Comparative Effects of Osmotic and Salt Stresses on Germination and Seedling Growth of Alfalfa: Physiological Responses Involved

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Summary

The comparative effect of salt (NaCl, KCl) and osmotic (PEG 6000) stresses on germination and early seedling growth of alfalfa seedlings is monitored. Seeds are germinated in Petri dishes under different salinity (0 to 500 mM) or PEG (0 to 50%) treatments for 9 days. Final germination percentage, germination rate index, mean daily germination, water uptake, ability to recover germination after exposure to severe stress, seedling length, seedling dry weight and vigor index are measured. In order to understand the specific response of alfalfa to salt and osmotic stresses, several biochemical parameters are evaluated by determining the contents of organic solutes (proline, amino acids, soluble sugars) and inorganic solutes (Na^+, K^+, Cl^-). The results obtained show that the two stresses at high concentration significantly decrease all attributes of germination and growth of seedlings while in the case of moderate stress this effect remains insignificant. These results, which reflect the tolerance of the Algerian cultivar, are confirmed by the high percentages of recovery obtained after exposure of the seeds to severe stress. The use of isotonic PEG solutions indicates that the effect of salinity occurs through both an osmotic and an ionic effect and that the latter effect is more inhibiting. In addition, alfalfa seedling growth is found to be more sensitive than seed germination. The obtained solute levels show that the osmoregulatory mechanisms involved under salt stress are induced primarily by ionic toxicity rather than osmotic toxicity.

Key words

drought, germination, osmotic adjustment, salt stress, seedling growth

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Introduction

Salt stress and water stress are recurrent abiotic stresses that cause a decline in plant productivity and agricultural yields (Munns and Tester, 2008). Indeed, more than 831 million hectares of land worldwide are affected by high salt levels (Flowers and Muscolo, 2015). In North Africa and particularly in Algeria, approximately 3.2 million hectares are affected by high salt levels (Belkhodja and Bidai, 2004). The first confrontation of plants with drought and salinity takes place during seed germination and the beginning of seedling growth. These stages are crucial for the successful establishment of plants in saline soils. It is therefore important to understand the mechanisms of seed tolerance to salt and osmotic stress. Indeed, at the germination stage, the response of seeds is more complex than that of plants because it depends on the availability of stored reserves. Salinity has many effects on the germination process: it alters seed imbibition, causes ion toxicity that alters enzyme activity, reduces the utilization of seed reserves (Othman et al., 2006), alters protein metabolism (Dantas et al., 2005), disturbs hormonal balance (Alboresi et al., 2005), and results in nutritional imbalance (Munns and Tester, 2008). The depressive effects of salt stress and drought on germination and seedling emergence are widely studied in many crops such as sunflower (Kaya et al., 2006), maize (Khayatnezhad and Gholamin, 2011) and sorghum (Patanè et al., 2013).

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops in arid and semi-arid areas (Djilianov et al., 2003). This plant is of major agronomic importance because of its high protein content and its ability to improve soil fertility through symbiotic interactions with atmospheric nitrogen-fixing Rhizobia. It is also considered moderately salt tolerant and relatively drought tolerant compared to many other crops (Munns and Tester, 2008). Therefore, alfalfa is an excellent choice for improving and exploiting salinity-affected lands. Numerous publications have described the effects of drought and salinity on alfalfa plants at the adult stage (Li et al., 2010; Wang et al., 2012; Baha and Bekki, 2015), while at the germination stage, very little data are found in the literature. This work is a contribution to the study of the effects of salt and water stress on alfalfa germination and seedling growth by comparing saline solutions (NaCl and KCl) with the iso-osmotic PEG 6000 solution. The ability of seeds to recover their germination ability after exposure to salt and water stresses is also studied. To determine difference in the mechanisms involved in their stress tolerance, the contribution of inorganic ions and organic solutes to the osmotic adjustment of alfalfa seedlings is also evaluated.

Materials and Methods

Plant material: Alfalfa (*Medicago sativa* L.) ecotype El Golea seeds are recovered from plants grown in an arid region of southern Algeria (El Menea located at 30°35'20" north and 2°52'47" east) where the average annual rainfall is 62 mm.

Germination tests: Alfalfa seeds are germinated in Petri dishes containing distilled water (control) or different solutions of NaCl, KCl or PEG 6000 at 25 °C in the dark for 9 days. Salt solutions are prepared by dissolving amounts of NaCl or KCl in distilled water to a final concentration of 100, 200, 300, 400 and 500 mM. The PEG solutions (12%, 20%, 30%, 40% and 50%) are prepared by dissolving PEG 6000 in distilled water (w/v). The osmotic potential of the different solutions is measured as described by Michel and Kaufman (1973).

Germination parameters: Germinated seeds are counted daily for 9 days. The parameters retained are the final germination percentage (FGP), the germination rate index (GRI) using the equation of Maguire (1962) and the mean daily germination (MDG) determined according to Scott et al. (1984). Stressed seeds that failed to germinate after exposure to salt and water stress were collected, washed with distilled water and then transferred to new Petri dishes containing distilled water and incubated under the same conditions for additional 9 days. The ability of these seeds to recover germination is determined using the formula of Khan and Gulzar (2003).

Water uptake: the imbibition curve of seeds in water and in stressed solutions is determined. Alfalfa seeds are weighed before (W_0) and after imbibition (W_1) of 1, 3, 5, 10, 24 and 72 hours. Seeds removed from the Petri dishes are dried with paper, weighed on a digital scale, and placed for further imbibition. Water uptake (WU) is calculated using a formula presented in Rahman et al. (2008): $WU\ \% = (W_1 - W_0) / W_0$.

Seedling growth measurements: Root and shoot length of each seedling are measured (mm/plant) and the vigor index is also determined (Abdul-Baki and Anderson, 1973) for PEG and salt treatments. The seedlings are then dried at 65 °C for 48 h for dry weight (SDW) measurement.

Measurement of root and hypocotyl elongation: In this test, ten alfalfa seeds are placed in each Petri dish maintained vertically at 25 °C in the dark for 7 days (Verslues et al., 2006). The resulting seedlings are transferred to new Petri dishes containing 0, 200 mM NaCl, 200 mM KCl or 20% PEG 6000 maintained vertically and rotated 180° to visualize new root and hypocotyl growth under stress.

