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EFFECTS OF DROUGHT STRESS AND FIELD TREATMENTS ON SEED GERMINATION AND EARLY GROWTH TRAITS OF PARENTAL BERSEEM CLOVER SEEDS

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ABSTRACT

Improving seed resistance to water shortage can enhance forage production and sustainability in arid and semi-arid agricultural lands. This three-year field, laboratory and greenhouse study proposes applying surfactant to effectively prevent the detrimental impacts of severe drought conditionson early plant development. Seeds retrieved from six parental plantsof berseem clover (Trifolium alexandrinum L.) developedunder I100 (100% irrigation water)/I100+s (irrigation water plus surfactant), I75/I75+s and I50/I50+s treatments in the field. In a germination experiment, drought stress was imposed through seven osmotic potentials (0, -0.2, -0.4, -0.6, -0.8, -1, and -1.2 MPa) created by using polyethylene glycol (PEG-6000). Comparative analyses evaluated the interaction between field treatments effects by drought stress and surfactant application on seed germination and early growth stage of berseem clover seedlings. Seeds developed in the field from moderately limited irrigation with surfactant (I75+s) attained a favorable seed germination percentage (78%) under a drought stress of -0.4 MPawhich acheived24.3% increase in germination percentage compared tocontrol (I100) without surfactant). Surfactant application across all fieldirrigation treatments hada constructive effect on the weighted germination index compared to counterpart treatments. Under -0.4 MPadrought stress + surfactant at gemination project, the highest seedling height (10.2 cm) was obtained from the seeds retrieved fromI75 and I50 field treatments. Seeds retrieved from I75+shada better performance under drought stress with the highest seedling height of 11.2 cm from irrigation water+surfactant. These results support the idea that adding surfactant to irrigation water can support the agricultural sustainability in arid and semi-arid regions.

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INTRODUCTION

Water deficit can seriously reduce the herbage and seed production (Chaichi *et al*. 2015) and decrease crop quality and quantity in dry and semi-dry agricultural lands (Flexas *et al*. 2010).Water stress, which originates from water shortage, can impact the crop growth. Responses of different crops to drought can vary depending on the intensity and duration of drought, growth stage, physiology, and genotype of crop specie (Costa *et al*., 2012).

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The authors reported that as the severity of limited irrigation increases, all the growth and development traits, e.g., seed yield, follow a decreasing trend (Daneshnia *et al*., 2015). Consequently, a fruitful crop production in dry regions mainly depends on the effective seed germination, which is strictly associated with the ability of seeds to germinate without adequate water (Arjenaki *et al*., 2011). Harsh parental environments can influence the seed development during the formation and maturation stages, performance and phenotypes of offspring in numerous plants (Herman and Sultan, 2016; Donohue *et al*, 2005). In some cases, stressed parents produce offspring with specific developmental alterations that mitigate that stress (Galloway and Etterson, 2007; Holeski, 2007; Salinas and Munch, 2012; Walsh, 2016). When these off springs encounter similar conditions, such alterations result in a heritable environmentally induced adaptation. There is currently great interest in the extent to which environmentally induced phenotypic change is adaptive. Development of the optimum phenotype may be constrained by environmental circumstances, for example, where low resource availability during prenatal or offspring development gives rise to a stunted, poorly performing individual. The authors reported that drought stress can improve the tolerance of barley (*Hordeum vulgare*) seeds againstosmotic stress in the grain filling stage (Maleki Farahani *et al*., 2010). Although water availability has direct effects on the quantitative and qualitativeresources provisioned to seeds (Gooding *et al*. 2003), studies on offspring tolerance to water stress have not yet been systematically conducted (Herman*et al*. 2012). Berseem clover (*Trifolium alexandrinum* L.) is a high-quality forage legume with a high growth rate and low bloating potential. It is widely cultivated in the Mediterranean region, Central Asia, and the United States (Amato *et al*., 2013). Berseem clover is considered as an important ‘strategic resource’ within sustainable forage systems due to its low input farming requirements (Iannucci *et al*., 2001). Consequently, major studies have directed towards the yield improvement of *Trifolium alexandrinum* L. in dry and semi dry regions (Ghollarata and Raiesi, 2007).

