A STUDY ABOUT THE EFFECT OF LIQUID NITROGEN (-196ºC) ON BARLEY GRAIN (*Hordeum vulgare* L.)

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Abstract. We followed the germination of barley grains, growth of plantlet vegetative organs - radishes, coleoptile and first leaves – after grain storage by immersion in liquid nitrogen (LN)(-196ºC), for five minutes, one hour, one day, one week respectively one month. The capacity of germination of barley grains with moisture content 7,24%, haven't been affected anyone was the time for immersion in LN. Barley grains which were treated with liquid nitrogen for 5 minutes and one hour were probably the most affected. Growth in the length of radishes was inhibited, irrespective of periods of time for immersion in LN. Physiological regulation in radishes growth was obviously from third to sixth day of germination. In third and fourth days of germination growth of coleoptile and first leaves was inhibited. After these, in fifth and sixth days of germination, was observed the physiological regulation in coleoptile and first leaves growth. However, have been registered in fifth day of germination, a better coleoptile and first leaves growth then the reference lots, after 5 minutes, one hour, or one month barley grain immersion into liquid nitrogen.

INTRODUCTION

Plant germplasm resources, for example seeds, are store in gene banks at 4ºC and -18ºC (Vlase, 1982; Străjeru, 1997; FAO,1996), or in cryogenic conditions using liquid nitrogen, at -196ºC (Walters, 1998, 2004; Wood et. all, 2003; Popov et.all, 2004; IPGRI,1996; NSSL, 2000).

În our research, we followed the germination of barley grains and the growth of plantlet vegetative organs - radishes, coleoptile and first leaves - after grain storage by immersion in liquid nitrogen (LN)(-196ºC), for five minutes, one hour, one day, one week respectively one month.

MATERIALS AND METHODS

In cloth sachets have been packed as many grains, exactly fifty grains/sack. Moisture content of barley grains was, in percentage of 7,24% and the capacity of germination was 85%. We plunked these sachets with dry grains in LN and after this rapid immersion the grains were stored for variable periods of time in LN, exactly for five minutes, one hour, one day, one week respectively one month. After this variable periods of time the sachets were pulled out and the defrost was making at room temperature. In transparent boxes, above a filter paper moisturized with 20 ml water, grains were putting on for germination at 23ºC. After three days of germination was established germination capacity and have been begun all measures. In four days, exactly third, fourth, fifth and sixth day of the germination, was measured the length of the plantlets radishes, coleoptils and first leaves at plantlets which were in the treated lots in comparative with the same parameter determined in the references lots, values that were considered 100%. Percent differences, it was registered graphically. All obtained values were controlled statistics. It was calculated arithmetic media (M), media deviation (б) , standard deviation (S), standard error (SE) and the coefficient of the variability (CV).

$$CV=б/Mx100$$

Interpretation of the coefficient:

0-15% - arithmetic media is representative and we have homogeneous lot.

15-30% - arithmetic media is enough representative and the lot enough homogeneous.

>30%- arithmetic media isn't representative and we have heterogeneous lot.

RESULTS AND DISCUSSION

At barley (*Hordeum vulgare* L.), experimental results were registered graphically in figure 1 and 2.

In four days, exactly third, fourth, fifth and sixth day of the germination, was measured the length of the plantlets radishes and coleoptile. In fifth and sixth days was measured supplementary the first leaves. In the graphics we can see percent difference between arithmetic media calculated for vegetative organs of plantlets results through germination of barley grain, treated with LN for variable periods of time, in comparative with the same parameter determined in the references lots, which was not immersed in LN, values that was considered 100%.

After three days of germination we established that capacity of germination haven't been affected, anyone was the time for immersion in LN, for five minutes, one hour, one day, one week respectively one month.

Coefficient of the variability (**CV**)(see table number 1) demonstrated that lots of plantlets results through germination of barley grain treated with LN were enough homogeneous.

