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EFFECT OF SALINITY ON *ARABIDOPSIS THALIANA* SEED GERMINATION AND ACID PHOSPHATASE ACTIVITY

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Abstract: The salt tolerance of four accessions of *Arabidopsis thaliana* (*COL* (*Columbia*), *NOK2, N1438* and *N1380*) was evaluated during germination by the capacity of seeds to germinate in the presence of 50 mM NaCl and to maintain adequate acid phosphatase activity. Our results show that saline conditions reduced the final germination percentage, speed of germination and delayed the germination processes of accessions *NOK2, N1438* and *N1380*. In contrast, 100% of germination was found in *COL* under salt-stress conditions. In the presence of NaCl 50 mM, acid phosphatase activity increased in the first 24 h, the activity reaching the control level in germinating seeds of *COL*, but in the three other accessions *NOK2, N1438* and *N1380,* acid phosphatase activity diminished under salt stress. These findings suggest that changes in the phosphatase enzymes might play an important role in the acclimation of *COL* seeds to the changing environmental conditions.

Key words: Acid phosphatase; Arabidopsis thaliana; germination; salinity

INTRODUCTION

Seed germination is an important developmental event in plants because it resolves the site of plant growth and thus plant dissemination (Fowler, 1991). Seed germination and reserve storage are highly controlled processes regulated by environmental factors such as temperature (Birendra et al., 2011), salt (Baghbani et al., 2013), water stress (Qayyum et al., 2011) and light (Benitez-Rodriguez et al., 2004). Studies of germination performance have indicated that the major effects of a saline environment on germination are the prevention of water uptake and ionic toxicity (Khajeh-Hosseini et al., 2003). However, salt stress affects germination percentage, germination rate and seedling growth in different ways, depending on the plant species. NaCl decreased the germination percentage, speed of germination and seedling dry matter in different types of rice (Khan et al., 1997). The germination percentage of wheat cultivars was significantly affected by the salt stress (Datta et al., 2009). It has also been shown that salinity caused a decrease in both the germination rate and germination percentage of pepper seeds (Yildirim and Guvenç, 2006). Moreover, during the germination phase, salinity can affect the activities of some enzymes that play a vital role in the remobilization of nutritive reserves (Dubey and Sharma, 1990). Adaptation to salt stress is associated with metabolic adjustments that lead to the modulation of different enzymes (Ehsanpour and Amini, 2003). Phosphatases are one among them, which are believed to be important for many physiological processes, including the regulation of soluble phosphorous (Pi) (Yan et al., 2001). Phosphorus (Pi) is an essential macronutrient for plant growth and development that plays a key role in many processes, including energy metabolism and the synthesis of nucleic acids and membranes (Ehsanpour and Amini, 2003). Acid phosphatases are constitutively expressed in seeds during germination and their activities increase with germination to release the reserve materials for the growing embryo (Biswas and Cundiff,

1991). Various researchers have studied the behaviors of many enzymes during germination, using cultivars differing in salt tolerance in order to find a possible correlation between enzyme activities and the degree of salt tolerance. Phosphatases contribute to salt tolerance in rice. In fact, acid phosphatase activity in the seeds of salt-tolerant rice is higher than in seeds of salt-sensitive rice and increased with increasing NaCl concentration (Dubey and Sharma, 1990). Salt stress has also been reported to enhance acid phosphatase activity in sorghum, pearl millet and lettuce seeds (Jain et al., 2004; Sharma et al., 2004; Nasri et al., 2015). Salinity increases acid phosphatase activities by maintaining a certain level of inorganic phosphate in plant cells (Olmos and Hellin, 1997).

Some studies have explored natural variation in seed germination constrained by osmotic and salt stresses in *Arabidopsis* (Quesada et al., 2002; Vallejo et al., 2010; Joosen et al., 2010). Vallejo et al. (2010) established that germination regulation under moderate salt and osmotic stresses involves the action of independent major loci, revealing the existence of loci specifically associated with the toxic component of salt and not just its osmotic effect.