Polyethylene glycol (PEG 6000) is used as an osmotic agent to create the drought stress and verify the effects of irrigation water deficit during the germination process. Reports show that high concentrations of PEG reduce the final germination percentages of lentil (Siahsar *et al*. 2010) and root length in Pantelleria and Ustica (Frazer *et al*. 1990), correlating with the suppression of cellular division and elongation during the germination. The adverse effects of osmotic pressure induced by PEG on germination has been reported for other crops such as wheat (Dhanda et al., 2004), sugar beet (Sadeghian and Yavari, 2004), sorghum (Gill et al., 2002), and sunflower (Mohammad et al., 2002). Da Silva *et al*. (2016) reported that as the water stress (induced by PEG solutions) increases, the percentage and germination index of Tamarind (*Tamarindus indica* L.) reduces; thus, the germination process ishaltedunder the potentials of ≥ -0.6 MPa. There have been numerous reports on the effects of drought stress on different stages of plant growth (Erda *et al*., 2011; Bajehbai, 2011), including seed germination and seedling establishment (Fethi *et al*., 2011; Wu *et al*., 2011). However, investigation about the parental effects (drought stress along with surfactant application) on subsequent drought tolerance at the seed germination stage and seedling establishment of legume forage crops, i.e., berseem clover is under evaluation. Thus,systematic experimental studies are required for such plants to increase water stress tolerance in arid and semi-arid environmental conditions for strategic farming. The objective ofthisthree-year study is to determine whether application of surfactant can effectively enhance berseem clover seed germination and early seedling growth under drought stress conditions.

MATERIALS AND METHOD

Seed materials

Berseem clover (*Trifolium alexandrinum* L.) seeds were obtained from field experiments during the 2013-2015 growing seasons at the Research Farm of the College of Agriculture, University of Tehran, Karaj, Iran (N 35°56’, E 50°58’) (Daneshnia *et al*., 2015; Daneshnia *et al*., 2016). The climate at this site is considered as arid to semi-arid 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 two years 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), and limited irrigation including I_{75} and I_{50}. Two types of water treatment, including water and water+surfactant (added at a rate of 1 ppm), were applied to the sub plots.

Seed quality tests under drought stress

The interaction of field treatments effects on six parental plants (three irrigation levels and two water treatments) under different drought conditions was systematically studied during the germination process. These experiments were performed at the Seed Research Laboratory of the College of Agriculture, University of Tehran. The drought stresses of seven osmotic potentials (0, -0.2, -0.4, -0.6, -0.8, -1, and -1.2 MPa) were created by respectively using0, 112.232, 169.425, 213.64, 251.023, 284.021, 313.881 g of PEG-6000 in demineralized water (Patane *et al*., 2016).50 seeds from each field treatment group were placed in 9 cm sterile Petri dishes containing two What man No.1 filter papers; they were moistened either with 13 ml of sterile demineralized water (control) or the same solution plus PEG-6000 contents (drought stress),and kept in a seed germinator at 20°C. The 42 treatments (6 parental treatments and 7 PEG levels) were arranged on a factorial base with a completely randomized design and three replications. During daily monitoring, demineralized water or PEG solution was added to the petri dishes as needed to maintain water and osmotic pressure near the targeted levels. The number of seeds that germinated was recorded at24 hours intervals for two weeks. A seed was considered to have germinated when its emerging radicle was longer than 2 mm. In laboratory study, germination parameters, such as final germination percentage (G%) and weighted germination index (WGI) (Bu*et al*., 2009),were measured and calculated with higher weights assigned to the seeds that germinated early and less to those that germinated late. The final values (after 14 days) were calculated by:

$$WGI = \{N\times n_1 + (N-1)\times n_2 + \cdots + 1\times n_{14}\}/N\times N' \quad \text{(1)}$$

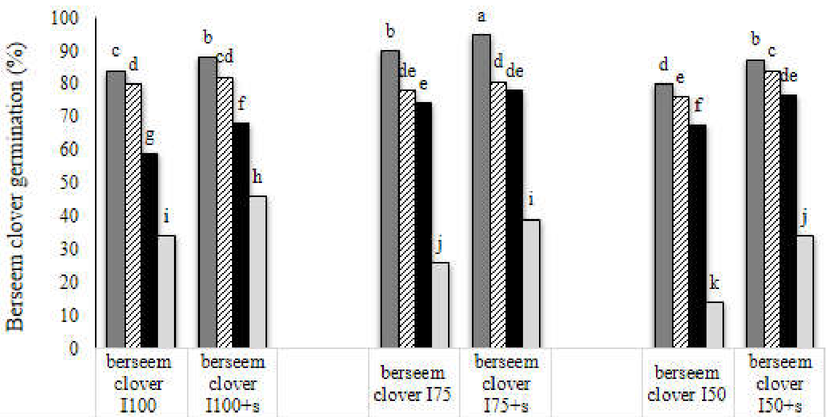
here, n_1, n_2, ..., n_{10} are the number of seeds that germinated on the 1st, 2nd, and subsequent days until the 10th day, respectively, N is the total number of days of the germination process, and N' is the total number of seeds placed in an incubator. In Eq. 1, the final *WGI* value is the product of the experimental value of *WGI* and the percentage of alive seeds in each treatment.

