JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2015. Vol. 9(3), p. 2059-2068

Evaluation of Drought Resistance and Yield in PGPR-Primed Seeds of *Festuca arundinacea* Schreb under Different Levels of Osmotic Potential and Field Capacity

Hadi Radnezhad[1]*, Forogh Mortazaeinezhad[2], Ali Asghar Naghipour[3], Behzad Behtari[4] and Maryam Foroughi Abari[1]

[1]Department of Environmental Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.
[2]Department of horticulture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.
[3]Ph.D Student in Range Sciences and Member of Young Researchers Club Islamic Azad University, Isfahan(khorasgan)Branch, Iran.
[4]Ph.D Candidate of Rangeland Science and Management, Faculty of Natural Resources, Sari Agricultural sciences and Natural Resources University (SANRU), Iran.

(Received: 23 May 2015; accepted: 08 July 2015)

Plant growth promoting rhizobacteria (PGPRs) are a group of bacteria that can actively colonize plant roots and can modulate plant growth. The present study was conducted to examine the effects of bio-priming with Azotobacter and Azospriliumon on the yield and resistance to drought stress of *F. arundinacea* Schreb seeds under different four levels of osmotic potential such as 0.5,1,1.5 and 2 Mpa that created using the polyethylene glycol. Seeds treated for 2 and 4 days. Drought stress on the field capacity, in the four levels of 100%, 75%, 50% and 25% of field capacity was applied during plant growth. The results showed that bio-priming treatments in the traits of root length, stem length and fresh stem weight improved yield and increased resistance to drought stress as compared to control. Both types of bacteria Azospirillum and Azotobacter significantly increased yield compared to control. The performance of Azotobacter was relatively higher than in Azospirillum. With regard to F. Arundinacea species seeds treated with Azotobacter 2 and 0.5 MPa for 2 days were identified as the superior treatments.

Key words: Azotobacter, Azosprilium, Bio-priming, Drought stress, field capacity.

Drought stress is common in many parts of the world, and more than 50 % of the globe is arid, semiarid, or subjected to some kind of drought stress (Mayaka *et al.*, 2004). Crop production in arid and semi-arid regions is restricted by soil salinity and soil deficiencies in moisture (ELSiddig *et al.*, 1998; Pessarakli, 2001). Growth reduction under drought stress conditions has been well characterized in several plant species, such as rice, barley, maize, and wheat (Kasim *et al.*, 2013). At present, the use of biological approaches is becoming more popular as an additive to chemical fertilizers for improving crop yield in an integrated plant nutrient management system. In this regard, the use of PGPR has found a potential role in developing sustainable systems in crop production (Sturz *et al.*, 2000; Shoebitz *et al.*, 2009). Many studies have reported on the efficiency of PGPR under determined conditions in protecting plants from the deleterious effects of environmental stresses (Enebak *et al.*, 1997; Glick *et al.*, 1997; Timmusk and Wagner, 1999). Timmusk and Wagner (1999) were the first persons who show that inoculation of Paenibacillus polymyxa confers drought tolerance in Arabidopsis thaliana through the induction of drought-responsive gene ERD15. Inoculation of Bacillus amyloliquefaciens 5113

* To whom all correspondence should be addressed.
E-mail: hradnezhad@yahoo.com

and Azospirillum brasilense NO40 significantly alleviated the deleterious effect of drought stress on wheat (Kasim *et al*., 2013). In addition, moisture stress at any stage of crop growth can cause an irreversible loss in yield potential (Reginato, 1993). One pragmatic approach to increase crop production is seed invigoration (Basra *et al*., 2004; Farooq *et al*., 2006; Lee and Kim, 2000). Seed invigoration strategies include hydropriming, osmoconditioning, osmohardening, hardening, hormonal-priming, matripriming, and others (Chiu *et al*., 2002; Kao *et al*., 2005; Windauer *et al*., 2007).