Therefore the aim of the present study was to assess the effect of salt on the germination and acid phosphatase activities in four genotypes of *Arabidopsis thaliana*. These genotypes were chosen from eight genotypes that were previously studied by Labidi et al. (2002).

MATERIALS AND METHODS

Plant material and chemicals

The seeds were purchased at Nottingham *Arabidopsis* Stock Center. Experiments were made with four accessions of *Arabidopsis thaliana*: *Columbia (COL), NOK2, N1438 and N1380*.

Seed germination

The seeds were germinated in Petri dishes containing filter paper moistened with distilled water (control) or 50 mM NaCl (saline conditions). Petri dishes were placed first at 4°C in the dark for 3 days to raise germination and then transferred at 25°C to light. Each treatment consisted of 50 seeds per Petri dishes and was replicated 3 times. Seeds were considered to be germinated with the emergence of the radical from the seed coat. The germination percentage was recorded every 4 h for the first 48 h and every 24 h again later up to 4 days.

A theoretical model was used for a more accurate monitoring of germination kinetics. Such a mathematical simulation, previously reported by Debez et al. (2004) for the oil seed halophyte, *Cakile maritima*, hypothesizes that germination has a latent period of duration (t_0), during which the seeds acquire the aptitude to germinate, followed by germination itself. After that latency, the probability (k) of germination per unit time is equal and constant with time for all seeds. This model is formulated as: $Y(t) = Ymax\ (1 + e^{-k(t - t_0)})$ (1), where Y(t) represents the percentage of sown seeds that germinate at time t and Ymax is the plateau (%) reached by Y(t). Germination kinetics was represented as the variations of germination percentage with time. The above empirical simulation model of germination kinetics allowed us to identify three parameters that describe seed germination behavior: germination capacity (Ym), germination rate constant (k) and latency time (t_0). The values of the three parameters were determined by a simulation of the observed values to the previous equation (1) using the non-linear regression method of *STATISTICA* software. The germination rate constant (k) determines the time interval between the end of the latency period and the beginning of the plateau.

After 4 days, non-germinated seeds under salt stress conditions were transferred to distilled water to test their ability to recover germination. The recovery germination percentages (RPs) were determined by the following formula: RP = (c/d) 100 (Shen et al., 2003), where c is the number of germinated seeds in the recovery experiment after 4 days, and d is the seed number total for the recovery germination experiment.

Enzyme extraction and activity

At differing hours of germination, acid phosphatase activity was assayed in germinating seeds of *A. thaliana* accessions. One hundred germinated seeds were extracted in 0.1 M sodium acetate buffer (pH 4.5). The homogenate was centrifuged at 12000 × g for 15 min, and the supernatant was collected for enzyme assays. All procedures were carried out at 4°C. Acid phosphatases activities were assayed by measuring the amount of p-nitrophenol produced. The assay mixture contained 5 mM p-nitrophenyl phosphate (pNPP), 50 mM acetate buffer (pH 5.0) and 0.04 ml enzyme in a total volume of 0.2 ml. After incubation for 30 min at 37°C, the reaction was stopped by the addition of 0.8 ml NaOH (0.1 N). The amount of p-nitrophenol (pNP) liberated was measured by recording absorbance at 400 nm in a spectrocolorimeter. One unit of enzyme activity is defined as the amount of enzyme liberating 1 nmol of p-nitrophenol per minute (Daman et al., 1989).

Statistics

The significance of salt treatment means was tested by one-way analysis of variance (ANOVA) using Statistica for Windows; the Student t-test was used to compare differences among treatment means ($P<0.05$). Results were expressed as means±standard error of the mean (SEM).

RESULTS

Germination of seeds differed according to genotype and salt treatment (Table 1). For *NOK2, N1438, N1380,* the final germination percentage (FG%) was strongly decreased by increasing salt concentration, germination being suppressed at 100 mM NaCl. For *COL*, FG% was 100% at 0 and 50 mM NaCl, but was slightly reduced at 75 mM and decreased to 40% at 100 mM NaCl. Further, a moderate concentration of salt (50 mM NaCl) was chosen to analyze the effect of salt on germination parameters and enzymatic activities in four genotypes of *A. thaliana*.

