PEROXIDASES ACTIVITY IN THE EMBRYOS OF GERMINATED BARLEY CARIOPSES, AFTER GRAIN CRYOPRESERVATION

Monica Angela ŞIPOŞ[*], Dorina CACHIŢĂ-COSMA[**]

[*] *University of Oradea, Faculty of Science, Department of Biology, Oradea, Romania*
[**] *"Vasile Goldiş" Western University of Arad, Department of Biology, Arad, Romania*
Corresponding author: Monica Angela Şipoş, University of Oradea, Faculty of Science, Department of Biology, 1 Universitatii Str., 410087 Oradea, Romania, tel.: 0040259408161, fax: 0040259408461, e-mail: siposmonica@yahoo.com

Abstract. This study following the peroxidase activity (PA) in the embryos of germinated barley caryopses (*Hordeum vulgare* L.), after 20, 30 and 44 hours of germination. The experimental variants were represented by grains that have been frozen in liquid nitrogen (LN) (-196ºC) for variable periods of time (5 minutes, 1 hour, 1 day, 1 week or 1 month). The results were represented in relation with the same parameter registered of the control lots (embryos of caryopses that were not subject of the treatment with LN). In all situations PA in the embryos was characterized especially by statistically insignificant inhibitions. The inhibitions of PA with statistical relevance were registered at 20 hours of germination, in the case of the experimental variants of 5 minutes, 1 hour and 1 day in LN. These results reveals a very well growth of the barley embryos after grains cryopreservation.

Keywords: peroxidases activity, embryos, barley, cariopses, cryopreservation, germination

INTRODUCTION

The peroxidases are basic and acid in accordance to Mäder classification [12]. Basic and acid peroxidases in the cellular walls are involved in their formation [1, 10, 13, 14]. The fact that the acid peroxidases are involved in lignification process of cellular walls is completly accepted [2, 3]. Thus, the peroxidases have a specific role in growth and in the cellular differentiation [8,9]. With the advance of the germination the peroxidasic activities in the cariopses were accelerated [4, 5, 6, 7].

The present study followed the peroxidase activity (PA) in the embryos of barley caryopses after grain submersion for 5 minutes, 1 hour, 1 day, 1 week or 1 month in LN (-196º) and its were germinated for 20, 30 and 44 hours. After cryopreservation and germination the caryopses were ex-embryonated and embryos were used for analyses.

MATERIALS AND METHODS

Lück's [11] colorimmetric method was adapted to the specific of our experiments. Certified seed material was employed in carrying out the research, having a germinative faculty established as being of 85%, and the humidity degree of the barley caryopses - parameter determined gravimetrically - was of 7.24%.

The principle of the method: p-phenylene-diamine in the presence of a vegetal extract containing peroxidases, is oxydated by enzymes. Further to the reaction there results a violet colour of the mixture. Between the intensity of the colouring and that of the PA there is a direct proportional relation.

The work stages: The PA was determined in the control embryos, as well as in those that were detached from germinated barley cariopses at 20, 30 and 44 hours of germination (Fig. 1A-C) after grains preservation in LN for 5 minutes, 1 hour, 1 day, 1 week or 1 month. The germination was performed in transparent casseroles made of plastic material, on filter paper humidified with 20 ml tap water, at 23°C temperature and in dark conditions. The caryopses were taken at 20, 30 and 44 hours of germination and the embryos were detached from them. The peroxidase activity was determined in 35 embryos/sample (3 samples were made, both for the control and for each experimental variants).

The enzyme extract was prepared. The 35 embryos/sample were ground with sand sterilized at 120°C. Over each homogenous product was added 4 ml of dilluted phosphate buffer (a concentration of 6.7x 10^{-3} M, pH=7 was dilluted 1 to 9 with distilled water). The samples were centrifuged at 6000 rotations /minute, for 20 minutes. The supernatant obtained, which represented the enzyme extract and was collected in test tubes and was preserved between ice cubes in the fridge. The samples were preserved in ice during the entire duration of the spectrophotometry performed.

The enzyme extract was introduced in the tub of the spectrophotometer, 0.5 ml for each sample, there was added 1ml solution phosphate buffer in a concentration of 6.7x 10^{-3} M, pH=7, 0.05 ml oxigenated water hyper-dilluted, 0.05 ml solution p-phenilene-diamine 1%, both of them freshly prepared.

