THE EFFECT OF CRIOPRESERVATION OF CARIOPSES UPON THE SUBSEQUENT GROWTH OF THE WHEAT, TRITICALE AND BARLEY PLANTLETS

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ABSTRACT. 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 wheat, triticale and barley 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. Maintaining the wheat cariopses for 5 minutes in liquid nitrogen has determined significant inhibitions of the growth in length of the organs of plantlets resulted further to germination of the embryos of these grains and, implicitly, of the whole plantlet; the inhibitions were reflected in the 3-rd day from placing the wheat grain to germinate, of percentage values that exceeded -50% but until the 6-th day of germination, these were not relevant statistically, of approximately -25%. The embryos of the triticale and barley caryopses have proved to be less sensitive compared to those of wheat, the inhibitions registered along the 6 days of germination - the growth in length of the plantlets and their organs after exposing the cariopses for 5 minutes in liquid nitrogen - have been non-significant from a statistical point of view, placed around -20% or below this value. Among the five experimental variants regarding the duration of submersing the cariopses of wheat, triticale and barley in liquid nitrogen, the most affected was the growth of plantlets from germination of the embryos of cariopses exposed for 5 minutes at -196°C. After 1 hour, 1day, 1 week and 1 month exposure of wheat, triticale and barley cariopses in LN, inhibitions registered in growth in length of the vegetative organs of plantlets resulted further to germination of the embryos of these grains and, implicitly, of the whole plantlet have been non-significant statistical.

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

INTRODUCTION

Numerous experiments in research work regarding the cryopreservation of different inocula were accomplished: cell suspensions, protoplasts, caulinar apex, axillary buds, somatic embryos, zygotic embryos detached from recalcitrant seeds or just the axes of zygotic embryos (Touchell and Walters, 2000; Cho et al., 2001, 2002; Kim et al., 2002; Gonzáles et al., 2002; Walters et al., 2002; Santos and Stushnoff, 2003; Corredoira et all, 2004), experiments in the domaig of pollen preservation in LN (-196ºC), of the recalcitrant seeds (Dussert et al., 2000; Thammasiri, 2000; Lambardi et al., 2004; Merritt et al., 2005), branches and buds, as well as an orthodox seeds (Gonzáles-Benito and Pérez-Garcia, 2001; Wood et al., 2003), which may be dehydrated just up to very little degree of humidity (3-4%).

The orthodox seeds are kept tens of years in the gene banks based on classic methods, at -18ºC. But, many banks have problems to keep the refrigerating installations and the remultiplying in experimental field - when the preserved samples viability diminishes – it is risky and expensive.

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 wheat, triticale and barley 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 water content of wheat, triticale and barley caryopses were established through gravimetric methods and was between 6 and 8%. The 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 wheat, triticale and barley 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 statistical processing carried out in the program SigmaPlot 2001 (exemplified in Graphic 1, in what concerns the primary root of wheat) has reflected the fact that these inhibitions have been significant or non-significant statistically.

There were registered - in the case of the experimental variant - 5 minutes of preserving the wheat cariopses in liquid nitrogen (-196°C) – in the 3-rd day of germination in which there were performed the biometric measurements, significant inhibitions from a statistical point of view – with relation to the control – of the growth of the plantlets organs (primary roots and adeventitious roots, coleoptile). These were above -50%, both in what concerns the growth in length of the vegetative organs (Tables 1,2 and 3), and of the whole plantlet (Table 4). At triticale and barley, in the case of the experimental variant of submersion for 5 minutes in liquid nitrogen, the inhibitions were non-relevant statistically beginning with the 3-rd day of germination, the values being placed around -20% (Tables 1,2,3 and 4).

At all 3 species taken into study the most affected were the embryos of the cariopses exposed for 5 minutes in liquid nitrogen, at this submersion duration there being registered higher inhibitions in what concerns the average growth in length of the roots (Table 1), of the coleoptile (Table 2), of the first small leaf (Table 3), as well as of the whole plantlet (Table 4), comparatively with the other variants of crio-preservation experimented within the same species.