Priming offers a means to raise seed performance in many crop species (Chiu *et al*., 2002). Heydecker *et al*. (1973) defined seed priming as a pre-sowing treatment in osmotic solution that allows seeds to imbibe water to proceed to the first stage of germination. Taylor *et al*. (1998) used a broader term, seed enhancement, which includes presoaking hydration (priming), coating technologies, and seed conditioning. Therefore, seed priming can be accomplished through different methods such as hydro priming(soaking in water), osmo-priming (soaking in osmotic solutions such as polyethylene glycol, potassium salts, e.g. KCl, K2SO4) solid matrix priming, and using plant growth regulators(PGRs) (Capron *et al*., 2000; Chiu *et al*., 2002; Dearman *et al*., 1987; Harris *et al*., 2002). Priming has been known as a viable technology to enhance rapid and uniform emergence, high vigor, and better yields mostly in vegetable and flower species (Bruggink *et al*., 1999; Dearman *et al*., 1987; Parera and Cantliffe, 1994) and some field crops (Basra *et al*., 1988; Chiu *et al*., 2002; Giri and Schillinger, 2003; Harris, 1999; Hartz and Caprile, 1995; Murungu *et al*., 2004). Seed priming has been a common seed treatment to reduce the time between seed sowing and seedling emergence and the synchronization of emergence (Parera and Cantliffe, 1994).

Forages are the backbone of sustainable agriculture and contribute extensively to the world economy (Barnes and Baylor, 1995). On a worldwide basis, grassland acreage is estimated to be twice that of cropland (Jauhar 1993). Tall fescue (*F. arundinacea*) is a cool-season forage grass that is widely grown throughout the temperate regions of the world (Sleper, 1985). It is the most important perennial forage species of the genus Fistula (Malay *et al*., 2005). Tall fescue (*F. arundinacea*) is native to Europe and North Africa (Hannaway *et al*., 1999). The use of tall fescue in forage, soil conservation and turf increased dramatically during the early 1980s (Sleper and West 1996). Tall fescue grows naturally in pastures in the northern, central and western of Iran, it plays an important role in the production of forage and soil conservation. Although tall fescue cultivation is not common in Iran, but it has large potential for the production of crops and pasture forage (Sharifi Tehrani *et al*., 2009)

This study evaluated the effectiveness of (i) bio-priming method on the species F. arundinacea Schreb using inoculation of Azotobacter and Azospirillum at different levels of osmotic pressure which is under drought stress and (ii) the effectiveness of bio-priming of Azotobacter compared with the effectiveness Azospirillum on F. arundinacea Schreb.

MATERIALS AND METHODS

This study was carried out as a factorial experiment based on completely randomized design (CRD) with three factors and three replacing in greenhouse of Isfahan (Khorasgan) Branch, Azad University of Iran, during 2014. Tall fescue (*F. arundinacea*) seeds were purchased from Pakan Seed Company of Isfahan. Bio-priming method is considered as one of the most common methods for seed priming. Tall fescue seed bio-priming carried out with -0.5, -1, -1.5 and -2 Mpa osmotic potential. This osmotic potentials had been used in previous studies on various grasses (Hardegree & Emmerich, 2000, Hardegree & Van Vactor ,2000). Time priming, according to studies Hardegree *et al*. (2002), was chosen 2 and 4 days in order to verify the short and long term effects of bio- priming. The first factor was seed bio-priming with Azotobacter and the second factor was bio-priming with Azospirillum at -0.5, -1, -1.5 and - 2 Mpa osmotic potentials levels and control (C1 and C2 without bio-priming). Poly ethylene glycol 6000 used to create osmotic potential.

