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## **Germination and Seedling Emergence of Primed Okra (*Abelmoschus esculentus* L.) Seeds under Salt Stress and Low Temperature**

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### **ABSTRACT**

This experiment was conducted to evaluate the effects of priming on the germination, emergence and seedling growth of *Abelmoschus esculentus* (cultivar Marsaouia) under low temperature and salinity conditions. Seeds were primed for 24 h at 20°C in three priming media (KCl 4%, mannitol 0.75M, CaCl<sub>2</sub> 10 mM) and control (non-primed seeds) and were examined at different salinity levels (0, 40 and 100 mM NaCl). Results indicated that KCl priming increased final germination percentage, radicle length and seedlings dry weight 100%, 40.94 mm and 0.03 g, respectively, as compared with non-primed seeds. Mannitol and CaCl<sub>2</sub> have been found to be better treatments for improving final emergence percentage. Overall increased NaCl level, led to the reductions in final germination and emergence percentage but these reductions were higher for non-primed compared to primed seeds. The increase in NaCl concentrations didn't show any significantly effect on cotyledons fresh weight of primed or non-primed seeds. Besides, our results proved that priming alleviated the adverse effects of salinity for seedlings biomass as compared to non-primed seeds.

**Key words:** *Abelmoschus esculentus*, seed priming, salinity, germination, emergence

### **INTRODUCTION**

Tunisia is essentially represented by arid and semi arid climate regions characterized by variable winter rainfall and with a salt water concentration in dams and wells altering from 2-6 g L<sup>-1</sup> and 4-7 g L<sup>-1</sup>, respectively.

Okra (*Abelmoschus esculentus* L.) is an annual plant that is widely grown in all regions of the world with a tropical or Mediterranean climate for its immature pods (Doymaz, 2005). In Tunisia, the productivity of this crop is limited because of the ignorance for its suitability with regards to soil salinization and climate. Ben Dkhil and Denden (2012) mentioned that environmental factors such as salinity, drought and temperature contributed to erratic germination of okra seeds.

Osmopriming or osmoconditioning is defined as a pre-sowing hydration treatment often used to accelerate seed germination. Sedghi *et al.* (2010) reported that priming is one of the seed enhancement methods that might be resulted in increasing seed performance (germination and emergence) under stress conditions such as salinity, temperature and drought stress. Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops (Perveen *et al.*, 2008; Mohammadi, 2009; Sedghi *et al.*, 2010; Binang *et al.*, 2012).

In priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing in enough water for radicle protrusion, thus suspending the seeds in the lag phase (Taylor *et al.*, 1998). Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence (Parera and Cantliffe, 1994). Mereddy *et al.* (2000) reported that improved germination performance of okra can be achieved by solid matrix priming, that priming could allow for greater membrane integrity in the embryo and the developing seedling reducing leakage through the membranes.

There is evidence that seed osmo-priming increased salinity tolerance of melon (Sivritepe *et al.*, 2003), wheat (Iqbal *et al.*, 2006), canola (Mohammadi, 2009) and sunflower (Bajehbaj, 2010). Priming of chickpea seeds with mannitol and water improved seedling growth (Kaur *et al.*, 2002) and gave high yield (Kaur *et al.*, 2002) under salt stressed conditions. Priming of tomato seeds with NaCl had been reported to improve seedling growth (Sarwar *et al.*, 2006).

The aim of this research was to evaluate the effects of KCl, mannitol and CaCl<sub>2</sub> priming on the germination, emergence dynamics and subsequent seedling growth of *Abelmoschus esculentus* cultivar 'Marsaouia' at lower temperature and under salinity condition.

## MATERIALS AND METHODS

Okra (*Abelmoschus esculentus* L. cv. 'Marsaouia') seeds were obtained from Baddar Seed Company, Tunisia, used in the present investigation. Sample seeds were selected and adjusted to the same size. The initial seed moisture was 14% (dry weight basis).

**Priming treatment:** Seeds were primed in aerated solutions of potassium chloride (KCl 4%), mannitol (0.75 M) and calcium chloride (CaCl<sub>2</sub> 10 mM) for 24 h at 20°C under dark conditions. After respective priming treatments for specific period, seeds were washed with distilled water. The seeds were then dried at ambient temperature on filter study for 24 h up to their original moisture contents. Dried seeds were then packed in polyethylene bags and stored in refrigerator (6±2°C) for further use.

