

THE ACTIVITY OF α -AMYLASE AND PEROXIDASE DURING GERMINATION OF SOME MAIZE CARYOPSES WITH DIFFERENT VIABILITIES

*Marcel AVRAMIUC

Ștefan cel Mare University of Suceava, Faculty of Food Engineering,
Str. Universității, no. 13, 720229, Suceava, România, e-mail: avramiucm@fia.usv.ro

*Corresponding author

Received 2 October 2011, accepted 15 November 2011

Abstract. *The paper examines, during pregermination and germination (after 48 and 96 hours), the activity of α -amylase and peroxidase in endospermum of maize caryopses with different viabilities (germination percentages) to emphasize what is the relationship between maize viability and activity of these enzymes, which belong to two different classes: oxidoreductases and hydrolases. The biological material, used in this work, was represented by six samples of maize caryopses belonging to a local variety, whose germination percentages ($G\%$) were between 34% and 95%. The data derived from experiences show that samples with $G \geq 70\%$ registered after 48 hours or after 96 hours of germination, growth between 18% and 242% of the activity of α -amylase, compared with the period before germination (0 h). At samples with $G \leq 70\%$ the α -amylase activity registered either increases with maximum 18% or reductions between 1% and 24%. The peroxidase activity has risen after 48 and 96 hours of germination, versus run-up (0 hours), also in the samples with $FG \geq 70\%$, but there was smaller (8% to 107%). In trials with $FG \leq 70\%$ the enzyme activity has recorded either increases between 12% and 18%, or fall between 8% and 32%. Corelațiile între viabilitate și activitatea peroxidazei, respectiv a α -amilazei cariopselor de porumb analizate au arătat că doar valorile activității peroxidazei în timpul germinației au fost în relație directă cu viabilitatea cariopselor. Correlation between caryopses viability and activity of peroxidase, respectively α -amylase, have shown that only the peroxidase activity values during germination were into a direct relationship with caryopses viability. The caryopses structural and functional damages, shown, indirectly, through their viability reduction, have influenced the both enzymes, leading to the decrease of their activity in samples with low germinations.*

Keywords: α -amylase, peroxidase, caryopse, sample, germination.

1. Introduction

Within the plants world, the beginning of a new vital cycle is marked of germination, physiological and biochemical complex process influencing the future plant body growth and development.

Under appropriate environmental conditions (temperature, humidity, pH, etc.), the substances stored in seed reserve tissues, suffer, under the influence of different enzymes, major transformations, qualitative and quantitative, evidenced by the emergence of biologically active compounds that positively affects cell activity and its equilibrium (1). The vitamins E și C biosynthesis, superoxid-dismutase, catalase and peroxidase activation are evidence of the antioxidant potential of germinated

seeds, which are used as protective foods in nutrition (2).

The germination process, through the study of seed compounds, on the one hand, and the enzymes involved in their transformations, on the other hand, can offer precious advice to farmers on some seed features and of the future plant (viability, productivity etc.).

This paper examines, during seeds pregermination and germination, the activity of peroxidase and α -amilase in reserve tissue of some maize caryopses with different viabilities to highlight what is the relationship between viability and activity of these enzymes, which belong to two different classes: oxidoreductases and hydrolases.

2. Experimental

The biological material used in this work, was represented by six samples of maize caryopses, belonging to a local variety, deriving from the last 10 years production. Both the new samples and the older ones (with smaller viabilities) have been stored without controlled temperature and humidity.

The samples were encoded, having specified the germination capacity values in parentheses (G%), as follows: PM (G = 95%); P1 (G = 80%); P2 (G = 56%); P3 (G = 70%); P4 (G = 34%); P5 (G = 85%).

To determine the germination capacity (G%), it have been used 4 repetitions of 50 seeds for each sample (3, 4). It have been used Petri plates of glass with special filter paper. The germination medium was distilled water that has soaked the filter paper, and temperature of 25°C. The maximum duration of the test was 4 days (96 hours).

The α -amilase activity was determined using the Noelting-Bernfeld method (5), based on free maltose forming through hydrolysis of starch solution 2% by α -amilase extracted from the sample to be analysed. The calculation of the results was done by means of a standard curve, and the α -amilase activity was expressed

in micromols (μ M) of maltose content, formed under the action of the enzyme, from 1 g of seed (flour).

Determination of peroxidase activity was performed with a colorimetric method (5). The enzyme extract was obtained through grinding of 0,1-0,5 g of caryopses with a buffer solution M/15 of NaH_2PO_4 and Na_2HPO_4 , with pH=6,7, followed by centrifugation. The activity dosing was made colorimetrically at 430 nm, using a pyrogalol solution (189 mg in 25 ml distilled water), hydrogen peroxide 0,15%, distilled water, phosphate buffer solution of sodium and enzyme extract. The peroxidase activity was calculated by the reaction speed, and was expressed in conventional units at 1 g of seeds (flour).

