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INFLUENCE OF SALINITY ON GERMINATION, SEEDLING GROWTH, ION CONTENT AND ACID PHOSPHATASE ACTIVITIES OF *Linum usitatissimum* L.

N. Nasri[*1•], S. Maatallah[2•], I. Saidi[1] and M. Lachaâl[1]

1Université de Tunis El Manar, Faculté des Sciences de Tunis, Unité de Physiologie et Biochimie de la Réponse des Plantes aux Contraintes Abiotiques, Campus Universitaire Farhat Hached d'El Manar, 1068 Tunis, Tunisia;
[2]Institution de la Recherche et de l'Enseignement Supérieur Agricole (IRESA). Centre Régional de Recherche en Agriculture, Sidi Bouzid. 9100, Tunisia
[*]Corresponding author E-mail address: nasrinawel@yahoo.fr
[•] These two authors contributed equally to this work

ABSTRACT

The aim of this work was to investigate the effect of different concentrations of NaCl (0 mM, 100 mM, 200 mM and 300 mM) on germination, seedling growth and acid phosphatase activity of *Linum usitatissimum* seeds. Flax seeds were germinated under control and saline (100, 200 and 300 mM NaCl) conditions and the effect of salt stress on germination and enzyme activity was determined. Final germination percentage was reduced at higher concentrations than 100 mM and was annulated at the highest salt concentration (300 mM NaCl). However, seedling growth was inhibited by all salt concentrations. The Na content of seeds after imbibitions significantly increased with increasing salinity but K^+ content decreased. The effect of saline treatment on hydrolytic enzymes activities varied, depending on the concentration of NaCl and the organ of seedlings. However, radicle acid phosphatase activity exhibited a decrease in flax seedlings after 48h and 96h of germination under 100 and 200 mM NaCl. In hypocotyl and cotyledons, this activity showed no difference compared with the control under salt stress after 48h and 96h of growth.

Key words: Germination, seedling growth, salinity, acid phosphatase activity, *Linum usitatissimum.*

INTRODUCTION

Flax seed (*Linum usitatissimum* L.) is a globally important agricultural crop grown both for its seed oil (Kurt and Bozkurt, 2006; Berti *et al.* 2010) as well as its stem fiber which are made into linen and other cloths (El-Nagdy *et al.* 2010). Moreover, consumption of the oil or seed has been reported to have beneficial effects on cardiovascular health and in the treatment of certain cancers, neurological and hormonal disorders and inflammatory diseases (Simopoulos, 2002).

Germination and seedling emergence may be influenced by temperature, sowing depth and seedbed conditions like available moisture and salinity (Couture *et al.* 2004; Kurt and Bozkurt, 2006). Salinity leads to delayed germination and emergence, low seedling survival, irregular crop stand and lower yield due to abnormal morphological, physiological and biochemical changes (Muhammad and Hussain, 2010). NaCl decreased germination percentage, speed of germination and seedling dry matter in different varieties of lettuce (Nasri *et al.* 2011) and in rice genotypes (Mondal *et al.* 2015).

In response to salt stress, the plant must develop adaptive mechanisms place to adjust the pressure through the internal osmotic and electrolyte to organic solutes. Adaptation to all these stresses is related to metabolic adjustments that escort to the inflection of diverse enzymes (Yan *et al.* 2001; Ehsanpour and Amini, 2003). Among these enzymes are phosphatases, which are assumed to be crucial for many physiological processes, including regulation of soluble phosphorous (Pi) (Yan *et al.* 2001). Phosphorus is one of the most important mineral nutrient for plant growth but least available. The efficient acquisition and utilization of phosphorus requires a ubiquitous class of enzymes known as phosphatases, which function to hydrolyse Pi from orthophosphate monoesters. Acid phosphatases are enzymes highly expressed in plants and especially in plant tissues such as roots, bulbs, seeds, tubers, coleoptiles and leaves (Yan *et al.* 2001; Tejera Garcia *et al.* 2004).

Acid phosphatase activity is known to contribute to resistance under salt and water stress by preserving a certain level of inorganic phosphate (Dubey and Sharma, 1990; Olmos and Hellin, 1997). The salinity stress on the canola seeds caused an increase in both acid phosphatase and alkaline phosphatase activity (Bybordi and Elnaz, 2011). Increasing salinity levels induced reduction in germination, delayed emergence, inhibited seedling growth, delayed degradation of seed lipids and fluctuating content of linolenic and linoleic acid in different flax varieties (Sebei *et al.* 2007; Kaya *et al.* 2011).

