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Full Length Research Paper

The effect of osmopriming on germination, seedling growth and phosphatase activities of lettuce under saline condition

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This experiment was conducted to evaluate the effects of osmopriming with KNO_3 on germination traits, seedling growth and phosphatase activities of lettuce (*Lactuca sativa* L.) seeds under salinity condition. Lettuce seeds (Var. Vista) were primed with KNO_3 (0.05%) for 2 h at 25°C in the dark. Primed and non-primed seeds were germinated on distilled water containing 0 or 100 mM NaCl, for four days. Results show that germination percentage, root and shoot length and seedling fresh weight of primed seeds was higher than that of non-primed seeds in saline condition. Priming also increased acid phosphatase and phytase activities in the roots, shoots and cotyledons under salt stress. It seems that seed priming can be used for improving performance of lettuce seeds and seedlings grown under saline conditions.

Key words: Osmopriming, lettuce, germination, salinity, acid phosphatase, phytase.

INTRODUCTION

Salinity is one major problem of increasing production in crop growing areas throughout the world. Numerous attempts have been made to improve the salt tolerance of crops by traditional breeding programmers, but commercial success has been very limited (Cuartero and Fernandez-Munoz, 1999). Soil salinity reduces water availability of plant roots via negative (low) osmotic potential, as well as decrease the germination dynamics of plant seeds by ionic toxicity of Na^+ and Cl^- (Khajeh-Hosseini et al., 2003). Seed priming is an efficient method for increasing seed vigor and improvement of germination and seedling growth (Mauromicale and Cavallaro, 1997).

Seed priming is a process in which seeds are imbibed in water or osmotic solutions followed by drying before radical emergence (McDonald, 2000). This process has been used to improve germination, reduce seedling germination time, improve stand establishment, increase emergence, and to induce earlier flowering and maturing, which result in higher grain yield (Basra et al., 2005a). Moreover, priming (osmo-conditioning) is one of the physiological methods, which improves seed performance and provides faster and synchronized germination (Sivritepe and Dourado, 1995). Priming affects the lag phase and causes early DNA replication (Bray et al., 1983), increased RNA and protein synthesis (Fu et al., 1988), greater ATP availability (Mazor et al., 1984) and accelerates embryo growth (Dahal et al., 1990). However, osmopriming has been shown to activate the processes related to germination, through affecting the oxidative metabolism such as increasing superoxide dismutase (SOD) and peroxidase (POD) (Jie et al., 2002) or by the activation of ATPase as well as acid phosphatase and RNA synthesis (Fu et al., 1988).

There are several reports that under diverse environmental stresses such as salinity, water deficiency and high and low temperatures, osmopriming leads to cellular, sub-cellular and molecular changes in seeds and subsequently promotes seed vigor during germination and emergence in different plant species (Numjun et al., 1997; Cuartero et al., 2006). There is evidence that seed

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osmopriming increased salinity tolerance of sunflower (*Helianthus annus* L.), melon (*Cucumis melo* L.) and tomato (*Lycopersicon esculentum* Mill.) (Jumsoon et al., 1996; Sivritepe et al., 2003; Kaya et al., 2006). Indeed, Sivritepe et al. (2003) demonstrated that primed melon seeds with NaCl solution for 3 days at 20°C, significantly increased seedling emergence percentage, emergence rate, and root dry weight under salinity conditions when compared to non-primed seeds. Osmo-conditioning of cucumber (*Cucmis sativus*) seed with mannitol has also been reported to alleviate the adverse effects of salt stress on germination and growth of seedlings (Passam and Kakouriotis, 1994). Morever, Afzal et al. (2006) suggests that seed halopriming pretreatments alleviated the adverse effect of salinity by improving germination and seedling growth of wheat. Similar results have been earlier reported to improve germination and seedling vigor in wheat cultivars by seed priming under saline conditions (Kamboh et al., 2000; Basra et al., 2005b). Sedghi et al. (2010) observed that priming with NaCl and GA_3 improves germination indices and seedling growth of two medicinal plants including pot marigold (*Calendula officinalis*) and sweet fennel (*Foeniculum vulgare*) under salinity stress.