At the other experimental variants taken into study - 1 hour, 1 day, 1 week or 1 month of submersing the cariopses in liquid nitrogen, in the 3-rd day from placing the grains to germinate, the growth in length of the plantlets resulted out of the embryos of the wheat cariopses, as well as of their organs – related to the control (considered 100%), represented by plantlets resulted following the germination of the grains that were not submersed in liquid nitrogn before being placed to germinate - seemed to be more inhibited (the percentage values were placed around -25%, even -30%), comparative to the same parameters determined at the triticale and barley (percentage values placed around -15%)(see Tables 1, 2 and 4), but the inhibitions registered at all the 3 species were non-relevant from a statistic point of view in the biometry days taken by us into study, i.e. in the 3-rd and 6-th day of germination.

Analysing the percentage data from tables 1, 2 and 4 – at the experimental variants above-mentioned – one can notice in most of the cases a physiological recovery concerning the growth in length of the plantlets resulted further to germination out of the embryos of wheat, triticale and barley cariopses, as well as their organs, from the 3-rd to the 6-th day of germination, with relation to the average size of the control plantlets, resulted out of cariopses that were not immersed in liquid nitrogen before being placed to germination.

Table 1

Percentage values (%) concerning the average growth in length, in the 3-rd and 6-th day of germination, of the roots of wheat plantlets (*Triticum aestivum* L. var. Turda), triticale (*Triticale*) and barley (*Hordeum vulgare* L.) resulted from the caryopses submersed for 5 minutes, 1 hour, 1 day, 1 week or 1 month in liquid nitrogen (-196^{0}C), with relation to the respective parameter biometrized at the plantlets of the control lot, non-immersed in liquid nitrogen, values regarded as 100%

Vegetative organs	**Primary roots**		**Adventitious roots**		**Primary roots**		**Adventitious roots**		**Primary roots**	
Species	**Wheat**				**Triticale**				**Barley**	
Day of germination	3-rd	6-th	3-rd	6-th	3-rd	6-th	3-rd	6-th	3-rd	6-th
Variants of the experiment										
5 minutes	-57,6%	-27,1%	-61%	-29%	-17,6%	-14,9%	-20,9%	-17,3%	-22,1%	-15,4%
1 hours	-33,1%	-15,4%	-29%	-14%	-12,5%	-10,7%	-14,5%	-15,4%	-15,3%	-16,9%
1 day	-25,1%	-19,2%	-28%	-21,8%	-11,9%	-9,4%	-15,3%	-8,3%	-17,6%	-11,6%
1 week	-29,1%	-15,6%	-25%	-10,7%	-11,5%	-11,7%	-16%	-8,3%	-14,4%	-11,7%
1 month	-24,7%	-21,2%	-15%	-19%	-13,7%	-12%	-20,4%	-14%	-17,6%	-14,5%

Table 2

Percentage values (%) concerning the average growth in length, in the 3-rd and 6-th day of germination, of the coleoptile of wheat plantlets (*Triticum aestivum* L. var.Turda), triticale (*Triticale*) and barley (*Hordeum vulgare* L.) resulted from the cariopses submersed for 5 minutes, 1 hour, 1 day, 1 week or 1 month in liquid nitrogen (-196^{0}C), with relation to the respective parameter biometrized at the plantlets of the control lot, non-immersed in liquid nitrogen, values regarded as 100%

Vegetative organ	**Coleoptile**					
Species	**Wheat**		**Triticale**		**Barley**	
Day of germination	3-rd	6-th	3-rd	6-th	3-rd	6-th
Variants of the experiment						
5 minutes	-52,3%	-16,6%	-20,8%	-9,9%	-24%	-7,2%
1 hour	-22,2%	-15,9%	-16,6%	-6,8%	-17%	-3%
1 day	-22,2%	-17,3%	-13,8%	-4,7%	-10,8%	-5,3%
1 week	-27%	-5%	-12,8%	-3%	-20,6%	-5%
1 month	-23%	-7,5%	-15,3%	-5%	-14,1%	-5,6%

