GROWTH OF THE TRITICALE PLANTLETS (x *TRITICOSECALE* Wittm.) AFTER CARIOPSES CRIOPRESERVATION

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Summary: Our research followed the growth of the triticale plantlets, in first 6 days of germination, after cariopses (with 6.89% degree of humidity) storage by immersion in liquid nitrogen (-196ºC), for variable periods of time: 5 minutes, 1 hour, 1 day, 1 week or 1 month, in proportion with the same parameters of the control lots, which were not treated with liquid nitrogen. After triticale grains immersion in liquid nitrogen and than defrosted and taken for germination we registered a statistically non-significant inhibition in the growth in length of the vegetative organs (primary and adventitious roots, coleoptils and first leaves) of the triticale plantlets.

Keywords: triticale, cariopses, criopreservation, germination, plantlets growth

INTRODUCTION

At present a particular importance is granted to the developing of techniques for the cryopreservation of genetic vegetal resources, techniques that could ensure the preservation over an unlimited period of time of the germoplasm (Engelmann, 2004). It is estimated that these procedures would resolve the urgent, pressing and costly problems posed by the gene banks in what concerns the preservation of seeds on a long term (FAO, 1996), eliminating altogether as well, the costs implied by the subcultures ,,in vitro", which require time and materials and could lead to a genetic deviation in population (Badea et al., 2000).

Our research has taken into study the growth in length - in the first 6 days of germination – of the vegetative organs (primary and adventitious roots, coleoptils and first leaves) of the triticale plantlets, further to the cryopreservation of the caryopses in liquid nitrogen (-196ºC) on variable durations of time: 5 minutes, 1 hour, 1 day, 1 week or 1 month.

MATERIALS AND METHODS

The triticale control caryopses – not subject to the treatment with liquid nitrogen – and the samples exposed for a certain period of time at -196°C, have been placed to germinate on a substratum consisting of a filter paper humidified with 20 ml tap water. This quantity of water has ensured the humidity in the germinators closed during the first 3 days of germination. The caryopses taken out of liquid nitrogen have been placed into germinators after a slow defrosting of these at the laboratory temperature. Subsequently, in the 4-th, 5-th and 6-th day from placing the caryopses to germinate the germinators were opened and the humidity has been maintained by daily adding 5 ml water. This was uniformly distributed on the filter paper from the germinators with the help of a syringe. For the biometric determinations the germinators were kept at normal light (at 23°C ±2°C). Placing the caryopses on the surface of the filter paper has been made in such a way as to place the embryonic zone in direct contact with this.

In order to observe the effects produced by the treatment with liquid nitrogen upon the viability of the embryos of the triticale caryopses there was followed - in the first 6 days of germination – the growth in length of the plantlets and their vegetative organs.

The measurements have been carried out with lots of approximately 150 plantlets – both at the control lots, and at those coming further to germination of the caryopses submersed for 5 minutes, 1 hour, 1 day, 1 week or 1 month in liquid nitrogen – beginning with the 3-rd day of germination and there were performed for four days. The individual data concerning the length of the primary root, of the first two adventitious roots – among which the average was done – of the coleoptil and the first leaf, there have been statistically processed with the help of a program determining the average and the standard deviation (Steinbach, 1961).

For the interpretation of the statistical significance of the average lengths of the organs of the plantlets coming by germination out of the cryopreserved caryopses – in relation to their control sample – there has been used the graphical representation in the program SigmaPlot 2001, utilising the standard deviations.

The biometric measurements referring to the average growth in length of the control plantlets and of their organs have been considered as reference values, of 100%, data to which there were reported in percentage the biometric determinations effected at the similar lots, but resulted further to the germination of the caryopses that have been submersed in liquid nitrogen.

RESULTS AND DISCUSSION

The percentage values inserted in figures 1-4 reflect the inhibitions registered with relation to the control sample in what concerns the growth in length of the organs of plantlets (primary and adventitious roots, coleoptils and first leaves)(fig.1-3) and of the whole plantlets (fig.4) resulted out of the embryos of the triticale caryopses that have been submersed in liquid nitrogen for 5 minutes, 1 hour, 1 day, 1 week or 1 month, then defrosted and set to germinate in optimum conditions. The statistical processing carried

out in the program SigmaPlot 2001 (exemplified in figure 5, in what concerns the primary root) has reflected the fact that these inhibitions have been non-significant statistically at all vegetative organs of the triticale plantlets, beginning with the 3-rd to the 6-th day of germination. It is important to underline the fact that there can be observed a physiologic re-establishment in the growth of plantlets and their organs as the germination advances (see fig.1-4).