Solutes determination: contents of free proline, soluble sugars and amino acids are measured by ninhydrin method (Troll and Lindsey 1954), anthrone method (McReadyet al., 1950) and method of Yemm and Cooking (1955) respectively. The contents of mineral ions (Na^+, K^+ and Cl^-) are determined by digesting the ground dry matter of each treatment in nitric acid (0.5 N) for 48 hours. After filtration on Whatman paper, K^+ and Na^+ ions are quantified using a flame spectrophotometer (Ogawa Seiki, Japan) and Cl^- ions are titrated by silver nitrate ($AgNO_3$).

Statistical analysis: The experimental setup is composed of two factors arranged in a completely randomized design with four replications (four Petri dishes) per treatment and 50 seeds per replication. The first factor is the chemical nature of the stress (NaCl, KCl and PEG solutions) and the second factor is the severity of the stress (concentration). Data presented as percentages are subjected to an arcsine transformation before statistical analysis. For all parameters studied, an analysis of variance is performed using the Statistica software package. Significant differences between the mean values are compared by the LSD test at $P < 0.05$.

Results and Discussion

The results of the two-way analysis of variance showed that the severity of stress, regardless of its nature (salt or drought), had a highly significant effect on final germination percentage ($P < 0.001$) and on all seedling growth parameters ($P < 0.001$).

Effect of salt and osmotic stresses on the germination capacity

The final percentage of germination (FGP) decreased significantly ($P < 0.05$) with increasing NaCl and KCl concentration. At the same low osmotic pressure (-0.4 MPa) developed by PEG (12%), NaCl (100 mM), and KCl 10 mM), the reductions in FGP were not significant and were 2%, 6.9%, and 10%, respectively, compared to the unstressed control (Fig. 1A, Fig. 2). In contrast, at moderate osmotic pressure -0.8 MPa, there were significant differences in GPF between water-stressed and salt-stressed seeds (Fig. 1C, Fig. 2). Subjected to a high osmotic pressure of -1.9 MPa, seeds were similarly inhibited by salt and PEG 6000 (Fig. 1E, Fig. 2) which does not clearly distinguish the ionic effect from the osmotic one (Fig. 1E, Fig. 2). The distinction between ionic and osmotic effect was only possible at moderate concentrations. The inhibition of seed germination by salt solution is attributed to osmotic stress and/or specific ionic toxicity (Zhang et al., 2010), whereas inhibition of germination by PEG 6000 is solely attributed to osmotic effects. Therefore, any recorded differences in germination between salt-treated seeds and PEG are generally attributed to the ionic effects of salt (Dodd and Donovan, 1999). The higher FGP obtained with PEG compared to NaCl or KCl at the same osmotic potential proves that it is the ionic component of this salt stress that is more inhibitor than its osmotic component. This inhibition of germination could be related to altered hydrolytic enzyme activity, hormonal imbalance at the seed level or altered seed imbibition process due to high osmotic potential which inhibits radicle emergence (Gill et al., 2003; Alboresi et al., 2005).

Speed of germination

Tthe results obtained (Table 1) clearly showed that stressed seeds were slower to germinate as indicated by the significant reduction in germination rate index (GRI) and MDG. These reductions were greater under salt stress than under PEG at all osmotic potentials used. This clearly revealed that salt stress was greater inhibitor than osmotic stress. Furthermore, the values obtained (Table 1) clearly showed that the effect of the applied stresses was more pronounced on the germination rate index than on FGP.

This result is consistent with other studies that have reported the same observations (Kaya et al., 2008). Indeed, the absorption of toxic ions during the imbibition phase of seeds in saline solutions would affect the activity of hydrolytic enzymes, thus prolonging the germination time without reducing the final germination percentage (Patané et al., 2013). Other authors also suggest that salt stress would delay germination by altering the translocation of reserves to developing embryos (De Lacerda et al., 2003).

Kinetics of water uptake

As for PEG 6000, the immediate effect of the salt is osmotic by creating an osmotic potential external to the seed preventing the absorption of water. On the long term, it is the ionic effect that is expressed following an accumulation of ions (Munns and Tester, 2008). Placed in distilled water or in stress solutions, the seeds displayed similar kinetics (Fig. 1D, 1E, 1F). They were all characterized by rapid uptake during the first few hours of imbibition followed by a phase where uptake slowed and ending with a third stationary phase where water uptake appeared to cease. At low osmotic potential - 0.4 MPa (Fig. 1D), the uptake kinetics in the different stressing solutions (NaCl, KCl, PEG 6000) were superimposed on that of the control reflecting the insignificant differences recorded for FGP in these solutions. At moderate (-0.8 MPa) and higher (-1.9 MPa) osmotic potential (Fig. 1E and 1F), the water contents absorbed between 1 h and 7 h by the seeds were lower compared to the control, especially those subjected to the PEG. At the end of the third day (72 h), the amounts of water uptake used by seeds treated with saline solutions were almost equal to that of the control, while their FGP in these solutions remained zero even after 9 days of incubation, confirming that the ionic stress imposed by these saline solutions was responsible for this inhibition.

Actually, in distilled water, the seed allows water to pass and the end of imbibition occurs when the two internal compartments of the seed and the external medium are balanced. In case of salt stress, water enters the interior of the seed together with Na^+, K^+ and Cl^- ions. The internal medium at this time remains even more concentrated than the external medium which allows water to continue to go into the seed and the ions accumulate creating toxicity (Kaya et al., 2006; Demir and Mavi, 2008). In the case of PEG 6000, which cannot penetrate the interior of the seed, water will continue to enter the seed until equilibrium between the outer and inner compartments is reached. The low seeds weight in PEG 30% solutions reflects a low water uptake, which justifies the low germination percentages (10.4%) recorded. On the contrary, the increase in seed weight during the imbibition in saline solutions (500 mM NaCl or KCl) would result from an influx of water and/or simply from an accumulation of absorbed ions inducing the total inhibition of germination. These results are in agreement with Sharma (1973) and Alam et al. (2003) who revealed that water uptake in NaCl solutions was greater than in iso-osmotic PEG solutions in seeds. It appears that the main reason for delayed germination and reduced FGP at -0.8 MPa is a greater accumulation of ions in the seeds rather than a lower water uptake. At -1.9 MPa, the ion concentration accumulated inside the cells would be so high that it completely inhibited seed germination (Zhang et al., 2010).

