# PEROXIDASES ACTIVITY IN THE GERMINATION TIME OF THE TRITICALE CARYOPSES AFTER THESE WERE CRYOPRESERVED

**Monica ŞIPOŞ[1]*, Dorina CACHIŢĂ-COSMA[2], Alina Dora SAMUEL[1]**
[1]Departament of Biology, University of Oradea, Romania
[2]Departament of Biology, "Vasile Goldiş" West University Arad, Romania

*** Correspondence:** Şipoş M., University of Oradea, Department of Biology, no.1 Universităţii St., Oradea, Romania, email: siposmonica@yahoo.com
Received: march 2008; Published: may 2008

**ABSTRACT.** We followed the peroxidases activity (PA) - in first 44 hours of germination - in the ex-embryonated caryopses of triticale, after grain storage by immersion in liquid nitrogen (LN)(-196ºC) for variable periods of time: 5 minutes, 1 hour, 1 day, 1 week or 1 month. The cryopreservation of the triticale grain for 5 minutes or 1 hour has made that at 6 hours of germination the PA to be significantly decreased in relation with corresponding parameter in the control ex-embryonated caryopses and after 20 and 30 hours from placing the triticale caryopses to germinate decreases did not present statistic relevance; at 44 hours of germination has determined statistically insignificant stimulations of the PA. The cryopreservation of the triticale grains for 1 day in LN has determined – with relation to the control lot – a statistically insignificant inhibition of AP, after 6 and 20 hours of germination; further on, to 30 and 44 hours of germination there have been recorded stimulations of the activity of this enzyme, statistically relevant only at 30 hours from placing the caryopses to germinate. The cryopreservation of the triticale caryopses for 1 week or 1 month has made that at 6 hours of germination the PA activity to be insignificantly decreased in relation with corresponding parameter in the control grains. However, after 20, 30 and 44 hours from placing the triticale caryopses to germinate, stimulations of the PA have been registered; statistical relevance having the stimulations only at 30 hours of germination.

**Keywords:** grains, *Triticale*, cryopreservation, liquid nitrogen (LN), peroxidases activity (PA)

## INTRODUCTION

The research work concerning cryopreservation of biological vegetal materials has developed because this method assures the infinity of the germplasm preservation (Engelmann, 2004). In the vegetal materials cryopreservation there is no standard protocol. Thus, experiments regarding the optimum protocol of cryopreservation for each type of vegetal material are performed. Anyhow, in cryopreservation technology of each type of vegetal material there are certain steps: the material preparation, cryoprotection (if it is necessary), the way of cooling, the defrost and the checking after defrosting of the survival degree, morphological integrity (Hornung et al., 2001), biochemical (Touchell and Walters, 2000), cytological (Mikula et al., 2005) and genetic integrity of them (Dixit et al., 2003; Gagliardi et al., 2003; Zhai et al., 2003; Harding, 2004).

Our researches were concerned with the influence of cryopreservation - for 5 minute, 1 hour, 1 day, 1 week or 1 month - on the triticale grains. The present study aims at following the effect of the extremely low temperatures upon the peroxidase activity (PA) in the triticale caryopses submersed in liquid nitrogen (LN)(-196º). After cryopreservation, the caryopses have been put to germinate and were ex-embryonated before being analysed. The research regarding the PA of these caryopses were carried out during the first 44 hours of germination. The PA were determined after cryopreservation in report with the control represented to the non-submersed grains in LN. The peroxidases were chosen because they are extremely induciable with external factors.

## MATERIALS AND METHODS

The degree of humidity of triticale grains used in our experiments were certified respecting methodological norms recommended by International Seed Testing Association (ISTA). The water content of caryopses were established through gravimetric methods and was between 6 and 8%. The cryopreservation of triticale grains were achieved after these were packed in small cotton sacks (containing 50 grains/sack) very easy penetrated from the LN. The sacks were placed inside container with LN and then were submersed directly in -196°C (quick cooling), without intermediary freezing temperature steps. The grains storage in our experimental variants were for different periods of time, for 5 minutes, 1 hour, 1 day, 1 week or 1 month. After this operation the sacks were extracted from container compartments and passed to defrosting through their maintenance to the room temperature (slow defrosting). Then the caryopses were germinated (50 grains/germinator) on filter paper humidified with 20 ml of tap water. The germination temperature was of 23°C and in dark conditions.

Lück's (1974) colorimmetric method of dosing the peroxidases was adapted to the specific of our experiments.