Our results are similiar with those of other authors. It is mentioned that the cryopreservation in liquid nitrogen does not affect the germinative faculty and the viability of the embryos (Stanwood, 1985; Gonzales-Benito et al.,1995; Pence,1996; Gonzales-Benito and Pérez-Garcia, 2001; Wood et al., 2003; Popov et al., 2004), but reduces the growth potential of plantlets (Keul et al., 1998). Such inhibitions can be an expression of the harms induced, probably, in the critical moments of the immersion in liquid nitrogen, respectively of defrosting, effects that manifest themselves in the following phases of germination.

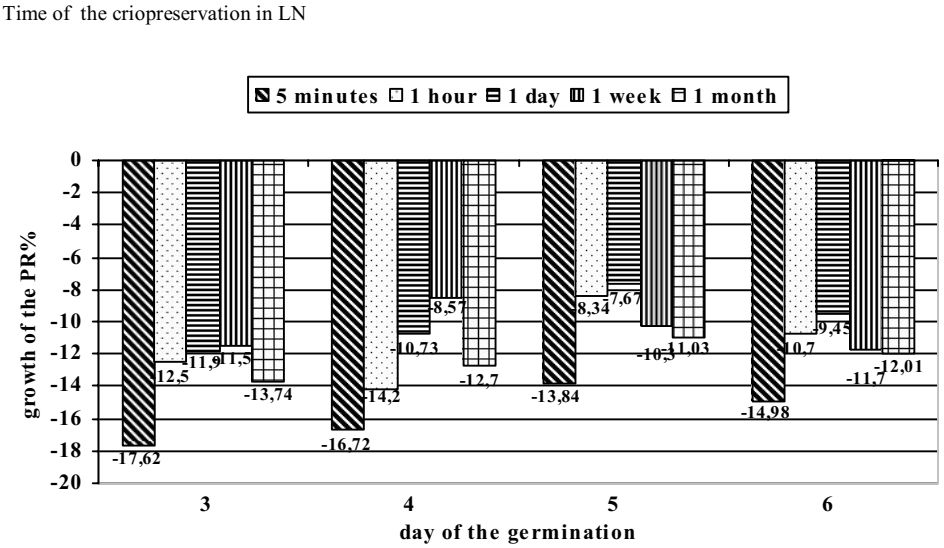

Figure 1. The procentual values reflecting the inhibitions regarding the average growth in length of the primary roots (PR) to the triticale plantlets coming from caryopses submersed in liquid nitrogen (LN ; -196^{0}C) for 5 minutes, 1 hour, 1 day, 1 week or 1 month, with relation to the growth of these vegetative organs at the plantlets of the control lot (considered 100%, graphically marked with 0).

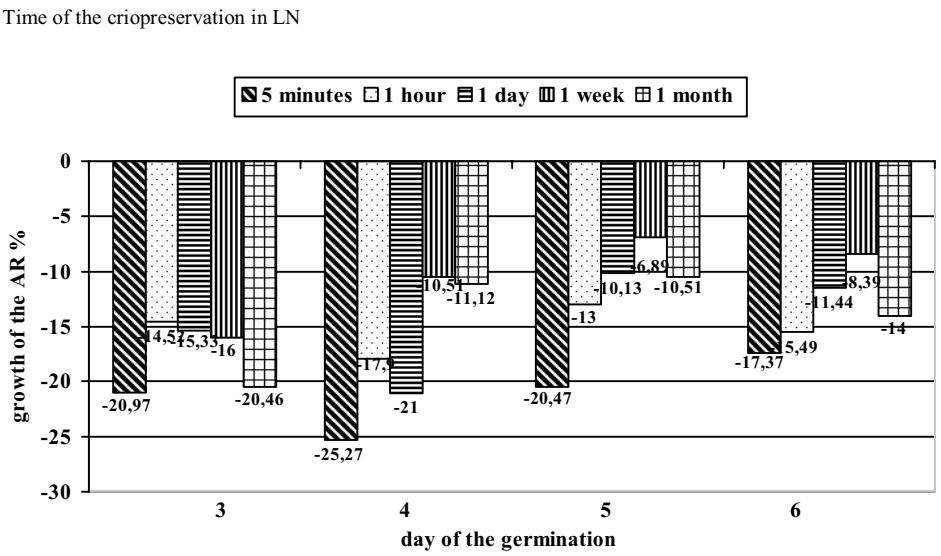

Figure 2. The procentual values reflecting the inhibitions regarding the average growth in length of the adventitious roots (AR) to the triticale plantlets coming from caryopses submersed in liquid nitrogen (LN ; -196^{0}C) for 5 minutes, 1 hour, 1 day, 1 week or 1 month, with relation to the growth of these vegetative organs at the plantlets of the control lot (considered 100%, graphically marked with 0).

