

ON THE ACTIVITY OF TOTAL AMYLASE IN SOME SPECIES OF THE *BROMUS* GENUS, DURING GERMINATION

ELENA CIORNEA^{1*}, GABRIELA VASILE¹, DUMITRU COJOCARU¹

Key words: total amylase, germination, *Bromus squarrossus*, *Bromus japonicus*, *Bromus sterilis*

Abstract: The study discusses the activity of total amylase in the germinated caryopses of the three species belonging to the *Bromus* genus, namely: *Bromus squarrossus*, *Bromus japonicus* and *Bromus sterilis*. Statistical analysis of the experimental results obtained shows that the enzymatic activity is strongly influenced by the germination time, negligible differences being recorded from one species to another.

INTRODUCTION

Seeds' germination represents an extremely complex biochemical and physiological process, in which the reserve substances are mobilized, at incredible speed, for assuring to the embryo the energy and the metabolic precursors necessary for biosynthetic processes (BURZO *et al.*, 1999).

One of the plants' main reserve substances is starch, a polysaccharide accumulated in seeds, bulbs, tubercles and other organs (FRANKOVA, 2003). Generally, the processes of starch's catalytic degradation occur at variable rates, in the vegetal tissues, especially during the multiplication period. From practical reasons, the amylolytic enzymes of vegetal origin have been more thoroughly studied in culture plants and less in the spontaneous flora (TÂNASE *et al.*, 2000).

MATERIALS AND METHOD

The experiments were developed on germinated caryopses of *Bromus squarrossus*, *Bromus japonicus* and *Bromus sterilis* harvested in 2006.

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 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.

As amylase's substrate is the starch, the concentration of this polysaccharide has been evaluated, for each series of samples, by the polarimetric method. Also, for evidencing enzyme's specific activity, the concentration of proteins was dosed by the Bradford method (ARTENIE *et al.*, 1981; COJOCARU, 2005).

RESULTS AND DISCUSSION

A first objective had in view in the determination of amylases' activity in the species under study was plotting of the standard curve for converting the extinction units. To this end, a series of reference samples - in which the concentration in maltose varied between 0.2 and 1.8 mg - has been employed. The values of extinction have been read at a wavelength equal to 540 nm (Fig. 1).

On the basis of the graph, the regression straight line has been drawn and its regression equation has been calculated. According to the equation, the amounts of maltose corresponding to the samples subjected to analysis have been subsequently established, and the values obtained were referred to the amount of tissue employed (μM maltose / g).

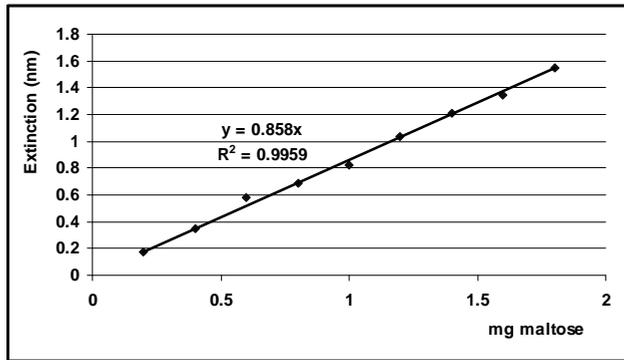


Fig.1. Standard curve for maltose dosing

In the case of *Bromus squarossus*, the amylasic activity in the first 24 hours from the beginning of germination is minimum (118.50 μ M maltose/g), a slow increase being recorded in the following 96 germination hours, after which a progressive leap of the enzymatic activity is recorded, with the maximum value attained after 216 hours of germination (869.25 μ M maltose/g) (Figs. 2-3).

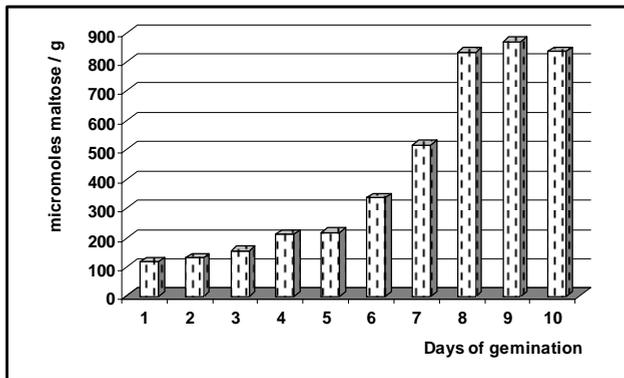


Fig. 2. Dynamics of the absolute activity of total amylase in the germination of *Bromus squarossus* seeds