During germination, the α -amilase and peroxidase activity dosages were made from caryopses reserve tissue, after excision of new appeared formations.

The data of experiments (consisting in 4 replicates for each determination) were statistically processed, using the mean values and standard deviations.

3. Results and Discussion

Table 1 reproduces the α -amilase activity mean values of maize samples before

germination (0 hours) and after 48 and 96 hours from seed germination start.

Table 1
The activity of α -amilase during germination of maize samples

Samples	G (%)	α -Amylase activity (μ M maltose / g DM)		
		0 hours*	48 hours*	96 hours*
PM	95	32.80	30.50	84.25
P1	80	20.55	70.30	18.50
P2	56	30.80	23.35	25.82
P3	70	25.69	45.68	14.51
P4	34	26.45	26.20	30.85
P5	85	24.58	49.46	16.42

* = germination process duration

The determination of α -amylase activity before germination has evidenced values between 20.55 μ M maltose/g DM at P1 sample (G = 80%) and 32.80 μ M maltose /g DM at PM sample (G = 95%). The caryopse samples P3 (70%), P4 (G = 34%) and P5 (G = 85%) have registered close values (24.58 – 26.45 μ M maltose/g DM) of α -amylase activity, while the P2 sample (G = 56%) had an enzyme activity close to PM sample (G = 95%).

After 48 hours of germination, the enzyme activity values have vacillated between 23.35 μ M maltose/g DM at sample P2 (G = 56%) and 70.30 μ M maltose/g DM la proba P1 (G = 80%).

As against the period before germination (0 hours), the enzyme activity values after 48 hours of germination have registered rises with 242% at P1 sample (G = 80%), with 101% at P5 sample (G = 85%), with 78% at P3 sample (70%) or reductions with 24% at P2 (FG = 56%), with 7% at PM (G = 95%) and with 1% at P4 (FG = 34%).

After 96 hours of germination, the enzyme activity values have vacillated between 14.51 μ M maltose/g DM at P3 sample (G = 70%) and 84.25 μ M maltose /g DM at PM sample (G = 95%).

Compared with the period before germination (0 h), the enzyme activity has grown with 176% at PM sample (G = 95%), with 18% at P4 sample (G = 34%) and reductions with 43% at P3 sample (G = 70%), with 33% at P5 sample (G = 85%) and with 16% at P2 sample (G = 56%).

As against the enzyme activity after 48 hours, the α -amylase activity values after 96 hours of germination have risen with 176% at PM, with 18% at P4, and with 10% at P2. In the same conditions, the α -amylase activity has reduced with 74% at P1, with 68% at P3 and with 67% at P5.

An analysis of the data in table 1 shows that samples with FG \geq 70% (PM, P1, P3 and P5) have registered either after 48 hours or after 96 hours of germination, growth between 18% and

242% of the α -amylase activity, compared with the period before germination (0 h). In samples with FG \leq 70% (P2 and P4) the enzyme activity has recorded either increases with maximum 18% (P4) or reductions between 1% (P4) and 24% (P2).

The viability (germination) reduction of some seeds is accompanied by reducing of the synthesis capacity of the hydrolytic enzymes (6). During germination of barley aged seeds the α -amylase activity was lower by 50-70% compared to the control group, represented by young seeds (6).

Figure 1 reproduces the correlations between the values of caryopses germination (G%) and the values of α -amylase activity, determined before (0 hours) and after 48 and 96 hours of germination.

As seen in graph, between germination (G%) and α -amylase activity at 0, 48 and 96 hours of germination, one could establish positive correlations, but insignificant, after 48 hours ($R^2 = 0,1235$), and 96 hours ($R^2 = 0,084$) of germination.