The aim of this work was to study the effect of salinity on germination, seedling growth and acid phosphatase of *Linum usitatissimum* seeds.

MATERIALS AND METHODS

Plant material and NaCl stress treatment: The seeds of flax seed (*Linum usitatissimum* L.) variety, ‘‘H52’’ were supplied by the “Institut National de la Recherche Agronomique de Tunis” (INRAT, Tunisia). For germination, seeds were soaked for 2 h in distilled water. The seeds were then placed in Petri dishes with double layer filter paper initially moistened with a solution of the respective salt concentration 0, 100, 200 and 300 mM. The Petri dishes were incubated for 4 days in the dark at room temperature (25 ± 2°C). Each treatment consisted of 25 seeds per Petri dish and was replicated 3 times. Seeds with emerged radicle were counted daily.

Germination and growth parameters: * Final germination percentage was calculated using the following formula: Germination percentage = Number of germinated seeds / number of total seed x 100.

* Germination rate: is a measure of rapidity of germination, it is calculated as follows: $GR = (n_1t_1)+(n_2t_2) + ... + (n_xt_x)/ Xn$ where n_1 is the number of germinates at the first day of germination , t_1 is the days from start – of first germination, and Xn is the total number of seeds germinated (Rubio-Casal *et al.* 2003).

* Mean Germination Time $MGT = \sum dn/\sum n$ (Kandil *et al.* 2012), where n is number of seeds which germinated on day d, d is number of days counted from the beginning of germination.

* Seedling vigour index was calculated based on the formula used by Hossein and Kasra (2011): Seedling vigour index = Germination % × Seedling dry weight.

Regarding morphological studies radicle length, hypocotyl length, fresh weight (FW) and dry weight (DW) were measured from 6 seedlings on 4th day after sowing (DAS). Plant material was dried at 60°C for 2 days and dry weights (DW) was measured. For the analysis of K^+ and Na^+, dried seedlings were digested in 0.1 N nitric acid and two cations in the extract were assayed by atomic absorption spectrophotometry.

Water content (WC) was calculated as: WC % = [(FW-DW) / FW] x100

Biochemical parameters: For biochemical study, acid phosphatase activities were analysed from physiologically active root, shoot and cotyledons and were carried out as previously described by Nasri *et al.* (2011).

So, acid phosphatase was extracted by grinding the tissues (radicle, hypocotyl and cotyledons) after 48 h and 96 h of germination at 4 °C using 0.1 M sodium acetate buffer (pH 4.5). The homogenate was centrifuged at 12000g for 15 min and supernatant was collected. A total reaction volume of 0.5 mL was prepared for each sample and incubated at 30°C for 30 minutes. The reaction was stopped with 1 mL of 0.2 M NaOH. Acid phosphatase activity was measured spectrophotometrically at 400 nm by monitoring the release of p-nitrophenol (pNP) from p-nitrophenyl phosphate. A standard curve was established with p-nitrophenol solutions. One unit of enzyme activity is defined as the amount of enzyme liberating 1 nmol of p-nitrophenol per minute (Saluja *et al.* 1989).

Statistical analysis: Statistical analysis including Analysis of variance (ANOVA), Duncan’s multiple range test was performed to study the significance of different salinity gradient on different parameters studied. Values were calculated at the $p \leq 0.05$ probability level.

RESULTS

Effect of NaCl stress on physiological parameters: The effects of different levels of NaCl on physiological parameters are represented in Table 1 and Table 2. For H52 seeds, final germination percentage was not affected at 100 mM NaCl compared to control but a reduction in FGP was observed at 200 mM and germination being suppressed at 300 mM NaCl (Table 1). Increasing NaCl concentration resulted in decrease in germination rate and seed vigor index of flax seeds. In fact, mean germination time of *Linum usitatissimum* seeds at 200 mM was higher than the control (Table 1).

The effect of salt on the radicle and hypocotyl elongation of flax seedlings was shown in Table 2. It shows that the gradual increase in the NaCl concentration reduces significantly the radicle and the hypocotyl length in flax variety. At 100 mM NaCl, the reduction is 63% and 55% compared to the control, respectively, in radicle and the hypocotyl. At 200 mM NaCl, this reduction is 78% in radicle and 73% in hypocotyl. Therefore, radicle growth was more affected by salt than was hypocotyl growth.

As the concentration increased, the dry weight of seedlings in *Linum usitatissimum* was reduced significantly. Whole seedling biomass was diminished by 18 % at 100 mM (6.06±0.20 mg DW. $plant^{-1}$) and by 34% at 200 mM (4.88±0.09 mg DW. $plant^{-1}$) (Table 2) compared to control (0 mM NaCl). A slight decrease in water content was observed by increasing NaCl levels in the investigated linseeds (Table 2).