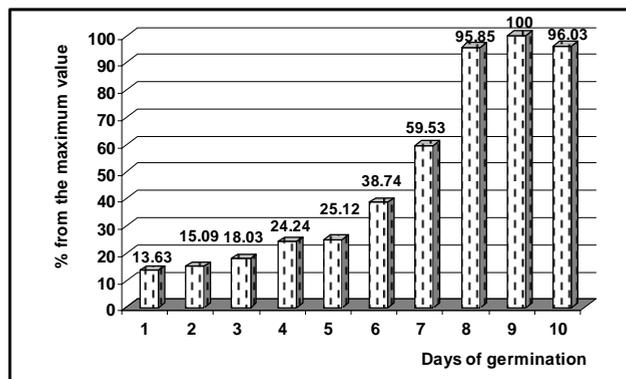


Fig.3. Dynamics of the relative activity of total amylase in the germination of *Bromus squarossus* seeds

As to the starch concentration, one may observe that, along the 10 days taken into study, it is hydrolyzed more and more proportionally to the amount of enzyme hydrolyzed in the seeds under germination (Fig. 4).

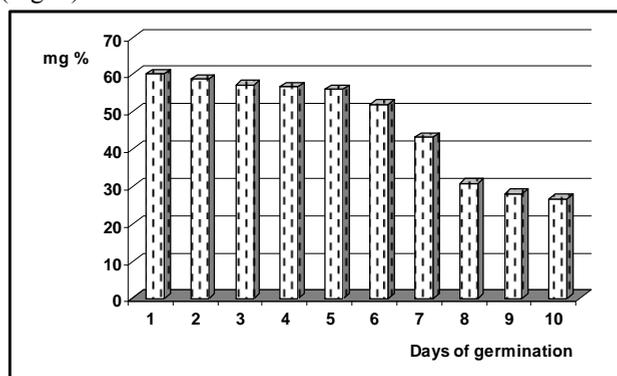


Fig.4. Starch concentration in germinated seeds of *Bromus squarossus*

The present study involved, too, determination of proteic concentration, once known that the amount of protein present in the seeds both - prior to and during germination - should be also considered for the calculation of the specific enzymatic activity, as well as for the elimination from the calculus of the proteins which are not enzymes.

A possible explanation for the increase of the proteins' content is that α -amylase is not present in seeds prior to their germination, the substance being synthesized in seeds' endosperm, during the germination process; on the other side, although β -amylase occurs in seeds in an inactive state, it is synthesized during seeds' germination. As to the decrease of protein concentration after its maximum threshold was attained, it might be explained by the reduction of the amount of starch in seed's endosperm, and by the hydrolysis of the reserve protein substances. In spite of the fact that, in this type of starchy seeds, the predominant reserve substances is the starch, still other additional sources of energy exist, usually employed when the main source is exhausted (NEAMȚU, 1981; BODEA, 1984).

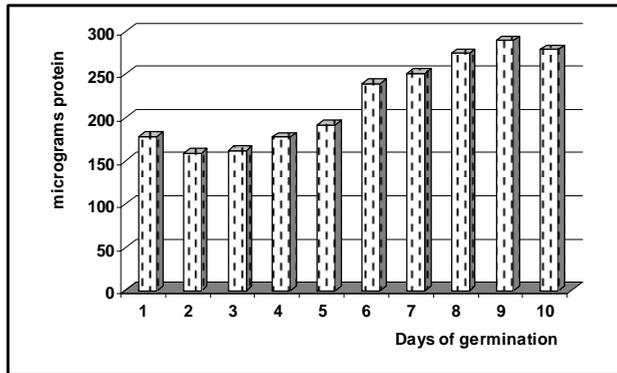


Fig.5. Dynamics of protein concentration in *Bromus squarossus*

When taking into consideration the concentration of proteins, as well, the graph of amylasic activity is modified, that is, although the maximum enzymatic activity is recorded, too, in the 8th, and not in the 9th day, a decrease of activity is noticed in the 5th day, to be followed by another increase (Fig. 6).

