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Effect of salinity stress on *in vitro* propagation of different Albanian wheat cultivars (*Triticum aestivum* L)

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Abstract

Salinity stress is a critical environmental constraint to crop productivity for most species. Wheat is one of the most important species of cereals used for food and feed, as well as in the bio ethanol industry, but is intolerable to high salinity conditions resulting in decreased yield. In the present study was evaluated salinity effect (NaCl) on five wheat cultivars (U2, U10/15, Progresi, Dajti and LVS). As primary explants were used zygotic embryos cultivated on MS media and for organogenesis induction were tested two types of PGRs, BAP and 2,4-D (2 mg/l each). The derived explants were cultivated on MS media combined with 2 mg/l BAP and 0.2 mg/l NAA. In this stage was evaluated salinity stress where were investigated three NaCl levels compared with control (0, 50, 100 and 200 mM). Significant differences were noticed among the cultivars followed by different NaCl levels. The salt stress significantly influenced the plantlets growth which was reduced gradually with the increase of salinity from 0 to 200 mM NaCl. All the cultivars survived at 50 mM NaCl concentration. Only the plantlets of Progresi and LVS survived 100 mM NaCl concentration, meanwhile none of them survived at higher concentrations of NaCl. In most cases, the control was found superior in growth characterized than rest of the tested NaCl levels.

Keywords: salt stress, NaCl, *in vitro* culture, wheat, MS medium, PGRs

1. Introduction

The lower agriculture crop productivity is mostly attributed to various abiotic stresses, which is a major area of concern to cope with the increasing food requirements [22]. Water deficit imposed by either drought or salinity is considered to be the major environmental factor limiting plant growth and productivity, especially in arid and semi-arid regions. Apart from drought, soil salinity is one of the most brutal environmental factors and a complex phenotypic and physiological phenomenon in plants imposing ion imbalance or disequilibrium, disrupting the overall metabolic activities and thus limiting the productivity of crop plants worldwide [15, 19].

Soil salinity levels increase during extended drought periods because less water is available to leach salts (salt already present in soil), which can lead to an abundance of concentrated salt. When soil salinity levels are high, water in the roots is pulled out and back into the soil, depriving the plant of any available moisture and causing potential loss in growth and productivity. The loss of farmable land due to either salinity or drought has posed a major challenge for maintaining world food supplies for the growing population [14].

Wheat is the most economically cereal crop in many parts of the world and considered as salt sensitive species. High concentrations of salts in soils account for large decreases in the yield of a wide variety of wheat culture all over the world [4, 5]. Wheat crops growing in both irrigated and rain fed environments commonly experience environmental stresses, among which drought is one of the most important contributors to yield reduction. Drought drives high salinity in soil, which is another major abiotic stress for wheat crops [18].

Albania has a Mediterranean clime, characterized by short - soft winters and hot - dry summers. During maturity stage of wheat crops, because of water insufficiency and high temperatures, the plants flourish earlier and the grains mature rapidly. In this period of year, in Albania are observed low rainfall values and high temperatures [9, 11, 1]. In

future, the summer is expected to come earlier and to end later. High temperatures and drought stress, that led to possibly high salinity levels in soil will reduce highly crop yield [2, 17].

Thus, there is an urgent need to develop varieties that can maintain optimum yield levels under abiotic stresses.

An option to cope with higher population densities in developing countries is to increase productivity of cultivated land. Plant cell and tissue culture has been a useful tool to study stress tolerance mechanisms under *in vitro* conditions [6]. Plant tissue culture techniques have been used to produce salt-tolerant cell lines and plants in several species [3, 12, 13]. Sodium chloride is a strong osmotic agent and it affects the growth when there is an increased concentration of it in a medium.

The objective of the present investigations was to screen 5 wheat genotypes (Dajti, LVS, Progresi, U2 and U10/15) for salt tolerance under *in vitro* conditions via direct and indirect regeneration.

2. Material and Methods

2.1. Plant material

The experiment included the examination of five wheat cultivars (Dajti, LVS, Progresi, U2, U10/15). The seeds were obtained from the Institute of Plant Genetic Resources, Agriculture University of Tirana. The experiment was set up in the laboratory of the Department of Biotechnology, Faculty of Natural Sciences, University of Tirana.

