MOBILIZATION OF RESERVES OF SUNFLOWER SEEDS UNDER SALINE STRESS

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SUMMARY: This study aimed to evaluating salt stress effects on germination of BRS-122 sunflower seeds, through determination of reserve mobilization by cytochemical and biochemical methods. The experiment consisted in the seed germination in control treatment (distilled water) and salt treatment (200 mM NaCl) in germination chamber (BOD) at 25 °C and 12h photoperiod of light. In lipid determination, there was an accumulation of these reserves in cotyledons, in salt conditions, demonstrating a delay in the use of these reserves. Regarding to protein mobilization, a 21.2% reduction was observed for these contents in control treatment at 2DAI, and on other hand, at the final germination period, it was verified an accumulation of proteins. The Na^+ content was higher in plants exposed to NaCl, whereas K^+ content was reduced over time in control treatment, however, in salt conditions, there was an accumulation of this ion. We assumed that the salinity exposure in sunflower seeds led to Na^+ ion accumulation, which negatively affected the reserve mobilization of these seeds.

KEYWORDS: Germination, *Helianthus annus* L., salinity

MOBILIZAÇÃO DE RESERVAS DE SEMENTES DE GIRASSOL SUBMETIDAS AO ESTRESSE SALINO

RESUMO: Nesse trabalho, objetivou-se estudar os efeitos do estresse salino sobre a germinação de sementes de girassol BRS-122, avaliando a mobilização de reservas ao longo da germinação por meio da determinação de compostos de reserva por métodos citoquímicos e bioquímicos. O experimento consistiu na germinação das sementes em tratamento controle (água destilada) e tratamento salino (200 mM de NaCl) em câmara de germinação (BOD) com temperatura de 25°C e fotoperíodo de 12h. Na determinação de lipídios, observou-se que no tratamento salino houve um acúmulo de lipídios nos cotilédones, demonstrando retardo na utilização dessas reservas. Em relação a mobilização proteica dos cotilédones para o eixo

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embrionário, foi observado uma redução de 21,2% nos teores de proteínas no tratamento controle e um acúmulo na fase final da germinação. O teor de Na^+ foi superior nas plantas expostas ao NaCl, enquanto que o teor de K^+ foi reduzido no decorrer do tempo no tratamento controle, porém, no tratamento salino, houve acúmulo deste íon. Pode-se verificar que a exposição à salinidade levou ao acúmulo de íons Na^+ nas sementes de girassol, o que afetou negativamente a mobilização de reservas dessas sementes.

PALAVRAS-CHAVE: Germinação, *Helianthus annuus* L., salinidade

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oleaginous species belonging to Compositae family, showing excellent quality of the oil extracted from its seed, as well the utilization of by-products of oil extraction in animal feed and their agronomic characteristics. This crop accounts for about 13% of all vegetable oil produced in the world, and in the last few years has increased its cultivation. It has a wide climatic adaptability, high drought tolerance and high oil yield of grains (PRADO & LEAL, 2006).

The Brazilian Northeast semiarid region shows high salt content in most of its water sources (GUILHERME *et al*., 2005), and its use in irrigation can compromise the germination and establishment of several crops. According to Lima Junior (2010), in semiarid regions, it is common the occurrence of soils with high salt concentrations, some of them, are naturally saline.

Soil salinity is one of the main environmental factors that limit agricultural productivity due to its effects on plant growth and development, which may be characterized by an ionic and/or osmotic components inhibiting the seed germination of various crops (MUNNS & TESTER, 2008). When seeds or seedlings are subjected to environmental stress, such as salinity, drought, low or high temperatures, these factors impose an osmotic stress that can lead to the cell turgor loss (KRASENSKY & JONAK, 2012).

Although the effects of saline stress on germination and seedlings establishment has already well characterized, the biochemical mechanisms involved in establishing seedlings under saline stress conditions are still poorly understood. However, it is known that the inhibition of germination by salinity usually coincides with reserve mobilization delay (VOIGT *et al*., 2009). In the case of sunflower, few studies about tolerance to the negative effects of salinity at the different development stages are related (DICKMANN *et al*., 2005). Therefore,

the aim of this study was to investigate the salt stress effects on sunflower seed germination, by the evaluating of cytochemicals and biochemical parameters, in order of knowing the processes involved in their reserve mobilization when submitted to salinity.