Recovery of germination

Ungar (1996) defines tolerant seeds as those that can germinate under stress and are able to recover their germination capacity upon stress removal. Indeed, only 60.29% and 19.13% of the seeds that failed to germinate in the 300 mM and 400 mM NaCl solutions, respectively, were able to recover their germination capacity (Table 1).

All seeds inhibited from germination by 500 mM NaCl, 300 mM KCl, 400 mM KCl, and 500 mM KCl were unable to recover their germination ability, despite the removal of salt stress. In contrast, the imposition of severe osmotic stress (PEG 30%, 40%, and 50%) did not reduce the viability of alfalfa seeds as the percentage of germination recovery reached 70.51%, 97.60%, and 95.9% respectively (Table 1).

PEG has no toxic effect, but however, salt stress at the same osmotic potential seems to cause embryo death. This proves that salt stress especially KCl reduces seed viability. These results show that seeds that have accumulated Na^+ ions at 200 mM are able to partially recover their germination because they could have mechanisms to allow some recirculation of Na^+ ions to the external environment or simply mechanisms for the cell to ensure the compartmentalization of Na^+ into vacuoles to escape their toxicity or to be used as inorganic solutes for osmotic adjustment (Sucre and Suarez, 2011). In the case of seeds subjected to KCl 200 mM, K^+, although essential for normal cell function, but accumulated in the cytosol at high concentrations cause embryo death which explains the non-recovery of germination by this class of seeds. Similar results have been obtained in other plants such as *Prosopis strombulifera* (Sosa et al., 2005). These observations confirm that it is the ionic component of salt stress that is largely responsible for the inhibitory effect of salt on alfalfa seed germination. These results are not in agreement with those reported by Chartzoulakis and Klapaki (2000) in pepper and Murillo-Amador et al. (2002) in cowpea, as they stated that the osmotic effect of salt is greater inhibitor than the ionic effect.

Table 1. Parameters related to germination and establishment of alfalfa seeds as a function of salt and osmotic stresses

	Germination attributes				Seedling growth attributes		
Treatment	FGP (%)	MDG (day)	GRI (seed/ day)	PR (%)	SD (mg/seedling)	SL (mm/seedling)	VI
Control	79.4 ae	8.8 a	158.9 a		1.5 ac	60.8 a	483.5 a
NaCl 100mM	71.4a	7.9 a	97.4 b		1.4 a	62.5 a	446.7 a
NaCl 200mM	59.3 b	6.5 b	56.4 c		1.4 ac	28.2 d	167.2 b
NaCl 300mM	10.5cf	1.1 c	2.9 d	60.2	1.6 ac	2.3 c	2.4 c
NaCl 400mM	0 df	0 d	0 e	19.1	0 b	0 c	0 c
NaCl 500mM	0 df	0 d	0 e	0	0 b	0 c	0 c
KCl 100mM	73.9 a	8.2 a	122.4 b		1.4 ac	52.5 b	388.9 a
KCl 200mM	56.5 b	6.2 b	70.1 e		0.2 b	7.5 c	17.8 c
KCl 300mM	15.8 c	1.7 c	13.5 d	0	0 b	0 c	0 c
KCl 400mM	6.2 cd	0.6 d	0.6 e	0	0 b	0 c	0 c
KCl 500mM	0 df	0 d	0 e	0	0 b	0 c	0 c
PEG 12%	77.8ae	8.6 a	114.9 b		1.6 ce	58.9 ab	458.9 a
PEG 20%	77.4 ae	8.6 a	78.0 c		2.3 d	41.3 e	320.6 a
PEG 30%	10.4 c	1.1c	4.7 d	70.5	1.9 e	14.9 f	15.5 b
PEG 40%	0 df	0 d	0 e	97.6	0 b	0 c	0 c
PEG 50%	0 df	0 d	0 e	95.9	0 b	0 c	0 c
Source of variation	FGP		SL		SDW		VI
Nature of stress (A)	ns		***		***		***
Severity of stress (B)	***		***		***		***
A x B (Interaction)	ns		***		***		***

(FGP: Final Germination Percentage; MDG: Mean Daily Germination; GRI: Germination Rate Index; PR: % of Recovery; SDW: Seedling Dry Weight; SL: Seedling Length; VI: Vigor Index).

Means sharing the same letter(s) in column do not differ significantly according to LSD range test at $P < 0.05$.

Ns, *, ** and *** - non significant,.significant at $P < 0.05$, 0.01 and 0.001 confidence level, respectively