Our results also indicated that ion uptake was strongly affected by all salinity treatments. High sodium amount was accumulated in flax seedling while K^+ content in seedlings was decreased relatively as NaCl concentration was increased (Table 2).

Effect of NaCl stress on biochemical parameters: The effect of NaCl treatment on acid phosphatase activity in flax seedlings was shown in Figure 1 (A and B). With increasing NaCl level in the growth medium a marked decrease in enzyme activity was observed in radicle. Therefore, with 100 mM NaCl treatment about 20 to 23% inhibition in enzyme activity was observed during 2-4 d of growth respectively. Whereas at 200 mM NaCl about 60 to 43% inhibition in enzyme activity was observed respectively after 48h and 96 h of growth. In hypocotyl, acid phosphatase activity was comparable to control under salinity (Figure 1 A and B). In cotyledons, acid phosphatase activity increased under salt stress condition (100 and 200 mM) at 48 h of growth and was comparable to control at 96 h of growth (Figure 1 A and B).

DISCUSSION

This research was carried out to observe the effects of salinity on germination and seedling growth of linum seeds H52 cultivar. The results showed that salt stress reduced germination capacity and delayed germination. However, high concentration of NaCl affected germination capacity and increased lightly the delay time. This is in line with the findings of El Goumi *et al.* (2014) that showed decreased germination index as salt concentration increased. Also, Bordi (2010) reported that the germination percentage in B. napus was significantly reduced at 150 and 200 mM NaCl. Moreover, Habtamu *et al.* (2013) reported that the rate of seed germination of chickpea significantly reduced by increasing salinity levels. Decreases in seedling vigour under salinity were also mentioned by Zapata *et al.* (2003) in nine lettuce varieties.

e and hypocotyl length of seedlings grown in salt solutions also showed decline, indicating that the salt stress not only affected germination but also the growth of seedlings. These observations are in accordance with the findings of Jamil and Rha (2004) for sugar beet and cabbage, Tambhale *et al.* (2011) for rice cultivars; Nasri *et al.* (2011) for lettuce varieties, Kumar *et al.* (2014) for *Avena sativa* and Patil *et al.* (2015) for *Linum usitatissimum* where increase of salinity levels induced reduction of root, shoot length and seedlings dry weight. NaCl affected seed germination by creating an external osmotic potential preventing water uptake. However, a number of studies have demonstrated that water uptake in triticale (Mehmet *et al.* 2006) and wheat cultivars (Akbarimoghaddam *et al.* 2011) is significantly reduced under salt stress conditions.

Na^+ from medium was absorbed by seeds. It is reported that increasing NaCl concentrations was resulted in increasing K^+ leakage from seeds (Mehmet *et al.* 2006). Bhivare and Nimbaker (1984) found that the reduction of K content and the increased of Na content in plant could be attributed to the effect of competition between Na and K ions on the absorptive sites of the plant. The reduction in K concentration causes a growth reduction by decrease the capacity of plants for osmotic adjustment and turgor maintenance or by the negative effects on metabolic functions as protein synthesis (Green Way and Munns, 1998).

Concerning enzymatic activity, In our study, acid phosphatase activity was decreased in radicle by salinity in linum seedlings. The decrease in acid phosphatases levels under salinity might lead to reduced rate of phosphate and energy liberation in the endosperms. This may escort to decreased in general metabolic status of germinating seeds causing the reduction in hydrolysis of endosperm reserves that eventually leads to diminution of seed germination and seedling vigour (Dubey and Sharma, 1990).

In hypocotyls and cotyledons of flax seedlings we distinguished an increase in acid phosphatase activities. These results were in accordance with the results of Nagesh Babu and Devaraj (2008) who indicated increased acid phosphatase activities under salt stress. In soybean seedlings, Oprica and Marius (2014) demonstrated that at four days after NaCl treatment 50 mM concentration diminished acid phosphatase activity (reduced rate -49%) whereas at 100 mM and 150 mM stimulation rate was high (87% and 33%, respectively). Oprica *et al.* (2011) showed that salt stress stimulated the acid phosphatase activity, more intense at 4 days and moderately at 7 days after treatment in *Brassica napus* seedlings. Under stress conditions, growth is restricted and delivery of phosphate is impaired, thus resulting in the activation of the cellular phosphatases that release soluble phosphate from its insoluble compounds inside or outside of the cells thereby modulates osmotic adjustment by free phosphate uptake mechanism (Fincher, 1989). According to Ehsanpour and Amini (2003) experiments when a plant increases its acid phosphatase activity under stress condition it becomes more resistant to harmful condition. Olmos and Hellin (1997) observed that acid phosphatase are known to act under salt and water stress by maintaining a certain level of inorganic phosphate which can be co-transported with H^+ along a gradient of proton motive force.