MATERIALS AND METHODS

Seeds were germinated on germitest paper pre-moistened with 5 mL of distilled water, 0 mM NaCl (control treatment) or 200 mM NaCl solution (salt treatment) in plastic boxes sealed with PVC film. The seeds were placed in a germination chamber, under constant temperature of 25 °C and a 12 h light photoperiod.

For cytochemical analyses, seeds were collected at different stages of development (quiescent seed (0), 2, 4, 6 and 7 days after imbibition). The material was fixed in a solution of 4% paraformaldehyde and 1% glutaraldehyde in a buffer of sodium phosphate 0.1 M, pH 7.2 for 24 hours and then maintained at 4 °C (KARNOVSKY, 1965). After dehydration, the material was included in resin (Historesin Embedding Kit-Jung), and in following, was sectioned at 5μm using an automatic microtome. The seed cross sections were subjected to the following cytochemical staining: 0.025% Toluidine Blue (TB) pH 4.0 to detect the anion radicals, which are cell wall components (VIDAL, 1970) and Xylidine Ponceau (XP) 0.1% pH 2.5 (VIDAL, 1977), allowing the identification of protein bodies. The staining with XP was performed for 15 min at ambient temperature, followed by washing in 3% acetic acid solution and in distilled water (CORTELAZZO & VIDAL, 1991).

For biochemical analyses, 10 seeds/seedlings were collected daily for six days and stored at -10 °C until use. Quiescent seeds were considered as day 0. The cotyledons of the seedlings were separated for use in the analyses. The obtained material was lyophilized; thereafter the determination of the dry mass, the samples were stored in closed containers and kept refrigerated until the extraction and quantification of reserves.

For lipid determination, the Bligh & Dyer (1959) method was used; the quantification of soluble proteins was done by the Bradford (1976) method, using bovine serum albumin (BSA) as standard. Inorganic compounds, Na^+ and K^+ ions were determined according to Malavolta *et al.* (1989), with the use of a flame photometer.

The experiments for biochemical analyses were carried out in a completely randomized design, in a 7x2 factorial scheme (7 germination times and 2 concentrations of NaCl). Each treatment contained four replicates of 25 seeds each. The results were submitted to variance

analysis (ANOVA), and the means were compared by the Tukey Test using the program ASSISTAT 7.7 ($P < 0.05$).

RESULTS AND DISCUSSION

Regarding Na^+ and K^+ contents in sunflower seeds, Na^+ content was observed to be higher in plants exposed to NaCl, as shown in Table 1. The Na^+ content at 6 days after imbibition (DAI) was 44.2% more in saline treatment compared to control, at the same period. The K^+ content was reduced over time in both treatments, however, its assimilation by the seeds exposed to the salt was significantly delayed (Table 1). The K^+ contents at 6 days after imbibition were 37.7 and 110.0 mmol g^{-1} DM for the control and salt treatments, respectively, which representing an intense decrease in both treatments. Alves *et al.* (2013) also observed a significant increase in Na^+ content and delay in the use of K^+ in cashew seedlings exposed to NaCl, demonstrating that the ionic component of saline stress seems to be responsible for inhibition of cotyledonary reserve depletion.

In organic compound determination, a reduction of 18.8% in lipid contents in the cotyledons was observed at 6 DAI in comparison to 0 DAI, in the control treatment, whereas in the saline treatment there was an lipid accumulation in the cotyledons, demonstrating a delay in the use of these reserves (Table 2). Similar results were found by other authors, such as Alencar *et al*. (2015), who observed delay in the reserve mobilization during the germination of *Jatropha curcas* seeds submitted to salt stress. The metabolic processes of lipid degradation can be highly sensitive to stresses, such as water deficit, salinity, among others (KRASENSKY & JONAK, 2012).