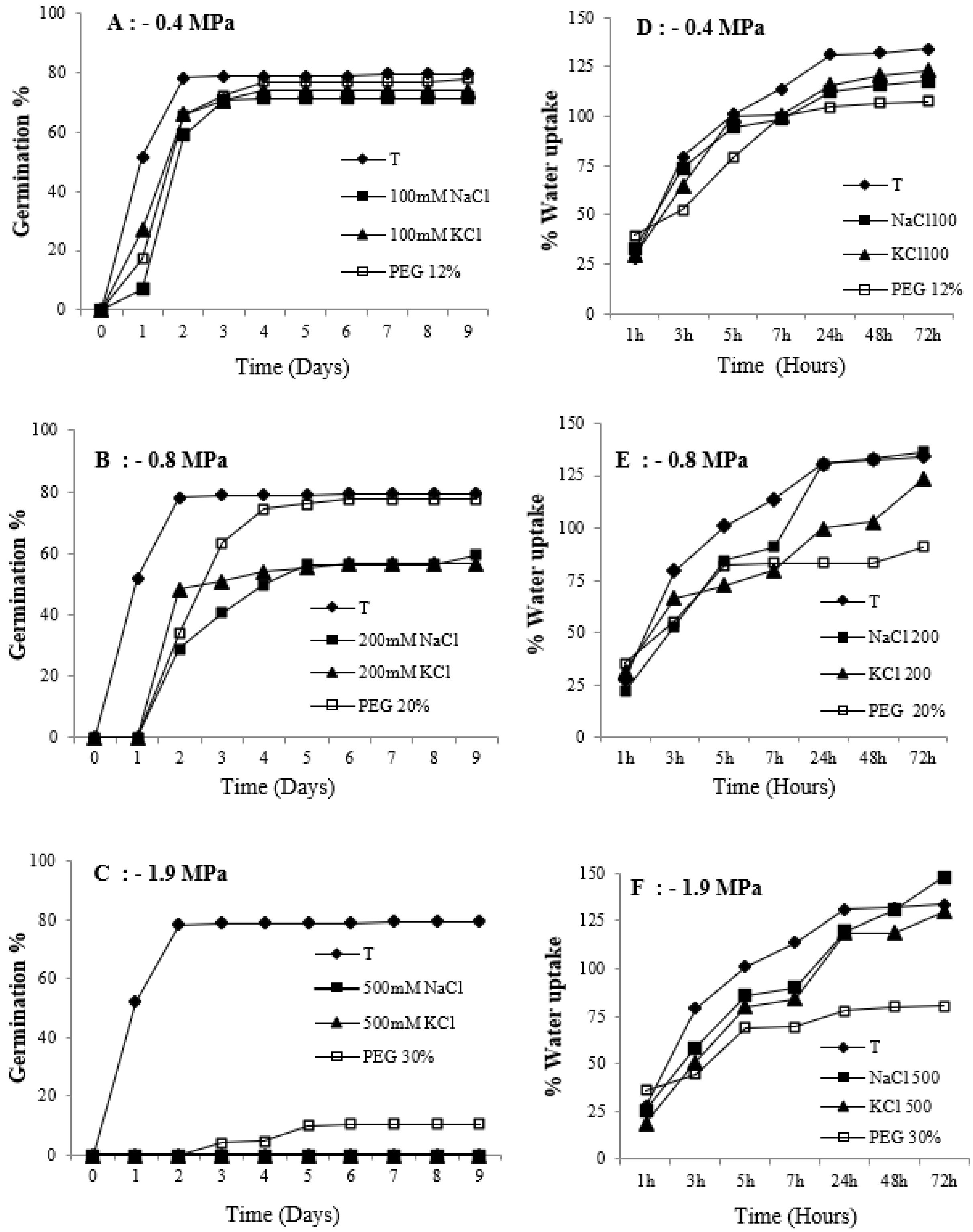

Figure 1. Cumulative germination percentage of *Medicago sativa* seeds during 9 days and kinetic of water uptake under iso-osmotic salt (NaCl, KCl) and water stresses (PEG 6000).

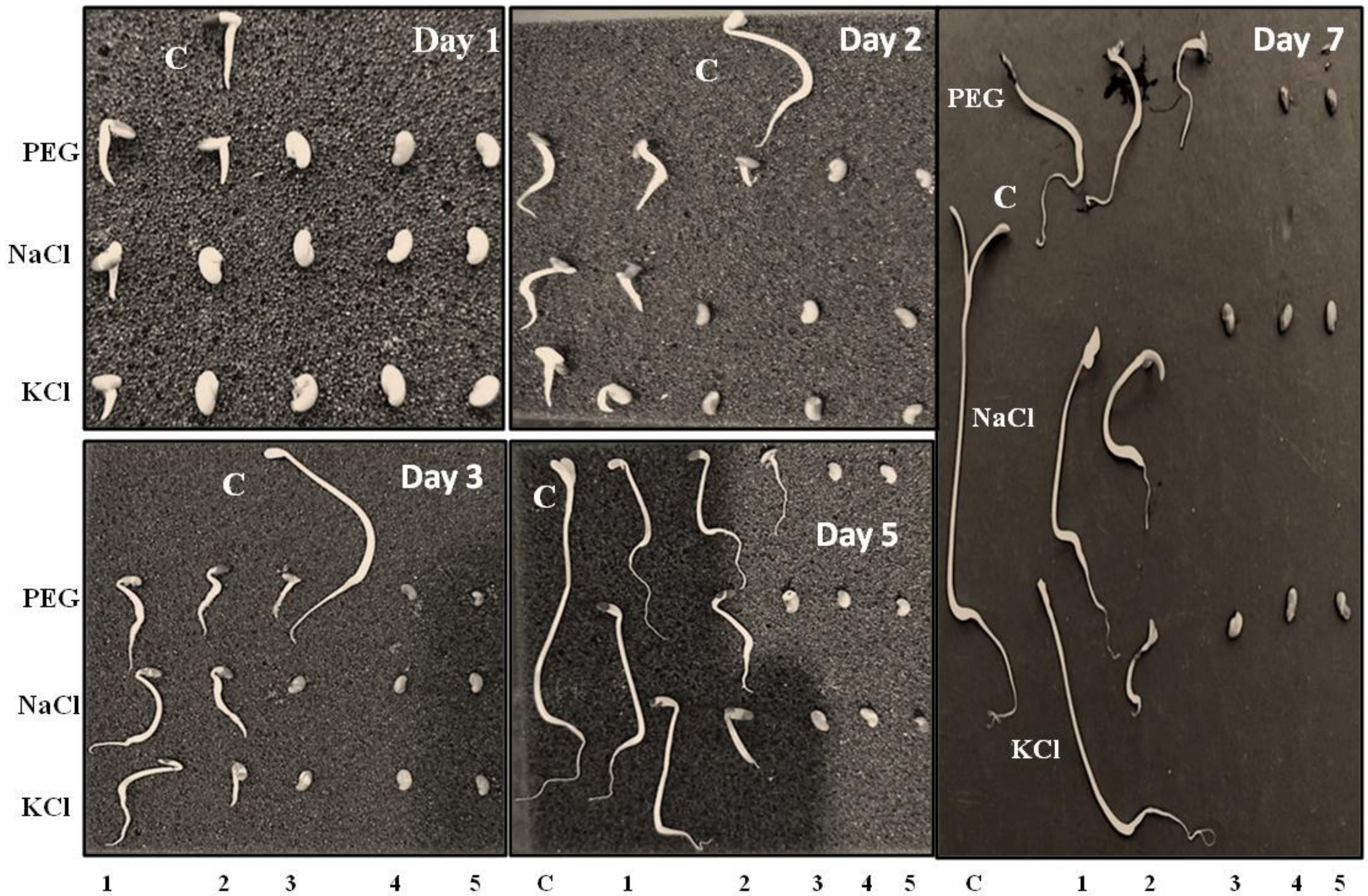

Figure 2. Morphologicals differences observed during germination and seedlings growth of *Medicago sativa* treated with PEG 6000, NaCl or KCl. C: Control, 1:100 mM (or 12% PEG), 2:200 mM (or 20% PEG), 3:300 mM (or 30% PEG), 4:400 mM (or 40% PEG) and 5:500 mM (or 50% PEG) of NaCl or KCl