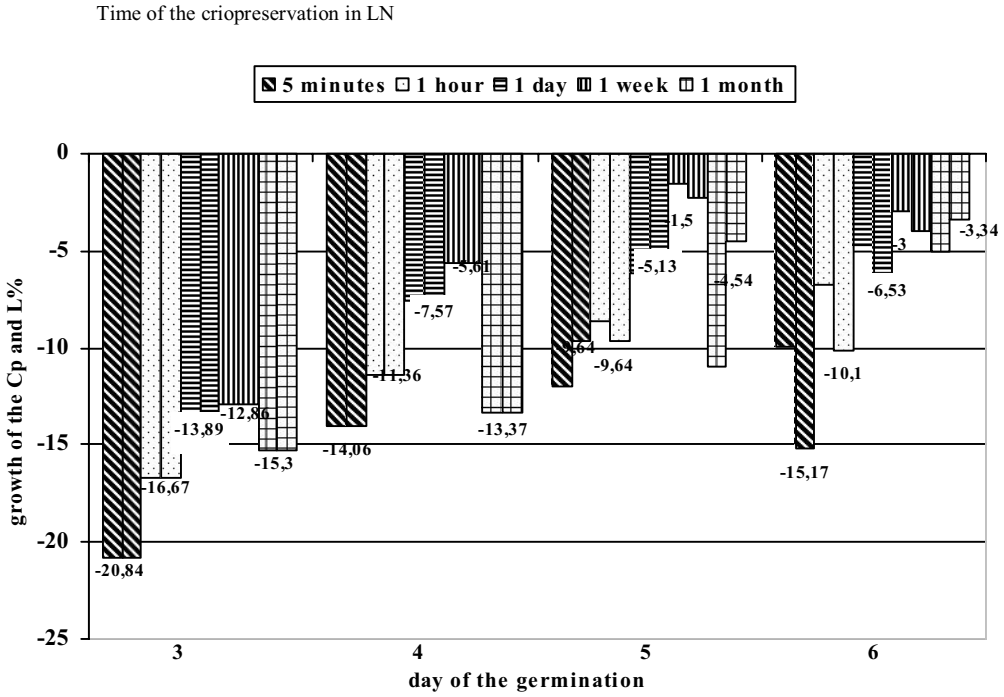

Figure 3. The procentual values reflecting the inhibitions regarding the average growth in length of the coleoptils (Cp) and first leaves (L) to the triticale plantlets coming from caryopses submersed in liquid nitrogen (LN ; -196^{0}C) for 5 minutes, 1 hour, 1 day, 1 week or 1 month, with relation to the growth of these vegetative organs at the plantlets of the control lot (considered 100%, graphically marked with 0).

