Abstract

Developing seeds with a high level of tolerance against salinity and water shortage can guarantee best seedling establishment in arid and semi-arid agricultural lands. This three-year field, laboratory and greenhouse study proposes the surfactant application to effectively prevent the detrimental impacts of severe environmental conditions on the development of parental plants. The germination rate of six parental seeds of berseem clovers (Trifolium alexandrinum L.) from I$_{100}$ (100% irrigation water)/I$_{100}$+s (irrigation water with surfactant, s), I$_{75}$/I$_{75}$+s and I$_{50}$/I$_{50}$+s treatments are studied under seven osmotic salinity potentials (0, -0.2, -0.4, -0.6, -0.8, -1, and -1.2MPa). Plants from seeds developed by surfactant in full and moderate limited irrigation (I$_{100}$ and I$_{75}$) show a favorable germination percentage when under saline stress treatments of -0.4 and -0.8MPa. The highest seedling lengths of 10.2 and 10.3cm were achieved for the seeds produced from I$_{75}$ and I$_{100}$+s treatments, respectively. Utilizing the surfactant across all field treatments had a positive effect on the weighted germination index compared to the counterpart treatment. In addition, Seedlings from seeds treated by surfactant in the germination tests have higher shoot/root ratios, which show the efficiency of surfactant application in promoting a better root and shoot development under saline/drought stresses.

Introduction

Seed performance refers to a capacity of seeds to germinate under various environmental conditions and relates to a critical period of a plant’s life cycle which is eminently important in ecological and agronomic plant growth [1]. There is a general agreement that the performance and viability of seeds during their early stages of germination may be related to the conditions under which seeds are formed, developed, and matured [2]. As seed germination is the beginning of a plant’s life cycle, proper seedling emergence is critically important for a decent establishment of plant population [3]. Many researchers have reported that adverse
environmental conditions affect crop growth and productivity, postponing the seed germination and reducing the germination rate [4-7]. Plants experience a variety of abiotic stresses such as high/low temperatures, drought and salinity stress during their lifecycles which have a profound effect on their viability, growth, morphology, and reproduction [8]. In arid and semi-arid regions, seed germination and emergence are considered as critical stages for plant establishment and crop growth, which can elevate the crops’ density and yields Shaygan 2017. In these regions, soil salinization is detrimental to plant growth and productivity [9,10].

Plants usually take up salt (Na⁺ and Cl⁻ ions) from water which is detrimental to germination [11-13]. Plant growth in saline dry and semi-dry soils can be further damaged by water scarcity due to high osmotic pressure, which prevents adequate water uptake [14]. (Tables 1 & 2) present the summary of recent reports on the adverse effects of salt/drought stress on seed germination and establishment [15-18]. For instance, the seed germination, emergence rate and growth of young seedlings of sugar beet (Beta vulgaris L.) [19,20], Phaseolus mungo [21] and Dianthus chinensis L. [22] decreased by NaCl treatments. Quantitatively, the salinity condition decreased the seed germination of nut (Jatropha curcas L.) by 50% [23], coriander (Coriandrum Sativum L) by 65.67%, cultivar Kalmiand Pant Haritma by 55.56% [24], oat (Avena sativa L) by 60.87% [25] etc. Berseem clover (Trifolium alexandrinum L.) by 50% [23], coriander (Coriandrum Sativum L) by 65.67%, cultivar Kalmiand Pant Haritma by 55.56% [24], oat (Avena sativa L) by 60.87% [25] etc. Berseem clover (Trifolium alexandrinum L.) is one of the best forage sources for feeding live stock and is considered as non-bloating and high-quality forage [26].

**Table 1:** Recent studies on the effects of salinity/drought stresses on qualitative and quantitative traits of plants. 