The inhibition of germination under stress and the ability of seeds to recover their germination is an adaptive strategy (Gill et al., 2003; Miransari and Smith, 2014) that can be associated with the establishment of seedlings in saline environments as soon as ecophysiological conditions become favorable (Debez et al., 2004).

Seedling length

The length of alfalfa seedlings was significantly affected by salt and osmotic stress (Table 1). At equal osmotic potential, KCl was greater inhibitor than NaCl, which in turn was greater inhibitor than PEG. The osmotic effect is less inhibitory because it results only from the restriction of water uptake that contributes to the cell turgor necessary for cell elongation and seedling growth. A reduction in root elongation of about 69%, 65%, and 39% was recorded, whereas for the hypocotyl this reduction in length was approximately 32%, 28%, and 24% in KCl, NaCl, and PEG, respectively. These results show that roots are more affected by salt and osmotic stress than hypocotyls (Fig. 3). These results confirm those found by other authors (Jamil et al., 2007; Khayatnezhad and Gholamin, 2011) and are contrary to the results of Kaya et al. (2008) and Meena et al. (2016) who found that hypocotyl growth was more affected than roots.

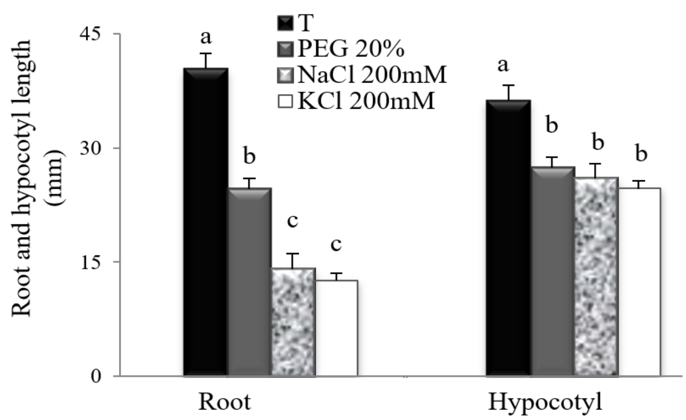

Figure 3. Effect of salt stress (NaCl and KCl) and osmotic stress (PEG 6000) on hypocotyl and root length growth (mm) of alfalfa seedlings. Errors bars represent the standard errors of the means. For each organ, different letters show significant differences at $P < 0.05$

Seedling dry weight and vigor index

Seedling dry weight (SDW) was significantly decreased only by severe osmotic stress (Table 1), whereas at moderate osmotic stress, SDW was significantly increased. Salt stress (100 and 200 mM NaCl) did not affect the SDW since the recorded reduction remained insignificant compared to the unstressed. In contrast, KCl at the same concentrations induced a drastic reduction of about 86%.

At higher NaCl concentration (300 mM), the increase in SDW would likely be due to accumulation of ions and/or osmolytes (Gill et al., 2003; Sivasankaramoorthy et al., 2010). In the presence of PEG, SDW is higher than in the presence of salt stress. Similar results are reported by Demir and Mavi (2008) and Piwowarczyk et al. (2017). Seedling vigor index (VI) was significantly reduced by both salt stresses at all concentrations, especially by KCl (Table 1). In the case of osmotic stress, the seedling vigor index was higher compared to the control, as the dry weights recorded under osmotic stress were also higher. These results are consistent with the findings of Khajeh Hosseini et al. (2002) for soybean and Kaya et al. (2008) for cicer. The growth of alfalfa seedlings seems to be more affected by stress than seed germination since the percentage reductions recorded for germination parameters are lower than those for growth. These results are consistent with those of (Gimmeno Gilles, 2009) which suggest that cell division, being responsible for seedling elongation, is more sensitive to stress than cell expansion which drives germination. Seedling growth as well as germination seems to be more affected by the ionic component of salt stress than by its osmotic component (Khayatnezhad and Gholamin, 2011).

Osmotic Adjustment

Organic solutes

At low osmotic potential (-0.4 MPa) developed by 100 mM NaCl, KCl or 12% PEG, the changes recorded in the contents of soluble sugars and proline remained insignificant (Fig. 4A and Fig. 4B). Under osmotic stress, these changes became significant only at an osmotic pressure of -1.9 MPa (30% PEG). In contrast, under salt stress, these variations in soluble sugars and proline were significant at a lower osmotic pressure -0.8 MPa developed by 200 mM (Fig. 4A and Fig. 4B). The greater accumulation of proline and soluble sugars in 30% PEG-treated seedlings was associated with stable amino acid content (Fig. 4C). This result suggests that osmotic adjustment in these seedlings involves proline and soluble sugars. This high accumulation could also explain the increase in dry biomass recorded under these conditions (Demir and Mavi, 2008). On the other hand, under salt stress (100 mM and 200 mM NaCl), significant reductions in amino acids levels were recorded, which could be explained by an increase in amino acid degradation or an inhibition of their synthesis (Da Silva et al., 2008).