Early growth characterization of berseem clover seedlings under drought condition

Following the germination tests, a series of pot experiments were conducted to evaluate the interaction between parental growth condition and effects of limited irrigation on the early growth and development of berseem clover seedlings. The greenhouse temperature, humidity and Photosynthetic Photon Flux Density (PPFD) of daily/night were in the range25±5°C, 65±5% and 300-600 µmol $m^{-2}s^{-1}$, respectively. The pots were sprinkled with fresh water either with or without surfactant to determine whether surfactant has a modifying effect on the adverse effects of drought stress during early berseem clover seedling growth. The experimental treatments comprised of six parental seeds of berseem clover obtained from the field experiments (Daneshnia *et al*., 2015) and the drought treatment was selected as -0.4 MPa, based on the optimum results of osmotic potential treatments from drought stress in the germination tests. All the pots were filled with 2.43 kg of air-dried soil collected from the field site. The soil was sieved (with a mesh diameter of 1 cm) and loaded in plastic pots with a diameter of 15 cm and height of 12 cm. 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) = 225 $mgkg^{-1}$. The soil in each pot was fertilized with 1 liter of basal nutrient solution which mainly consisted of both ammonium (NH_4) and nitrate (NO_3) as the sources of nitrogen (Wu *et al*., 2003). The irrigation treatments were based on maximum allowable depletion or deficiency (*MAD*) over a soil depth (Behera and Panda, 2009). *MAD*s were calculated using Eq. 2 (Mokhtassi-Bidgoli *et al*., 2013):

$$MAD = \frac{1}{n}\sum_{1}^{n}\frac{FCi-\theta i}{FCi-PWPi} \qquad \text{.......................................(2)}$$

n is the number of layers in the effective rooting depth used for the soil moisture sampling, *FCi* is the soil volumetric moisture (calculated from the pressure plate test) at a field capacity of i^{th} layer, θi is the soil volumetric moisture in thei^{th} layer which (calculated from the pressure plate test), and *PWPi*is the soil volumetric moisture at the permanent wilting point of thei^{th} layer. The amount of *FCi*, θi and *PWPi* were calculated as 18.78, 14.43 and 8.92 (%), respectively. Based on the predefined *MAD*, the percentage of maximum allowable depletion of available soil water (*ASW*) and the volume of required water in the effective root zone (V_d) were estimated by Eqs. 3 and 4:



	berseem clover I100	berseem clover I100+s	berseem clover I75	berseem clover I75+s	berseem clover I50	berseem clover I50+s
Normal condition	84	88	90	94.667	80	87.333
Water potential -0.2 MPa	80	82	78	80.667	76	84
Water potential -0.4 MPa	59	68	74	78	67.333	76.667
Water potential -0.6 MPa	34	46	26	39	14	34

Fig. 1.

$$ASW = \frac{1}{n}\sum_{1}^{n} FCi - PWPi \qquad \text{.......................................(3)}$$

$$V_d = MAD \times ASW \times R_z \times 10 \qquad \text{.......................................(4)}$$

here, *ASW* is equal to 9.86 cm m^{-1}, $V_d$is in mm, R_z is the effective rooting depth (0.12 m), and 10 is the cm to mm conversion coefficient. A non-ionic surfactant with the commercial name of Golden Irrig. Aid was added to the irrigation water at the rate of 1 mg per liter (1 ppm). The surfactant was provided by Aquatrols Corporation, New Jersey, USA, with the following chemical components: 10% Alkoxylatedpolyols, 7% Glucoethers, and inter ingredient 83% water (Mitra*et al*., 2006; Karcher and Landrerth, 2003).

Statistical Analysis

For statistical analysis, the ANOVA technique for a randomized design with three replications was used through the Proc. GLM procedure SAS (SAS, 1996). Mean comparison was implemented using Duncan's test at the 95% level of probability. All calculated differences are significant at $p \leq 0.05$ unless otherwise stated.

