PHYSIOLOGICAL QUALITY OF BARLEY SEEDS SUBMITTED TO SALINE STRESS [1]

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ABSTRACT - The objective of the experiments was to determine the influence of the effects of different salt levels (zero, 15, 30, 45 and 60mM NaCl) on the physiological quality of seeds of two barley cultivars (BRS 195 and AF 98067). Assays were conducted to evaluate salt stress on germination and vigor. The germination and germination rate of the barley seeds decreased as salt levels increased, reducing the seed viability and vigor. The salinity affected the membrane integrity, mainly in AF 98067 that showed more sensitivity to salt stress.

Index terms: *Hordeum vulgare*, germination, vigor, salt.

QUALIDADE FISIOLÓGICA DE SEMENTES DE CEVADA SUBMETIDAS A ESTRESSE SALINO[1]

RESUMO – O objetivo dos experimentos foi determinar a influência de diferentes níveis de sal (zero, 15, 30, 45 e 60mM NaCl) sobre a qualidade fisiológica de duas cultivares de cevada (BRS 195 e AF 98067). Ensaios foram feitos para avaliar o efeito do estresse salino na germinação e vigor nas sementes de cevada. A germinação e a velocidade de germinação de sementes de cevada decresceram com aumento da salinidade, reduzindo a viabilidade e o vigor das sementes. A salinidade afetou a integridade das membranas, principalmente da cv. AF 98067 que mostrou mais sensibilidade ao estresse salino.

Termos para indexação: *Hordeum vulgare*, germinação, vigor, sal.

INTRODUCTION

The saline effects in the germinative development of several seeds have been known for long time, increasing considerably the germination period for the presence of soluble salts in the soil (Campos and Assumption, 1990). Halophytes and glycophytes respond similarly to the increased salt stress, reducing the total number of germinated seeds and the germination speed (Pérez and Tambelini, 1995). The saline concentration capable of causing delay and reduction in the number of germinated seeds depends on the tolerance of each species (Ungar, 1982).

The germination and growth patterns are programmed by the genetic constitution of the species. The eventual expression of this pattern is frequently modified by the conditions of the atmosphere environment under which the seeds grow (Santos et al., 1992). The saline and sodic soils do not always offer to the seeds favorable conditions for their germination, as happens naturally in arid areas, semi-arid and in coastal sandbanks or areas irrigated with salt water.

The vigor tests are considered by many researchers as the most appropriate to evaluate the physiologic attributes of

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seeds and their potential to resist adverse conditions, complementing the information of the germination test (Krzyzanowski et al., 1991). Vigor determination indicates the seed capacity to resist specific stress.

The vigor of the seed is also evaluated by the electric conductivity test, which has as objective to determine the amount of ions present in the soaking water and, indirectly, the vigor of the seeds, based on the fact that vigor is related to the integrity of the cellular membranes system (Marcos Filho et al., 1987). Several processes occur during the seed germination, such as: seed imbibition and activation of the metabolism, breaking of the tegument, emission of the radicle and seedlings growth. The initial stage is mainly a function of water absorption, while the following stages depend on mobilization of the seed reserves (Prisco et al., 1981). Thus, in the imbibition process, citoplasmatic solutes are released in a proportional intensity to the state of disorganization of the membranes. The more deteriorated or damaged seeds release larger amounts of exsudates**.**

The water absorption decreases with the excess of soluble salts that reduce the water potential of the soil. This reduction in the water potential associated to the deleterious effects of the salts interferes initially in the process of water absorption by the seeds, influencing the germination (Bewley and Black, 1978). The smallest water absorption by seeds acts reducing the speed of physiologic and biochemical processes and, therefore, the seedlings show little development, characterized by smaller lengths and lower dry matter accumulation (Sá, 1987).

According to Prisco and O'Leary (1970) the high concentration of salts in the soil, especially sodium chloride, can inhibit the germination, not only due to physiological drought, but also because of the decrease in the water potential, caused by the increase in ion concentration in the embryo, and thus producing a phytotoxic effect.