**Germination test:** Primed and un-primed seeds were surface-sterilized for 20 min in sodium hypochlorite solution (15%) and rinsed three times in sterile deionised water. The germination tests were carried out in Petri dishes containing two layers of filter study moistened with saline solutions containing different NaCl levels [0 (control), 40 and 100 mM], kept at 20°C in an incubator under dark conditions.

**Data collection:** Seeds with visible radicle were considered as germinated. Data were recorded on daily basis for germination rate for 10 days. Seeds with visible cotyledons were considered as emerged. Final germination and emergence percentage was calculated at the end of each experiment.

Moisture in germinated okra seeds was estimated by recording the difference in its wet weight and the weight recorded after drying them in an oven at 80°C.

The weight of mobilized seed reserve (g per seed) was calculated as the dry weight of primed and non-primed germinated seed at 0, 40 and 100 mM NaCl.

Ten normal seedlings from each replication were taken at random at final count and radicle length was measured. Seedlings (Cotyledons+embryonic axes) fresh weight were obtained using ten normal seedlings from each replication after 15 days of sowing and dry weight was obtained by drying at 80°C for 48 h in oven.

**Statistical analysis:** The experiment was arranged according to completely randomized design with four replicates, each replicate having 25 seeds. Data recorded were analyzed statistically using Duncan's Univariate Range Test at 5% probability level to compare the differences among treatment means.

## RESULTS

**Final germination percentage:** Results for Final Germination Percentage (FGP) were found to be significant for all treatments under saline conditions (Table 1). Salinity reduced the final germination percentage of both primed and non-primed seeds with more intensity as salinity levels were increased. However, our results confirmed that priming reduced the adverse effects of salinity on okra germination as compared to non-primed seeds. The Lowest germination percentage (19.5%) was observed at high salinity treatment (100 mM NaCl) for un-primed seeds (control). It was observed also that priming with mannitol, CaCl<sub>2</sub> and KCl increased germination of okra seeds at 40 and 100 mM NaCl. The highest value (100%) was obtained with KCl primed seeds at 40 mM NaCl.

At 100 mM NaCl, final germination percentage was improved by seeds primed with CaCl<sub>2</sub> and mannitol which reached as a consequence the highest value 83.88%.

**Final emergence percentage:** Final emergence percentage was also accelerated by priming treatment, even though, salinity had adverse effects on okra seeds emergence (Table 2).

Under control condition the lowest emergence percentage (64%) was observed in non-primed seeds, while the highest one (90%) was determined by seeds primed with mannitol and CaCl<sub>2</sub>.

Salinity reduced the final emergence percentage of both the primed and non-primed seeds. The intensity of this decrease was accentuated by salinity levels, final emergence percentage regressed from 64% (control) to 52 and 48% at 40 and 100 mM NaCl, respectively.

Emergence was found to be much enhanced in primed seeds as compared to non-primed ones at all the salinity levels. KCl priming enhanced final emergence percentage to 79.5% at 0 mM NaCl, 78% at 40 mM NaCl and 70% at 100 mM NaCl.

Mannitol priming showed the best results, final emergence percentage was significantly ( $p < 0.05$ ) affected by the interaction of this treatment with salinity levels. Emergence percentage was ranged between 79.8 and 76% at 40 and 100 mM NaCl, respectively.

Table 1: Priming effects on final germination percentage of okra seeds subjected to various NaCl treatments

Salinity levels NaCl (mM)	Control	KCl (4%)	Mannitol (0.75 M)	CaCl <sub>2</sub> (10 mM)
0	88.0 <sup>a</sup> %	100.00 <sup>a</sup> %	96.97 <sup>a</sup> %	94.44 <sup>a</sup> %
40	73.5 <sup>b</sup> %	100.00 <sup>a</sup> %	90.60 <sup>ab</sup> %	84.84 <sup>b</sup> %
100	19.5 <sup>c</sup> %	81.11 <sup>b</sup> %	83.33 <sup>b</sup> %	83.33 <sup>b</sup> %

Within columns, means with different letters are significantly different at  $p < 0.05$

Table 2: Priming effects on final emergence percentage of okra seeds subjected to various NaCl treatments