| Plant | Objectives | Stress | Osmotic potential | Results | Applied condition to overcome stresses | References |
|-------|------------|--------|-------------------|---------|----------------------------------------|------------|
| Alfa (Medicago sativa) | Analyze activity of four antioxidant enzymes for adaptability to salt and drought stresses during laboratory-based germination | Salinity/ drought | 35% PEG or 200 mM NaCl | Higher POD in roots and CAT in shoots; higher SOD and APX and lower levels of H₂O₂ and lipid peroxidation in Xinmu No.1 tissues treated with NaCl or PEG. GR of Xinmu No.1 (stress-tolerant): 90-95%, Northstar (sensitive cultivars): 52.7% under 200 mM NaCl treatment and 72% under 35% PEG treatment | - | Wang et al. [78] |
| Canola (Brassica napus L.) | Compare iso-osmotic stresses induced by NaCl and PEG on germination components during laboratory-based germination | Salinity/ drought | PEG and NaCl: 0, -0.1, -0.2, -0.3, -0.4, -0.6, -0.8 MPa | Compared to NaCl condition, G% reduced under -0.6 and -0.8 MPa PEG; highest G% (72%) gained at 0 MPa and decreased to 54.2 and 26.1% in -0.8 MPa for NaCl and PEG | - | Torabi & ghehsareh. [77] |
| Sorghum (Sorghum bicolor L. Moench) | Evaluate seed germination and seedling establishment tolerance to moisture and salt stresses in saline and semi-arid regions of San Joaquin Valley of California during laboratory/field-based growth | Salinity/ drought | PEG: 0 to -5.56 MPa, NaCl: 0-25 dSm⁻¹ | Higher grain hybrid G% at higher salinity level; higher tolerance of forage sorghum to moisture stress than grain sorghum. G% reduced by 50% in SS405 at -2.5 MPa and 19.3 dS m⁻¹and in NK5418 at -1.5 MPa, but NK5418 had 70% germination even at 25 dS m⁻¹ | - | Shrestha et al. [75] |
| Medicago sativa L. | Evaluate coupling effects of drought and salt stresses on germination during laboratory-based growth by using an orthogonal design to evaluate the tolerance | Salinity/ drought | PEG: 5, 10, 15%, NaCl: 50, 100, 150 mM | G% of M. sativa and A. adsurgens were higher (57.55 and 39.56%, respectively) than C. varia (14%) under both stresses. G% of M. sativa decreased (13.33%) upon the increase in 15% PEG+150 mM NaCl | - | Wu et al. [79] |
| Artemisia scopulorum (L.) | Effects of drought and salinity on seed growth during laboratory-based germination | Salinity/ drought | PEG: 0, 10%, 15%, 20%, 25%, NaCl: 0, 50, 100, 150 mM | G%, GR, root length, shoot length, RDW and SDW had 55.29%, 76.17%, 95.4%, 86.63% and 100% reduction from 0 to 20% PEG. G%, GR, root length, shoot length, RDW and SDW had 2.3%, 39.18%, 4.51, 48.12, 35.34% and 12.96% reduction in 0-150 mMNaCl | - | Jorenush & Rajabi [71] |
Pennisetum divisum (Gmel.)

Effects of four alternative temperature regimes (10/20, 15/25, 20/35, 25/40°C under 12 h-light), drought (PEG8000) and salt stress on seed growth during laboratory-based germination

Salinity/drought

PEG/NaCl: 0, -0.2, -0.4, -0.6, -0.8 MPa

Optimum G% was attained at 15/25°C; G% decreased at -0.6 MPa, no germination occurred at -0.8 MPa by NaCl; Seedling growth was more sensitive to NaCl; G% increased to 80% when temperature raised to 15/25°C and decreased to 30% under 25/40°C

- Al Taisan, WA [60]

Mugwort (Artemisia vulgaris L.)

Effects of salt and water stresses on seed and seedling growth, fresh and dry weight during laboratory-based germination

Salinity/drought

PEG/NaCl: 0, -0.05, -0.1, -0.15, -0.2, -0.25 MPa, NaCl: 0, 5, 10, 25, 50, 100 mM

G% decreased to 70% at 100 mM. G% reduced at -0.25 MPa of PEG; Root length, shoot length, fresh weight and dry weight obtained 88.88/94.44%, 85.48/94.62%, 63.63/81.81%, 66.66/96.66% reduction at 100 mM NaCl/-0.25 MPa PEG

- Ebadi Almas et al. [64]

Sesame (Sesamum indicum): Moroccan cultivars (yellow/brown seeds), American accessions (black/white seeds)

Evaluate and compare effects of salt and drought stresses on germination and early seedling growth, and various cultivars characterized by different seed colors during laboratory-based germination

Salinity/drought

PEG/NaCl: 0, -0.2, -0.4, -0.6, -0.8, -1.2, -1.4 MPa

40%, 56% and 100% GP for black, white and yellow/brown seeds and 19, 26, 99 and 98% GR for control; G of all cultivars ceased at -1.0 MPa of PEG; reduction in GP and GR was gradual for yellow and brown seeds and drastic for white and black ones at -0.2 MPa of PEG and NaCl; seedling growth of yellow and brown seeds more affected by both stresses than G

- El Harfi et al. [66]

Common Yarrow (Achillea millefolium L.)

Evaluate effects of salinity and drought on seed germination and seedling growth during laboratory-based germination

Salinity/drought

PEG: 0, 10%, 15%, 20%, 25%, NaCl: 0, 50, 100, 150 mM

G% decreased from 96.00% (control) to 56.67% at 50 mM NaCl

- Fetri et al. [67]

Wheat (Triticum aestivum L.)