RESULTS

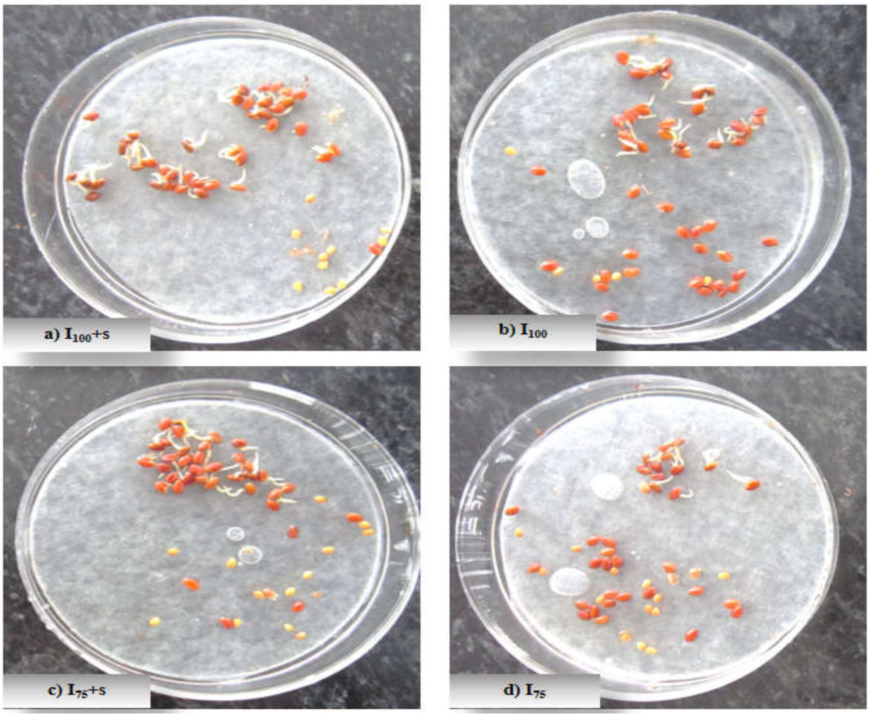
Germination test under drought conditions

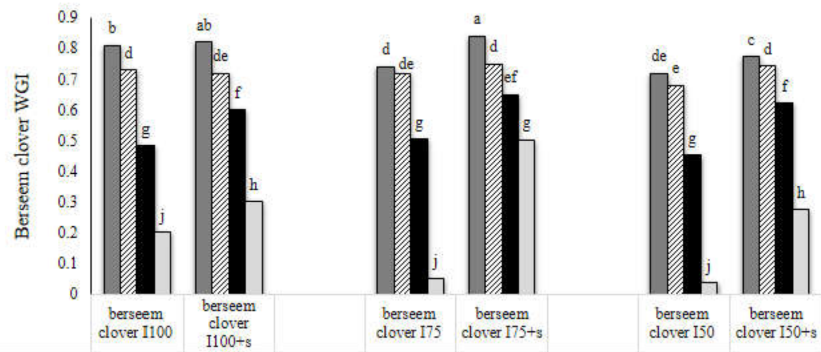
The results showed that germination was suppressed at-0.8, -1 and-1.2 MPadrought stress induced by the PEG solution. The seeds produced from limited irrigation treatments under various water treatments (with/without surfactant application) were influenced by PEG levels. The highest G% (94.7) was for the parental seeds developed at the moderately limited irrigation of I_{75}+s under the normal condition (without stress) (Fig.1). As the severity of the stress increased, the G% followed a decreasing trend in all parental seed treatments. Either with or without surfactant, as the severity of osmotic pressure increased, the seeds subjected to moderate or severe stress became more tolerant to stress conditions during the seed development, compared to those produced under full irrigation. This observation showed that the subsequent seeds produced from the fields under drought stress develop the tolerance against drought stress. By increasing the PEG concentration from the normal condition to -0.6 MPa, a better G% was observed among the parental seeds treated by surfactant (across all drought stress levels), compared with no surfactant treatment (Fig. 1). Under a drought stress of -0.4 MPa, seeds developed by moderately limited irrigation were more tolerant to osmotic pressure upon adding the surfactant to the system (I_{75}+s) and a reasonable G%(78) was obtained. As shown in Fig. 2a-d, seeds developed with surfactant in full and

Table 1. Parental effects (water stress along with water treatment: I_{100}/I_{100}+s, I_{75}/I_{75}+s and I_{50}/I_{50}+s) on berseem clover seedling establishment and early growth characteristics under saline condition of -0.4 MPa

Treatment	Normal condition plant height (cm)	Drought stress (-0.4 MPa) plant height (cm)	Normal condition shoot/root (ratio)	Drought stress (-0.4 MPa) shoot/root (ratio)
Irrigation treatment on parental plants				
100%	6.6 b	8.9 b	6.82 a	1.99 b
75%	7.74 a	9.9 a	5.28 b	2.24 a
50%	7.82 a	9.8 a	3.96 c	2.52 a
Water treatment on parental plants				
Water	6.80 b	8.0 b	6.25 a	2.30 a
Water+ surfactant	8.14 a	10.02 a	4.69 b	2.90 a
Water treatment on seeds from parental plants				
Control	6.71 b	9.0 b	5.92 a	3.43 a
Water+ surfactant	8.24 a	10.83 a	5.03 a	1.77 b

Mean comparison was implemented using Duncan's test at the 95% level of probability. Different letters in each column indicate significant differences.