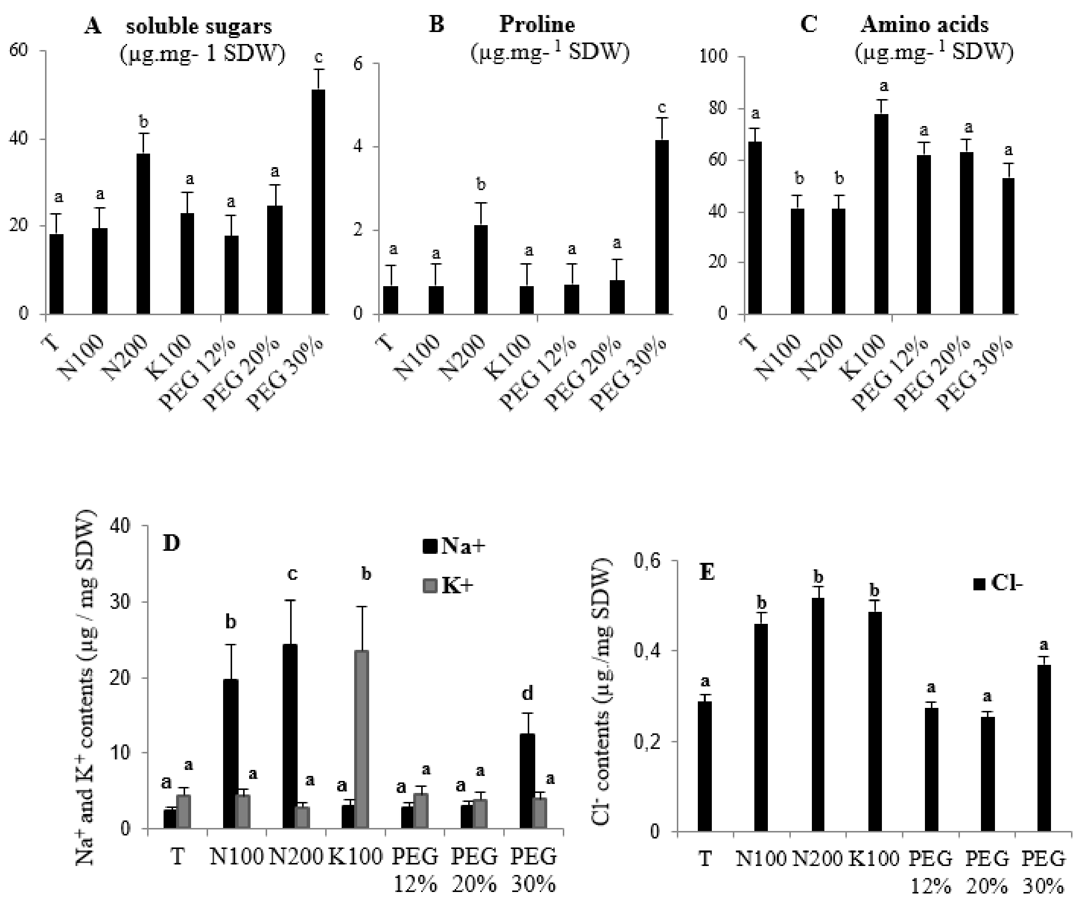

Figure 4. Effect of salt (NaCl and KCl) and osmotic (PEG 6000) stresses on organic and inorganic osmolyte accumulation by alfalfa seedlings. A: Soluble sugars, B: proline, C: amino acids, D: Na^+ and K^+, E: Cl^- µg/mg SDW). Errors bars represent the standard errors of the means. For each osmolyte, different letters show significant differences at $P < 0.05$

It could also be due to their assimilation in the synthesis of stress proteins that have an osmoprotective role of cellular structures, especially since osmoregulation seems to be ensured by the accumulation of proline and soluble sugars. These results show that applied stresses affect osmolyte concentrations differently depending on the chemical nature of the stress and the severity of the stress as also demonstrated by Muscolo et al. (2015). This suggests that salinity and drought stresses, both considered inducers of osmotic stress, activate different biochemical pathways to enhance tolerance in alfalfa seedlings.

Inorganics solutes

In seedlings exposed to NaCl, Na^+ accumulation was proportional to NaCl concentration while K^+ levels remained almost stable. In contrast, seedlings subjected to 100 mM KCl, showed increased K^+ accumulation (Fig. 4D). It should be noted that these seedlings were not greatly affected at this concentration of KCl, suggesting that these accumulated K^+ are not toxic at this concentration and are probably used as an inorganic solute that could neutralize the negative charges of the accumulated amino acids in abundance (Morgan, 1984). The *de novo* synthesis of organic solutes is costly to the cells, especially under stressful conditions. This suggests that these seedlings would use these Na^+ and K^+ ions as osmolytes to adjust their osmotic pressure. The same findings are reported by Hariadi et al. (2011) who attributed 95% of the osmotic adjustment in Quinoa to these ions.

Cl^- ions, on the other hand, increased significantly and comparably in seedlings treated with NaCl or KCl (Fig. 4E), suggesting that this Cl^- ion would not be responsible for the significant difference between the negative effects induced by NaCl and KCl on germination and growth parameters. Indeed, Cl^- ions are reported in forage plants to be less detrimental than Na^+ and K^+ ions (Teakle and Tyerman, 2010). Similar results were previously reported in alfalfa by Wang et al. (2012) and Baha and Bekki (2015).

Thus, alfalfa seedlings in the presence of osmotic stress seem to use proline and soluble sugars to adjust their osmotic pressure while in the presence of salt stress, both its ionic and osmotic components would lead to simultaneous accumulation of organic solutes (proline , soluble sugars and amino acids) and inorganic solutes (Na^+, K^+ and Cl^-). These results are supported by similar observations by Quéro et al. (2014).

Conclusion

This work shows that salt and drought stresses through their osmotic and ionic components have a depressive effect on seed germination and seedling growth as indicated by the different parameters monitored. However, they do not affect them in the same way, depending on the intensity and nature of the stress. It also seems that ionic stress is more inhibiting than osmotic stress, especially K^+ ions. In addition, the growth is more sensitive to salinity than seed germination.

The results also suggest that salt and drought stresses, both considered inducers of osmotic stress, activate different biochemical pathways to improve seedling tolerance.

The high recovery percentages obtained after exposure to severe stress clearly show that seeds of the local cultivar *Medicago sativa* El Golea have the ability to dodge unfavorable conditions, which is an adaptive strategy that will allow this seed to become established as soon as ecophysiological conditions become favorable. This suggests that Algerian alfalfa could be used for the rehabilitation of degraded arid areas.