Several enzymes are activated during seed germination including acid phosphatase implicated in the remobilization of phosphorus reserves (Biswas and Cundiff, 1991) and phytases, phytate-specific phosphatase that hydrolysis phytate to inositol and free orthophosphate (Greiner et al., 1998). 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 regulation of soluble phosphorous (Yan et al., 2001). Phosphorolytic enzymes have received little attention while conducting salt tolerance studies in rice (Dubey and Sharma, 1990). Salt stress has been reported to increase acid phosphatase activities by maintaining a certain level of inorganic phosphate in plant cell (Olmos and Hellin, 1997).

The aim of this research was to evaluate the effects of osmopriming on the germination dynamics, subsequent seedling growth and phosphatases activities of *Lactuca sativa* L. variety Vista under saline condition. This lettuce variety, Vista, employed in this study was selected as representative of the most sensitive variety to salt under salt treatment in germination step (Nasri et al., 2011).

MATERIALS AND METHODS

The seeds of lettuce (variety Vista) used in this investigation were provided by the Seed Laboratory of Tunisian Ministry of Agriculture.

Seed treatments

For osmopriming, lettuce seeds were immersed in 0.05% KNO_3 solution at 25°C for 2 h in the dark. Thereafter, seeds were rinsed with tap water three times. The treated seeds were surface dried and dried back to their original moisture content at room temperature for two days.

Germination tests

Primed (P) and non-primed (NP) seeds were soaked for 2 h in distilled water or salt solution (100 mM NaCl). After imbibitions, seeds were placed in Petri dishes with double-layer filter paper initially moistened with the same solutions. 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 three times. Seeds with emerged radicle were counted daily. Final germination percentage (FG%) was calculated as 100 × number of germinated seeds divided by number of sown seeds. Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981): $MGT=\Sigma(Dn)/\Sigma n$, where n is the number of seeds which germinate on day D and D is the number of days counted from the beginning of germination test.

After 4 days, seedlings were divided into root, shoot and cotyledons for determination of growth parameters. Fresh weights (FW) of all samples were recorded.

Extraction and assay of acid phosphatase and phytase

Both acid phosphatases and phytase were extracted by grinding the tissues (root, shoot and cotyledons) at 4°C using 0.1 M sodium acetate buffer (pH 4.5) for the first enzyme and 0.1 M acetate buffer (pH 5.4) for the second one. The homogenate was centrifuged at 12,000 g for 15 min and supernatant was collected. Acid phosphatase activity was measured spectrophotometrically at 400 nm by monitoring the release of *p*-nitrophenol (pNP) from *p*-nitrophenyl phosphate. One unit of enzyme activity is defined as amount of enzyme liberating 1 nmol of *p*-nitrophenol per minute (Saluja et al., 1989). Phytase activity was performed by measuring the release of phosphate from sodium phytate and was carried as previously described (Nasri et al., 2011).

The acid phosphatase and phytase activities were expressed in nmol of P_i released per min per µg of protein.

Determination of total protein concentration

Total protein concentration of the supernatant was determined according to the method described by Bradford (1976) with bovine serum albumin as a standard.

Statistics

Data were presented as the mean of three repetitions for germination test, four seedlings for enzymes activities and six seedlings for roots and shoots growth. Significant differences between treatments were analyzed using ANOVA and mean comparison with Duncan test (Statistica®). Values were calculated at the $P \leq 0.05$ probability level.

RESULTS

The effect of osmopriming and salinity on germination kinetics of lettuce seeds was examined (Figure 1). Salinity (NaCl 100 mM) not only decreased the germination but also delayed the germination initiation. Therefore, osmopriming with KNO_3 increased