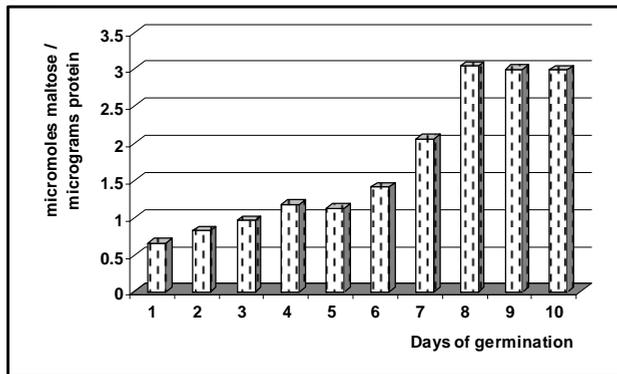


Fig.6. Dynamics of the specific activity of total amylase in the germination of *Bromus squarossus* seeds

In *Bromus japonicus*, during the 240 germination hours taken into study, the amylasic activity follows an ascending curve, ranging in the 146.50 - 862.50 μ M maltose/g interval, while the maximum threshold is recorded after 192 hours of germination (Figs. 7 - 8).

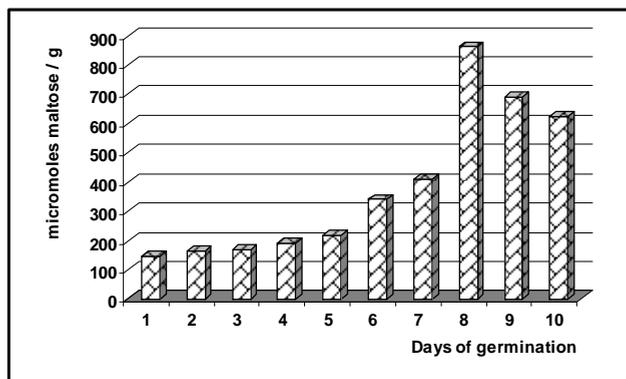


Fig.7. Dynamics of the absolute activity of total amylase in the germination of *Bromus japonicus* seeds

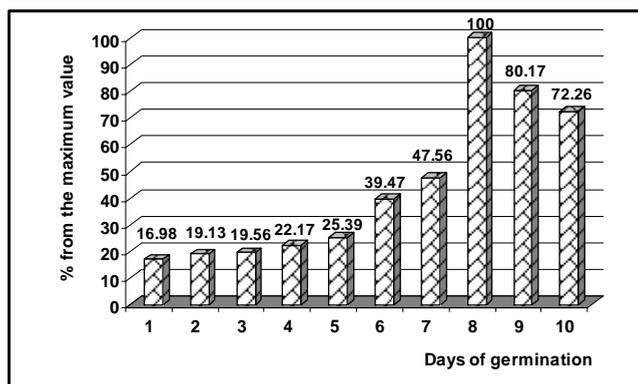


Fig.8. Dynamics of the relative activity of total amylase in the germination of *Bromus japonicus* seeds

The concentration of starch follows a descending curve, the values here recorded evidencing somewhat lower values (24.8 - 57.4 mg %) than those of *Bromus squarossus* (26.7 - 60.1 mg %) (Fig.9).

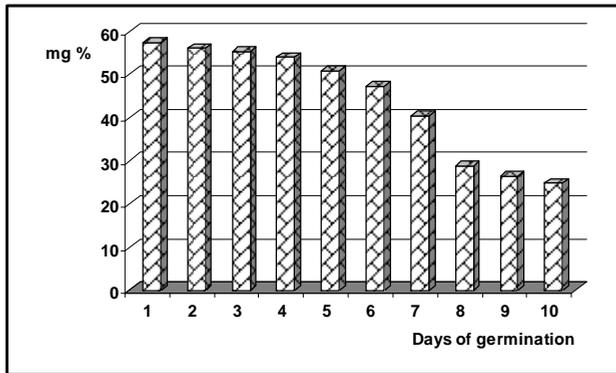


Fig.9. Starch concentration in germinated seeds of *Bromus japonicus*

In the first three germination days, the proteic concentration decreases, after which, starting with the 4th day up to the last one taken into study, a significant increase is recorded from one germination day to another (Fig. 10).