References

Abdul-Baki A. A., Anderson J. D. (1973). Vigor Determination in Soybean Seed by Multiple Criteria.Crop Sci 13: 630-633. doi:10.2135/cropsci1973.0011183X001300060013x

Alboresi A., Gestin C., Leydecker M. T., Bedu M., Meyer C., Truong H. N. (2006). Nitrate a Signal Relieving Seed Dormancy in *Arabidopsis*. Plant cell Environ 28: 500-512. doi:10.1111/j.1365-3040.2005.01292.x

Alam Z., Stuchbury T., Naylor R. E. L., Rashid M. A. (2003). Water Uptake and Germination Pattern of Rice Seeds under Iso- Osmotic Solutions of NaCl and PEG Different Concentrations of $CaCl_2$ and Combinations of NaCl and $CaCl_2$. Pak J Biol Sci. 6: 1059-1066. doi:10.3923/pjbs.2003.1059.1066

Baha N., Bekki A. E. K. (2015). An Approach of Improving Plant Salt Tolerance of Lucerne (*Medicago sativa* L.) Grown under Salt Stress: Use of Bio-Inoculants. J Plant Growth Regul. 34: 169-182. doi:10.1007/s00344-014-9455-8

Belkhodja M., Bidai Y. (2004). Réponse des graines d'*Atriplex halimus* L. à la salinité au stade de la germination. Secheresse 15: 331-335

Chartzoulakis K., Klapaki G. (2000). Response of Two Different Greenhouse Pepper Hybrids to NaCl Salinity during Different Growth Stages. Sci Hortic 86: 247-260. doi:10.1016/S0304-4238(00)00151-5

Dantas B. F., Ribeiro L. D. S., Arago C. A. (2005). Physiological Response of Cowpea Seeds to Salinity Stress. Rev Bras Sementes 27:144-148. doi:10.1590/S0101-31222005000100018

Da Silva E. C., Nogueira R. J. M. C., De Araujo F. P., De Melo N. F., Azevedo Neto A. D. (2008). Physiological Responses to Salt Stress in Young Umbu Plants. Environ Exp Bot. 63: 147-157. doi:10.1016/j.envexpbot.2007.11.010

Debez A., Ben Hamed K., Grignon C., Abdelly C. (2004). Salinity Effects on Germination, Growth and Seed Production of the Halophyte *Cakile maritima*. Plant and Soil 262: 179-189. doi:10.1023/B:PLSO.0000037034.47247.67

De Lacerda C. F, Cambraia J., Oliva M. A., Ruiz H. A., Prisco J. T. (2003). Solute Accumulation and Distribution during Shoot and Leaf Devlopment in Two Sorghum Genotypes under Salt Stress. Environ Exp Bot 49: 107-120. doi:10.1016/S0098-8472(02)00064-3

Demir I., Mavi K. (2008). Effect of Salt and Osmotic Stresses on the Germination of Pepper Seeds of Different Maturation Stages. Braz Arch Biol Technol. 51: 897-902. doi:10.1590/S1516-89132008000500004

Djilianov D., Prinsen E., Oden S., Van Onckelen A.,Muller J. (2003). Nodulation under Salt Stress of Alfalfa Lines Obtained after *in vitro* Selection for Osmotic Tolerance. Plant Sci. 165: 887-894. doi:10.1016/S0168-9452(03)00291-7

Dodd G. L., Donovan L. A. (1999). Water Potential and Ionic Effects on Germination and Seedling Growth of Two Desert Shrubs. Am J Bot. 86: 1146-1153.

Flowers T. J., Muscolo A.(2015). Introduction to Special Issue:Halophytes in a Changing World. AoB Plants 7: plv020. doi:10.1093/aobpla/plv020.

Gill P. K., Sharma A. D., Singh P., Bhullar S. S. (2003). Changes in Germination Growth and Soluble Sugar Contents of *Sorghum bicolor* L. Seeds under Various Abiotic Stresses. Plant Growth Regul. 40: 157-162. doi:10.1023/A:1024252222376

Gimmeno Gilles C. (2009). Etude cellulaire et moléculaire de la germination chez *Medicagotruncatula*. University of Angers, Angers, France

Hariadi Y., Marandon K., Tian Y., Jacobsen S. E., Shabala S. (2011). Ionic and Osmotic Relations in Quinoa (*Cheopodium quinoa* Willd.) Plants Grown at Various Salinity Levels. J Exp Bot. 62: 185-193. doi:10.1093/jxb/erq257

Jamil M., Lee K. B., Jung K. Y., Lee D. B., Han M. S., Rha E. S. (2007). Salt Stress Inhibits Germination and Early Seedling Growth in Cabbage (*Brassica oleracea capitata* L.). Pak J Biol Sci. 10: 910-914. doi:10.3923/pjbs.2007.910.914

Kaya M., Kaya G., Kaya M. D., Atak M., Saglam S., Khawar K. M., Ciftci C.Y. (2008). Interaction between Seed Size and NaCl on Germination and Early Seedling Growth of Some Turkish Cultivars of Chickpea (*Cicer arietinum* L.). J Zhejiang Univ SciB 9:371-377.doi: 10.1631/jzus.B0720268

Kaya M.D., Okçu G., Atak M., Cikih Y., Kolsariçi O. (2006). Seed Treatments to Overcome Salt and Drought Stress during Germination in Sunflower (*Helianthus annus* L.). EuroJ Agron24: 291-295. doi:10.1016/j.eja.2005.08.001

Khajeh Hosseini M., Powell A.A., Bingham I.J. (2002). Comparison of the Seed Germination and Early Seedling Growth of Soybean in Saline Conditions. Seed Sci Res 12:162-172.doi: 10.1079/SSR2002108

Khan M.A., Gulzar S. (2003). Light, Salinity and Temperature Effects on the Seed Germination of Perennial Grasses. Am J Bot90:131-134. doi:10.3732/ajb.90.1.131

Khayatnezhad M., Gholamin R. (2011). Effects of Salt Stress levels on Five Maize (*Zea mays* L.) Cultivars at Germination Stage. Afr J Biotechnol 10:12909-12915.doi:10.5897/AJB11.1568

Li R., Shi F., Fukuda K., Yang Y. (2010). Effects of Salt and Alkali Stresses on Germination, Growth, Photosynthesis and Ion Accumulation in Alfalfa (*Medicago sativa* L.). Soil Sci Plant Nutr 56:725-733. doi:10.1079/SSR2002108