Table 1. Effect of salinity (0, 50, 75 and 100 mM NaCl) on final germination percentage of *Arabidopsis thaliana* accessions *NOK2, N1438, N1380* and *Columbia* (*COL*).

NaCl (mM)	0	50	75	100
NOK2	84	36	0	0
N1438	87	44	0	0
N1380	86	66	22	0
COL	100	100	86	46

Table 2. Effect of salinity (0 and 50 mM NaCl) on germination parameters of *Arabidopsis thaliana* accessions *NOK2, N1438, N1380* and *Columbia (COL)*.

	Rate of Germination (%)		Constancy of speed k		Time of latency t0 (h)	
NaCl (mM)	0	50	0	50	0	50
NOK2	84	36	0.05	0.05	17	32
N1438	87	44	0.10	0.07	14	6.5
N1380	86	66	0.05	0.04	14	14
COL	100	100	0.35	0.16	0	0

The evolution of seed germination rates of four *A. thaliana* accessions under control and saline condition (50 mM NaCl) allows the distinction of three phases (Fig. 1): (i) the first phase of latency corresponds to period of imbibition (uptake of water necessary for metabolic activity); (ii) the second ascending phase is represented by the increase in germination speed. It is valued by speed constancy deduced from the empiric model. (iii) The third phase is a landing, which represents the final rate of germination in the adopted applied conditions.

The parameters of germination for *NOK2, N1438, N1380* and *COL* in the absence as well as presence of salt (50 mM NaCl) are given in Table 2. When seeds are imbibed in distilled water, the final germination percentage is high; it was around 84% in accessions *NOK2, N1438* and *N1380* and 100% in the *COL* accession. The salt stress caused a decrease in final germination percentage in the first three accessions and this reduction was more pronounced in *NOK2* and *N1438* (50%) and less significant in *N1380* (15%). The results presented in Table 2 also show a reduction in germination speed and an increase in the days of latency in these accessions. We observed that the *COL* accession is more tolerant to salt at the stage of germination than the three other accessions. Indeed, 50 mM

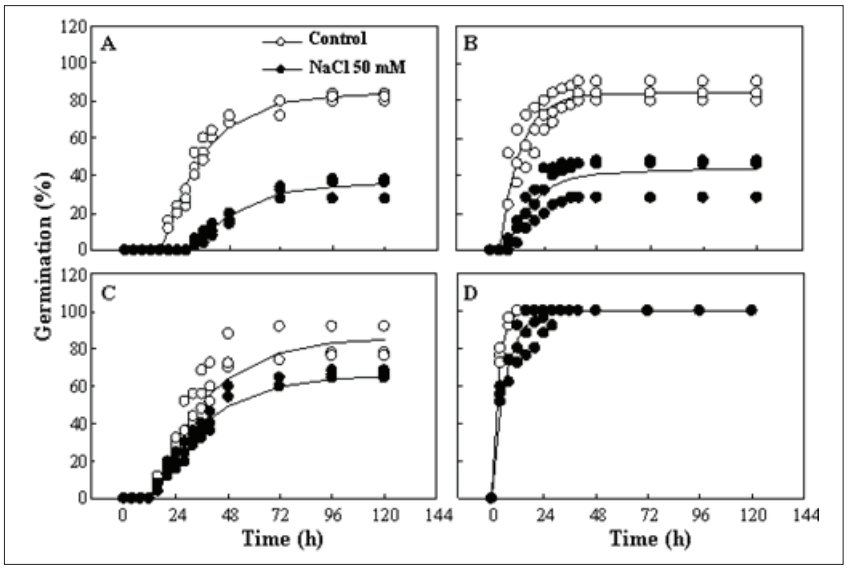

Fig. 1. Evolution of germination percentage of four *Arabidopsis thaliana* accessions: *NOK2* (A), *N1438*(B), *N1380* (C) and *Columbia* (D) under control and conditions of increased salinity (50 mM, NaCl). Data are means from three samples containing 50 seeds/sample.