The protein mobilization of cotyledons showed similar pattern to lipid mobilization (Table 2). A reduction of 21.2% in protein contents was observed in the control treatment, however, in the salt treatment, there was a reduction of only 9.3% during the germination. The values of protein contents in cotyledons were 40.6 mg g^{-1} DM at 6 days after imbibition for the control treatment and 50.2 mg g^{-1} DM for salt treatment. Corte *et al.* (2006) analysing the mobilization of reserves during the germination of *Caesalpinia peltophoroides* Benth., observed that the soluble protein contents decreased during germination, indicating that the protein mobilization is occurring.

Toluidine Blue (TB) staining was used for the morphological analysis of the cotyledonary cells of the sunflower seeds (Figure 1). The cells presented irregular shapes, ranging from elliptic to rounded. Fine cell walls were observed, and difference in coloration in seeds of day

2 of the saline treatment (S - D2). Additionally, the presence of colored material in the cellular cytoplasm was also verified, which probably indicates the presence of cell division. Other authors have also used microscopy techniques to analyse the changes that occur in cell contents during germination and seedling development (GALLÃO *et al*., 2007).

Cytochemical analyses with Xylidine Ponceau (XP) detected the presence of proteins in the cytoplasm of the cotyledonary cells of sunflower seeds (Figure 2). These protein bodies appeared as small globular corpuscles, distributed throughout the cytoplasm, stored in reserve vacuoles. It was observed that in the control treatment these protein globules were degraded during the germination, however, in the salt treatment these globules remained practically unchanged. These data corroborate with biochemical analyses, which showed a delay in the use of cotyledonary protein reserves during germination in salt stress. Lopes *et al*. (2013) also observed reduction of these protein bodies along the germination in seeds of *Jatropha*. The presence of XP stained globular protein bodies was also observed in seeds of *Sorghum bicolor* (OLIVEIRA *et al*., 2011) and *Cereus jamacaru* (ALENCAR *et al*., 2012).

CONCLUSIONS

The reserve mobilization of sunflower seeds occurs rapidly under control conditions, in other hand it is retarding by salt stress. Moreover, Na^+ accumulation in sunflower cotyledons can be related to the delay in the consumption of seed reserves during germination due to osmotic imbalance, mainly lipid reserves. In addition, the cytochemical study confirmed the biochemical analyses, demonstrating delay in the cotyledonary degradation of protein reserves.

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Table 1. Na^+ and K^+ contents in sunflower seed cotyledons during germination under control and salt conditions

	Na^+ (mmol g^{-1} cotyledon dry mass)		K^+ (mmol g^{-1} cotyledon dry mass)	
DAI	Control	Salt	Control	Salt
0	501.4 ± 27.6 aA	523.2 ± 6.7 aA	232.8 ± 6.1 bB	243.0 ± 3.3 aA
1	528.3 ± 7.6 aA	534.7 ± 4.4 aA	246.9 ± 2.4 aA	246.9 ± 3.2 aA
2	84.7 ± 2.1 bB	538.5 ± 5.7 aA	164.4 ± 0.8 bC	251.9 ± 4.4 aA
3	88.3 ± 1.2 bB	544.9 ± 5.3 aA	175.6 ± 0.9 bC	214.9 ± 3.6 aB
4	22.9 ± 1.2 bC	55.0 ± 1.9 aB	35.2 ± 0.4 bD	191.2 ± 4.3 aC
5	39.0 ± 0.4 aC	56.9 ± 0.3 aB	36.3 ± 0.2 bD	108.1 ± 1.3 aD
6	56.5 ± 0.5 bBC	81.5 ± 3.4 aB	37.7 ± 0.4 bD	110.0 ± 2.5 aD

Values are means ± standart error (n=4). Differents letters indicate significant difference at $P \leq 0.05$ according Tukey Test. DAI = Days after imbibition.