This study was carried out in order to evaluate the physiological quality of two barley seeds, cultivars BRS 195 and AF 98067, submitted to different salt concentrations.

MATERIAL AND METHODS

The present work was conducted at the Seeds Physiology Laboratory of the Department of Botany at UFPel, using barley seeds of the cultivars BRS 195 and AF 98067.

The germination test - the seeds were soaked for one hour in salt solutions (0, 15, 30, 45 and 60 mM of NaCl). The test was realized taking into account the Rules of Seed Analysis (Brasil, 1992), 400 seeds were used (four sub samples of 100 seeds) and three statistical replications, totalizing 1200 seeds per treatment and 6000 seeds per genotype. Germitest paper was used as substratum, previously moistened with distilled water, in the proportion of 2.5 times of its initial mass (Brasil, 1992). The germinator temperature was 25ºC ± 2ºC. **The first count** was made on the fourth day, and the final count on the eighth day, and seeds were considered germinated that presented radicle protrusion of 2 mm. The results were expressed in the germination percentage.

The electric conductivity was measured in conductivity meter after three and 24 hours of soaking, in four sub-samples of 25 seeds, that were incubated for 1h in a solution of NaCl (0, 15, 30, 45 and 60mM), with three statistical repetitions for treatment. Afterwards the seed masses were checked and put in a beaker with 80mL distilled and deionized water (maintained to 20ºC in advance of 24 hours) and taken to the germinator with constant temperature of 25ºC ± 2ºC. The electric conductivity was measured bya conductivity meter SCOTT CG and the result divided by the mass of the sample, expressed in $\mu S.cm^{-1}.g^{-1}$.

The speed germination index (GSI) - was determined according to Popinigis (1985). The evaluations were made daily at the same time for a period of 8 days.

The experimental design was made completely randomized in a factorial scheme (2 x 5), and consisted of two cultivars and five salt concentrations, with three replications. The regression analysis was performed between salt concentrations and germination and vigor tests.

RESULTS AND DISCUSSION

The averages of the germination tests, first count and the germination speed index are shown in Figure 1. The control seeds presented germination of 97.5% (BRS 195) and 95.5% (AF 98067), indicating excellent physiological conditions for germination (Figure 1A). In both cultivars, the results of the first count (4 days) and final count (8 days) of the germination showed that the salt stress produced a negative effect on the germination process (Figure 1A), decreasing the germination percentage as the salt increased, suggesting that NaCl reduced the viability of the seeds.

The results found for first counting didn't among themselves, in the concentrations of 15 and 30mM, for both cultivars, however the germination percentage (Figure 1A) and the germination speed index (Figure 1C) were shown decreased in tolerances. In both cultivars, the germination of the seeds did not present significant differences, at the concentrations 45 and 60 µM of NaCl.

The germination speed index (GSI) of seeds of *Leucaena leucocephala* (Lam.) de Wit decreased with the increase in the NaCl concentration, leading to the conclusion that as the salinity increased the GSI decreased and, therefore, the vigor of the seeds as well (Cavalcante, 1993). Salt sensitivity of rice, barley, tomato and wheat seeds increased after the germination (Maas and Hoffman, 1977). In this experiment similar results were obtained that can justify the high rates of germination and the GSI obtained.