Evaluate seed germination and seedling establishment tolerance of genotypic variability to drought and salinity stresses during laboratory-based germination

Salinity

NaCl: 4, 8, 16 dSm-1

Average G% showed 13, 41 and 84% and germination rate showed 22, 43 and 110% reduction of control at 4, 8 and 16 dSm1 respectively

- Bahrami & Haghigh, [62]

Berseem clover (Trifolium alexandrinum L.)

Sustainably reduce severe effects of saline and drought stresses

Salinity/drought

NaCl: 0, -0.2, -0.4, -0.6, -0.8, -1.2 MPa

Seeds developed by 1100+s and 75+s had better G% under -0.4 and -0.8 MPa NaCl; utilizing surfactant across all parental treatments had a positive effect on WGI; surfactant application on parental plants induces a lower shoot/root ratio; under -0.6 MPa salinity and 150, seeds had a reasonable G(75%) when applying surfactant; under -0.4 MPa salinity, surfactant increased seedling height by 43.7 and 31.5% in I100 and I75

Adding surfactant to irrigation water at a rate of 1 mg per liter (1 ppm)

This study

G% = Germination percentage, GR= Germination Rate, SDW= Seedling Dry Weight, RDW= Root Dry Weight, SOD= Superoxide of Dismutase, CAT = Chloramphenicol Acetyltransferase, APX = Ascorbate Peroxidase, PEG = Polyethylene Glycol

It is moderately tolerant to salinity compared to wheat and strawberry clover [27]. Despite the advantages, studies on the effects of salt stress have been mostly carried out on agricultural crops [28-30] (Tables 1 & 2). Thus, there is limited information on the effect of field treatments (seed production conditions) on subsequent salt stress tolerance at the germination stage and
seedling establishment of legume forage crops such as berseem clover. Water shortage and saline soil are known as one of the main natural hazards especially in arid and semiarid regions which delay seed germination and seedling establishment [31]. The aims of this three-year study are to determine factors responsible for the failure of germination and early growth characteristics of berseem clover seeds under saline/osmotic conditions. (Tables 1 & 2) present the results of recent studies on the reduction effect of salinity and drought conditions on different plants traits (Tables 1 & 2). Compared to these results, the present study proposes a co-friendly surfactant solution to mix with irrigation water at a very low rate of 1 mg per liter (1 ppm) to substantially reduce the severe effects of salinity and drought stresses on seed germination and growth of berseem clover.

Table 2: Recent studies on the effects of salinity/drought stresses on qualitative and quantitative traits of plants.