Table 2. Lipids and proteins contents in sunflower seed cotyledons during germination under control and salt conditions

	Lipids (mg g^{-1} cotyledon dry mass)		Proteins (mg g^{-1} cotyledon dry mass)	
DAI	Control	Salt	Control	Salt
0	46.9 ± 3.2 aAB	38.0 ± 1.5 bB	51.5 ± 3.8 aA	55.3 ± 3.1 aBCD
1	53.7 ± 3.7 aA	53.0 ± 0.9 aA	48.5 ± 2.8 bAB	87.6 ± 0.9 aA
2	49.1 ± 3.3 aAB	43.1 ± 3.9 aAB	20.2 ± 1.7 bC	64.0 ± 7.3 aBC
3	49.8 ± 3.5 aAB	34.9 ± 0.3 bB	48.5 ± 3.9 bAB	70.3 ± 5.9 aAB
4	43.1 ± 4.0 aAB	36.2 ± 0.4 aB	32.9 ± 1.5 bBC	51.4 ± 5.4 aCD
5	45.1 ± 3.9 aAB	43.7 ± 1.5 aAB	31.9 ± 5.1 bBC	44.4 ± 1.3 aD
6	38.1 ± 1.4 aB	41.5 ± 1.6 aAB	40.6 ± 3.0 aAB	50.2 ± 4.1 aCD

Values are means ± standart error (n=4). Differents letters indicate significant difference at $P \leq 0.05$ according Tukey Test. DAI = Days after imbibition.