Conclusions: In conclusion, our findings revealed that Flax genotype "H52" exhibited sensibility to salt stress at germination stage. So, we note reduction in germination percentage, seedling growth. Salt stress induced a decrease of acid phosphatase activity in radicle of flax seedlings after 48h and 96h of germination. In hypocotyl and cotyledons, this activity showed no difference compared with the control under salt stress after 48h and 96h of growth. The accumulation of Na^+ in the seeds may have an adverse effect on seed germination resulting in delaying mean germination time in linseeds. It seems that the delay in seed germination was related more to Na^+ accumulation in seeds rather than lower water content in flax seedlings. Under salt stress, the Na^+ content of germinating seeds gradually increased, while K^+ content diminished.

REFERENCES

Akbarimoghaddam, H., M. Galavi, A. Ghanbar, and N. Panjehkeh (2011). Salinity effects on seed germination and seedling growth of bread wheat cultivars. Trakia J. Sci. 9 (1): 43-50.

Berti, M., S. Fischer, R. Wilckens, F. Hevia, and B. Johnson (2010). Adaptation and genotype × environment interaction of flax seed (*Linum usitatissimum* L.) genotypes in South Central Chile. Chil. J. Agri. Res. 70: 345-356.

Bhivare, V.N. and J.D. Nimbalkar (1984). Salt stress effect on growth and nutrition of French beans. Plant Soil 80: 91-98.

Bordi, A. (2010). The influence of salt stress on seed germination, growth and yield of canola cultivars. Not. Bot. Hort. Agrobot Cluj. 38:128-133.

Bybordi, A. and E. Elnaz (2011) Effect of salinity stress on activity involved in nitrogen and phosphorous metabolism case study: canola (*Brassica napus* L.). Asian J. Agri. Res. 5 (3): 208-214.

Couture, S.J., A. DiTommaso, W.L. Asbil, and A.K. Watson (2004). Influence of seedling depth and seedbed preparation on establishment, growth and yield of fibre flax (*Linum usitatissimum* L.) in Eastern Canada. J. Agro. Crop Sci. 190: 184-190.

Dubey, R.S. and K.N. Sharma (1990). Behaviour of phosphatases in germinating rice in relation to salt tolerance. Plant. Physiol. Biochem. 28: 17-26.

Ehsanpour, A.A. and F. Amini (2003). Effect of salt and drought stresses on acid phosphatase activities in alfalfa (*Medicago sativa* L.) explants under in vitro culture. Afr. J. Biotechnol. 2: 133-135.

El Goumi, Y., M. Fakiri, O. Lamsaouri, and M. Benchekroun (2014). Salt stress effect on seed germination and some physiological traits in three Moroccan barley (*Hordeum vulgare* L.) cultivars. J. Mater. Environ. Sci. 5 (2) 625-632.

El-Nagdy, G.A., D.M.A. Nassar, E.A. El-Kady and G.S.A. El-Yamanee (2010). Response of flax plant (*Linum usitatissimum* L.) to treatments with mineral and bio-fertilizers from nitrogen and phosphorus. J. Ame. Sci. 6: 207-217.

Fincher, G.B. (1989). Molecular and cellular biology association with endosperm mobilization in germinating cereal grains. Annual. Rev. Plant. Physiol. Plant Mol. Biol. 40: 305-346.

GreenWay, H. and R. Munns (1998). Mechanisms of salt tolerance in non halophytes. Annual Rev. Plant. Physiol. 81: 149-190.

Habtamu, A., A.H. Ibrahim, F. Eden, and T. Fikadu (2013). Effect of salinity stress on germination and seedling vigour of chickpea (*Cicer arietinum* L.) cultivars. Academia. J. Agric. Res. 1(9): 161-166.

Hossein, A.F. and M. Kasra (2011). Effect of hydropriming on seedling vigour in Basil (*Ocimum Basilicum* L.) under salinity conditions. Adv. Environ. Biol. 5(5): 828-833.

Jamil, M. and E.S. Rha (2004). The effect of salinity (NaCl) on the germination and seedling of sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea* L.). Korean. J. Plant Res. 7: 226-232.