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 caryopses, as well as in those that were preserved in LN for 5 minutes, 1 hour, 1 day, 1 week or 1 month. The germinated caryopses were taken at 6, 20, 30 and 44 hours from placing the caryopses to germinate, and the embryos were detached from them. The peroxidase activity was determined in 2 grams of ex-embryonated caryopses /sample (3 samples were made, both for the control and for the experimental variant taken into study).

The enzyme extract was prepared by pounding without liquid the 2 grams of ex-embryonated caryopses. The pounding was made with sand well washed and sterilized beforehand at the drying stove at 120°C. Over each homogenous product obtained by pounding the ex-embryonated caryopses there was added further on, 16 ml of dilluted phosphate (the phosphate in 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 spectro-photommetry performed.

Out of the enzyme extract there was introduced in the tub of the spectro-photometer 0,5 ml for each sample, there was added 1ml solution phosphate in a concentration of $6,7x\ 10^{-3}$ M, pH=7 plus 0,05 ml oxigenated water hyper-dilluted, plus 0,05 ml solution p-phenilene-diamine 1%, that has to be 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 was utilised in the bio-chemical analyses.

The solutions of oxigenated water do not have stability in time, deteriorate very quickly, fact for which they have to be prepared the moment spectro-photommetry begins.

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 spectro-photometric readings regarding the values of the extinction were performed at 1 minute, since the colour of the mixture intensifies in time.

The average values of the extinctions were calculated, both for the control sample and for the experimental variant taken into study, the existing differences being statistically interpreted with the help of test „t" (Steinbach, 1975).

## RESULTS AND DISCUSSION

The average (mean values) of the extinctions regarding the PA, as well as the statistical significance of the results – both in the control ex-embryonated caryopses, and in those coming from the lots of triticale grains submersed in LN - prior to placing them to germinate have been inserted in Table 1.

The experimental data were expressed also 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 control samples, coming from controls not subject to submersion in LN (values regarded as being 100%). In the histograms presented in Graphic 1 there are rendered in percentage values (%), the pluses or minuses registered with relation to the control, graphically marked with 0.

After immersing the triticale caryopses in LN for 5 minutes there were registered - at 6 hours of germination - statistically significant inhibitions of the PA ($p<0,05$)(Table 1), of -16,5% (Graphic 1); at 20 and 30 de hours from placing the grains to germinate the minuses registered with relation to the control (-6,85%, respectively -6,13%) (Graphic 1) no longer presented statistical relevance. Non-relevant statistically was also the stimulation of the PA of +9,06 % registered at 44 hours of germination (Table 1 and Graphic 1). The situation was similar also after a submersion for 1 hour of the triticale caryopses in LN (see Table 1 and Graphic 1).

The cryopreservation of the triticale cariopses for 1 day in LN has determined after 6 and 20 hours of germination - with relation to the control - a statistically insignificant inhibition ($p>0,05$)(Table 1) of PA of -8,2%, respectively -6,57% (Graphic 1). At 30 and 44 hours of germination there were registered stimulations of the activity of this enzyme, statistically relevant (($p<0,05$) at 30 hours (+14,95%) ( Table 1 and Graphic 1).

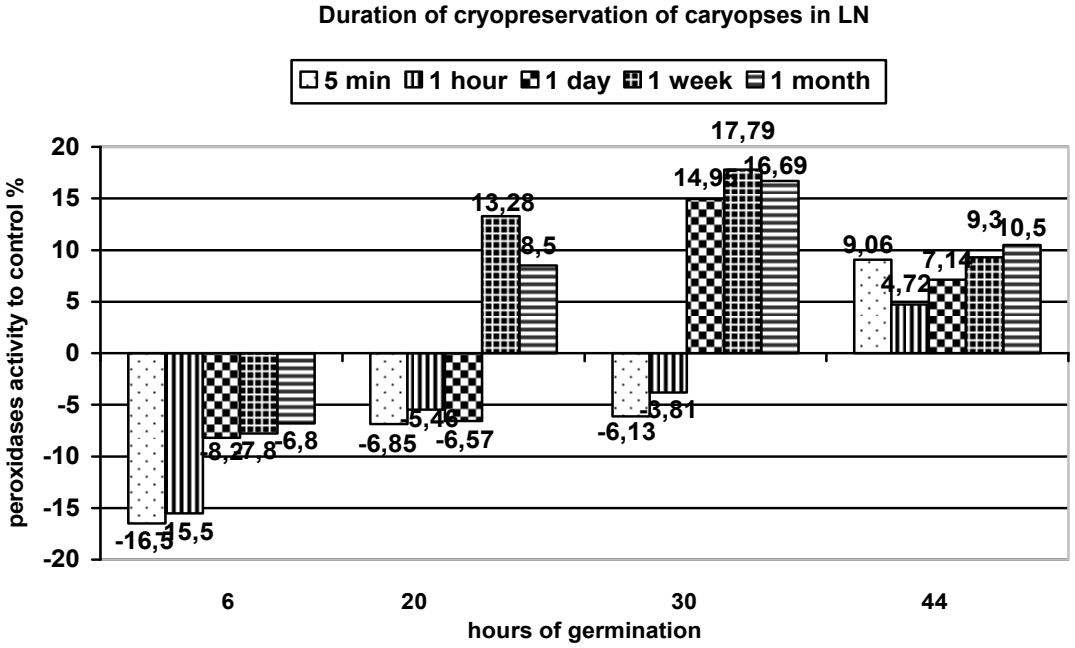
After maintaining the triticale caryopses for 1 week in LN – after 6 hours of germination - PA was non-significantly decreased (-7,8%)($p>0,05$) (Graphic 1 and Table 1) as a corresponding parameter in the control grains, non-immersed in LN. However, after 20, 30 and 44 hours from placing the triticale caryopses to germinate, there were registered stimulations of the PA; statistically significant being only those at 30 hours from germination (+17,79 %) (Graphic 1). The situation was similar also after a submersion for 1 month of the triticale caryopses in LN (see Table 1 and Graphic 1).