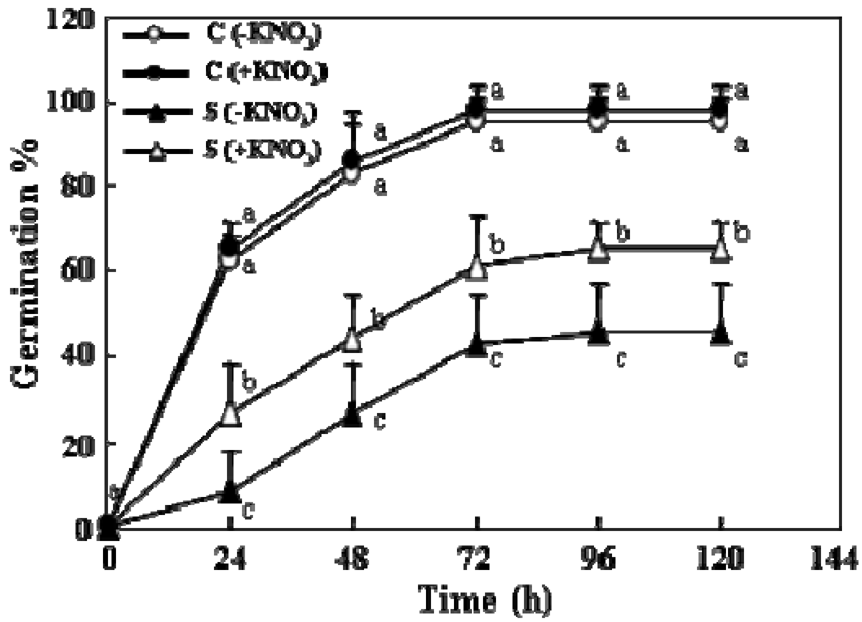

Figure 1. Effect of osmopriming with KNO_3 on germination percentage of lettuce seed under normal (control (C), 0 mM NaCl) or saline (*S* 100 mM NaCl) conditions. Data are the mean of three samples of 25 seedlings each one of (-KNO_3: NP; + KNO_3: P).

Table 1. Effect of KNO_3 priming on final germination percentage (FG%) and mean germination time (MGT) of non-primed (NP) and primed (P) seeds of lettuce under normal (0 mM NaCl) or saline (100 mM NaCl) conditions.

NaCl (mM)	KNO_3 Priming	FG%	MGT (days)
0	NP	$95±5.7^{a}$	$1.9±0.1^{a}$
	P	$97±5.7^{a}$	$1.5±0.2^{b}$
100	NP	$43±11.4^{c}$	$2.4±0.6^{c}$
	P	$65±5.7^{b}$	$1.9±0.4^{a}$

Different letters in columns show significant difference based on Duncan's multiple range tests at $p \leq 0.05$.

germination percentage under salt stress as compared with non-primed seeds. Final germination percentage (FG%) decreased with salinity in both primed and non-primed seeds (Table 1). Meanwhile, final germination percentage in primed seeds under salt stress was higher than that of non-primed seeds; it was 65 and 45% respectively. Like germination percentage, prime seeds had lower mean germination time (MGT) compared with non-primed seeds under salt stress (Table 1).

Salt stress (100 mM NaCl) decreased root and shoot length of NP seeds with greater reduction in the growth of root compared to shoots (74 and 24%, respectively). However, priming with KNO_3 enhanced length of root and shoot in salt conditions as compared to seedlings grown from NP seeds (Figure 2). A significant reduction in fresh weight of root and shoot of lettuce seedlings was observed under saline conditions. Nevertheless, the seedlings of the P group had a higher value for fresh weight of roots and shoots then NP group under salt stress (Figure 3).

Acid phosphatase activity (APA) was measured in three parts of lettuce seedling derived from primed (P) and non-primed (NP) seeds: root, shoot and cotyledons. In the presence of salt, this enzyme activity decreased in the three parts of lettuce seedling derived from NP seeds by 19.5, 15.4 and 30.3% respectively in roots, shoots and cotyledons. Priming with KNO_3 resulted in an enhancement of APA at a level close to that of the control in roots, shoots and cotyledons under saline conditions (Figure 4).

Phytase activity was also stimulated during seed germination (Figure 5). 100 mM NaCl decreased phytase activity only in the root derived from NP seeds but this activity was increased in shoots and was independent on salt in the cotyledons in NP group. Priming treatment increased significantly phytase activity in the roots and

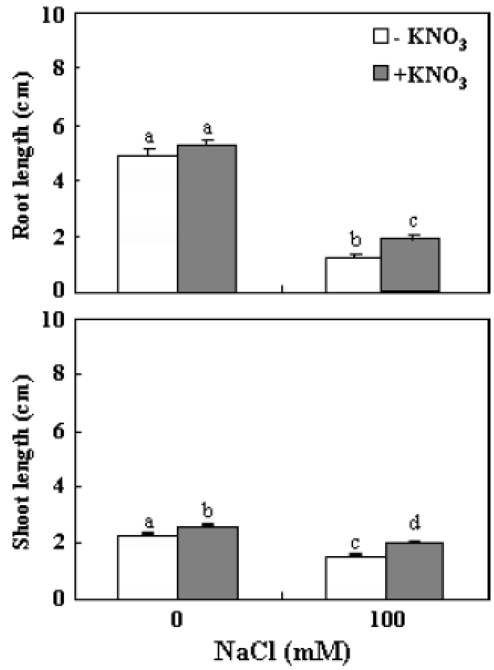

Figure 2. Effect of salinity (NaCl 100 mM) on root and shoot length in lettuce seedlings derived from P (+KNO_3) and NP (-KNO_3) seeds. Values are means of six replicates ± SD. Means not sharing a common letters (a, b, c or d) are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