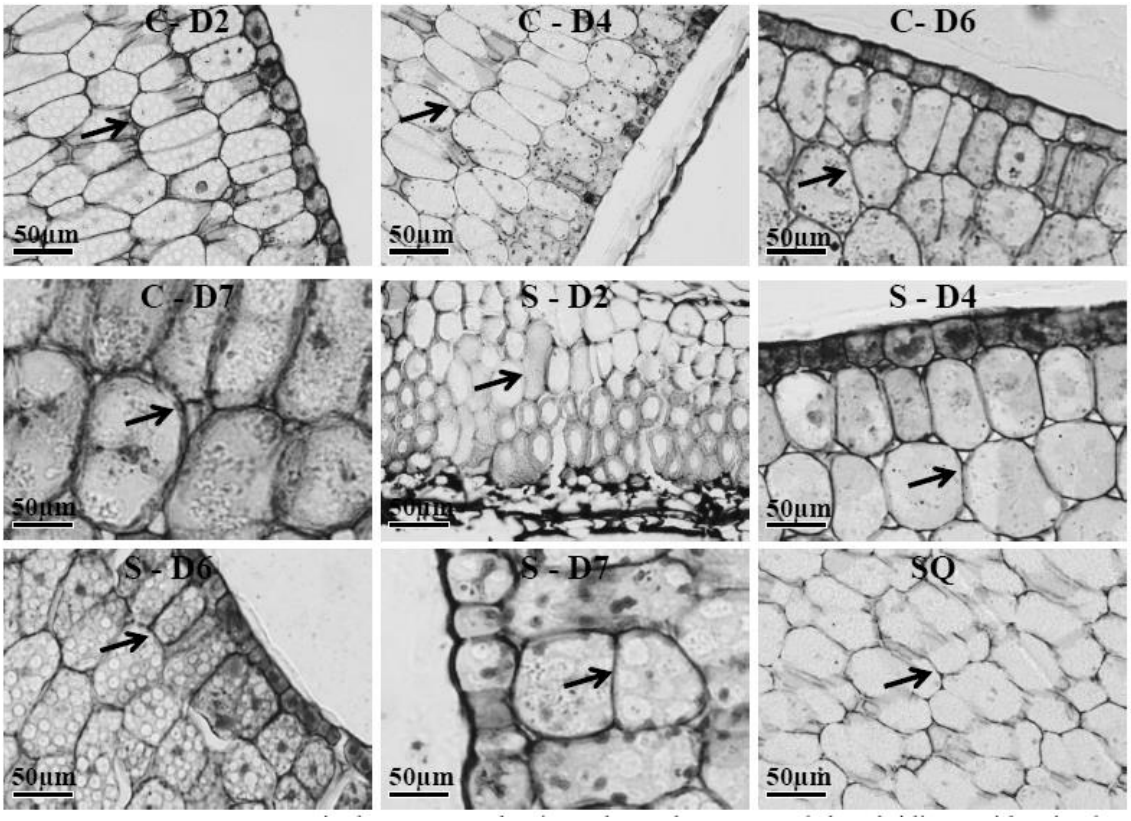

Figure 1. Transversal sections of sunflower seeds stained with toluidine blue, emphasizing the cell wall and size of cells (→). C – control treatment (distilled water); S – treatment with 200mM NaCl; D – days after imbibition; SQ – quiescent seed.

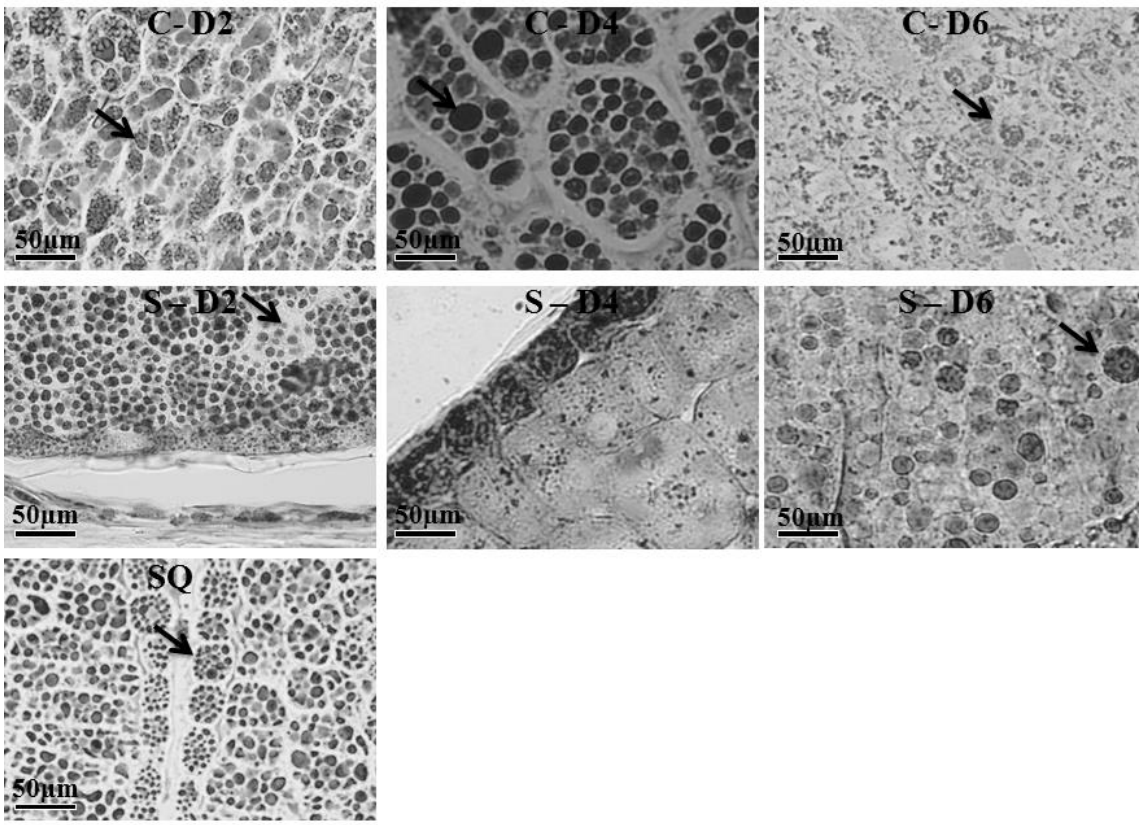

Figure 2. Transversal sections of sunflower seeds stained with xylidine ponceau, emphasizing the protein bodies in cotiledonary cells (→). C – control treatment (distilled water); S – treatment with 200mM NaCl; D – days after imbibition; SQ – quiescent seed.