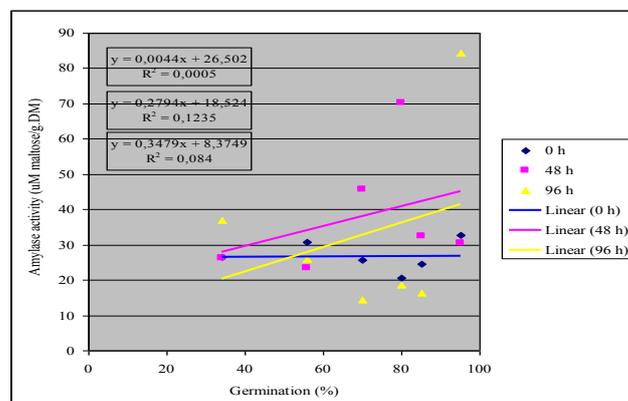


Fig. 1 - Linear regression for the correlation between Germination (%) and Amylase activity (μ M maltose/g. DM)

Researching the dynamics of starch content and α -amylase activity during germination of maize caryopses with different viabilities, Poroch-Seritan and Avramiuc (2002) have found the greatest values of enzyme activity after 24, 48, 72 or 96 hours of germination in samples with $G > 80\%$. Calculating the correlation between changes in the levels of starch and α -amylase activity during seed germination, the same authors have argued that when the viability

($G\%$) decreased the enzymatic activity efficiency decreased too, leading to the reduction of the caryopses capacity of using this reserve sugar.

The table 2 reproduces the peroxidase activity mean values of maize samples, before germination (0 hours) as well as during germination, after 48 and 96 hours.

Table 2
The activity of the peroxidase during germination of maize samples

Samples	G (%)	Peroxidase activity (UI/g.DM)		
		0 hours*	48 hours*	96 hours*
PM	95	15.56	20.03	31.98
P1	80	12.46	21.02	24.45
P2	56	14.40	16.55	15.83
P3	70	20.58	19.03	28.25
P4	34	13.84	9.34	12.70
P5	85	17.65	19,14	34.85

* = germination process duration

The determination of peroxidase activity before germination shows values between 12.35 IU/g. DM at P1 ($G = 80\%$) and 20.58 IU/g. DM at P3 ($FG = 70\%$). The caryopses samples P4 (34%), P2 ($FG = 56\%$), PM (95%) and P5 (85%) have registered close values of peroxidase activity (13,84 – 17,65 IU/g. DM)

After 48 hours of germination, the enzyme activity values have vacillated between 9,34 IU/g. DM at P4 ($G = 34\%$) and 21.02 IU/g. DM at P1 ($G = 80\%$). At the same germination interval, four samples (P1, P3, P5 and PM) have recorded very close values of this enzyme activity.

As against the period before germination (0 hours), the enzyme activity values after 48 hours of germination have registered rises with 66% at P1 sample ($G = 80\%$), with 27% at PM sample ($G = 95\%$), with 18% at P2 sample ($G = 56\%$), and with 8% at P5 sample ($G = 85\%$). The samples P4 and P3 have registered reductions with 32% and 7%.

After 96 hours of germination, the peroxidase activity values have vacillated between 12.70 UI/g.DM at P4 sample ($G = 34\%$) and 34.85 UI/g.DM at P5 sample ($G = 85\%$).

Compared with the period before germination (0 h), the enzyme activity has grown with 107% at PM sample ($G = 95\%$), with 97% at P5 sample ($G = 85\%$) and P1 sample ($G = 80\%$), with 37% at P3 sample ($G = 70\%$) and with 12% at P2 sample ($G = 56\%$). P4 sample has registered a reduction of activity with 8%.

As against the enzyme activity after 48 hours, the peroxidase activity values after 96 hours of germination have risen with 63% at PM, with 82% at P5, with 48% at P3, with 36% at P4, and with 18% at P1. The only one reduction of the peroxidase activity (with 4%) has been registered in P2 sample.

The analysis of the data in table 2 shows that samples with $FG \geq 70\%$ (PM, P1, P3 and P5) have recorded increases in activity of peroxidase after 48 and 96 hours of germination, compared to the period before

germination (0 hours), but there were smaller (8% to 107%). In samples with $FG \leq 70\%$ (P2 and P4) the enzyme activity has recorded either increases between 12% and 18% (P2) or fall between 8% and 32% (P4).

Fig. 2 reproduces the correlations between the values of caryopses germination (G%) and the values of peroxidase activity, determined

before (0 hours) and after 48 and 96 hours of germination.

As can be seen in the graph, between germination (G%) and peroxidase activity at 0, 48 and 96 hours of germination one could establish positive correlations, significant for 48 hours ($R^2 = 0,8255$) and 96 hours ($R^2 = 0,8319$) of germination.