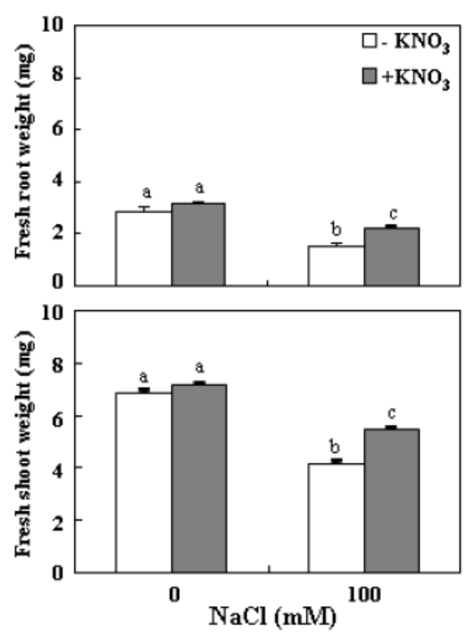

Figure 3. Effect of salinity (NaCl 100 mM) on root and shoot fresh weight in lettuce seedling derived from P (+KNO_3) and NP (-KNO_3) seeds. Values are means of six replicates ± SD. Means not sharing a common letters (a, b, or c) are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

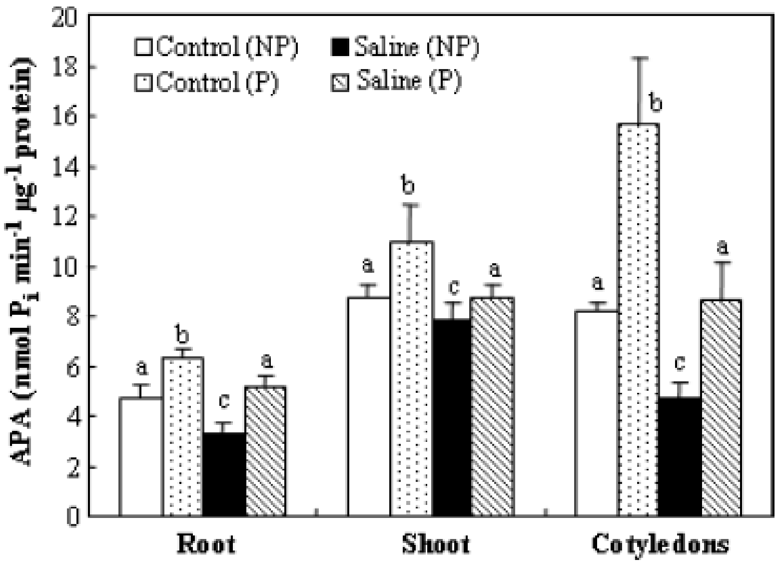

Figure 4. Effect of NaCl on acid phosphatase activity (APA) in root, shoot and cotyledons of lettuce seedlings derived from primed (P) and non-primed (NP) seeds. Values are means of four replicates ± SD. Means not sharing a common letters (a, b, or c) are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