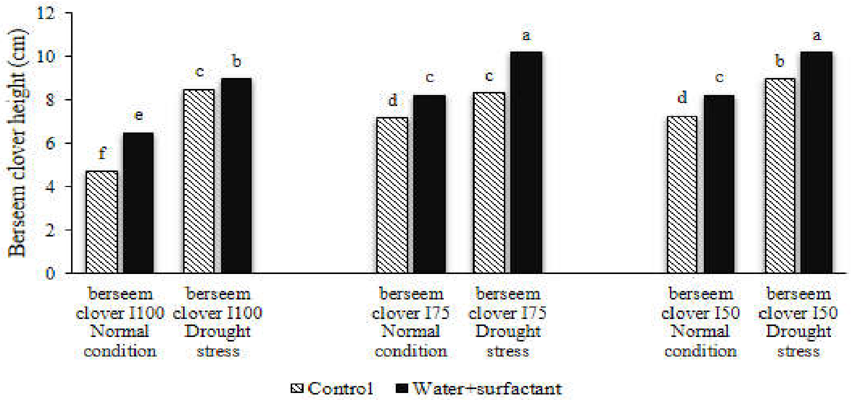
	berseem clover I100	berseem clover I100+s	berseem clover I75	berseem clover I75+s	berseem clover I50	berseem clover I50+s
Normal condition	0.81	0.82	0.74	0.84	0.71833	0.7725
Water potential -0.2 MPa	0.73	0.71917	0.7175	0.74917	0.68	0.74167
Water potential -0.4 MPa	0.48417	0.6	0.50333	0.65	0.4525	0.62
Water potential -0.6 MPa	0.2	0.3	0.05167	0.5025	0.0375	0.27417

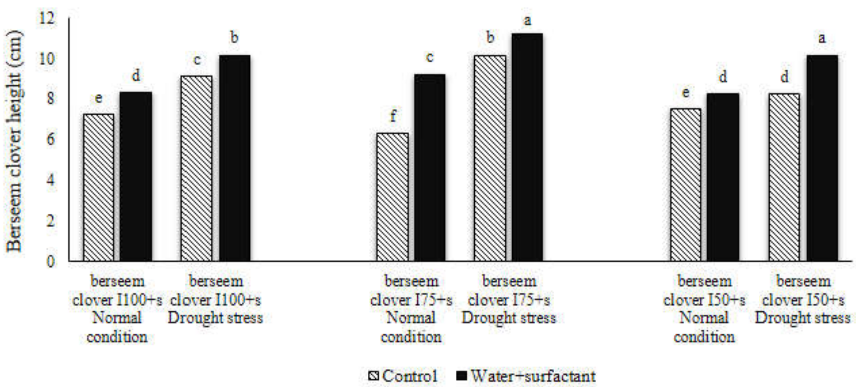
moderately limited irrigation treatments (I_{100} and I_{75}) under moderate drought stress (-0.4 MPa) had higher germination percentages of 20 and 25%, respectively, compared to their counterparts. For the highest osmotic potential (-0.6 MPa), the measured values of G(%) showed a decreasing trend in all parental treatments in the absence of surfactant; byusing the surfactant, a higher G(%)was created which reflects a favorable level of resistance against drought stress. In the germination tests, WGI values followed a decreasing trend across all parental treatments, in response to the drought stress at higher levels, e.g., -0.6 MPa (Fig. 3).As the severity of stressincreased, the WGI values followed a decreasing trend in all parental seed treatments, although, the application of surfactant in parental plants during the grain filling periodinduceda higher WGI compared to the non-surfactant setup. The highest WGI values (0.84) belonged to the parental seeds developed underI_{75}+s and normal condition (without stress). In Fig. 3, for the drought stress of -0.6 MPa, the surfactant application had notable and constructive effects on the WGI values in medium (I_{75}) and severely limited (I_{50}) irrigation treatments.