| Plant | Objectives | Stress | Osmotic potential | Results | Applied condition to overcome stresses | References |
|-------|------------|--------|-------------------|---------|----------------------------------------|------------|
| Wheat (Triticum aestivum L.) | Evaluate seed germination and seedling establishment tolerance of genotypic variability to drought and salinity stresses during laboratory-based germination | Salinity | NaCl: 4, 8, 16 dSm⁻¹ | Average 0% showed 13.41 and 64% and germination rate showed 22.43 and 11.0% reduction of control at 4, 8 and 16 dSm⁻¹ respectively | - | Bahrani and Haghjoo [62] |
| Cabbage (Brassica oleracea capitata), cauliflower (Brassica oleracea botrytis) and canola (Brassicanapus) | Investigate extent of salinity on germination and early seedling growth using different varieties during field-based growth | Salinity | NaCl: 0, 4, 7, 9.4, 14.1 dSm⁻¹ | 6% reduction in 14.1 dSm⁻¹; 76.5% in Cabbage, 79.51% in Cauliflower and 43.5% in Canola | - | Jamil et al. [70] |
| Radish (Raphanus sativus L.), Cabbage (Brassica oleracea capitata L.), Mustard (Brassica juncea) and Water spinach (Ipomoea aquatica) | Effect of salinity on germination, seed viability index, seedling and root length and weight of different seed varieties during laboratory-based growth | Salinity | NaCl: 0, 2, 4, 8, 16 dSm⁻¹ | 8 and 16 dSm⁻¹ salinity reduced all traits yield; lowest (61%) and highest (85%) 6% observed in mustard and radish; 6% of radish, cabbage, mustard and water spinach reduced by 47, 47.42 and 37% respectively, at 16 dSm⁻¹ NaCl | - | Sarker et al. [74] |
| Cowpea (Vigna unguiculata L.)- Sadiany, Chinese red, Kaha 1, TVU 21 and Black eye Crowder | Physiological and morphological response to salinity during field-based growth | Salinity | NaCl:153 (as control), 2500, 3500, 4800 ppm | 4800 ppm water salinity decreased germination rate; 6% shoot length, root length, SFW, SDW, RWC, chlorophyll content and ion leakage reduced by 18.960, 94.800, 8.936, 3.808, 5.128, 5.33, 11.116, 69.936 and 11.35% | - | El-Shaieym & Hakeem, 2015 |
| Bean (Phaseolus vulgaris L.) | Effects of salinization on germination during laboratory-based growth | Salinity | NaCl: 0, 0.3, 0.6, 0.9, 1.2, 1.5 MPa | MGT had no differences with increasing NaCl Maximum/ minimum GP and SW were 90% /60% and 867.0 /290.3 in control and 1.5 MPa NaCl respectively. | - | Cokkiegin [63] |
| Rice (Oryza sativa L.): IR20, BR29, BR40, Pokkali, MR33, MR68, MR84, MR52, MR211, MR219, MR220 and MR232 | Determine the salt-tolerance of rice variety(s) at early seedling establishment and their salt tolerance levels. | Salinity | NaCl: 0, 4, 8, 12, 16, 20 dSm⁻¹ | At 4 dSm⁻¹ 6% of MR52, MR211, MR219, MR232, BR40 and Pokkali were more than 90%; at 12 dSm-16% of IR20, MR33, MR68 and MR94 were less than 80%; at 4 dSm-1Pokkali, MR211, MR219 and BR40 showed better SG; at 8 dSm-1IR20, MR84, MR52, MR211, MR219, MR232 and BR40 had higher SG at 12 dSm⁻¹ IR20, MR232, MR211 and BR40 showed higher SG; at 16 dSm-1IR20 germination reduced to 70% | - | Hakim et al. [68] |
| Lettuce (Lactuca sativa L.) | Effect of salinity on seed germination, seedling growth and acid phosphates activities during laborotory-based growth | Salinity | NaCl: 100 mM | G% and GR in Vista decreased but Romaine’s G% remained 100%; length and fresh root and shoot weights reduced significantly in two varieties; acid phosphates activity in root increased in Romaine and decreased in Vista and had no changes in shoot but in cotyledons decreased in both varieties | Nasri et al [72] |
| Lettuce (Lactuca sativa L.) | Evaluate effects of osmo priming on germination dynamics, subsequent seedling growth and phosphases activities by analyzing activity of acid phosphatase | Salinity | NaCl: 0 or 100 mM | G% (was 65%), root and shoot length SFW of primed seeds was higher than non-primed seeds in saline condition; priming also increased acid phosphatase and phytase activities in roots, shoots and cotyledons under salt stress | Nasri et al [73] |
| Brassicanapus (L.): Heros and Eagle | Evaluate effects of osmo-priming on germination dynamics and subsequent seedling growth under different range of salinity stresses | Salinity | NaCl: 0.2, 5, 10, 15, 20 dSm-1 | Osmo priming with KNO3 (0.05%) for 2 h at 25°C in the dark | Hassanpouraghdam et al. [69] |
| Physalis ixocarpa and Physalis peruviana | Evaluate the effect of NaCl on germination and emergence and establish genetic potential for salt tolerance | Salinity | NaCl: 0, 30, 60, 90, 120, 180 mM | Highest G% (72%) obtained from P. peruviana at 180 mM NaCl, elevated NaCl concentrations 0-180 mM increased MGT in both species; FEP values of P. peruviana and P. ixocarpa reduced by 59% - 47% at 30 mM and 100% - 62% at 60 mM | Yildirim et al. [80] |

G% = Germination percentage, GR = Germination Rate, MGT = Mean Germination Time, FEP = Final Emergence Percentage, SVI = Seed Vigor Index, SFW = Seedling Fresh Weight, SDW = Seedling Dry Weight, RWC = Relative Water Content, SG = Speed of Germination.

**Materials and Method**

**Seed materials**

Berseem clover (Trifolium alexandrinum L.) seeds obtained from our field experiments during the 2013-2015 growing seasons at the Research Farm of the College of Agriculture, University of Tehran, Karaj, Iran (N 35º56' , E 50º58') [32] Daneshnia 2016. The climate at this site is considered as arid to semiarid with a long-term (50 years) mean air temperature of 13.5°C, soil temperature of 14.5°C, and 262 mm of mean annual rainfall. In the field experiments, three levels of irrigation were applied to the main plots, including normal irrigation (I_{100}), (replenishment of 100% of weekly evaporation and plant water requirement), moderate irrigation (I_{60}) and limited irrigation (I_{0}). Two types of water treatment, including tap water and water + surfactant (added at a rate of 1ppm), were applied to the subplots. The experiments were carried out in split-split plots based on a completely randomized block design with three replications. Likewise, the irrigation treatments (I_{100}, I_{60} and I_{0}) were scheduled based on the common practice of the area, once a week, when the plants reached the 4 to 6-leaf growth stage. The amount of required irrigation water was calculated using the following equation [26,32].

\[
I_n = 0.623 \times A \times K_c \times ET_0 \times IE \times 1
\]

Where In is the volume of irrigation water (gallons), 0.623 is a constant, A is the canopy area (sq.ft.), Kc is the crop coefficient, ET0 is the weekly potential evapo transpiration (inches), and IE is the irrigation efficiency.