Salinity levels NaCl (mM)	Control	KCl (4%)	Mannitol (0.75 M)	CaCl <sub>2</sub> (10 mM)
0	64 <sup>a</sup> %	79.5 <sup>a</sup> %	90.0 <sup>a</sup> %	90 <sup>a</sup> %
40	52 <sup>b</sup> %	78.0 <sup>a</sup> %	79.8 <sup>ab</sup> %	79 <sup>b</sup> %
100	48 <sup>c</sup> %	70.0 <sup>b</sup> %	76.0 <sup>b</sup> %	55 <sup>c</sup> %

Within columns, means with different letters are significantly different at  $p < 0.05$

Table 3: Priming effects on radicle length of emerged okra seeds subjected to various NaCl treatments

Salinity levels	Control	KCl (4%)	Mannitol (0.75 M)	CaCl <sub>2</sub> (10 mM)
<b>NaCl (mM)</b>				
0	11.86 <sup>a</sup>	40.94 <sup>a</sup>	26.79 <sup>a</sup>	20.07 <sup>a</sup>
40	13.82 <sup>a</sup>	32.76 <sup>b</sup>	25.42 <sup>a</sup>	21.12 <sup>a</sup>
100	12.52 <sup>a</sup>	16.22 <sup>c</sup>	23.44 <sup>a</sup>	22.89 <sup>a</sup>

Within columns, means with different letters are significantly different at p<0.05

Table 4: Changes in cotyledons (coty) and embryonic axes (axes) fresh weight (g) under different salinity levels during seedling growth of primed and non primed seeds

Salinity levels	Control		KCl (4%)		Mannitol (0.75 M)		CaCl <sub>2</sub> (10 mM)	
	Coty	Axes	Coty	Axes	Coty	Axes	Coty	Axes
<b>NaCl (mM)</b>								
0	0.06 <sup>a</sup>	0.048 <sup>a</sup>	0.06 <sup>a</sup>	0.05 <sup>a</sup>	0.06 <sup>a</sup>	0.057 <sup>a</sup>	0.05 <sup>a</sup>	0.06 <sup>a</sup>
40	0.06 <sup>a</sup>	0.046 <sup>b</sup>	0.07 <sup>a</sup>	0.05 <sup>a</sup>	0.06 <sup>a</sup>	0.052 <sup>b</sup>	0.05 <sup>a</sup>	0.05 <sup>a</sup>
100	0.05 <sup>a</sup>	0.043 <sup>c</sup>	0.06 <sup>a</sup>	0.05 <sup>a</sup>	0.05 <sup>a</sup>	0.048 <sup>c</sup>	0.06 <sup>a</sup>	0.05 <sup>a</sup>

Within columns, means with same letters are not significantly different at p<0.05

Table 5: Changes in cotyledons (coty) and embryonic axes (axes) dry weight (g) under different salinity levels during seedling growth of primed and non primed seeds

Salinity levels	Control		KCl (4%)		Mannitol (0.75 M)		CaCl <sub>2</sub> (10 mM)	
	Coty	Axes	Coty	Axes	Coty	Axes	Coty	Axes
<b>NaCl (mM)</b>								
0	0.01 <sup>a</sup>	0.006 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>
40	0.01 <sup>a</sup>	0.006 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.02 <sup>ab</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>
100	0.01 <sup>a</sup>	0.005 <sup>a</sup>	0.01 <sup>a</sup>	0.02 <sup>a</sup>	0.03 <sup>b</sup>	0.01 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>

Within columns, means with same letters are not significantly different at p<0.05

CaCl<sub>2</sub> priming partially alleviated the adverse effects of 100 mM NaCl by increasing final emergence percentage from 48% (non-primed seeds) to 55%.

**Seedling growth:** Seedling growth was recorded in terms of radicle length, cotyledons and embryonic axes biomass of primed and non-primed seeds at different levels of NaCl.

Radicle length was improved by osmopriming as compared to non-primed seed and gradually increased from 11.86 mm (control) to 20.07 (CaCl<sub>2</sub> priming), 26.79 mm (mannitol priming) and 40.94 mm (KCl priming) (Table 3).