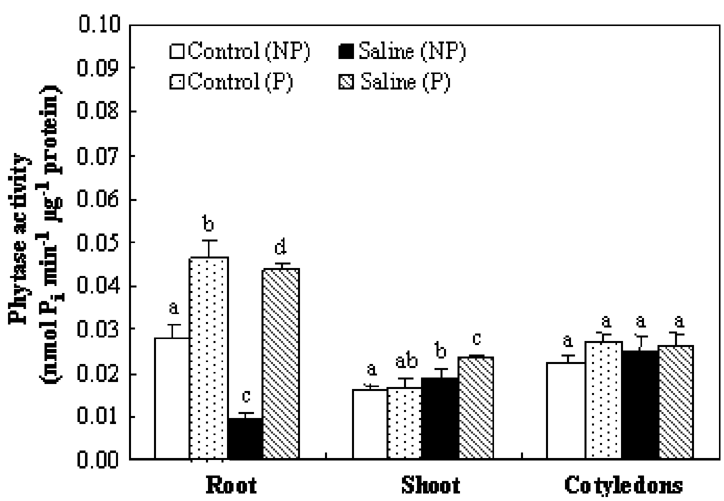

Figure 5. Effect of NaCl on phytase activity in root, shoot and cotyledons of lettuce seedlings derived from primed (P) and non-primed (NP) seeds. Values are means of four replicates ± SD. Means not sharing a common letters (a, b, c or d) are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range tests.

shoots of lettuce seedlings in saline condition (Figure 5).

DISCUSSION

In this study, salinity significantly reduced germination and seedling vigor of lettuce (variety Vista) (Figures 1, 2 and 3). However, osmopriming of lettuce seeds with KNO_3 reduced the inhibiting effect of salinity on germination and seedling growth of this variety. The biomass of roots and shoots of seedlings grown from seeds treated with KNO_3 were higher then those of untreated seeds sown under salt stress. In other plants such as canola (Hassanpouraghdam et al., 2009), melon

(Farooq et al., 2007) and chickpea (Sarwar et al., 2006), priming with KNO_3 increased also germination percentage and seedling growth under salt stressed conditions. These positive effects are probably due to the stimulatory effects of priming at the early stages of the germination process by mediation of cell division in germinating seeds (Sivritepe et al., 2003). In fact, KNO_3 has been proposed to stimulate germination by acting as an osmoticum thus enhancing water uptake (McIntyre et al., 1996). Primed seeds have better efficiency for water absorption from imbibitions medium and it is obvious that metabolic activities in seed during the germination process commence much earlier than the emergence of the radicle and plumule (Ascherman-Koch et al., 1992). Bajehbaj (2010) showed that KNO_3 primed seeds increased seed germination percentage and seedlings growth in sunflower cultivars under salinity conditions by promoting K and Ca accumulation and inducing osmoregulation by the accumulation of proline. A positive effect of KNO_3 on germination in saline condition was also documented in halophytes like *Suaeda salsa* (Li et al., 2005) and *Crithmum maritimum* (Atia et al., 2009).

Activities of several enzymes associated with the germination process have been proven to change in response to seed priming. These include increases in the activities of acid phosphatase and esterase in lettuce (Khan et al., 1978), α-amylase in rice (Farooq et al., 2006) and antioxidant enzymes in wheat (Afzal et al., 2006). Our results show significant improvement in acid phosphatase and phytase activities in roots, shoots and cotyledons due to osmopriming treatment. These enzymes activities were greater in primed seeds than in non-primed seeds in saline conditions (Figures 4 and 5). The stimulation of phosphatases activities appears to maintain higher cell metabolic status by providing a higher rate of phosphate release and active transport and biosynthetic events in growing embryoaxes (Dubey and Sharma, 1990). Kaur et al. (2002) established that priming may increase the activities of enzymes involved in carbohydrate metabolism. The activities of enzymes, like amylase, invertase (acid and alkaline), sucrose synthase and sucrose phosphate synthase in shoots, roots and cotyledons increased in primed stressed seedling as compared to the non-primed stressed seedling, which help in germination and crop establishment. Seed priming has positive effects on germination characteristic of amaranth cultivars such as speed of germination and root length, which was associated to increased peroxidase activity (Moosavi et al., 2009). It was suggested that higher activity of antioxidant enzymes could increase tolerance of primed seeds to environmental stresses such as salinity.

Conclusion

The results of this study demonstrate that osmopriming with KNO_3 is effective for the improvement of germination and early seedling growth of lettuce (sensitive variety Vista) by stimulated phosphatases activities. Since phosphatases are key enzymes which regulate energetic metabolism and the level of inorganic phosphate in germinating seeds, higher activities of these enzymes under salinity conditions provide higher rate of phosphate and maintain the much needed energy requirement of the cell to cope with adverse condition of salinity, which ultimately leads to increased seed germination and seedling growth. Therefore, priming with KNO_3 may be an efficient method to overcome seed germination problems and to improve seedlings growth of crops especially under salinity conditions.

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