Table 3

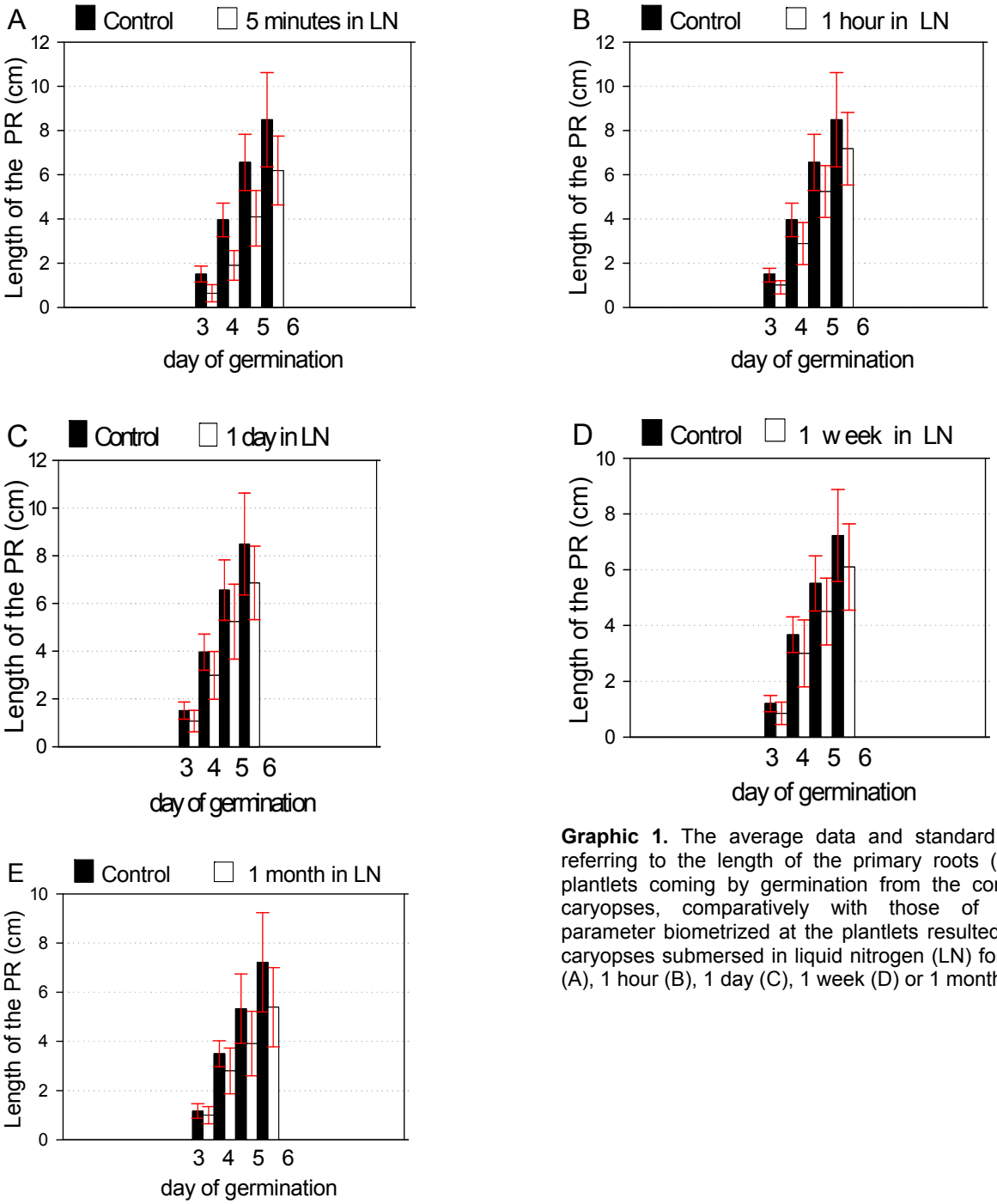
Percentage values (%) concerning the average growth in length, in the 3-rd and 6-th day of germination, of the first small leaf of the wheat plantlet (*Triticum aestivum* L. var. Turda), triticale (*Triticale*) and barley (*Hordeum vulgare* L.) resulted from the cariopses submersed for 5 minutes, 1 hour, 1 day, 1 week or 1 month in liquid nitrogen (-196^{0}C), with relation to the respective parameter biometrized at the plantlets of the control lot, non-immersed in liquid nitrogen, values regarded as 100%