NaCl did not reduce the final germination percentage or time of latency, but the speed of germination was decreased by more than 50%. Four days later, non-germinated seeds were moved from NaCl stress to a distilled water solution; the recovery germination percentage of *Arabidopsis* accessions was high (Fig. 2) and exceeded 80%, i.e., 88.2% for *N1380*, 85.7% for *N1438* and 81.2% for *NOK2*.

The effect of salt (NaCl, 50 mM) on acid phosphatase activity was studied in germinated seeds of *A. thaliana*. In salt-sensitive accessions (*NOK2, N1438* and *N1380*), the acid phosphatase activity increased during the early hours of germination and decreased thereafter (Fig. 3). Salt stress (50 mM NaCl) caused inhibition in acid phosphatase activity of these accessions. However, in salt-tolerant *COL*, there appeared an increase in acid phosphatase activity at 8 h and 24 h, after which it decreased. Salinity induced an increase in acid phosphatase activity in the 8 to 24 h period and decreased thereafter.

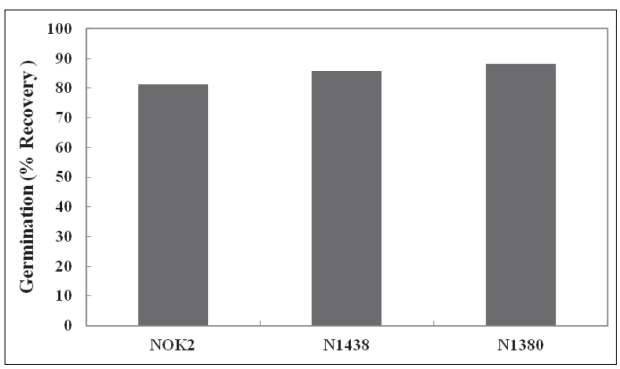

Fig. 2. Percentage of germination recovery of *A. thaliana* accessions (*NOK2*, *N1438* and *N1380*) following their transfer to distilled water.

DISCUSSION

The sensitivity of plants to salinity depends on plant species and their developmental stage (Prado et al., 2000). Our results show differences in sensitivity to salt of four *A. thaliana* accessions during germination. Indeed, three accessions of *A. thaliana* (*NOK2, N1438* and *N1380*) presented sensitivity to salt stress, manifested by a significant reduction in germination. NaCl decreased the final germination percentage and speed of germination, and increased the latency time. A similar decrease in final germination percentage has been reported in all *Brassica* species as salinity levels increased (Jamil et al., 2005). Furthermore, Jamil and Rha (2004) reported that the germination of sugar

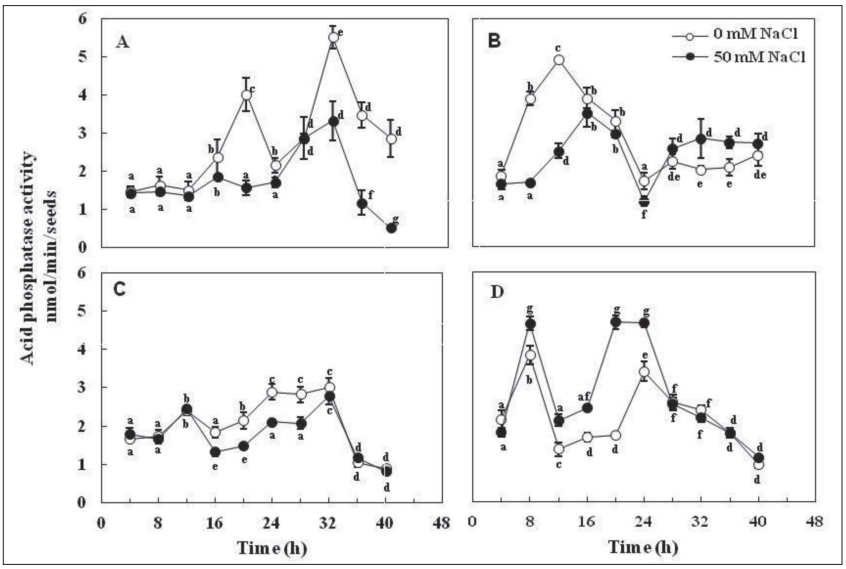

Fig. 3. Acid phosphatase activity of four *Arabidopsis thaliana* accessions *NOK2* (A), *N1438* (B), *N1380* (C) and *Columbia* (D) during germination in distilled water (white circles) or salt solution (black circles). The values presented are means for 3 replicates (SE – interval of security to 5%). Values are means of three replicates ± SD. Means not sharing a common letters (a, b, c, d, e, f or g) are significantly different ($p \leq 0.05$) as assessed by Student t-tests.