2.2. Organogenesis induction

In order to establish an effective protocol for disinfection and sterilization, the seeds were treated primarily with ethanol 70% for 3 minutes, and after were treated 0.01% $HgCl_2$ for 5 minutes. Finally, the seeds were rinsed three times with sterile distilled water. The zygotic embryos were exceeded from mature seeds using a stereomicroscope under laminar flow.

MS [16] culture medium supplemented with 3% sucrose, 0.6 % agar and pH 5.7 was used as the basal medium for all *in vitro* treatments. For organogenesis induction were tested two types of PGRs, 2 mg/l 2,4-D for callus induction and 2mg/l BAP (for shoot/root induction). The medium was autoclaved at 121ºC for 20 min and the explants were incubated at 25ºC in growth chamber. Growth morphological parameters such as callus proliferation (%), shoot/root proliferation (%) were determined after two weeks.

2.3. Organogenesis under salinity stress conditions

The explants produced by both direct and indirect organogenesis were used in experiments to determine the effects of different salt concentrations on *in vitro* growth and morphogenesis. The explants were subcultured to MS media combined with 2 mg/l BAP and 0.2 mg/l NAA. Three NaCl levels (50, 100 and 200 mM) compared with control (0 mM) were investigated. For all wheat cultivars under study, survival rate (%) and plant height (cm) were determined in different periods during subculture.

2.4. Data elaboration

All experiments are repeated at least three times with a minimum of 25 test tubes for each of them**.** The experiment was laid out in a completely randomized design with two factors: wheat cultivars and salinity stress. All data were statistically processed using Analysis of variance (ANOVA). Differences between treatments in each cultivar and differences between cultivars within treatments were tested with Student's test. All the analyses were done using JMP 7.0 statistical software.

3. Results and Discussion

3.1. The effect of growth regulators during organogenesis induction

In order to establish the organogenesis pathway which produces explants with a high survival rate in saline media, are tested two types of induction media. Different types of organogenesis induction are observed during cultivation of mature embryos on MS media containing BAP or 2,4-D. Besides PGRs type,

there are observed differences between wheat cultivars for germination rate parameter.

Mature embryo culture supplemented with 2,4-D gives good callus growth in all cultivars under examination (Figure 1a). Callus is proliferated rapidly and there is not observed any infection of the explants. During all cultivation period in 2,4-D containing media callus proliferation rate is evaluated for all cultivars, and there are observed significant differences among them (Figure 2). Progresi cultivar shows the best performance with a callus proliferation rate of 97.4 % on 2,4-D containing media. The lower value for this parameter is observed for U2 cultivar, with only 33.2 %. During this stage, calli of all cultivars remain in the same development level.

The embryos cultivated in BAP containing media develop via direct organogenesis (Figure 1b). The plantlets don't show signs of infections or necroses. Regarding to morphological parameters, there are observed differences between cultivars for shoot/root proliferation (Figure 2). Even during direct organogenesis, Progresi cultivar gives the higher rate (100 %) and U2 cultivar the lower one (26 %).

Auxins induce indirect organogenesis and cytokinins induce the direct one in a very high shoot/root proliferation percentage. In most cases, is preferred direct organogenesis because callusogenesis often drives to somaclonal variations and other abnormalities in cultured explants [7, 21, 26].

Figure 1. a) Callusogenesis in wheat cultivars embryos cultivated in 2,4-D containing media; b) Shoot/root organogenesis in wheat cultivars embryos cultivated in BAP containing media