Table 1

Day of the germination	Lots with hundred plantlets	**CV** for radishes	**CV** for coleoptile	**CV** for leaves
Third	Reference	27.84	28.57	
	5 minutes	29.53	27.97	
	1 hour	34.30	31.61	
	Reference 1 day	25.83	24.73	
	1 day	27.34	28.21	
	Reference 1 week	21.41	20.73	
	1 week	25.63	25.84	
	Reference1 month	23.45	21.82	
	1 month	25.70	24.73	
Fourth	Reference	16.04	16.81	
	5 minutes	21.13	16.83	
	1 hour	22.94	29.58	
	Reference 1 day	14.91	19.10	
	1 day	18.95	13.26	
	Reference 1 week	18.46	12.17	
	1 week	20.93	22.95	
	Reference1 month	16.16	14.90	
	1 month	17.50	19.30	
Fifth	Reference	19.14	18.35	25.96
	5 minutes	20.79	12.66	25.90
	1 hour	14.98	12.04	30.90
	Reference 1 day	15.62	18.65	25.41
	1 day	24.17	22.80	27.83
	Reference 1 week	20.95	8	17.50
	1 week	18.44	6.6	16.29
	Reference1 month	12.62	9.09	17.76
	1 month	17.88	11.19	17.94
Sixth	Reference	22.47	9.51	24.14
	5 minutes	18.55	14.74	25.25
	1 hour	21.95	11.43	29.26
	Reference 1 day	17.16	13.37	21.34
	1 day	21.58	16.71	24.16
	Reference 1 week	18.56	8.13	11.27
	1 week	20.30	8.40	14.21
	Reference1 month	17.64	8.90	19.74
	1 month	21	7.70	21.09

If you will analyze figure 1, you can observe that the length of radishes of the plantlets results through germination of barley grain immersed in LN for five minutes, one hour, one day, one week respectively one month, was negative placed in comparative with the same parameter registered for reference plantlets, results after germination of the barley grains which were not storage in LN, values which were considered 100%. Growth in the length of radishes was inhibited, anyone was the duration time of immersion in LN.

After barley grain treatment with LN for 5 minutes and one hour, the growth in the length of radishes was affected the most. However, if in third day of germination was registered these negative percent difference: -15,3% after 5 minutes treatment with LN, -22,1% after one hour, -5,74% after one day, -11,4% after one week and -8,1% after one month, in sixth day of germination percent difference was: -8,8% after 5 minutes, -6,8% after one hour, -5,1% after one day, -7,5% after one week and –5,2% after one month (see figure 1). In conclusion, physiological regulation in radishes growth was obviously from third to sixth days of germination.

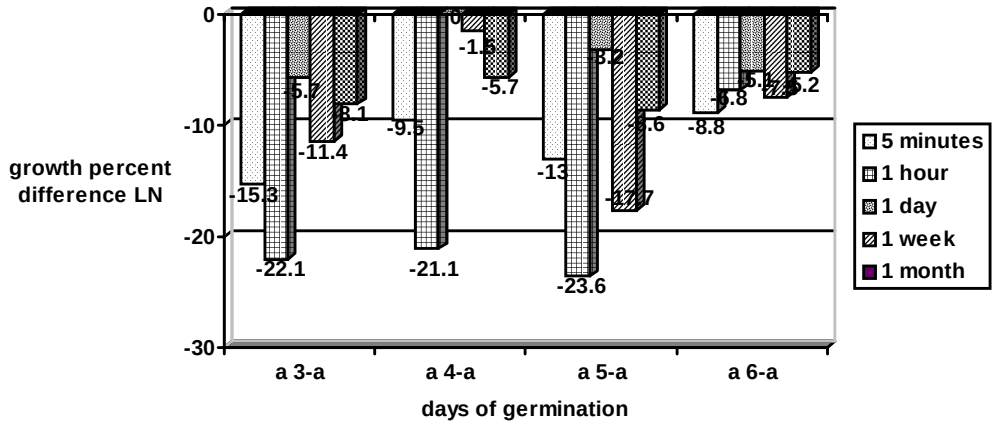

Figure 1. Percent difference between arithmetic media calculated for growth in the length of radishes of plantlets resulted after barley grain germination, treated with LN for variable periods of time, for 5 minutes, one hour, one day, one week or one month, in comparative with the same parameter determined in the references lots, which were not immersed in LN, values that were considered 100%.