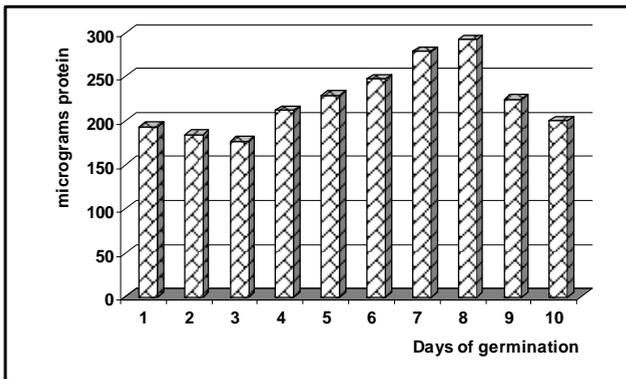


Fig.10. Dynamics of protein concentration in *Bromus japonicus*

As to the specific activity of total amylase in *Bromus japonicus*, a considerable increase is recorded starting from the 6th germination up to the 10th germination day, when the maximum threshold is being attained (3.12 μ M maltose/ μ g protein) (Fig. 11).

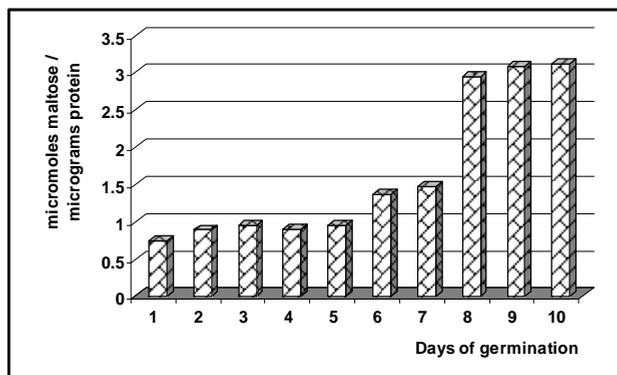


Fig.11. Dynamics of the specific activity of total amylase in the germination of *Bromus japonicus* seeds

In *Bromus sterilis*, the activity of total amylase remains relatively constant in the first three germination days while, starting with the fourth day, significant increases are observed from one germination day to another, up to the eighth one, when the maximum value (910.50 μM maltose/g) is attained, followed by a slow decrease until the last germination day under analysis (Figs. 12 - 13).

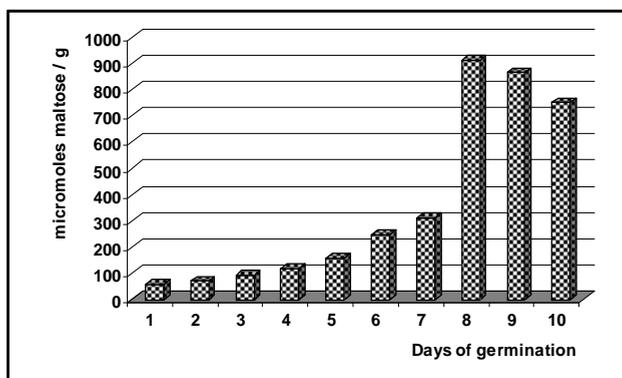


Fig.12. Dynamics of the absolute activity of total amylase in the germination of *Bromus sterilis* seeds

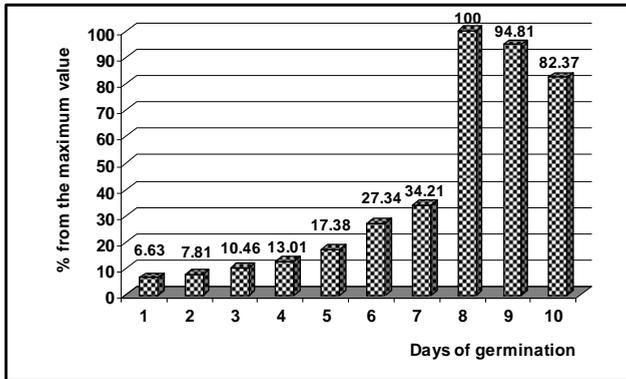


Fig.13. Dynamics of the relative activity of total amylase in the germination of *Bromus sterilis* seeds

In this situation, too, the starch concentration follows a descending curve, oscillating over the 20.6 - 55.4 mg % interval (Fig. 14).

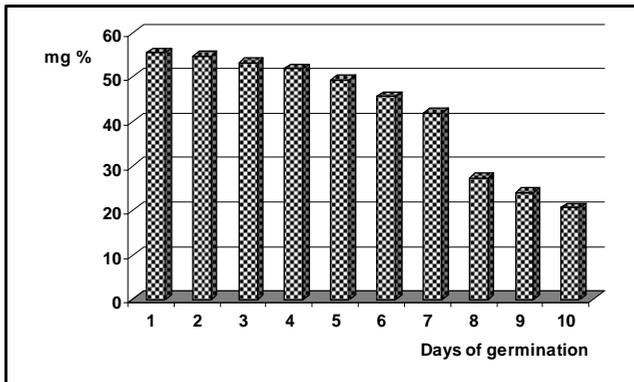


Fig.14. Starch concentration in germinated seeds of *Bromus sterilis*

As to the proteic concentration, it is seen as decreasing in the first 3 germination days, while increasing values are recorded starting with the 4th until the 8th germination day, to be followed by a gradual decrease in the last 2 days taken into study (Fig. 15).