Comparing these high germination percentages with the imbibition curves (Figure 2) allowed inference that cultivars BRS 195 and AF 98067 began germination around the 300

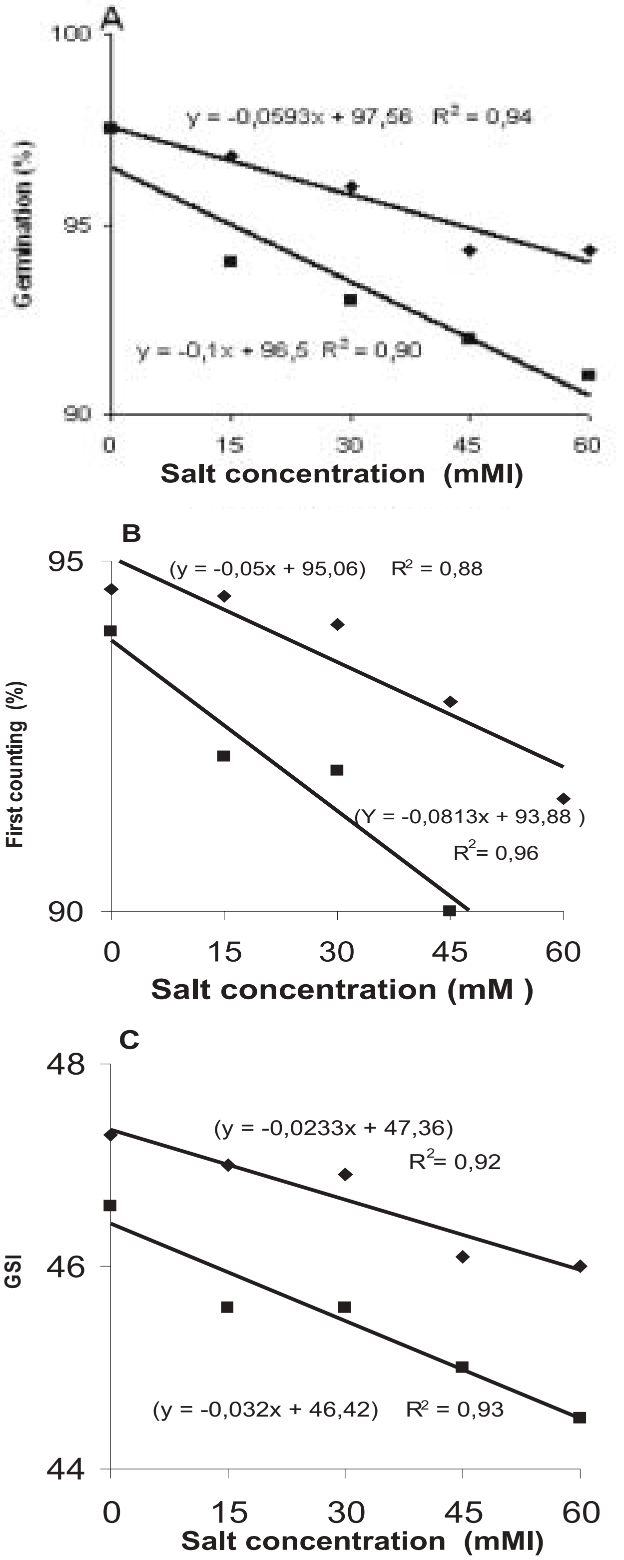

FIGURE 1. Germination percentage (A), first counting (B) and speed germination index (C) of barley seeds cultivars BRS 195 (◆) and AF 98067 (■) as function of the NaCl concentration.