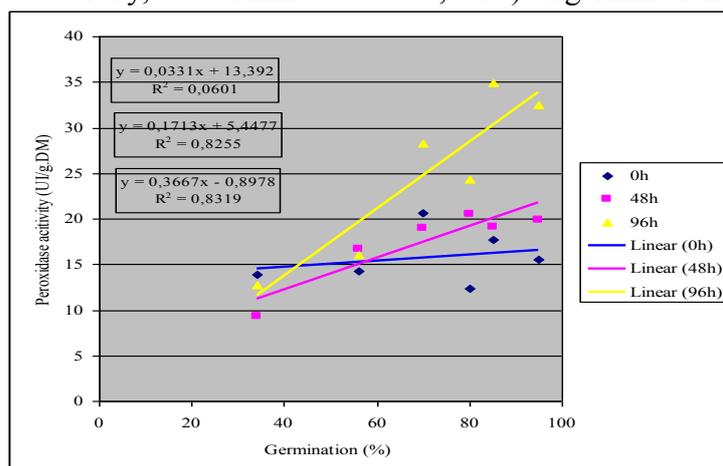


Fig. 2. Linear regression for the correlation between Germination (%) and Peroxidase activity (UI/g. DM)

Researching the peroxidase activity in caryopses of 4 samples of maize (with germination from 0 to 95%), Avramiuc (2004) found on pregermination period small differences between enzyme values. After 48 and 96 hours of germination the differences between samples have emphasized, the peroxidase activity values placing on a ascending path (apart from the sample with G =

0%), in direct correlation with gemination values of the analysed.samples. Computing the correlations between germination values and enzyme activity at 0, 48 and 96 hours of germination, the author has found direct correlations (significant and very significant) between germination capacity (G%) and peroxidase activity only at 48 and 96 hours of germination.

4. Conclusions

The research of the α -amylase and peroxidase activity of some samples of maize caryopses with different viabilities in pregermination (0 hours) and at 48, and 96 hours of germination, has revealed certain differences between samples depending on caryopses viability values.

The study of α -amylase has shown that samples of cariopse with germination capacity $\geq 70\%$ have registered, either after 48 hours or

after 96 hours of germination, increases between 18% and 242% of the enzyme activity, compared with the period before germination (0 h). In samples with $FG \leq 70\%$ the α -amylase activity has recorded either increases with maximum 18% or reductions between 1% and 24%.

The analysis of peroxidase showed that the maize samples with germination capacity $\geq 70\%$ have registered rises between 8% and

107% of this enzyme activity after 48 and 96 hours of germination compared to period before germination (0 hours). In samples with $FG \leq 70\%$ the enzyme activity has recorded either increases between 12% and 18%, or fall between 8% and 32%.

The germination process has stimulated the activity of the both enzymes (α -amylase – from hydrolase class, and peroxidase – from oxidoreductase class) in samples of caryopses with germination capacity $\geq 70\%$, after 48, respectively 96 hours of germination, compared to pregermination period. In these samples of caryopses, α -amylase has registered, at the

same time intervals, more increases of its activity as against peroxidase.

The correlations between viability (G%) and the peroxidase, respectively α -amylase activity, in analysed maize caryopses, have shown that only peroxidase activity values (during germination) have been in direct relationship with caryopses viability.

The caryopses structural and functional damages, shown, indirectly, through their viability reduction, have influenced the both enzymes, leading to the decrease of their activity in samples with low germinations.

5. References

1. Segal R., Segal B., Teodoru V., Gheorghe V., 1982 - *Valoarea nutritivă a produselor agroalimentare*. Ed. Ceres, București.
2. Segal R., Fulea V., 1998 - *Acțiunea antioxidantă a grâului germinat*, „Noutăți în microbiologie și biotehnologie”. Ed. Corson, Iași, 96-104.
3. Anghel G., Raianu Maria, Matei C., Bucurescu N., Rădulescu I., Anganu I., Velea C., 1959 - *Determinarea calității semințelor*. Ed. Acad. R.P.R., București.
4. Ellis R.H., Hong T.D., Roberts E.H., 1985 - *Handbook of Seed Technology for Genebanks*. International Board for Plant Genetic Resources, vol. II, Rome.
5. Arteni V., Tănase Elvira, 1981 - *Practicum de biochimie generală*. Centrul de Multiplicare al Univ. “Al. I. Cuza” Iași, 138-140.
6. Anderson J.D., 1973 - *Metabolic changes associated with senescence*. Seed Sci. Techn., I, 401-416.
7. Avramiuc M., 2004 - *Research on seed exudate indices and peroxidase activity in some maize and broad bean seeds*. Analele Universității “Ștefan cel Mare” Suceava, Anul III, Nr.2-2004, 5-8, ISSN 1583-2295.
8. Poroș-Serișan Maria, Avramiuc M., 2002 - *Dinamica conținutului de amidon și a activității α -amilazei în timpul germinăției unor cariopse de porumb*. Analele Universității “Ștefan cel Mare” Suceava, Sect. Ing. Alim., Anul I, Nr.2-2002, 14-17, ISSN 1583-2295.