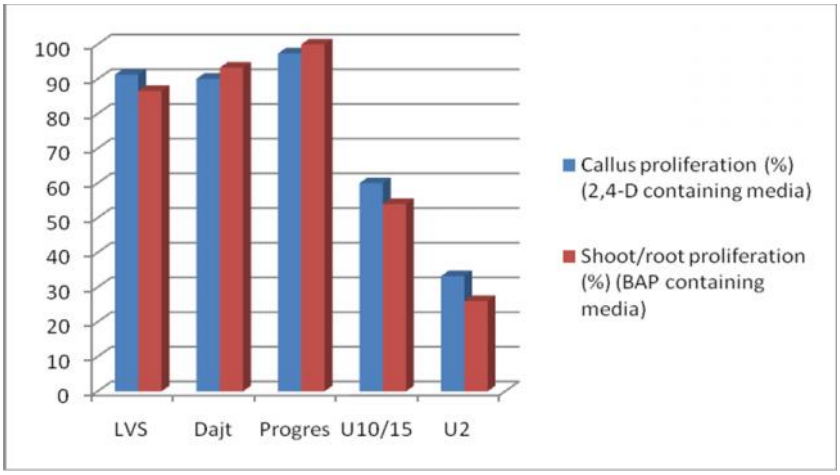

Figure 2. Germination rate and type of organogenesis for 5 albanian wheat cultivars on *in vitro* culture

3.2. The effect of NaCl stress on explants development

The obtained explants from direct or indirect organogenesis are exposed in different salt treatments in order to evaluate their tolerance regarding to salinity in nutrient media.

Increase of salt concentration in the culture medium significantly decreases callus growth in all

cultivars. 100 mM and 200 mM results detrimental for callus explants for all wheat cultivars in the first days of culture and none of them survive. Cultivation on 50 mM NaCl concentration results fatal after two weeks of cultivation with the formation of brownish calli.

Regarding to the explants derived from direct organogenesis, the survival rate is reduced with increasing of NaCl level in the culture medium during a subculture cultivation period for two weeks and four weeks. The effects of NaCl treatments are different between 5 wheat cultivars and within groups for a cultivation period of 2 and 4 weeks (Figure 3a, b).

The results of NaCl effect between groups for 2 week cultivation period shows that except U2 cultivar, all the others survive in a high rate at 100 mM NaCl concentration. In 200 mM NaCl concentration, for a 2 week cultivation period, survive only the plantlets of LVS, Progres and Dajt. The reduction in survival rate increases not only by the increase of NaCl concentration, but is affected even by the continuance of the culture. After 4 weeks of cultivation, only LVS and Progres plantlets survive in a relatively low percentage in 100 mM NaCl, and either of them doesn't survive at 200 mM NaCl concentration. Regarding to their survival in different NaCl treatment, U2 cultivar can be classified as the most sensitive one, U10/15 and Dajt cultivars can be classified as moderate and LVS and Progres as tolerant till 100 mM NaCl during a 4 week cultivation period.