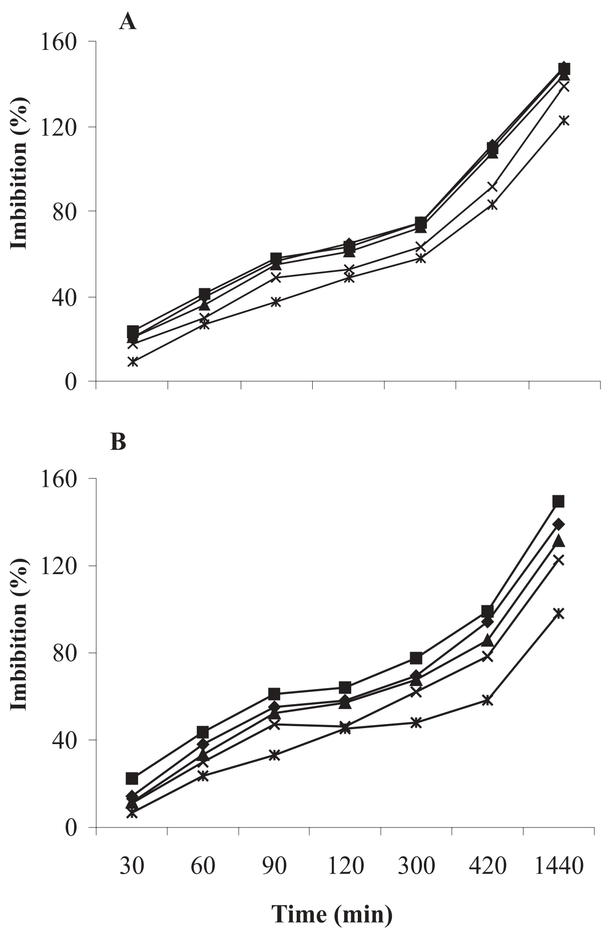

FIGURE 2. Curve of imbibition of barley seeds BRS 195 (A) and AF 98067 (B) submetted the different saline concentrations, being control (■), 15 (◆), 30 (^{2}%), 45 (▲) e 60 mM (X) de NaCl.

minutes, presenting high values at first count, final count and GSI.

The germination speed index decreased as function of the increase in the NaCl concentration (Figure 1B), showing similar results to those reported for *Cnidosculus phyllacanthus* (Silva et al., 2001) and *Prosopis juliflora* (Freire et al., 2001), concluding that the GSI decreases with concomitant reduction in seed vigor. These results were similar to reports for rice seeds (Lima et al., 2005**)** and wheat seeds (Damiani et al., 2003).

According to Bewley and Black (1978) the imbibition curve (Figure 2) follows a sigmoid pattern**,** where the initial phase of the process (phase I) constitutes an essentially physical phenomenon, which can be completed in 1 or 2 hours in the cotiledonares seeds, regardless of their physiological condition. The second stage (phase II) of slow water absorption was 8 to 10 times longer than the previous, which involved series of preparatory metabolic events for the emission of the primary root, that is the mark of the establishment of phase III, represented by the visible beginning of germination. Imbibition during phase I is accompanied by the release of sugars, amino acids and electrolytes in variable amounts with the state of organization of the system of membranes. Several authors (Simon and Raja-Harun, 1972; Becwar et al., 1982; Bewley and Black, 1985) had detected that the electrolyte release rate is very high at the beginning of soaking; however, as time elapses this situation changes, as the reorganization of the cellular membranes takes place.

The time of the seed imbibition influences the capacity of the conductivity tests to distinguish quality differences among lots (Dias and Marcos Filho, 1996). The electric conductivity increased, as much the 3 hours as the 24 hours, in function of the increase in the salt concentration and among the genotypes studied, suggesting that NaCl affected negatively the speed of rearrangement of the cellular membranes, allowing the lixiviation of mineral salts, sugars, proteins and other components from the seed (Figure 3). Electric conductivity was always larger in the cv. AF 98067 in comparison with the cv. BRS 195, suggesting that the integrity of the membranes of AF 98067 was more damaged by NaCl than those of the cv. BRS 195.