Vegetative organ	**The first small leaf**					
Species	**Wheat**		**Triticale**		**Barley**	
Day of germination	5th	6-th	5-th	6-th	5-th	6-th
Variants of the experiment						
5 minutes	-39,4%	-26,1%	-9,6%	-15,1%	-9,6%	-7,1%
1 hour	-21,2%	-15,6%	-9,6%	-10,1%	-5,4%	-4,1%
1 day	-21,6%	-26,1%	-5,1%	-6,5%	-6,9%	-3,5%
1 week	-3%	-9,4%	-2,2%	-4%	-9,3%	-2,5%
1 month	-2%	-0,32%	-4,5%	-3,3%	-10,6%	-2,4%

Table 4

Percentage values (%) concerning the average growth in length, in the 3-rd and 6-th day of germination, of the whole wheat plantlet (*Triticum aestivum* L. var. Turda), of triticale (*Triticale*) and barley (*Hordeum vulgare* L.) resulted from the embryos of cariopses submersed for 5 minutes, 1 hour, 1 day, 1 week or 1 month in liquid nitrogen (-196^{0}C), with relation to the respective parameter biometrized at the plantlets of the control lot, non-immersed in liquid nitrogen, values regarded as 100%

	The whole plantlet					
Species	**Wheat**		**Triticale**		**Barley**	
Day of germination	3-rd	6-th	3-rd	6-th	3-rd	6-th
Variants of the experiment						
5 minutes	-56%	-26,6%	-18,6%	-15%	-22,9%	-10,7%
1 hour	-30%	-15,5%	-13,8%	-10,5%	-16%	-9,7%
1 day	-24,3%	-22,5%	-12,5%	-8,3%	-14,7%	-7,1%
1 week	-28,3%	-12,3%	-11,9%	-8,6%	-17,1%	-6,5%
1 month	-24,1%	-11,5%	-14,2%	-8,7%	-16,5%	-7,8%



Graphic 1. The average data and standard deviations referring to the length of the primary roots (PR) of the plantlets coming by germination from the control wheat 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).

CONCLUSIONS

Maintaining the wheat cariopses for 5 minutes in liquid nitrogen has determined significant inhibitions of the growth in length of the organs of plantlets resulted further to germination of the embryos of these grains and, implicitly, of the whole plantlet; the inhibitions were reflected in the 3-rd day from placing the wheat grain to germinate, of percentage values that exceeded -50% but until the 6-th day of germination, these were not relevant statistically, of approximately -25%.

The embryos of the triticale and barley caryopses have proved to be less sensitive compared to those of wheat, the inhibitions registered along the 6 days of germination - the growth in length of the plantlets and their organs after exposing the cariopses for 5 minutes in liquid nitrogen - have been non-significant from a statistical point of view, placed around -20% or below this value.

Among the five experimental variants regarding the duration of submersing the cariopses of wheat, triticale and barley in liquid nitrogen, the most affected was the growth of plantlets from germination of the embryos of cariopses exposed for 5 minutes at -196°C.

After 1 hour, 1day, 1 week and 1 month exposure of wheat, triticale and barley cariopses in LN, inhibitions registered in growth in length of the vegetative organs of plantlets resulted further to

germination of the embryos of these grains and, implicitly, of the whole plantlet have been non-significant statistical.

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