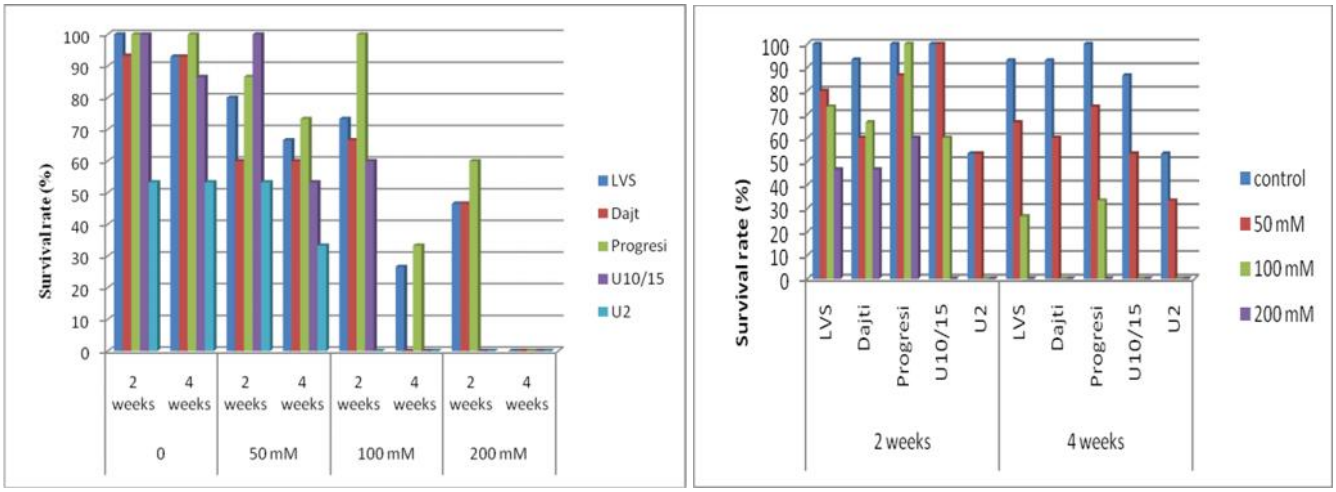

Figure 3. Survival rate (%) of wheat cultivars plantlets exposed to various concentrations of NaCl after 2 and 4 weeks a) comparison between groups b) effect of NaCl stress within groups

As seen in Table 1, there are significant differences between cultivars within a treatment (within groups), and between treatments within cultivars for plant height parameter. Growth dynamics of wheat seedlings related to the salt concentrations during 2 weeks and 4 weeks cultivation period showes that for all the wheat cultivars the development rate decreases with the increase of salt concentrations, relative to the control.

Progres cultivar shows the best response for plant height parameter for all NaCl treatments, with a high value for a 2 week cultivation period in 200 mM NaCl (1.79 cm). After 4 weeks of cultivation in different NaCl treatments, 100 mM NaCl treatment results tolerant for LVS and Progres cultivar with a plantlet height of 2.17 and 4.06 cm respectively.

U10/15 cultivar shows the lower plantlet height value after 2 weeks of cultivation at 100 mM (0.9), with no significant differences with the values for this parameter at 200 mM NaCl cultivation for LVS and Dajt cultivars (0.77 and 0.5 respectively). U2 cultivar shows the lower values in all NaCl treatments including control for both periods of cultivation.

It can be noticed that the plantlets height increase with extend of the culture period for all cultivars under study in control group and 50 mM NaCl concentration. For 100 mM NaCl treatment this parameters also increases but slightly for LVS and

Progres plantlets. During cultivation on 200 mM NaCl, extension of culture period in 4 weeks resulted fatal for all cultivars survived formerly (Figure 4).

Table 1. Average lengths of wheat cultivars plantlets exposed to various concentrations of NaCl after 2 weeks and 4 weeks

	Control	50 mM	100 mM	200 mM
	After 2 weeks			
LVS	3.62 ± 0.24^{cde}	3.26 ± 0.27^{def}	2.01 ± 0.28^{gh}	0.77 ± 0.35^{j}
Dajti	4.32 ± 0.25^{b}	2.45 ± 0.31^{fgh}	1.11 ± 0.29^{ij}	0.5 ± 0.35^{j}
Progresi	5.17 ± 0.24^{a}	4.16 ± 0.24^{bc}	3.9 ± 0.24^{bcd}	1.79 ± 0.25^{hi}
U10/15	3.19 ± 0.24^{ef}	1.70 ± 0.24^{hi}	0.90 ± 0.27^{j}	-
U2	2.85 ± 0.33^{efg}	1.15 ± 0.33^{ij}	-	-
	After 4 weeks			
LVS	4.01 ± 0.26^{c}	3.5 ± 0.30^{cd}	2.17 ± 0.48^{efg}	-
Dajti	4.80 ± 0.25^{b}	2.59 ± 0.32^{ef}	-	-
Progresi	5.7 ± 0.25^{a}	4.97 ± 0.23^{ab}	4.06 ± 0.43^{bcd}	-
U10/15	3.66 ± 0.26^{cd}	1.86 ± 0.34^{fg}	-	-
U2	3.05 ± 0.34^{de}	1.46 ± 0.43^{g}	-	-

Levels not connected by same letter are significantly different according to Student's T ($\alpha < 0.05$)

