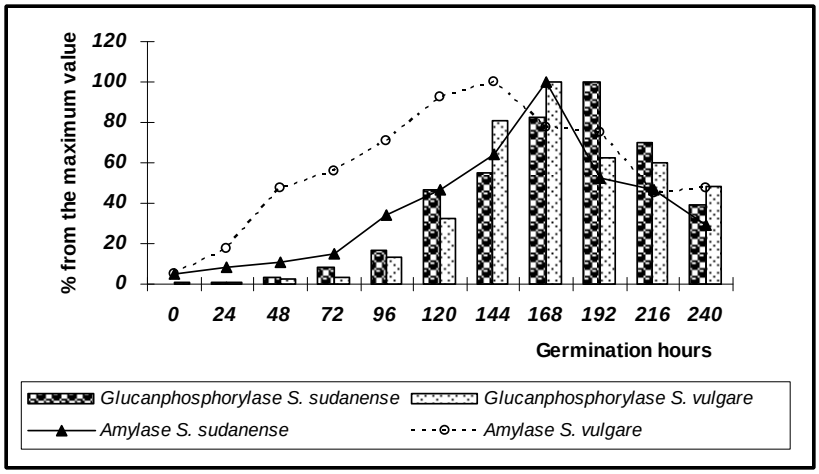

Fig.7. Comparative representation of amylase and α-glucanphosphorylase activity in *Sorghum sudanense* and *Sorghum vulgare* germinated caryopses

CONCLUSIONS

The germination extent of the caryopses belonging to the species under investigation is different (from 68 % in *Sorghum vulgare* at 91% in *Sorghum sudanense*), which may be probably explained by a different ripening extent, yet closely related to species specificity.

The optimum action pH (ranging between 4 and 5.8) of the enzymatic activity was registered in the acid and weakly acid zone, in spite of the fact that the activity remains at high values over a somewhat larger pH interval, which agrees with the literature data, according to which the optimum action pH is substantially different in the vegetal world.

The maximum values of the amylase and α-glucanphosphorylase activity were recorded at temperatures ranging between 40 - 45ºC.

Determination of the starch amount evidences a slight, progressive decrease, along the whole duration of the experiments, which confirms the fact that mobilization of the reserve starch occurs both hydrolytically - under the action of amylases - and phosphorolytically - under the action of α-glucanphosphorylase, the starch concentration recording a dynamics reversely proportional to the enzymatic activity.

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