**Salt stress germination test**

The effects of six parental treatments (three irrigation levels and two water treatments) under salinity conditions were systematically studied during the germination process. These experiments were performed at the Seed Research Laboratory.

_Citation: F Daneshnia1 and MR Chaichi. Field Treatment Effects on Seed Germination and Early Growth Traits of Berseem Clover under Salinity Stress Conditions. Curr Inves Agri Curr Res 2(1), 2018. CIACR.MS.ID.000127._
of the College of Agriculture, University of Tehran. The salinity treatment was built with seven osmotic potentials of 0, -0.2, -0.4, -0.6, -0.8, -1 and -1.2 MPa by, respectively, using 0, 2.66, 5.3, 7.99, 10.65, 13.31 and 15.98 gr/kg NaCl in dematerialized water. 50 seeds from each parental treatment were placed in 9cm sterile Petri dishes which contained two what man No.1 filter papers, and then placed in seed germination at 20°C. 10ml solution from each test was poured onto the plate; the papers were altered once after every 2 days to prevent salt accumulation [33]. The 42 treatments (6 parental treatments and 7 salinity levels) were arranged on a factorial base with a completely randomized design and three replications. To maintain water and salt concentrations near the target levels, required dematerialized or saline water was added to the Petri dishes during the daily monitoring. The number of germinated seeds was recorded every 24 hours for two weeks. A seed was considered as germinated when an emerging radical was longer than 2mm. Then, the germination parameters, such as final germination percentage (G%) and weighted germination index (WGI) [34], were determined with higher weights assigned to the seeds that germinated early and less to those that germinated late. The final WGI values (after 14 days) were calculated using the method proposed by Bu [34]

\[
WGI = \left( \frac{N \times n_1 + (N-1) \times n_2 + \cdots + 1 \times n_{14}}{N \times N'} \right)
\]

Here, \(n_1, n_2, \ldots, n_{14}\) is the number of seeds that germinated on the 1st, 2nd and subsequent days until the 10th day, respectively, N is the total number of days of germination process, and N' is the total number of seeds placed in incubation. In Eq.1, the final WGI value is the product of the experimental value of WGI and the percentage of a live seeds in each treatment.

Early growth characterization of berseem clover seedlings under saline condition

Following the salt stress germination test, a series of pot experiments were conducted to evaluate the interaction between field treatment, salinity, and surfactant in the early growth of berseem clover seedlings. These experiments were performed at the Research Greenhouse of the College of Agriculture, University of Tehran. The greenhouse temperature, humidity and photosynthetic photon flux density (PPFD) for day/night were 25/20°C, 65/60%, and 300-600 µmol m⁻² s⁻¹, respectively. The saline treatments comprised of either intact soil from the field (soil with an electrical conductivity (EC) of 0.075 MPa and a primary osmotic potential of (-0.083MPa) or saline soil with an osmotic potential of -0.4MPa. The osmotic potential of salinity stress (-0.4MPa) was chosen on the basis of the optimum results of osmotic potential treatments from saline stress in the germination tests. In the following, the salinity level of the pots was kept at -0.4MPa throughout the experimental period. After a few preliminary tests, the EC of soil was adjusted to -0.4MPa by adding a solution of 5.3g NaCl+100 ml distilled water to all the pots which contained the saline-treated soil. All the pots were filled with 2.43 kg of air-dried soil collected from the field. The soil of the pots was sieved (with a mesh diameter of 1 cm) and loaded in plastic pots 15cm in diameter and 12cm in height.

The field soil texture was clay loam with the following physico-chemical characteristics: pH=7.8, EC=2.31 dSm⁻¹, total nitrogen (N)=0.09%, available phosphorus (P)=8.87 mgkg⁻¹, and available potassium (K)=225mgkg⁻¹. The soil in each pot was fertilized with 1 liter of basal nutrient solution (BNS) which mainly consisted of both ammoniums (NH₄) and nitrate (NO₃) as the source of nitrogen [35]. To study the effect of surfactant on seedling establishment of parental seeds in saline environments, the pots were sprinkled with different levels of fresh water with/without surfactant additive. The soil was watered once every three days to replenish 100% of the soil moisture to ensure the plants were under no drought stress. The seeds used in this experiment were the same as used in the germination test. The nonionic surfactant marketed with the name Golden Irrig. Aid was provided by Aquatrols Corporations, New Jersey, USA (10% Alkoxylated polyols, 7% Glucoethers, Inter ingredient 83% water). The surfactant was applied at a rate of 1mg per liter (1ppm) to the irrigation water for water treatment purposes [36,37].