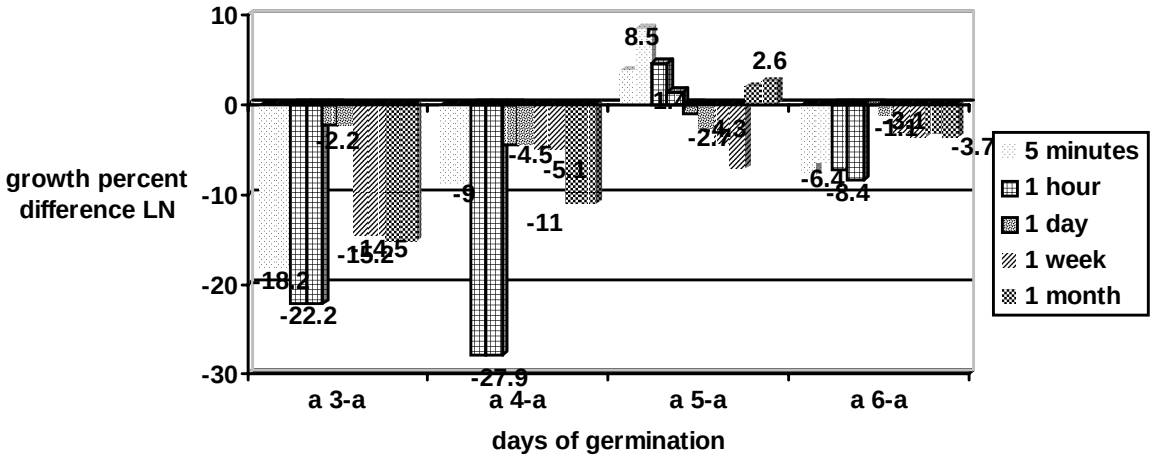

Figure 2. Percent difference between arithmetic media calculated for growth in the length of coleoptile and first leaves of plantlets resulted after barley grain germination, treated with LN for variable periods of time, for 5 minutes, one hour, one day, one week or one month, in comparative with the same parameter determined in the references lots, which were not immersed in LN, values that were considered 100%.

In the case of barley have been registered (see figure 2) in third and fourth days of germination that coleoptile and first leaves growth was inhibited, anyone was the duration of time immersion in LN. Percent differences between arithmetic media calculated for growth in the length of coleoptile and first leaves of plantlets results after germination of barley grain treated with LN, in comparative with the same parameter determined in the references lots, were in third day: -18,2% after 5 minutes treatment, -22,2% after one hour, -2,2% after one day, -14,5% after one week, -15,2% after one month, and in fourth day: - 9% after 5 minutes treatment, -27,9% after one hour, -4,5% after one day, -5,1% after one week, -11% after one month. After these, in fifth and sixth days of germination, we can observe (see figure 2.) physiological regulation in coleoptile and first leaves growth. We have registered, in fifth day of germination, a better coleoptile and first leaves growth then the reference lots, after 5 minutes, one hour, or one month barley grain immersion into LN. In sixth day of germination was registered, in all cases, negative difference between growth of plant vegetative organ results after germination of barley grain treated with LN for variable periods of time, for 5 minutes, one hour, one day, one week or one month, in comparative with the same parameter determined in the references lots.

CONCLUSION

1.The capacity of germination of barley grains with moisture content 7,24%, haven't been affected anyone was the time for immersion in liquid nitrogen (-196ºC).

2. Barley grains which were treated with liquid nitrogen for 5 minutes and one hour were probably the most affected because growth in the length of radishes and coleoptile, in third and fourth day of germination, registered the biggest negative percent difference, in comparative with the same parameter determined in the references lots, which were not immersed in LN, values that were considered 100%.

3. Growth in the length of radishes was inhibited, anyone was periods of time for immersion in liquid nitrogen, for 5 minutes, one hour, one day, one week or one month. Physiological regulation in radishes growth was obviously from third to sixth days of germination.

4. In third and fourth days of germination growth of coleoptile and first leaves was inhibited, anyone was the duration of time immersion in liquid nitrogen. After these, in fifth and sixth days of germination, was observed the physiological regulation in coleoptile and first leaves growth. However, have been registered in fifth day of germination, a better coleoptile and first leaves growth then the reference lots, after 5 minutes, one hour, or one month barley grain immersion into liquid nitrogen.

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