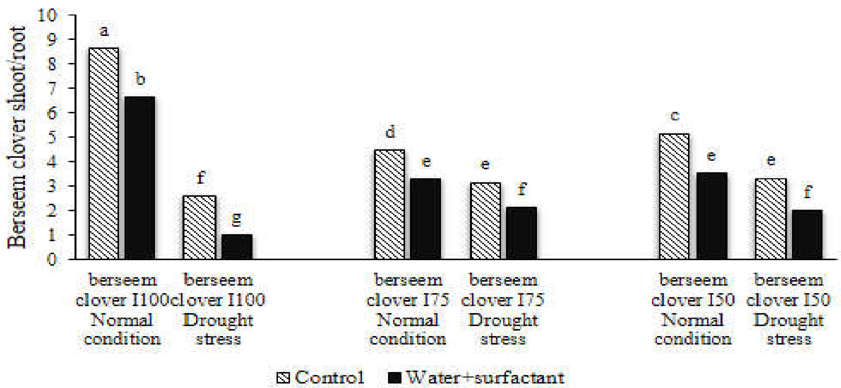
Field treatmenteffects on berseem clover seedling establishment and growth under drought stress of -0.4 MPa

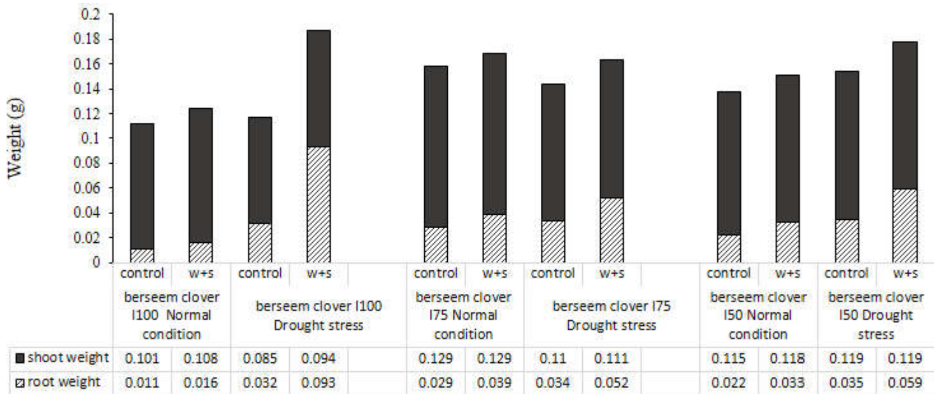
Results indicated that plant height and shoot/root ratio were significantly affected by field treatments and seedling growth conditions (Table 1).

Despite the fact that in our pot experiments significant differences were not observed between parental water treatments, but utilization of water + surfactant in pots irrigation resulted in the remediation of adverse effects of drought stress (-0.4 MPa). Consequently, plant height increased by 16.9%, when pots irrigated with water+surfactant compared with the control (sprinkling with water) (Table 1). As shown in Fig.4, seeds produced under limited irrigation (I_{75} and I_{50}) had a better performance under drought stress when