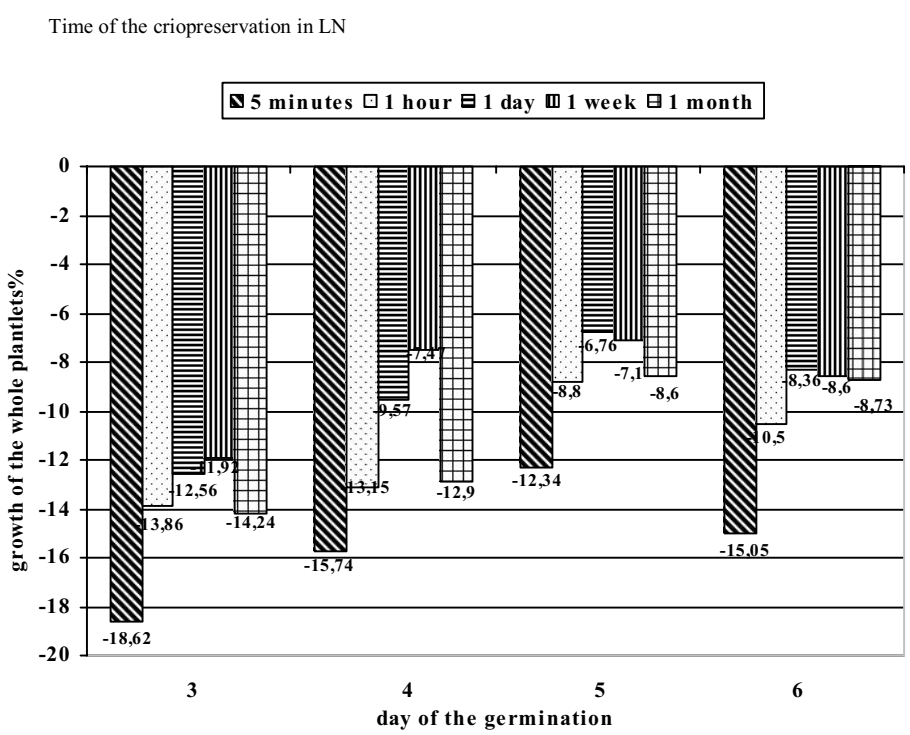

Figure 4. The procentual values reflecting the inhibitions regarding the average growth in length of the whole triticale plantlets coming from caryopses submersed in liquid nitrogen (LN ; -196^{0}C) for 5 minutes, 1 hour, 1 day, 1 week or 1 month, with relation to the growth of the control plantlets (considered 100%, graphically marked with 0).

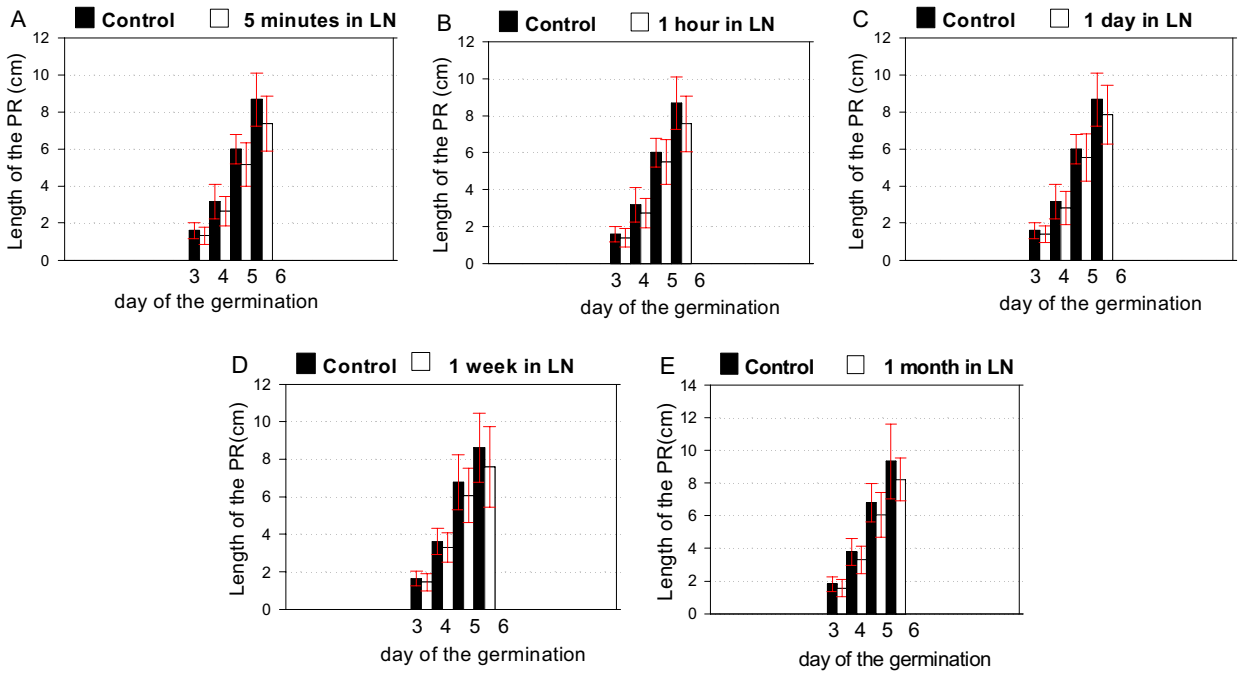

Figure 5. The average data and standard deviations referring to the length of the primary roots (PR) of the plantlets coming by germination from the control triticale caryopses, comparatively with those of the same parameter biometrized at the plantlets resulted out of the caryopses submersed in liquid nitrogen (LN) for 5 minutes (A), 1 hour (B), 1 day (C), 1 week (D) or 1 month (E).

CONCLUSION

After triticale cariopses immersion in liquid nitrogen for variable periods of time: 5 minutes, 1 hour, 1 day, 1 week or 1 month and than defrosted and taken for germination, we registered - in first 6 days of germination - a statistically non-significant inhibition in the growth in length of the vegetative organs (primary and adventitious roots, coleoptiles and first leaves) of the triticale plantlets.

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