Preparation of bio-osmotic solution priming

Michel and Kaufmann (1972) stated that the effects of concentration and temperature on osmotic potential of PEG-6000 solutions differ from those for most salts and sugars and apparently are related to structural changes in the PEG polymer and an empirical equation (Equation 1) permits calculation of Osmotic potential; from known concentrations of PEG-6000 over a temperature range of 15 to 35 C. Viscometery and gravimetric analysis are convenient methods by which the concentrations of PEG-6000 solutions may be measured. In this study, equation 1 was used to determine the exact amount of osmotic potential.

$$\Psi=-(1.18\times10^{-2})\,C-(1.18\times10^{-4})\,C^2+(2.67\times10^{-4})\,CT+(8.39\times10^{-7})\,C^2T \quad ...(1)$$

ψ= osmotic potential of PEG-6000

C= Weight of PEG-6000 in grams in a kilogram of water

T= the temperature of the solution to C°

Preparation of seed samples

Mass of seeds were randomly selected and weighed and poured into mesh bag. The two bags seeds were considered as controls that kept at 20 °C, and the remaining bags were used for priming. The seeds were rinsed with distilled water for two minutes and seed were air dried until the moisture level comes back to its original. Inoculation process was carried out using *rhizobacteria* powder that was prepared by the Institute of Soil and Water Research in Tehran. Plastic pots with 19 cm height and 15 cm diameter was prepared, drainage of the pots was normal. A height of 16.5 cm of soil was placed in each pot; this was in accordance with the winter wheat seed priming that was done by Giri and Schillinger (2003). Each pot was consisted of three parts: sand, soil and leaf soil with ratio of 2, 1 and 1 respectively. There were sprayed 25 seeds in each pot evenly. The seeds were covered with 1.5 cm of soil and pressed a little. The pots were placed in greenhouse at 20°C in several completely random rows. With regard to the water holding capacity in pots, drought stress was considered as third factor. Four levels of drought stress ,in the amount of 100% of field capacity (without stress)- 75%, 50% and 25% of field capacity, were applied.

The amount of drought stress according to water holding capacity of the soil in each pot was reviewed and applied every day. According to the last day of counting, emergence percentage was calculated for each treatment. Emergence rate was calculated using equation (2)

$$\mathrm{GR}=\sum_{I=1}^{n}\frac{n}{t} \quad ...(2)$$

n is the number of grown seeds in time t and t is the number of days since the start of experiment(Reyes *et al.,* 2002).

Mean germination time was calculated using equation (3)

$$MGT=\frac{A_1D_1A_2D_2\ A_nD_n}{A_1A_2\ ...\ A_n} \quad ...(3)$$

A is the number of seeds that germinated during the D and n is the number of days until the last day of counting (Cantliffe, 1991).

Three months after planting, 10 plants were randomly selected from each pot and stem length was measured. Wet and dry biomass of stems were measured with a digital scale.

To determine the root biomass, pots were shaken in the water for a long time, the soil and other materials were removed from the root. Fresh weight of separated roots was measured after the initial impounding.

The analysis of variance of the data was performed using SPSS and MSTATC software and if the variance was significant, mean comparison with Duncan's multiple range test was performed at p=5%.

RESULTS

Analysis of variance main effects, bio-priming and levels of drought stress and the interaction effects between the treatments' bio-priming at stress levels showed significant differences in studied traits in F. arundinacea species (Table 1).Comparison of the mean length of roots and shoots of the main effects of treatments bio-priming on F.arundinacea species is presented in Figure1. The maximum length of shoot (34.37 cm) obtained by 5.1 MPa Azospirillium brasilense treatment for 4 days. The difference was statistically significant (P <0.05) with the control. The minimum shoot length (28.13 cm) related to the control treatment. The maximum length of the roots (29.23 cm) obtained by the treatment of Azotobacter 2 MPa for 4 days, which was significantly different from control treatment (22.96 cm). Minimum root length (22.95 cm) created by the treatment of

Azospirillium brasilense 2 MPa for 4 days; it not showed significant different compared with control. Root length of the other treatments of Azospirillium 2 MPa for 4 days showed higher mean value than the control.