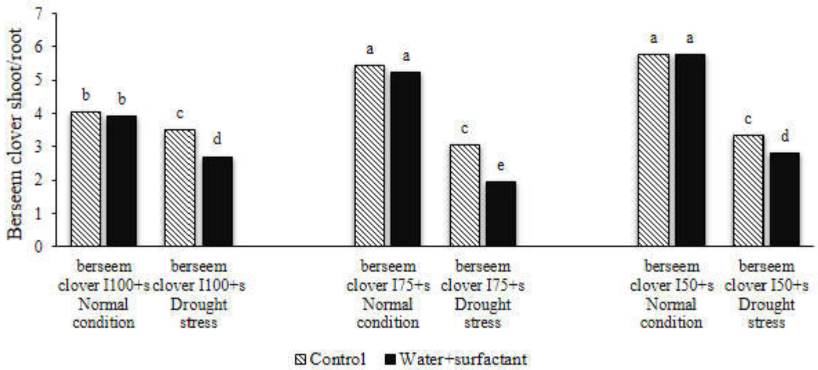


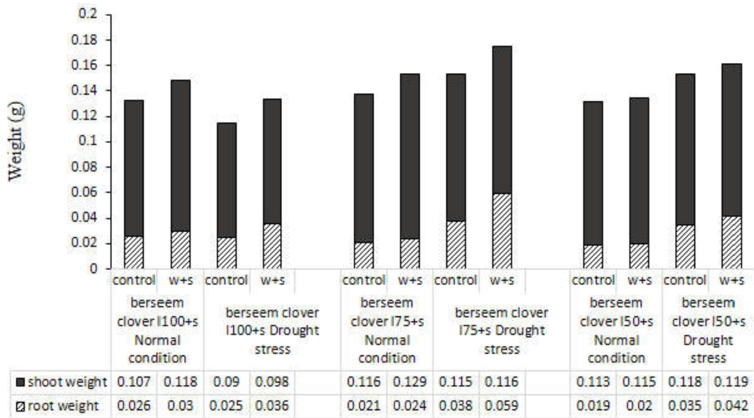






the pots were sprinkled withwater+surfactant, and consequently, the highest height of 10.2cm was recorded for both I_{75} and I_{50}irrigation treatments. Fig.5 showed that the application of surfactant (whether developing plants in the field or sprinkling the seeds in pots using surfactant/water compound) under drought stress conditions significantly increased the berseem clover height across all field treatments compared with the control. The highest height (11.2 cm) was obtained from the seeds, produced by I_{75}+s, when their corresponding pots were irrigated with water+surfactant. In general, declines were seen in the shoot/root ratio of plants developed from seeds under limited irrigation treatments (I_{50} and I_{75}), compared with plants produced from full irrigation treatments in both normal and drought conditions (Fig. 6).





Highlights

- Surfactant enhanced parental seeds tolerance todrought conditions
- Seeds from I_{75}+s attained a favorable germination (78%)under -0.4 MPa
- Highest seedling height (11.2cm) obtained from I_{75}+s, sprinkled by water+surfactant
- Shoot/root ratio enhanced in seeds irrigated with surfactant under drought stress

This phenomenon explained the higher potential of seeds developed by I_{100} to increase the plants vegetative growth (Fig. 7). However, in the presence of surfactant, the plant's roots can find water in a wider soil profile which leads to better vegetative and generative growths. Figs. 8a and b clearly proved the favorable effects of sparkling with water+surfactant on increasing the root growth under the stress condition of -0.4 MPa, resulting in lower ratio values. Fig. 9showedthat seeds from deficit irrigation plus surfactant produced plants with a higher shoot/root ratio in comparison with the seeds from full irrigation conditions. Moreover, the shoot/root ratio decreased in drought stress condition upon adding the surfactant to the system. However, in normal condition, the application of surfactant had no effect onshoot/root ratio across all filed treatments. Under the drought condition of -0.4 MPa, the root weight noticeably improved for the parental seeds developed by surfactant, especially when irrigated by surfactant, compared with their counterpart treatments (Fig. 10).Despite, the root weight showed no differences when pots irrigated with surfactant in the normal condition.

DISCUSSION

Offspring germination and drought tolerance

Growing plants (usually maternal) in environments with severe drought stress (Lack of moisture in soil)can remarkably influence some traits in offspring; in some cases, resulting in phenotypes that are more adaptive to the induced stress (Herman et al., 2012). The harsh parental growth conditions (usually maternal) were initially expected to directly reflectin resource levels, and consequently, the production of offspring is obtained with low quality (Falconer 1981; Roach and Wulff 1987; Donohue and Schmitt 1998). However, in this study (either with or without surfactant), as the severity of osmotic pressure increased (induced by PEG solution), the seeds from the plants subjected to moderate or severe stress became more tolerant to stress conditions during the seed development, compared to those produced under full irrigation. These results correspond with recent findings by authors, in which drought stress led to more drought tolerance in seeds during the grain filling stage (Maleki Farahani *et al*., 2010). At the early stages of imbibition, the seed respiration and metabolic processes are highly correlated with water uptake (Patanè *et al*., 2006). It has been identified that among various mechanisms, osmotic adjustment and induction of dehydrin may confer tolerance against drought injuries by maintaining a high tissue water potential (Turner *et al.*, 2001).

With the accumulation of solutes, the osmotic potential of the cell is lowered; this attracts water into the cell and helps with turgor maintenance. The osmotic adjustment maintains the cell water balance with an active accumulation of solutes in the cytoplasm, thereby minimizing the harmful effects of drought (Farooq *et al*., 2009). Our previous field study suggested that developing plants with surfactant can be considered a promising strategy, which may help to modify the adverse effects of drought stress on growth and development of crops (Daneshnia *et al*., 2015). We are assuming that the offspring of those plants treated by surfactant are more tolerant to drought stress conditions in terms of both germination and seedling growth establishment stages. As the osmotic potential increased from control to-0.6 MPa, the measured values of G (%) showed a decreasing trend in all parental treatments in the absence of surfactant, however by using the surfactant a higher G (%) is gained which assumes a favorable level of resistance against drought stress. The authors have shown that the surfactant decreases the surface tension of water and, in this way, the penetration of water through the soil surface becomes easier under a larger wetting area in soil (Mehrvarz*et al*., 2013). Therefore, the plant roots find water in a wider soil profile which helps to lessen the adverse effects of drought stress (Daneshnia et al., 2015). Results clearly proves the idea that eliciting the plant development with surfactant can guarantee the reasonable germination and subsequently convincing yield in arid and semi-arid regions. As shows in Figure 3, for higher levels drought stress, e.g., -0.6 MPa, the surfactant application has notable and constructive effects on plants developed under medium (I_{75}) and severely limited (I_{50}) irrigation treatments, resulted in more tolerant seeds and higher WGI values compared to the non-surfactant setup. Under osmotic pressure, the seed imbibition proceeds slower, thus, seed's metabolism activation is delayed, and seed germination is postponed (Cavallaro*et al*., 2016). Fenner (2000) proposed that, during the seed maturation stage, drought may affect seed germinability by changing the properties of the maternal tissues around the seed. This can cause seeds to switch from the seed-developing stage to the seed-germinating stage (Kermode*et al*., 1986). In this stage, the germination is suppressed by osmotic stress probably because of a higher inhibition in seed hydration under higher osmotic potentials (-0.4 and -0.6 MPa) (Maleki Farahani *et al*., 2010). Thus, upon increasing the internal osmotic potential, more water is absorbed which leads to more germination under high osmotic potentials (Heikal and Shaddad, 1982; Dodd and Donovan, 1991; Huang and Redman, 1995; Almansouri*et al*., 2001).