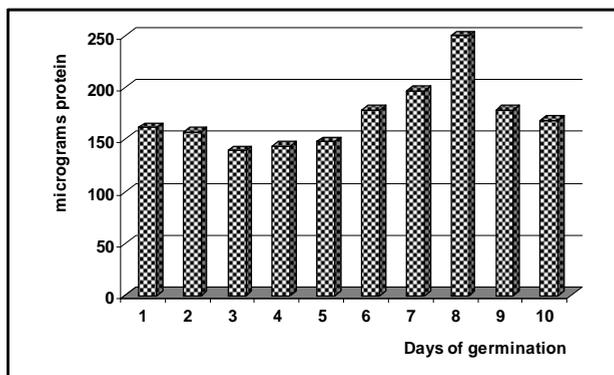


Fig.15. Dynamics of protein concentration in *Bromus sterilis*

The specific activity of total amylase follows the same curve with that of the absolute activity, the maximum enzymatic activity being recorded - this time - in the 9th day (4.84 μ M maltose/ μ g protein) (Fig. 16).

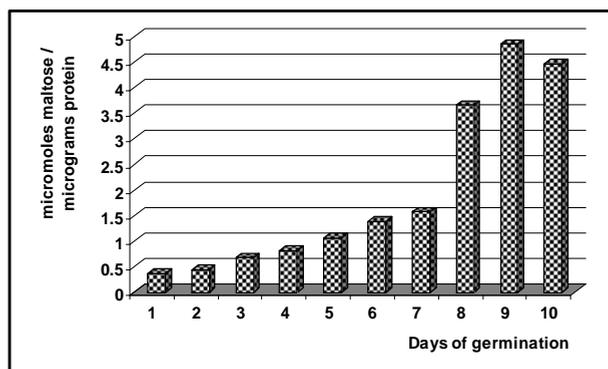


Fig.16. Dynamics of the specific activity of total amylase in the germination of *Bromus sterilis* seeds

To evidence the possible differences or similarities in the activity of total amylase of the three species belonging to the *Bromus* genus under analysis, the **ANOVA test - bifactorial I pattern**, with an equal number of observations in the cell, was applied (FOWLER *et al.*, 2000).

The test permitted to calculate the square sums, on the basis of variability sources (on columns, rows, on the total and internal interaction), the factors values (on columns, rows and of the interaction), as well as their critical values.

The results of the test showed that the activity of total amylase is strongly influenced by the germination time, being hardly differentiated from one species to another (Fig. 17).

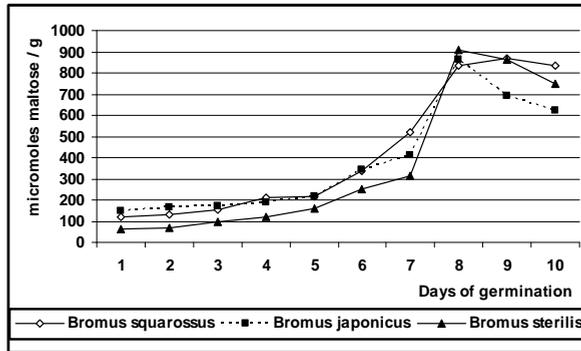


Fig.17. Comparative representation of the total amylase activity in the *Bromus* genus species

CONCLUSIONS

The results obtained in the study led to the following general conclusions:

In all species of the *Bromus* genus under investigation, the minimum value of the total amylase activity is registered in the 1st germination day, with a maximum in the 8th (*Bromus japonicus*, *Bromus sterilis*) and, respectively, 9th day (*Bromus squarossus*).

Statistical analysis of the experimental results obtained showed that the enzymatic activity is strongly influenced by the germination time, being hardly differentiated from one species to another.

The concentration of starch, as a reserve polyglucides, present in high ratios in the seeds of the plants belonging to the *Poaceae* family, decreases progressively along the whole period of seed's germination.

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1) “Alexandru Ioan Cuza” University of Jassy, Faculty of Biology
*) ciornea@uaic.ro