Maguire J. D. (1962). Speed of Germination Aid in Selection and Evaluation for Seedling Emergence and Vigor. Crop Sci. 2: 176-177. doi: 10.2135/cropsci1962.0011183X00020002003x

McReady R. M., Guggoz J., Silver V., Owensh S. (1950). Determination of Starch and Amylase in Vegetables. Anal Chem 22: 1156-116.doi: 10.1021/ac60045a016

Meena H. N., Meena M., Yadav R. S. (2016). Comparative Performance of Seed Types on Yield potential of peanut (*Arachis hypogaea* L.) under Saline Irrigation. Field Cr Res. 196: 305-310. doi:10.1016/gcr.2016.06.006

Michel B. E., Kaufmann M. (1973). The Osmotic potential of Polyethylene Glycol 6000. Plant Physiol. 51: 914-916. doi: 10.1104/pp.51.5.914

Miransari M., Smith D. L. (2014). Plant Hormones and Seed Germination. Environ Exp Bot. 99: 110-121. doi: 10.1016/j.envexpbot.2013.11.005

Munns R., Tester M. (2008). Mechanisms of Salinity Tolerance. Ann Rev Plant Biol. 59:651-68. doi: 10.1146/annurev.arplant.59.032607.092911

Murillo- Amador B., Lopez-Aguilar R., Kaya C., Larrinaga-Mayoral J., Flores-Hernandez A. (2002). Comparative Effect of NaCl and PEG on Germination Emergence and Seedling Growth of Cowpea. J Agr Crop Sci. 188: 235-247. doi:10.1046/j.1439-037X.2002.005

Muscolo A., Junker A., Klukas C., Weigelt-Fischer K., Riewe D., Altmann T. (2015). Phenotypic and Metabolic Responses to Drought and Salinity of Four Contrasting Lentil Accessions. J Expl Bot. 66: 5467-5480. doi: 10.1093/jxb/erv208

Othman Y., Al-Karaki G., Al Tawaha A.R., Al Horani A. (2006). Variation in Germination and Ion Uptake in Barley Genotypes under Salinity Conditions. World J Agric Sci. 2:11-15

Patanè C., Saita A., Sortino O. (2013). Comparative Effects of Salt and Water Stress on Seed Germination and Early Embryo Growth in Two Cultivars of Sweet Sorghum. J Agron Crop Sci. 199: 30-37. doi:10.1111/j.1439-037X.2012.00531.x

Piwowarczyk B., Tokarz K., Makowski W., Lukasiewicz A. (2017). Different Acclimatization Mechanisms of Two Grass Pea Cultivars to Osmotic Stress in *in vitro* Culture. Acta Physiol Plant. 39:1-5. doi: 10.1007/s11738-017-2389-6

Quéro A., Molinié R., Elboutachfaiti R., Petit E., Pau Roblot C., Guillot X., Mesnard F., Courtois J. (2014). Osmotic Stress Alters the Balance between Organic and Inorganic Solutes in Flax (*Linumusitatissimum*). J Plant Physiol. 171: 55-64. doi: 10.1016/j.jplph.2013.07.006.

Rahman M.U., Soomro U.A., Haq M.Z.U., Gul S. (2008).Effects of NaCl Salinity on Wheat (*Triticum aestivum* L.) Cultivars. World J Agri Sci. 4: 398-403

Scott S.J., Jones R.A., Willams W.A. (1984). Review of Data Analysis Methods for Seed Germination. Crop Sci. 24: 1192-1199. doi: 10.2135/cropsci1984

Sharma M. L. (1973). Stimulation of Drought and Its Effect on Germination of Five Pasture Species. Agron J. 65: 423-431. doi: 10.2134/agronj1973.00021962006500060041x

Sivasankaramoorthy S., Balasubramanian T., Amuthavalli P., Sivaraman P. (2010). The Effect of NaCl Salinity on Seed Germination of *Excoecariaagallocha* L. Curr Bot. 1:10-12

Sosa L., Llanes A., Reinosp H., Reginato M., Luna V. (2005). Osmotic and Specific Ion Effects on the Germination of *Prosopis strombulifera*. Ann Bot. 96: 261-267. doi: 10.1093/aob/mci173

Sucre B., Suarez N. (2011). Effect of Salinity and PEG Induced Water Stress on Water Status, Gas Exchange, Solute Accumulation and Leaf Growth in *Ipomoea pescaprae*. Environ Exp Bot. 70: 192-203. doi:10.1016/j.envexpbot.2010.09.004

Teakle N. L., Tyerman S. D. (2010). Mechanisms of Cl^- Transport Contributing to Salt Tolerance. Plant Cell Environ. 33 :566–589.doi: 10.1111/j.1365-3040.2009.02060.x

Troll W., Lindsey J. (1955). A Photometric Method for the Determination of Proline. J Biol Chem. 215: 655-660

Unguar I. (1996). Effect of Salinity on Seed Germination, Growth and Ion Accumulation of *Atriplex patuta*. Am J Bot. 83: 604-607. doi: 10.2307/2445919

Verslues P. E., Agarwal M., Katiyar- Agarwal S., Zhu J., Zhu J. K. (2006). Methods and Concepts in Quantifying Resistance to Drought, Salt and Freezing Abiotic Stresses That Affect Plant Water Status. The Plant J. 45: 523-539. doi: 10.1111/j.1365-313X.2005.02593.x

Wang X., Chen W., Zhou Y., Han J., Zhao J., Shi D., Yang C. (2012). Comparison of Adaptive Strategies of Alfalfa (*Medicago sativa* L.) to Salt and Alkali Stress. Aust J Crop Sci. 6: 309-315

Yemm E.W., Cooking E. C. (1955). The Determination of Amino Acids with Ninhydrine. Analyst 80: 209-213.doi:10.1039/AN9558000209

Zhang H., Irving L.J., McGill C., Matthew C., Zhou D., Kemp P. (2010). The Effects of Salinity and osmotic Stress on barley Germination Rate: Sodium as an Osmotic Regulator. Ann Bot. 106: 1027-1035.doi: 10.1093/aob/mcq204

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