Comparison of the main effects of bio-priming treatments on fresh weight root is presented in Fig 2A.The highest fresh root weight (44 g) was obtained in the treatment of Azotobacter 0.5 MPa for 4 days. The difference was statistically significant ($P < 0.05$) with the control. This treatment didn't show significant difference by fresh root weight compared with treatments of Azotobacter 5.0 MPa for 2 days (39.40 gr), Azotobacter 1 MPa for 4 days (42.91 gr), Azotobacter 2 MPa for 2 days (29.75 gr), and azosprilium 1 MPa for 2 and 4 days by values of 35.5gr and 29.45 gr. Treatment of control didn't show significant difference with treatments of Azospirillium 0.5 MPa for 2 days and Azotobacter 2 MPa for 4 days. Difference was

Table 1. Comparison of the mean of the interaction effects of bio-priming treatments at drought stress levels in the stem and root length (cm) and shoot fresh weight (gr) of Festuca arundinacea species

Root length		Fresh shoot weight		Stem length		Time	Osmotic	Drought
Azospirillum	Azotobacter	Azospirillum	Azotobacter	Azospirillum	Azotobacter		potential	stress
31.7 b-l	27.5 g-l	20 b-f	16.6 def	25 c-j	21.36g-k	2days	0.5 Mpa	
28.3 e-l	26.9 h-l	15 def	11.6f	24.9 c-j	18.1k	4days		
28.5 d-l	25.6 kl	16.6 def	18.3 c-f	20.4ijk	23d-k	2days	1 Mpa	
28.6 d-l	29.8 d-l	15 def	16.6 def	24.2 c-k	22.1e-k	4days		25% FC
32.7 b-j	26.5 ijkl	16.6 def	25 b-f	24.1c-k	24.6 c-k	2days	1.5 Mpa	
30.9 b-l	29.1 d-l	21.6 b-f	23.3 b-f	24.1c-k	23.5 d-k	4days		
28.8 d-l	28.4 e-l	21.6 b-f	23 b-f	21.7 f-k	23.7d-k	2days	2Mpa	
28.5 d-l	29.8 d-l	13.3 ef	21.6 b-f	18.7jk	26.5c-i	4days		
	25.7 jl		16def		23.1 d-k	-	Control	
30.4 b-l	25.3 l	23.3 b-f	15.3 def	24.1 c-k	24 d-k	2days	0.5 Mpa	
32 b-l	30 d-l	25 b-f	28.3 b-f	20.4 ijk	23.6 d-k	4days		
30.4 b-l	33.4 b-i	21.2 b-f	21.6 b-f	28.4 a-f	26.9 b-i	2days	1 Mpa	
30.2 c-l	26.5 ijkl	20 b-f	23.3 b-f	24.7 c- k	28.1 a-f	4days		50%FC
25.5 kl	33.2 b-i	20 b-f	30 abcde	22.6 e-k	25.5 c-i	2days	1.5 Mpa	
31.5 b-l	27.3 g-l	20.3 b-f	21.6 b-f	28 b-g	24.7 c-k	4days		
29.7 d-l	33.2 b-i	23.3 b-f	23.3 b-f	28.3 a-f	27.2 b-h	2days	2Mpa	
27.7 f-l	33.3 b-i	31.6 a-d	30.3 a-d	24.2 c-k	26.6 b-i	4days		
	26.9 h-ll		15.3 def		23 d-k	-	Control	
32.8 b-i	32.5 b-k	26.6 b-f	35 abc	27.8 b-h	27.6 b-h	2days	0.5 Mpa	
34.4 b-g	35.5 b-d	28.3 b-f	30 abcde	26.2 c-i	23.7 d-k	4days		
34.1 b-g	32.3 b-l	31.6 a-d	28.3 b-f	26.4 c-i	29.5 abcd	2days	1 Mpa	
31.6 b-l	33.4 b-i	26.6 b-f	28.3 b-f	24.4 c-k	23.9 d-k	4days		75%FC
33.1 b-i	31.7 b-l	25 b-f	26.6 b-f	25.7 c-i	24.5 c-k	2days	1.5 Mpa	
33.2 b-i	30.6 b-l	26.6 b-f	23.3 b-f	22.9 d-k	22.9 d-k	4days		
31.3 bl	32.4 b-k	28.3 b-f	35.6 ab	27.7 b-h	25.6 c-i	2days	2Mpa	
31.4 b-l	35.1 b-e	36.6 ab	35 abc	25 c-j	29.5 abcd	4days		
	31.7 b-l		21 b-f		21 b-k	-	Control	
30.9 b-l	33.7 b-h	28.3 b-f	30 abcde	22.1 e-k	28.5 a-e	2days	0.5 Mpa	
34.4 b-g	35.1 e-b	30 abcde	30 abcde	26.6 b-i	27.3 b-h	4days		
33.6 b-i	34.8 b-f	31.6 a-d	28.3 b-f	26.9 b-i	33 ab	2days	1 Mpa	100%
33.2 b-i	37.2 abc	31.6 a-d	23.3 b-f	24.4 c-k	25.1 c-j	4days		
29.5 d-l	37.4 ab	26.6 b-f	26.6 b-f	24.7 c-k	25.7 c-i	2days	1.5 Mpa	
41.7 a	33.4 b-i	31.6 a-d	30 abcde	25 c-j	23.2 d-k	4days		
32.8 b-i	33.5 b-i	26.6 b-f	45.3 a	24.3 c-k	23.5 d-k	2days	2Mpa	
29.9 d-l	32 b-l	25 b-f	18.3 c-f	23.8 d-k	34.3 a	4days		
	28.1 e-l		26.3 b-f		24.5 c-k	-	Control	