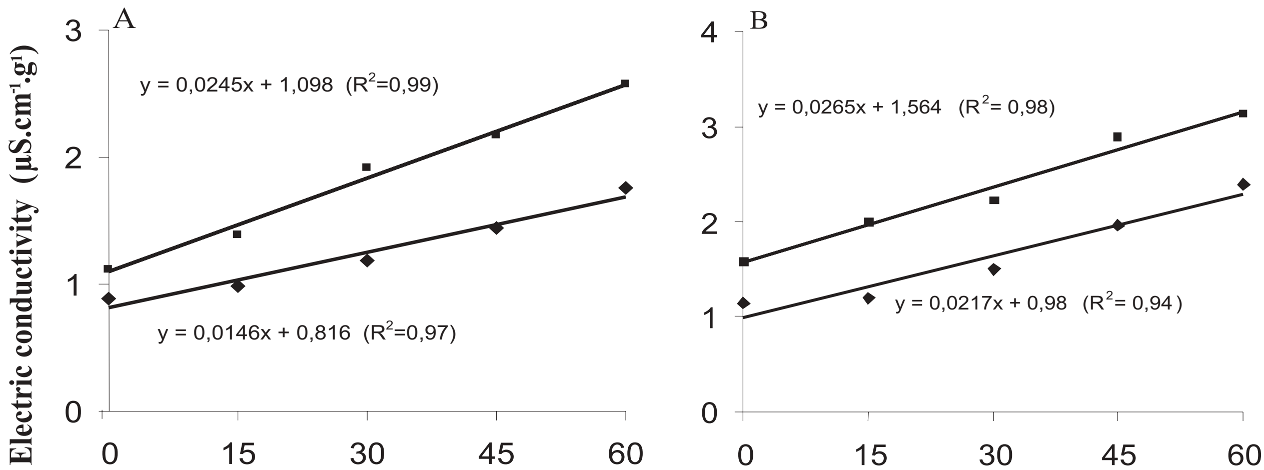

FIGURE 3. Electric conductivity of wheat seeds, cultivars BRS 195 (A) and AF 98067 (B), in function of the salt concentration determined the 3 (◆) and 24 h (■)

The greatest tolerance to salt was observed in e BRS 195 compared to cultivar AF 98067.

CONCLUSIONS

The increase in salinity reduced the seed viability and vigor of barley cvs. BRS 195 and AF 98067.

The cultivar BRS 195 had a higher germination percentage and germination speed index than the cultivar AF 98067 in all the saline treatments. The increment in the salinity caused increase in the electric conductivity, in both the barley cultivars.

REFERENCES

BECWAR, M.R.; STANWOOD, P.C.; ROOS, E.E. Rehydration effects on imbibitional leakage from desiccation-sensitive seeds.

Plant Physiology, Rockville, v.69, n.5, p. 1132-1135, 1982.

BEWLEY, J.D.; BLACK, M. **Physiology and biochemistry of seeds in relation to germination. Development, germination and growth.** v.1. Berlim: Springer Verlag, 1978, 306 p.

BEWLEY, J.D. ;BLACK, M. **Seeds.** Physiology of development and germination. New York: Plenum Press, 1985. 367p.

BRASIL. Ministério de agricultura e reforma agrária**. Regras para análise de sementes**. Brasília: SNDA/DNDV/CLAV. 1992. 365p.

CAMPOS, I.S.; ASSUNÇÃO, M.V. Efeito do cloreto de sódio na germinação e vigor plântulas de arroz. **Pesquisa Agropecuária Brasileira**, Brasília, v.25, n.6, p.837- 843, 1990.

CAVALCANTE, A.M.B. **Germinação de sementes de *Leucaena leucocephala* (Lam.) de Wit**. 1993. 92f. Dissertação (Mestrado)- Faculdade, Universidade Federal de São Carlos, São Carlos, 1993.

DAMIANI, C.R.; MORAES, D.M LOPES, N. F.; ABREU, C.M. Alterações bioquímicas e fisiológicas em sementes de trigo (*Triticum aestivum* L.) induzidas por reguladores de crescimento vegetal. **Revista Brasileira de Agrociência**, Pelotas, v. 9, n. 4, p. 347-352, 2003.

DIAS, D.C.F.S.; FILHO, M.J. Testes de condutividade elétrica para avaliação do vigor de sementes de soja (Glicine Max (L) Merril). **Scientia Agricola**, Piracicaba, v. 53, n. 1, p. 43-50, 1996.