The oxigenated water utilised in our experiments was hyper-dilluted and was prepared using peroxide 33%. Thus, at 100 ml distilled water there were added 0.3 ml peroxide. Then there was made a dillution of 1:9 with distilled water in the solution of oxigenated water above-mentioned – 10 ml solution oxigenated water + 90 ml distilled water – and the latter one was utilised in the biochemical analyses. The solutions of oxigenated water do not have stability in time.

The violet colouration of the mixture resulted, produced in the moment of introducing the solution of p-phenilene-diamine in the reaction tub, it was read at a spectrophotometer type Spekol 11 with a filter adjusted at a wavelength of 483 nm. The spectrophotometric readings regarding the values of the extinction were performed at 30 seconds.

The average values of the extinctions were calculated, both for the control sample and for the experimental variants taken into study. The existing differences were statistically interpreted after Steinbach [15], with the help of test „t".

RESULTS

The average values of the extinctions regarding the PA - as well as the statistical significance of the results - in the control embryos and in those coming from the lots of grains submersed in LN, have been inserted in Table 1. In the Figure 2 the experimental data regarding PA were expressed in percentage values. These reflect the differences (%) between the extinctions of the samples obtained from the vegetal material that was cryopreserved and the extinctions of the samples coming from control lots that were not subject to submersion in LN (these values were considered 100% and graphically marked with 0).

After immersing the barley caryopses in LN for 5 minutes there were registered - at 20 hours of germination - statistically significant inhibitions of the PA in the embryos ($p<0.05$) (Table 1), of -18.7% (Fig. 2). At 30 and 44 de hours from placing the grains to germinate the minuses registered with relation to the control (-12.5%, respectively -10.4%) (Fig. 2) no presented statistical relevance. The same results regarding the PA in the embryos detached from germinated cariopses, before these was submersed in LN for 1 hour or 1 day, were registered (Table 1, Fig. 2). After maintaining the barley caryopses for 1 week in LN – after 20, 30 and 44 hours of germination – PA was non-significantly decreased (-8.8 %, -10.2% and -8.9%) ($p>0.05$) (Fig. 2, Table 1) as a corresponding parameter determined in the control embryos. The situation was similar after cryopreservation for 1 month of the barley caryopses (Table 1, Fig. 2).

The PA in the embryos was characterized especially by statistically insignificant inhibitions in comparison with control, in all experimental variants in LN. The inhibitions of PA with statistical relevance were registered at 20 hours of germination, in the case of the experimental variants of 5 minutes, 1 hour and 1 day in LN.

DISCUSSIONS

At the passage of the embryo from a state of division (Fig. 1A), to elongation (Fig. 1B) and to differentiation (Fig. 1C) the total peroxidase activity rises considerably (Table 1). In accordance with Cochrane researches [4, 5] or with those that were made by Fraignier and al. [7] we can observed that total peroxidase activity increased from 20 to 44 hour of germination.

The peroxidases are involved in growth and in the cellular differentiation [2, 9], in cellular walls formation. Thus, Barcelo and al. [1], Perrey and al. [14] at *Lupinus* sp., Mäder and Walters [14] at *Nicotiana tabacum* were studied basic and acid peroxidases that were involved in the formation of cellular walls.

The fact that PA in the barley embryos was characterized especially by statistically insignificant inhibitions in comparison with control reveals a very well growth and differentiation of the barley embryos after cariopses cryopreservation.