beet and cabbage decreased as salinity increased and salinity also delayed the germination rate. The decrease in germination rate, particularly under drought and salt stress conditions, may be due to the fact that seeds seemingly develop an osmotically enforced "dormancy" under water-stress conditions. This may be an adaptive strategy of seeds to prevent germination in stressful environments, thereby ensuring the proper establishment of seedlings (Gill et al., 2003).

Unlike the three other accessions, *COL* was distinguished by a good precocity of germination, an elevated speed and a high final germination percentage (100%) in control and in salt treatment as well. The faculty of *Columbia* to germinate on a salt solution reflects a better capacity of hydration in spite of salt presence. It suggests that Na^+ and Cl^- ions are accumulated in the seeds to compatible levels with their metabolic activities. The accumulation of Na^+ would certainly permit water absorption, although this should be accompanied by ionic sequestration in the vacuole to prevent metabolic toxicity, as well as the accumulation of compatible solutes in the cytoplasm (Reuveni et al., 1991). This behavior of *Columbia* is reminiscent of that of *Arabidopsis thaliana* mutants, e.g., mutant rs (resistant to salt) identified by Saleiki et al. (1993) and mutant rss (reduced salt sensitivity) identified by Werner and Finkelstein (1995). These mutants expressed reduced sensitivity to salt and osmotic stress during germination.

In the present study, the total germination of *A. thaliana* seeds after recovery in distilled water did not differ significantly from germination in distilled water (Fig. 2). This result indicates that the effect of NaCl is more likely to be a reversible osmotic inhibition of germination rather than ion specific toxicity (Keiffer and Ungar, 1995). Similar results have been recorded in several salt-tolerant species including *Suaeda fruticosa* (Khan and Ungar, 1998), *Atriplex prostrata* (Egan et al., 1997) and *Panicum turgidum* (El-Keblawy, 2004).

In our study, acid phosphatase activity was stimulated by salinity in salt-tolerant accession *Columbia* during germination. The higher activities of these enzymes under salt stress in this accession suggest

their direct role in maintaining the much desired energy requirement of the cell to cope with the unfavorable condition of salinity. Since phosphatases are key enzymes that regulate energetic metabolism and the level of inorganic phosphate in germinating seeds, the activated phosphatase appears to maintain a higher metabolic status of a cell by providing a higher rate of phosphate liberation and active transport and biosynthetic events in growing embryo axes (Dubey and Sharma, 1990). Salt stress has also been reported to enhance acid phosphatase activity in sorghum and pearl millet seeds (Jain et al., 2004; Sharma et al., 2004). Unlike the salt-tolerant *Columbia,* salinity decreased acid phosphatase activity in the germinating seeds of sensitive accessions *NOK2, N1438* and *N1380*. Nasri et al. (2015) established that acid phosphatase activity in lettuce is stimulated by salinity in a salt-tolerant variety and decreased in a salt-sensitive one during the germination stage. The decreased levels of acid phosphatases under salinity might lead to a reduced rate of phosphate and energy liberation in the endosperms. This may lead to a decrease in the general metabolic status of germinating seeds, causing diminished hydrolysis of endosperm reserves that eventually leads to a reduction in seed germination and seedling vigor (Dubey and Sharma, 1990).

CONCLUSIONS

Based on the present study we can conclude that *Columbia* is more tolerant to salinity than *NOK2, N1438* and *N1380* at the germination stage. The reduction of germination under saline conditions of these three accessions is possibly due to the altered activities of phosphorolytic enzymes (acid phosphatase). Thus, the salt-tolerance ability is correlated with higher activity of acid phosphatases and their activation under salt stress.

Authors' contribution: NN and SM contributed equally to this work

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