Significant (p<0.05) results were found for KCl treatment under saline conditions for radicle growth. Roots were elongated deep to 40.94 mm under control condition, while their growth was averaged to 32.76 mm at 40 mM NaCl and to 16.22 mm under 100 mM NaCl which means a 20 and 40%, respectively reduction in root growth.

No-significant (p<0.05) results were found in primed and non-primed seeds for cotyledons fresh weight (Table 4); the increase in NaCl concentrations didn't show any significantly effect on cotyledons fresh weight of primed or non-primed seeds. Maximum fresh weight was 0.07 g; it was obtained at 40 mM NaCl in KCl primed seeds.

Meanwhile, primed seeds enhanced fresh weight of embryonic axes as compared with non-primed seeds, there was a considerable effect of CaCl<sub>2</sub> (Table 4).

The reduction in fresh biomass production under high salt stress (100 mM NaCl) was greater in the seeds primed with mannitol which was 16% comparative to 10% in non-primed seeds.

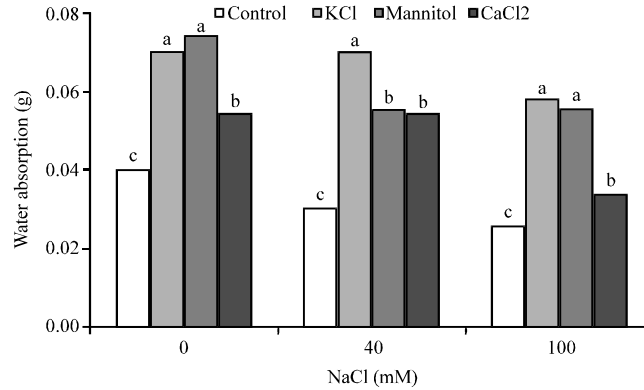


Fig. 1: Water absorption of primed seeds germinated under different salinity levels. Means with different letters are significantly different at  $p < 0.05$

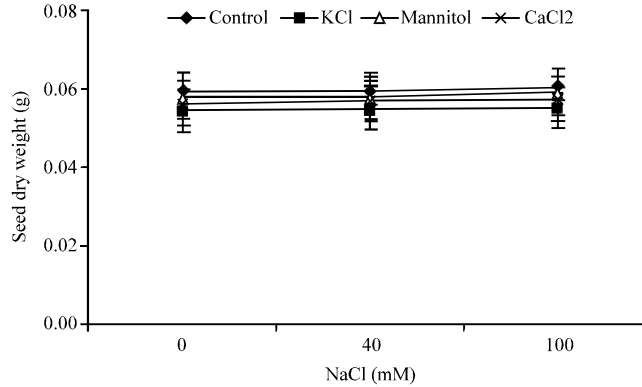


Fig. 2: Dry weight of primed germinated seed under different salinity levels. Means with different letters are significantly different at  $p < 0.05$

Cotyledons dry weight was higher in KCl priming seeds which reached 0.02 g in non saline condition (0 mM NaCl) (Table 5).

Cotyledons dry weight was not significantly affected by increasing salt levels in untreated seeds under control condition (0 mM NaCl). While, mannitol priming enhanced cotyledons dry mass production in both salinity levels (40 and 100 mM NaCl) to 0.02 and 0.03 g, respectively. The effect of salinity on dry weight of embryonic axes wasn't significant for primed and non-primed seeds; while, priming increased biomass production. The maximum dry weight of embryonic axes obtained at the priming with KCl (4%) was 0.02 g.

**Water absorption:** Water absorption was significantly ( $p < 0.05$ ) affected by seed priming treatment (Fig. 1). The maximum water content (0.07 g) in germinated seed was attained in KCl (4%) and mannitol (0.75 M) priming. The CaCl<sub>2</sub> (10 mM) priming seed increased water content as compared with non-primed one; the amount of this increase was 35%.

Increased NaCl levels, led to the decrease in water absorption. KCl primed seeds had better efficiency for water absorption from salty growing media than other treatments. Minimum reduction in water uptake was recorded at 40 and 100 mM NaCl with KCl priming as compared with other treatments and with respect to control.

**Seed reserve mobilization:** Across salinity treatments, the difference between priming treatments was significant for weight of mobilized seed reserve (Fig. 2). Seed dry weight was found to be slightly enhanced in mannitol and CaCl<sub>2</sub> primed seeds as compared to non-primed seeds at all the salinity levels. KCl priming seeds provided the highest seed reserve mobilization even at saline and non saline conditions. Non primed seeds had the greatest dry weight which implicated a lower weight of mobilized reserve.