*Table 1*

**The average values of the extinction that outline the intensity of the peroxidase activity in the control triticale caryopses as well as in those immersed in LN (-196°C) for 5 minutes, 1 hour, 1 day, 1 week or 1 month, at 6, 20, 30 and 44 hours of germination.**

| Variants in LN / Hour of germin | Control | 5 min | Control | 1 hour | Control | 1 day | Control | 1 week | Control | 1month |
|---|---|---|---|---|---|---|---|---|---|---|
| *6* | 0,515 | 0,430 | 0,495 | 0,418 | 0,488 | 0,448 | 0,460 | 0,424 | 0,483 | 0,450 |
| | p<0,05 | | p<0,05 | | p>0,05 | | p>0,05 | | p>0,05 | |
| *20* | 0,526 | 0,490 | 0,550 | 0,520 | 0,533 | 0,498 | 0,512 | 0,580 | 0,525 | 0,570 |
| | p>0,05 | | p>0,05 | | p>0,05 | | p>0,05 | | p>0,05 | |
| *30* | 0,588 | 0,552 | 0,605 | 0,582 | 0,595 | 0,684 | 0,590 | 0,695 | 0,587 | 0,685 |
| | p>0,05 | | p>0,05 | | p<0,05 | | p<0,05 | | p<0,05 | |
| *44* | 0,717 | 0,782 | 0,720 | 0,754 | 0,700 | 0,750 | 0,763 | 0,834 | 0,736 | 0,814 |
| | p>0,05 | | p>0,05 | | p>0,05 | | p>0,05 | | p>0,05 | |

*Note: p = significance threshold*




**Graphic 1.** Expressing in percentage values of the peroxidase activity in the triticale caryopses immersed in LN (-196ºC) for 5 minutes, 1 hour, 1 day, 1 week (s) or 1 month, with relation to the same parameter determined in the control caryopses (value regarded as being 100% and graphically marked with 0), at 6, 20, 30 and 44 hours of germination.

## CONCLUSIONS

The cryopreservation of the triticale grain for 5 minutes or 1 hour has made that at 6 hours of germination the PA to be significantly decreased in relation with corresponding parameter in the control ex-embryonated caryopses and after 20 and 30 hours from placing the triticale caryopses to germinate decreases did not present statistic relevance; at 44 hours of germination has determined statistically insignificant stimulations of the PA.

Exposure of the triticale grains for 1 day in LN has determined – with relation to the control lot – a statistically insignificant inhibition of AP, after 6 and 20 hours of germination; further on, to 30 and 44 hours of germination there have been recorded stimulations of the activity of this enzyme, statistically relevant only at 30 hours from placing the caryopses to germinate.

Cryopreservation of the triticale caryopses for 1 week or 1 month has made that at 6 hours of germination the PA activity to be insignificantly decreased in relation with corresponding parameter in the control grains. However, after 20, 30 and 44 hours from placing the triticale caryopses to germinate, the stimulations of the PA have been registered; statistical relevance having the stimulations only at 30 hours of germination.

The cryopreservation of the triticale caryopses for 1 day, 1 week or 1 month in LN has determined - at 30 hours of germination - significant stimulation of the PA. These were not of duration since already at 44 hours from placing the grains to germinate, the differences incurred did not present statistical relevance.

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