Similar letters are indicative of no significant difference between the means (Duncan test p= 5%)

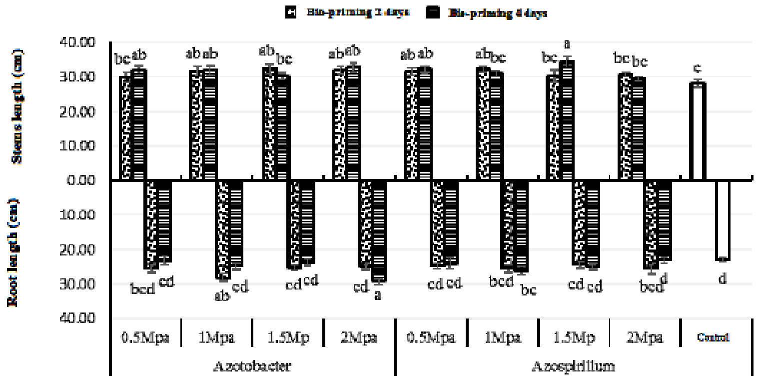

Fig. 1. Comparison of the mean (± standard error) of the bio-priming main effects of treatments on stems and root length (cm) of *Festuca arundinacea*

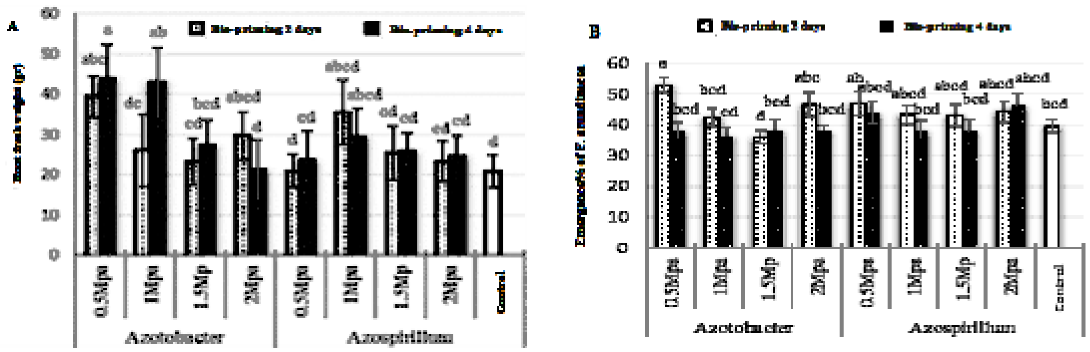

Fig. 2. Comparison of the mean (± standard error) of the bio-priming main effects of treatments in root fresh weight (gr) and emergence% of *F. arundinacea* species