Statistical Analysis

The ANOVA technique with three replications was applied to the randomized design using the Proc GLM procedure SAS [38]. Mean comparison was implemented using Duncan’s test at the 95% level of probability.

Results

Salt stress germination test

The laboratory experimental results showed that the germination is completely suppressed by -1 and -1.2 MPa a salinity stress in the presence of the highest concentrations of NaCl. As the salinity increases, the germination percentage followed a decreasing trend across all field treatments. However, berseem clover can tolerate NaCl up to -0.4 MPa saline stresses with a moderate decrease in its germination stage (Figure 1). As the salinity stress increased from -0.4 to -0.6 MPa, G(%) diminished significantly in all seed sources; the least (8.3%) and highest (33.3%) reductions were observed in I₅₀ (with and without surfactant) and full irrigation (I₁₀₀) (with and without surfactant) treatments, respectively. It is not able that under a higher salinity stress (-0.8 MPa), germination percentage increased across all parental seeds compared with -0.6MP a salinity stress. Parental seeds, collected from limited seed sources; the least (8.3%) and highest (33.3%) reductions were observed in I₅₀ (with and without surfactant) and full irrigation (I₁₀₀) (with and without surfactant) treatments, respectively. It is not able that under a higher salinity stress (-0.8 MPa), germination percentage increased across all parental seeds compared with -0.6MP a salinity stress. Parental seeds, collected from limited seed sources; the least (8.3%) and highest (33.3%) reductions were observed in I₅₀ (with and without surfactant) and full irrigation (I₁₀₀) (with and without surfactant) treatments, respectively.

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This showed a reasonable germination percentage (75%) when the utilized surfactant was added to the system. As the level of salinity stress increased, the application of surfactant led to a better performance in the preservation of the seeds germination under harsh conditions. As shown in Figure 2 (a-d), seeds developed with irrigation water plus surfactant in both full and moderately limited irrigation treatments ($I_{100}$ and $I_{75}$) have better germination rates, compared with their counterpart treatments with no added surfactant under saline stress (-0.4 and -0.8 MPa). In all parental treatments, as salinity stress intensity increased from 0 to -1.2 MPa, WGI followed a decreasing trend (Figure 3). The highest WGI (87) was gained from $I_{100}/+8$ under a moderate salinity stress (-0.4 MPa). Under higher osmotic stresses, seeds produced by surfactant had a higher WGI across all parental treatments compared to their
counter parts from non-surfactant treatments. This is the indication of an enhanced germination rate in parental seeds upon the application of surfactant in irrigation water. The WGI in moderate water potentials of -0.2 and -0.4 MPA, induced by NaCl, was higher compared to that under more stressed levels (Figure 3).

**Parental effects on berseem clover seedling establishment and growth under saline condition**

Table 3 indicates that the seeds produced under deficit irrigation regimes (75% and 50%) with surfactant application (parental treatments) have better tolerance to salinity stress. This is also seen in Figure 4 where seeds, collected from plants grown with the irrigation treatments of $I_{75}$ and $I_{50}$ have a better performance under salinity stress. The highest seedling height (10.2cm) is gained from $I_{75}+s$ where the pots are water reducing surfactant. Figure 5 presents the evidence where the application of surfactant significantly increased the berseem clover seedling height when subjected to saline stress treatments, compared to the control. Under saline conditions, surfactant application increased the seedling height by 43.7 and 31.5% in $I_{100}$ and $I_{50}$ respectively, compared to control. In fact, after the application of a surfactant, berseem clover height significantly increased across all treatments. Figure 6 shows that the seeds developed under moderate water stress had a better seedling growth and development with surfactant application ($I_{75}+s$), compared to the control.

| Treatment | Normal condition plant height (cm) | Saline stress (-0.4 MPA) plant height (cm) | Normal condition shoot/root (ratio) | Saline stress (-0.4 MPA) shoot/root (ratio) |
|-----------|----------------------------------|------------------------------------------|----------------------------------|------------------------------------------|
| Irrigation treatment on parental plants | | | | |
| 100% | 6.6 b | 7 b | 6.8 a | 3.6 a |
| 75% | 7.7 a | 8.0 a | 5.3 b | 3.3 a |
| 50% | 7.8 a | 8.0 a | 4.0c | 3.5 a |
| Significance | * | ** | ** | ** |

**Figure 4:** Parental effect (water stress along with water treatment: $I_{100}$, $I_{75}$, and $I_{50}$) and surfactant application on berseem clover seedling height under the saline condition of -0.4MPa in comparison with the normal condition.