Survival of seedlings in drought stress under filed treatments and utilizing surfactant

Developing seeds with surfactant resulted in more resistance to the stress conditions throughout the course of their growth and development (Table 1). Under deficit irrigation treatments, surfactants increase water retention which can be explained by the mechanism which is done when the surfactant is applied to the system. Surfactants reduce the surface tension of water and help water infiltrate into the pore spaces of soil which are not generally accessible without the surfactants (Leinauer, 2002). Consequently, when the surfactant is added to the system the crops can explore a wider soil profile; this brings higher efficiency in the use of resources (e.g., light) by plant (Daneshnia *et al*., 2016). Developing the plant under limited irrigation (I_{75} and I_{50}) resulted in a seed's better performance under drought stress when the pots were sprinkled with surfactant+water (Fig. 4). In the recent study by authors, seeds produced under severe stress during development geminated faster and displayed a shorter mean germination time (MGT) compared with seeds produced under full irrigation (Maleki Farahani *et al.,* 2010). The treatment with surfactant had a positive effect on soil water movement and increased water storage in soil, which improved the plant growth (Demie *et al*., 2007). Soldat *et al*. (2010) concluded that surfactants in drought conditions had a positive effect on increasing the uniformity of water content in soil. By utilizing the seeds produced from I_{75}+s, the highest height (11.2 cm) was obtained when surfactant was added to the pots. Our results are in agreement with those of Herman *et al*. (2012) and Germain *et al.* (2013), who explained that offspring's performance improved under drought stresses when parents already

experienced the same conditions. Reductions are seen in the shoot/root ratio of seeds from plants treated under limited irrigation treatments (I_{50} and I_{75}), compared with plants produced from full irrigation treatments in both normal and drought conditions (Fig. 6). When the water content was inadequate to facilitate nutrient uptake by roots, plants faced difficulties in absorbing essential nutrients such as nitrogen and phosphorus, which resulted in a reduction in seed yield (García *et al*., 2008). The authors have found that, by using the surfactant, water movement through the soil becomes easier because of the reduction in water tension (Chaichi *et al*., 2015). Thus, the plant roots can find water in a wider soil profile which leads to better vegetative and generative growth.
Utilizing surfactant along with applying deficit irrigations (I_{75} and I_{50}) during the growth season, produced seed witha characteristicof higher shoot/root ratio in comparison with the seeds from full irrigation conditions. When the soil wettability is less than optimal, surfactants in combination with appropriate irrigation can improve soil hydrological behavior, resulting in an improved irrigation efficiency and water conservation (Kostka *et al*., 2007), uniformity of soil moisture distribution, and root zone moisture holding capacity (Wolkowski *et al*., 1985). Moreover, the shoot/root ratio decreased in drought stress condition upon sprinkling pots with water+surfactant (Fig. 9). This fact confirms that the effective delivery of water to the root zone is the result of surfactant usage (Brumbaugh and Petersen, 2001); consequently, developed plant roots caused a reduction in the shoot/root ratio. Due to the need to conduct physiological studies, the authors are currently investigating the contribution of surfactants in the metabolism of plants and seeds under drought stress conditions.

Conclusion

According to the results of this project, surfactant application at the field experiment enabled berseem clover seeds to efficiently cope with limited irrigation levels in the germination mode. In other words, seeds developed under water shortage conditions were more tolerant to fluctuating environmental conditions when surfactant was added to irrigating water.

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