## DISCUSSION

On the basis of previous experiments, Ben Dkhil and Denden (2012) suggested that okra seeds germinated better in the intermediate incubation temperature (25°C) which was optimum for the final germination percentage and on the establishment of okra seedlings. Whereas, seeds incubated under lower temperature seemed to be subject to more environmental stress such as salinity. This study was undertaken to improve germination and seedling growth of okra under inappropriate temperature (20°C) and salt stress condition through the hydro priming technique.

The present study involving three different treatments (KCl, mannitol and CaCl<sub>2</sub>) which revealed substantial improvement in final germination percentage. Similar findings were reported with NaCl priming in hot pepper (Khan *et al.*, 2009), hydropriming and ZnSO<sub>4</sub> priming in cumin (Neamatollahi *et al.*, 2009) and NaCl priming in sunflower (Bajehbaj, 2010).

Priming with KCl enhanced germination of okra seeds soaked in distilled water and either in 40 mM NaCl as compared with un-primed seeds. Misra and Dwibedi (1980) suggested that potassium chloride was an osmoticum which has shown good potential to enhance germination, emergence, growth and/or grain yield of wheat.

At 100 mM NaCl, priming of seeds with mannitol and CaCl<sub>2</sub> gave the highest final germination percentage which was 83%. Osmo-conditioning of cucumber (*Cucumis sativus*) seed with mannitol had been reported to alleviate the adverse effects of salt stress on germination and growth of seedlings (Passam and Kakouriotis, 1994).

Furthermore, priming with KCl alleviated the adverse effects of both NaCl levels (40 and 100 mM) on water absorption in germinated seeds. These findings were explained as reported by Taiz and Zeiger (2002) to the osmotic advantage of K<sup>+</sup> which has an improving cell water statue and also in that it acts as cofactors in the activities of numerous enzymes. Moreover, Bajehbaj (2010) has reported that primed seeds had better efficiency for water absorption from growing media.

Priming of seed was an effective tool in enhancing the emergence under both saline and non saline conditions. Final emergence percentage of seedlings from both primed and non-primed seeds decreased with increasing salinity. However, this reduction was higher for non-primed seeds, compared to KCl and mannitol priming seeds. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Sivritepe *et al.*, 2003).

Our results are in line with the findings of Sarwar *et al.* (2006) who indicated the beneficial effects of mannitol and water priming on the early emergence of chickpea seedlings.

Root length of primed seeds was greater than that of un-primed ones; it was clear that osmopriming with KCl proved superiority over other treatments in enhancing seedling vigor as indicated by increased radicle length. This result wasn't in agreement with Yari *et al.* (2010) who reported that KCl soaked seeds didn't cause the maximum radicle length of wheat.

The inhibitory effect of 100 mM NaCl was significant in seeds osmoprimed with 4% KCl. However, non-significant results were found for control, mannitol and CaCl<sub>2</sub> treatments under

saline conditions for radicle length. These results diverged from those of Sarwar *et al.* (2006) who reported that the treatment of seeds with 2 and 4% mannitol increased the radicle length of chickpea seedlings as compared to non-primed controls under salt stressed conditions.

Effect of priming was statistically non significant on cotyledons fresh weight. Whereas, it was observed that KCl priming is effective in increasing seedlings dry biomass. Bajehbaj (2010) also reported that sunflower seedlings derived from primed seeds with NaCl and KNO<sub>3</sub> had higher dry weight than those derived from non-primed seeds. Moreover, Ahmadi *et al.* (2007) proposed that measurement of seedling dry weight may be a proper approach for early screening of wheat drought resistant genotypes.

## CONCLUSION

It was evident from this study that priming led to improve germination and emergence of okra seeds incubated at unsuitable temperature (20°C) under saline conditions. The efficient water uptake revealed KCl priming under the highest salinity level (100 mM NaCl) was correlated to the improvement in seedling growth. KCl alleviated the adverse effects of salinity by increasing the seed reserve mobilization as compared with un-primed seeds.

Further studies are needed to investigate the effects of KCl, mannitol and CaCl<sub>2</sub> priming on later growth and development stages of okra.

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