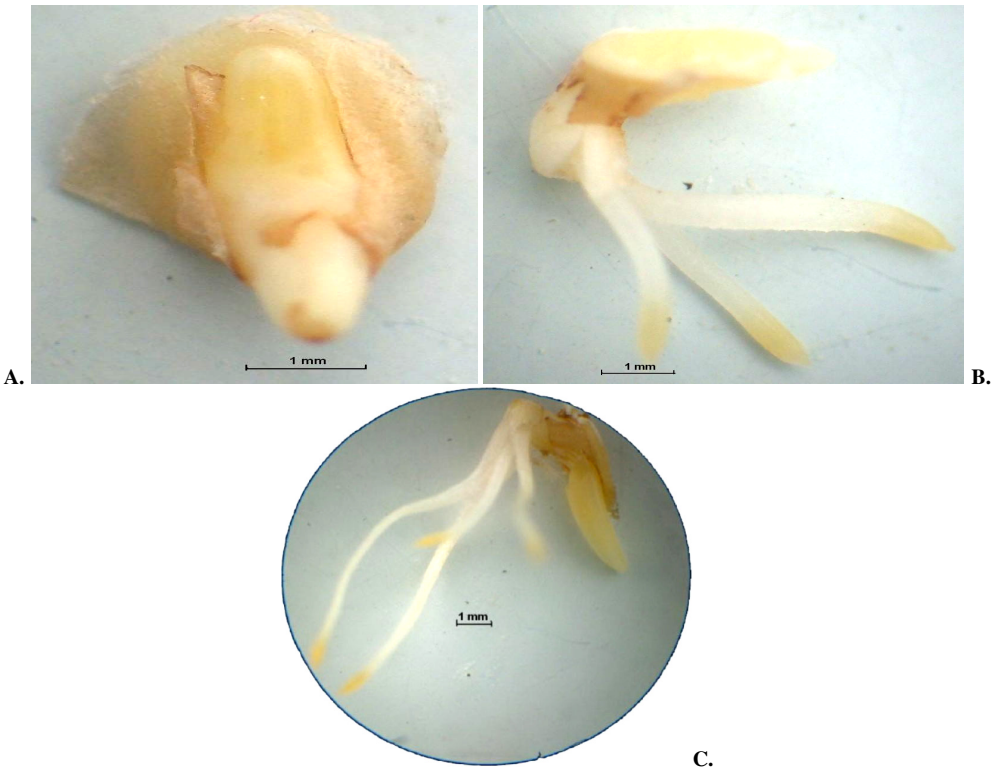

Figure 1. Morfological aspects of the barley embryos that were detached from germinated cariopses at 20 hours (A) şi la 30 hours (B) and 44 hours (C) of germination (foto: oc.10x and ob 1,6x).

Table 1. The average values of the extinction that reveals the intensity of the peroxidases activity in the embryos that were detached at 20, 30 and 44 hours of germination from barley caryopses (Hordeum vulgare L.) that were immersed in LN (-196ºC) for 5 minutes, 1 hour, 1 day, 1 week or 1 month, with relation to the same parameter determined in the control embryos.

Variants in LN / Hours of germination	Control	5 min	Control	1 hour	Control	1 day	Control	1 week	Control	1month
20	0.640	0.520	0.580	0.466	0.550	0.457	0.620	0.565	0.590	0.530
	p<0.05		p<0.05		p<0.05		p>0.05		p>0.05	
30	1.240	1.085	1.360	1.210	1.187	1.042	1.320	1.185	1.280	1.127
	p>0.05		p>0.05		p>0.05		p>0.05		p>0.05	
44	1.765	1.580	1.620	1.480	1.803	1.675	1.700	1.548	1.802	1.635
	p>0.05		p>0.05		p>0.05		p>0.05		p>0.05	

Note: p = significance threshold

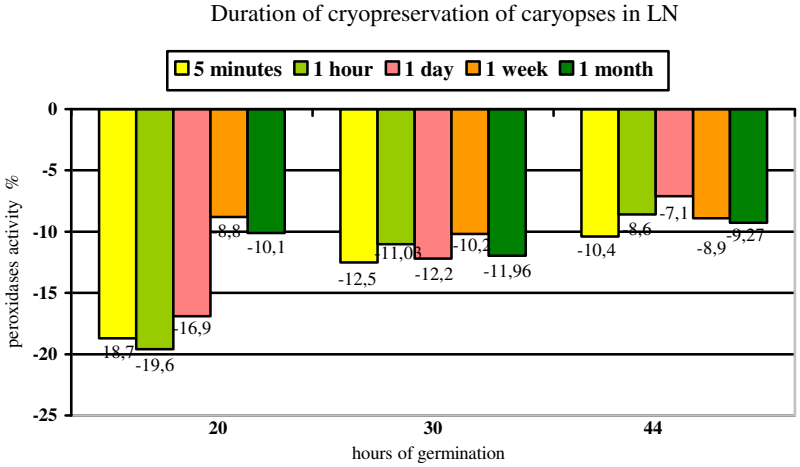

Figure 2. The percentage values of the peroxidases activity in the embryos that were detachet at 20, 30 and 44 hours of germination from barley caryopses (*Hordeum vulgare* L.) that were immersed in LN (-196ºC) for 5 minutes, 1 hour, 1 day, 1 week or 1 month, with relation to the same parameter determined in the control embryos (this value being 100% and graphically marked with 0).