**Figure 5:** Parental effect (water stress along with water treatment: $I_{100}+s$, $I_{75}+s$, and $I_{50}+s$) and surfactant application on berseem clover seedling height under the saline condition of -0.4MPa in comparison to normal condition.
### Table 1

| Water treatment on parental plants | 6.8 b | 6 b | 6.2 a | 4.0 a |
|-----------------------------------|-------|-----|-------|-------|
| Water + Surfactant                | 8.1 a | 7.9 a | 4.7 b | 4.1 b |
| Significance **                   | **    | **  | **    | **    |

### Table 2

| Water treatment on seeds from parental plants | 6.7 b | 5.8 b | 5.9 a | 4.8 a |
|-----------------------------------------------|-------|-------|-------|-------|
| Control                                       | 8.2 a | 9.8 a | 5.0 a | 3.2 b |
| Significance **                               | **    | **    | **    | **    |

Mean comparison was implemented using Duncan test at the 95% level of probability. Different letters at each column indicate significant difference at p≤0.05, 0.01. *, ** show the significance at p≤0.05, 0.01, respectively.

**Figure 6:** Berseem clover (I75+s) seedling growth under the saline stress of -0.4MPa: (a) irrigation with surfactant, (b) irrigation without surfactant.

Seedlings from seeds developed under limited irrigation treatments (I50 and I75), had lower shoot/root ratios compared to those developed in I100 treatment (Figure 7). This phenomenon verifies the higher vegetative growth potential of seeds developed under full irrigation treatment (I100) (Figure 8). It is assumed that when the soil water content is inadequate to facilitate nutrient uptake by roots, plants face difficulties in absorbing the nutrients necessary for their growth. The higher vegetative growth potential of seeds is confirmed in (Figure 8) where the developed seeds have a lower root weight under a full irrigation treatment (I100). As shown in Figure 8, when the pots were watered with a surfactant, the plants’ shoot/root ratio significantly reduced by the favorable effects of surfactant on increasing the root growth under different growing conditions. Figure 9 clearly shows that seeds, developed under deficit irrigations (I75 and I50 with surfactant), produced plants with higher shoot/root ratios compared to fully irrigated seeds. Using surfactant during the grain filling in parental plants induced a higher shoot/root ratio in developed seedlings, compared to their counterpart treatments. Figures 7 & 9 give clear evidence for this phenomenon which can be explained by the promoting effects of surfactant on better root development in the experiments.

**Figure 7:** Parental effect (water stress along with water treatment: I 100, I 75 , and I 50 ) and surfactant application on berseem clover’s shoot/root ratio under the saline condition of -0.4MPa in comparison to normal condition.
Figure 8: Parental effect (water stress along with water treatment: I100, I75, and I50) and surfactant application on berseem clover’s seedling biomass (shoot and root) under saline condition of -0.4MPa in comparison to normal condition.

Figure 9: Parental effect (water stress along with water treatment: I100+s, I75+s, and I50+s) on berseem clover’s shoot/root ratio under the saline condition of –0.4MPa in comparison to normal condition.

Discussion

It was previously reported that the application of surfactant had a modifying effect against the adverse condition of severely limited irrigation (I50) compared to full irrigation (I100); this resulted in maintaining the forage quality and quantity [26,32]. In this study, we conducted systematic experiments to specify the response of parental seeds (from deficit irrigation field treatments) to salinity stress.

Increased Salinity Tolerance of Seeds Under Field Treatments

Drought and salinity create multifaceted stresses that cause a significant reduction in crops yield depending on the plant growth stage, stress duration and severity Muscolo 2014. Germination is the most critical and sensitive stage in the life cycles of plants [38,39]. Seeds, exposed to unfavorable environmental conditions such as salinity and drought, may exhibit a change in the subsequent seedling establishment processes [40,41]. Our results highlighted the effects of field treatments (drought stress and surfactant application) on increasing seed germination under salinity stress. This study demonstrated that the seeds, developed under drought stress during grain filling, are more tolerant to osmotic stress which agrees with the report from Maleki [42]. The inhibitory effect of NaCl is because of the osmotic stress imposed on the seed/plant during the early phases of germination. At the early stages of imbibitions, the seed respiration and metabolic processes are highly correlated with the water uptake [43]. A threshold level of hydration is required in the initial steps of germination and their subsequent radical elongation [44]. By ensuring turgor maintenance, ‘osmotic adjustment’ may reduce sensitivity to water stress or allow the plant growth to continue even at a slow rate under stress conditions [45].