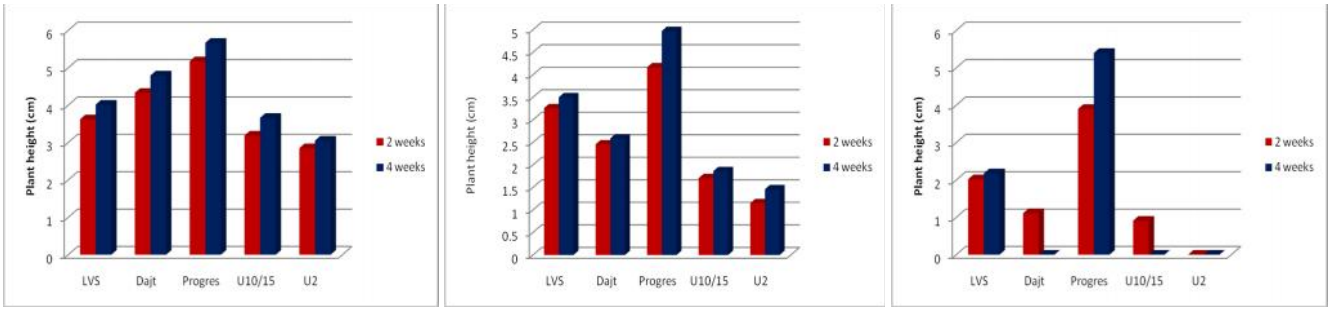

Figure 4. Growth dynamics for all wheat cultivars for 2 and 4 weeks of cultivation in 0, 50mM and 100 mM NaCl cc.

In vitro culture has been widely adopted as the most adequate technique in cereals for the selection of salt tolerant genotypes. This technique allows obtaining plantlets by direct regeneration [21] or by indirect regeneration *via* callus induction [10, 21]. The stressing agent could be added at the beginning of the culture or during later culture stages. Salinity strongly reduces growth and morphogenesis and the rhythm of regeneration is reduced gradually by the increase of NaCl concentration. Similar results are reported by other authors [20, 3].

Depending to plant species and/or genotypes, the salinity stress used can be low, medium or high [23, 24, 25]. According to other reports, the most appropriate salinity stress levels are 100-150 mM NaCl for bread wheat [8], 150 - 200 mM NaCl for barley [23], and 250-300 mM NaCl for tolerant crops as durum wheat [24] etj.

4. Conclusions

In conclusion, these results demonstrate that:

- Regarding to their survival in different NaCl treatment, U2 cultivar can be classified as the most sensitive one, U10/15 and Dajt cultivars can be classified as moderate and LVS and Progres as tolerant till 100 mM NaCl during a 4 week cultivation period;
- Most of wheat cultivars under study are tolerant up to 50 mM NaCL concentration for a 4 week cultivation period.
- Plantlets height increase with extend of the culture period for all cultivars under study;

- Growth parameters decrease strongly with the increase of NaCl concentration;