REFERENCES

[1] Barcelo, A.R., Pedreno, M.A., Munoz, R., Sabater, F. (1988): Lupin peroxidases II. Binding of acidic peroxidases to cell walls, Plant Physiol. 71: 238-244.

[2] Cattesson, A.M. (1992): Plant Peroxidases and Cell Diferentiation: cito- and histological aspects, pp. 117-124, In: Penel, C., Gaspar, Th., Greppin, H., (eds.) Plant Peroxidases 1980-1990: Topics and Detalied Literature on Molecular Biochemical and Physiological Aspects. Geneva University Press.

[3] Catesson, A.M., Imberty, A., Goldberg, R., Czaninski, Y. (1986): Nature, localization and specificity of peroxidases involved in lignification processes, pp. 189-198, In: Greppin, H., Penel, C., Gaspar, Th., (eds.) Molecular and Physiological Aspects of Plant Peroxidases, Geneva University Press.

[4] Cochrane, M.P. (1994): Observations on the germ aleurone of barley. Morphology and histochemistry, Annals of Botany 73: 113-119.

[5] Cochrane, M.P. (1994): Observation on the germ aleurone of barley. Phenol oxidase and Peroxidase activity, Annals of Botany 73: 121-128.

[6] Dencheva, A.V., Klisourska, D.Y. (1976): Activity and isoenzyme composition of the peroxidase in the zones of growth differentiation of the cells in shoots of maize, C.R. Acad. Bulg. Sci. 29: 1179-1182.

[7] Fraignier, M.-P., Michelle-Ferrière, N., Kobrehel, K. (2000): Distribution of peroxidases in durum wheat (*Triticum durum*), Cereal Chem. 77: 11-17.

[8] Gaspar, Th. (1986): Integrated Relationships of Biochemical and Physiological Peroxidase Activities, pp. 455-468, In: Greppin, H., Penel, C., Gaspar, Th., (eds.) Molecular and Physiological Aspects of Plant Peroxidases, Geneva University Press.

[9] Gaspar, Th., Penel. C., Hagège, D., Greppin, H., (1991): Peroxidases in plant growth, differentiation, and development processes, pp. 249-280, In: Lobarzewski, J., Greppin, H., Penel, C., Gaspar, Th., (eds.) Biochemical, Molecular, and Physiological Aspects of Plant peroxidases, Univ M. Curie-Sklodowska, Lublin & Univ Geneva, Switzerland Press.

[10] Hu, C., Van Huystee, R.B., (1989): Immunochemical relatedness of two peroxidase isozymes from peanut cell culture, Biochem. Cell. Biol. 67: 371-376.

[11] Lück, H. (1974): Peroxidaza, pp. 895-897, In: Bergmeyer, H.U. (ed.) Methods of Enzymatic Analysis, Academic Press New York.

[12] Mäder, M., (1992): Compartmentation of peroxidase izoenzymes in plant cells, pp. 37-46, In: Penel, C., Gaspar, Th., Greppin, H., (eds.) Plant Peroxidases, 1980-1990, Topics and Detailed Literature on Molecular, Biochemical and Physiological Aspects, Geneva University Press.

[13] Mäder, M., Walter, C., (1986): De-novo synthesis and release of peroxidases in cell suspension cultures of *Nicotiana tabacum* L., Planta, 169: 273-277.

[14] Perrey, R., Hauser, M.T., Wink, M. (1989): Cellular and subcellular localization of peroxidase izoenzymes in plant and cell suspension cultures from *Lupinus polyphyllus*, Z. Naturforch 44c :931-936.

[15] Steinbach, M. (1961): Prelucrarea statistică în medicină şi biologie, Edit. Academiei R.P.R., Bucharest.