Salinity can affect the germination of seeds by disruption to the structure of enzymes and other macromolecules, damaging the cell organelles and the plasma membrane, disrupting the respiration, photosynthesis, and protein synthesis [46-48]. In the salinity stress of -0.6 to -1.2 MPa, a sharp reduction of WGI was observed across all parental seed resources. These results corresponded with the findings of Cavallaroa [49], who asserted that, due to osmotic pressure, the seed imbibitions becomes slower; thus, seed metabolism activation is postponed, and germination is taken place with a delay [50]. The amount of WGI resulted from the osmotic pressure of -0.2 and -0.4MPa, induced by NaCl, is higher compared to that under more stress levels. This phenomenon can be the

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result of increases in the expression of aquaporin and Adenosinetri
phosphates (ATPase), as well as a rise in protease and lipase activities [51,52].

**Improved seedling growth under parental treatments**

Plants grown in the field under \( I_{50} \) surfactant application had the highest seedling height (10.2cm). As explained by Maleki Farahani 2010, seeds germinate faster under severe water stress and have a shorter mean germination time (MGT), compared with the seeds produced under full irrigation. Probably, the increased physiological activity, due to the greater absorption of moisture by the treated seeds, is responsible for the improvement of germination and seedling. These results support the idea that the surfactant application in irrigating water in dry regions with salinity problems can maintain agricultural sustainability. The surfactant has a constructive modifying effect on the adverse effects of salinity stress compared with control (Table 3). According to a report from Demie [53], the positive effects of surfactant on improving the water movement and storage in soil could explain the results of the present study in the field. As such, surfactants have the positive effect of increasing the uniformity of water content in soil under drought conditions [54].

Researchers have reported that in response to soil salinity, the seedling growth, leaf area, root and shoot biomass are all reduced [55]. However, in the present study, the irrigation with surfactant successfully maintained a higher resistance of the seeds to saline stress conditions throughout the course of their growth and development (Table 3). As presented in Figure 5, the application of surfactant increased the berseem clover seedling height significantly while subjected to saline stress treatments, compared to the control. When the soil wet ability is less than the water requirement of plants, the use of surfactant in combination with appropriate irrigation and soil cultivation practices improves the soil hydrological behavior; this also improves the irrigation efficiency and water conservation [56,57]. Reported that salinity reduced water potential in the leaves of clovers (Trifolium sp.); shortened the length and lowered the dry mass of the stem, and undesirably affected the length and conductivity of plant roots. However, in this study, when the pots were treated with a surfactant, the plants’ shoot/root ratio significantly reduced under all growing conditions. This result can be explained by the favorable effects of surfactant on increasing the root growth in plants (Figure 8).

Poulter [58] stated that the surfactants decrease the surface tension of water and, in this way, the penetration of water into the soil profile becomes easier, thus, the wetting area of soil increases. With the addition of a surfactant, the plant roots can find water in a wider soil profile, which leads to a better vegetative and generative growth and a higher efficiency in water usage. Figures 7 & 9 are a witness for promoting effects of surfactant on better root development in this experiment [59]. These results clearly show the efficiency of surfactant in increasing both the aboveground and underground components of plants in semi-arid regions where water is limited [60-64]. Our data indicate that those plants irrigated by surfactant and produced from seeds developed with surfactant have a higher shoot/root ratio under saline stress condition. The authors are currently carrying out physiological studies to better understand the function of surfactants in plants and its role in seed germination by tracking the absorption of surfactant into plants and seeds (Figure 10). This investigation is necessary to determine the contribution of surfactants to the metabolism of plants and seeds and other internal changes which lead to a better performance under saline stress conditions [65-71].

![Figure 10](image)

**Figure 10:** Parental effect (water stress along with water treatment: \( I_{100} + s, I_{75} + s, \) and \( I_{50} + s \)) on berseem clover’s seedling biomass (shoot and root) under the saline condition of –0.4MPa in comparison to normal condition.

**Conclusion**

Improving the seed tolerance to saline soil or water shortage can enhance forage production and agricultural sustain ability in arid and semi-arid areas. In this study, the application of surfactant increased the tolerance of berseem clover seeds to salinity stress under full, \( I_{100} \), medium, \( I_{75} \), and severe, \( I_{50} \) irrigation treatments. Parental seeds developed under the severely limited irrigation in the field had a higher germination percentage (75%) compared to normal and moderately irrigated seeds [72,73]. Enhanced WGI of seeds, produced by surfactant across all parental treatments, indicated the desired effect of surfactant application. Plants
treated by surfactant in irrigation water had a higher shoot/root ratio under saline stress condition. The results clearly showed the enhancement effect of surfactant application on improving seed performance and plant components in semi-arid regions where limited irrigation water and salinity are the main issues [74-81].

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