5. References

1. Acclimatise, Worley Parsons and World Bank. **World Bank workshop on climate change vulnerability and adaptation assessment of Albania's energy infrastructure**. Workshop 2 report, 2009, Tirana International Hotel, Tirana, Albania.
2. Acevedo E, Silva P, Silva H: **Wheat growth and physiology** In: Bread wheat improvement and production. FAO Plant Production and Protection Series No. 30 Rome, 2002, 567pp.
3. Alvarez I, Tomaro LM, Benavides PM: **Changes in polyamines, proline and ethylene in sunflower calluses treated with NaCl**. Plant Cell Tiss. Org. Cult. 2003, **00**: 1-9.
4. Ashraf M, Leary JWO: Responses of some newly evolved salt-tolerant genotypes of spring wheat to salt stress. I. Yield components and ion distribution. J. Agron. Crop Sci. 1996, 176: 91-101.
5. Ayolié K, El Yacoubi H, Atmane R, Kouakou KL, Kouakou TH: NaCl stress-induced growth, water and ions contents changes on in vitro selection of salt tolerant and salt sensitive callus of wheat (Triticum durum Desf.). Intern. J. Biosci. 2011, 1: 12-25.
6. Bajji M, Lutts S, Kinet JM: **Physiological Changes after Exposure to and Recovery from Polyethylene Glycol-Induced Water Deficit in Callus Culture Issued from Durum Wheat (Triticum durum) Cultivars Differing in Drought Resistance**. Plant Physiology 2000, **156**: 75-83.
7. Cutler S, Bonetta D: Plant Hormones. **Methods and Protocols.** Second edition. Humana Press. 2009.
8. Dreccer MF, Ogbonnaya FC, Borgognone MG: **Sodium exclusion in primary synthetic wheats. Proceedings of 54th Australian Cereal Chemistry**. Conference and 11th Wheat Breeding Assembly, C.K. Black, J.F. Panozzo & G.J. Rebetzke, (Eds.), 2004, Royal Australian Chemical Institute, Melbourne, Australia, 118-121.
9. Fitoteknia (1984). Grup autorësh, U.B.,Tiranë.Gorham J: **Salt tolerance in the Triticeae: K/Na discrimination in synthetic hexaploid wheats.** Journal of Experimental Botany, 1990, **41 (5)**: 623-627.
10. Hassanein AM**: Effect of relatively high concentrations of mannitol and sodium chloride on regeneration and gene expression of stress tolerant (*Alhagi Graecorum*) and stress sensitive (*Lycopersicon Esculentum* L.) plant species.** Bulg. J. Plant Physiol. 2004, **30**: 19-36.
11. Laska A: **Elementet klimatike dhe modeli statistikor i prodhimt bimor.** Monografi, 2009, pp. 11-32, 78-89.
12. Manoj KR, Rajwant KK, Rohtas S,, Manu PG, Dhawan AK: **Developing stress tolerant plants through in vitro selection—An overview of the recent progress.** Environmental and Experimental Botany, 2011, **71**: 89–98.
13. Messer T, Tiltscher C, Schmauss M. **Polypharmacy in the treatment of schizophrenia**. Fortschr. Neurol. Psychiatr. 2006, **74**: 377-391.
14. Munns R, Schachtman DP, Condon AG: **The significance of the two-phase growth response to salinity in wheat and barley**. Australian Journal of Plant Physiology 1995, **22**: 561-569.
15. Munns R, Tester M: **Mechanisms of salinity tolerance.** Annu Rev Plant Biol. 2008, **59**: 51-81.
16. Murashige T, Skoog F: **A revised medium for rapid growth and bioassays with tobacco tissue cultures.** Physiol. Plant. 1962, **115**: 473-97.
17. Passioura JB: **The drought environment: physical, biological and agricultural perspectives.** J.Exp.Bot. 2007, **58**: 113-117.
18. Rajaram S: Prospects and promise of wheat breeding in the 21st century. Euphytica 2001, 119: 3. doi:10.1023/A:1017538304429
19. Rozema J, Flowers T: **Crops for a salinized world**. Science 2008, **322 (5907)**: 1478-1480.
20. Saleem MY, Mukhtar Z, Cheema AA, Atta BM**: Induced mutation and *in vitro* techniques as a method to induce salt tolerance in Basmati rice (*Oryza sativa* L.).** Int. J. Environ. Sci. Tech. 2005, **2**: 141-145.
21. Satyavathi VV, Jauhar PP, Elias EM, Rao MB: **Effects of growth regulators on *in vitro* plant regeneration in durum wheat.** Crop Sci. Soc. Am. 2004, **44**: 1839-1846.
22. Shanker A, Venkateswarlu B: **Abiotic stress in plants - Mechanisms and adaptations.**

Agricultural and Biological Sciences 2011, ISBN: 978-953-307-394-1.

23. Shavrukov Y, Gupta NK, Miyazaki J, Baho MN, Chalmers KJ, Tester M, Langridge P, Collins NC: **HvNax3 – a locus controlling shoot sodium exclusion derived from wild barley (Hordeum vulgare ssp. spontaneum).** Functional and Integrative Genomics, 2010a, **10 (2)**: 277-291.

24. Shavrukov Y, Langridge P, Tester M, Nevo E: **Wide genetic diversity of salinity tolerance, sodium exclusion and growth in wild emmer wheat, Triticum dicoccoides.** Breeding Science, 2010b, **60 (4)**: 426-435.

25. Shavrukov Y, Langridge P, Tester M: **Salinity tolerance and sodium exclusion in genus Triticum.** Breeding Science, 2009, **59 (5)**: 671–678.

26. Tech V, Seiler JR: **Forest Biology and and Dendrology**, 2004. (Chapter 5 - Plant Growth and Regulators).