Kandil, A.A., A.E. Sharief, and S.R.H. Ahmed (2012). Germination and seedling growth of some chickpea cultivars (*Cicer arietinum*) under salinity stress. J. Basic. App. Sci. 8: 561-571.

Kaya, M.D., S., Day, Y. Cikili, and N. Arslan (2011). Classification of some linseed (*Linum usitatissimum* L.) genotypes for salinity tolerance using germination, seedling growth, and ion content. Chilean J. Agricult. Res. 72(1): 27 32.

Kumar, A., S. Agarwal, and A. Singh (2014). Salinity effects the germination and seedling growth in some cultivars of oat (*Avena sativa* L.). Indian J. Advances Plant Res. 1(2): 1-10.

Kurt, O. and D. Bozkurt, (2006). Effect of temperature and photoperiod on seedling emergence of flax (*Linum usitatissimum* L.). J. Agro. 5: 541-545.

Mehmet, A., M.D. Kaya, and G. Kaya (2006). Effects of NaCl on the germination, seedling growth and water uptake of Triticale. Turk. J. Agric. 30: 39-47.

Mondal, P., R.N. Remme, D. Das, Y. Ali, and E. Kabir (2015). Germination and seedling growth of indigenous Aman rice under NaCl salinity. Int. J. Multidiscip. Res. Dev. 2(5): 251-257.

Muhammad, Z. and F. Hussain (2010). Effect of NaCl salinity on the germination and seedling growth of some medicinal plants. Pakistan J. Bot. 42(2): 889-897.

Nagesh Babu, R. and V.R. Devaraj (2008). High temperature and salt stress response in French bean (*Phaseolus vulgaris*). Aust. J. Crop Sci. 2(2): 40-48.

Nasri, N., R. Kaddour, H. Mahmoudi, O. Baâtour, N. Bouraoui and M. Lachaâl (2011). The effect of osmopriming on germination, seedling growth and phosphatase activities of lettuce under saline condition. Afr. J. Biotech. 10(69): 14366-14372.

Olmos, E. and E. Hellin (1997). Cytochemical localization of ATPase plasma membrane and acid phosphatase by cerium based in a salt-adapted cell line of *Pisum sativum*. J. Exp. Bot. 48: 1529-1535.

Oprica L., S. Marius (2014). Evaluation of morphological and biochemical parameters of soybean seedlings induced by saline stress. Romanian. Biotechnol. Letters. 19 (4): 9615-9624.

Oprica L., Z. Olteanu, E. Truta, G. Voichita (2011). Early biochemical responses of *Brassica napus* var Exagone seed germination at salt treatment. Analele Stiinifice ale Universitatii Alexandru Ioan Cuza, Sectiunea Genetica si Biologie Moleculara, TOM XII.

Patil, N.M., S.S. Datir, and P.V. Shah (2015). Salt-induced physiological and biochemical changes in two varieties of *Linum usitatissimum* L. Int. J. Curr. Microbiol. App. Sci. 4(9): 296-304.

Rubio-Casal, A.E., J.M. Castillo, C.J. Luque, and M.E. Figueroa (2003). Influence of salinity on germination and seeds viability of two primary colonizers of Mediterranean salt pans. J. Arid. Environ. 53: 145-154.

Sebei K, Debez A, Herchi W, Boukhchina S, Kallel H. (2007). Germination kinetics and seed reserve mobilization in two flax (*Linum usitatissimum* L.) cultivars under moderate salt stress. J. Plant Biol. 50: 447–454.

Simopoulos (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. J. Am. Coll. Nutr. 21(6): 495 505.

Tambhale, S., V. Kumar, and V. Shriramc (2011). Differential response of two scented Indica rice (*Oryza sativa*) cultivars under salt stress. J. Stress. Physiol. Biochem. 7(4): 387-397.

Tejera Garcia, N.A., M. Olivera, C. Iribarne, and C. Liuch (2004). Partial purification and characterization of a non-specific acid phosphatase in leaves and root nodules of *Phaseolus vulgaris*. Plant. Physiol. Biochem. 42: 585–591.

Yan, X., H. Liao, M.C. Trull, S.E. Beebe, and J.P. Lynch (2001). Induction of a major leaf acid phosphatase does not confer adaptation to low phosphorus availability in common bean. Plant. Physiol. 125: 1901-1911.

Zapata, P.J., M. Serrano, M.S. Pretel, A. Amoros and M.A. Botella. (2003). Changes in ethylene evolution and polyamine profiles of seedlings of nine cultivars of *Lactuca sativa* L. in response to salt stress during germination. Plant Sci. 164: 557–563.