FREIRE, A.L.O.; RODRIGUES, T.J.D.; SOUSA FILHO, G.M. Efeitos da salinidade do substrato na germinação de sementes de algarobeira (Prosópis juliflora (SW) D.C.) In: CONGRESSO NACIONAL DE BOTÂNICA, 52. 2001, João Pessoa. **Anais...** João Pessoa: Sociedade Botânica do Brasil/Universidade Federal da Paraíba, 2001.p.47.

KRZYZANOWSKI, F.C.; FRANÇA-NETO, J.B.; HENNING, A.A**.** Relato dos testes de vigor disponíveis para grandes culturas. **Informativo ABRATES**, Londrina, v.1, n.2, p.15-50, 1991.

LIMA, M. G. S.; LOPES, N. F.; MORAES, D.M.; ABREU, C. M. . Germinação e vigor de sementes de arroz submetidas à estresse salino. **Revista Brasileira de Sementes**, Pelotas, v. 27, n. 1, p. 54-61, 2005.

MARCOS FILHO, J.; CÍCERO, S.M.; SILVA,W.R**. Avaliação da qualidade fisiológica das sementes**. Piracicaba: FEALQ, 1987. 230p.

MAAS, E.V.; HOFFMAN, G.J. Crop salt tolerance-current assessment. **ASCE Journal of Irrigation and Drainage Division**, New York, v. 103, n. 1, p. 115-134, 1977.

PEREZ, S.C.J.G.A.; TAMBELINI, M. Efeito dos estresses salinos e hídricos e do envelhecimento precoce na germinação de algarobeira**. Pesquisa Agropecuária Brasileira**, Brasília, v. 30, n.11, p.1289-1295, 1995.

POPINIGIS, F. **Fisiologia da semente**. 2. ed. Brasília: AGIPLAN, 1985. 289 p.

PRISCO, J.T.; ENÉAS FILHO, J.R.; GOMES FILHO, E. Effect of NaCl on Cotyledon starch mobilization during germination of Vigna unguiculata (L) Walp seeds **Revista Brasileira de Botânica**, São Paulo, v. 4, n. 1, p. 63-71,1981.

PRISCO, J.T.; O'LEARY, J.W. Effect of salt and water stress on protein synthesizing capacity of embryo-axis of germinating *Phaseolus vulgaris* L. seeds. **Revista Brasileira de Biologia**, v. 30, n. 2, p.317-331,1970.

SÁ, M.E. **Relações entre qualidade fisiológica, disponibilidade hídrica e desempenho de sementes de soja** (*Glycine Max* **(L.) Merrill**). 1987. 147 f. (Doutorado em Agronomia) – Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 1987.

SANTOS, V. L.M.; CALIL, A.C.; RUIZ, H. A.; ALVARENGA, E.M.; SANTOS, C.M. Efeito do estresse salino e hídrico na germinação e vigor de sementes de soja. **Revista Brasileira de Sementes**, Brasília, v. 14, n.2, p.189-194. 1992.

SILVA, L.M.M.; AGUIAR, I.B.; RODRIGUES, T.J.D**.** Efeito do estresse salino na germinação de sementes de faveleira (*Cnidosculus phyllacanthus* Pax & K. Hoffm.). In: CONGRESSO NACIONAL DE BOTÂNICA, 52. João Pessoa, **Anais...** João Pessoa: Sociedade Botânica do Brasil/Universidade Federal da Paraíba, 2001. p. 47.

SIMON, E.W.; RAJA-HARUN, R.M. Leakage during seed imbibition. **Journal of Experimental Botany**, Oxford, v.23, n.77, p. 1076-1085, 1972.

UNGAR, I.A. Germination ecology of halophytes. In: D.N. SEN and K.S. Rajpurohit, editors, Contribuition to the ecology of halophytes. **Asks for vegetation science**, v.2, S.1. W. Junk Publishers, The Hague, Netherlands, 1982. p.143-154.

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