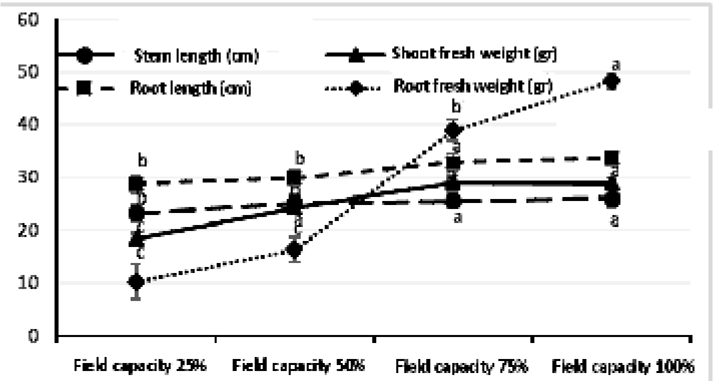

Fig. 3. Comparison of the mean (± standard error) the effects of drought stress (based on field capacity) in Stem length, root and shoot and root weight (cm) the species Festuca arundinacea

significant with other treatments.

Figure 2B offers emergence percentage of the seeds in the treatments. The highest emergence percentage (53%) was obtained by the Azotobacter 0.5 MPa for 2 days. it showed significant difference than treatments of the control (39.5%) and Azotobacter 2 MPa for 2 days.

Difference was not significant between the Azotobacter 0.5 MPa for 2 days compared with Azospirillium 0.5 MPa for 2 and 4 days, the

Azospirillium 1.5 MPa for 2 days and Azospirillium 2 MPa for 2 and 4 days, however the impact of these treatments was higher than control. Lowest emergence percentage (36%) was created by Azotobacter 1.5 Mpa for 2 days, there was not significant difference compared to control.

Figure 3 provided comparison of the effects of drought stress (based on field capacity) in shoot and root length, and fresh shoot and root weight. The figure shows a decreasing trend with increasing drought stress in all traits. With increase in stress level reduction trend in the fresh weight of root was more severe compared with other traits.

Fig3. Comparison of the mean (± standard error) the effects of drought stress (based on field capacity) in root and shoot length (cm) and fresh root and stem weight (gr) F. arundinacea species.

Bio-priming treatments comparing means of interactions at the level of drought stress in stem length are presented in Table 1. Stem length (34.33 cm) was highest at 100% of field capacity (without stress) and treatment of Azotobacter 2 MPa for 4 days. This treatment had significant difference compared with control treatment and other treatments; also its effect was incremental at all levels of drought stress. Minimum stem length (18.16 cm) related to drought stress treatment 25% of field capacity of Azotobacter 0.5 MPa for 4 days. This treatment didn't show significant difference with control. The mean stem length of bio-priming treatments was higher than the control.

Comparing the means of interaction effects of bio- priming treatments in levels of drought stress in stem and root length and fresh shoot weight is presented in Table 1. The stem length was higher at 100% of field capacity (without stress). The highest amount of stem length (34.33 cm) at 100% of field capacity (without stress) related to the treatment of Azotobacter 2 MPa for 4 days. This treatment showed significant difference compared with control and many other treatments of different drought stress levels. The stem length was less of drought stress at 25% of field capacity. The lowest stem length (18.16 cm) related to Azotobacter treatment of 5.0 MPa for 4 days. This treatment showed no significant difference with control of drought stress at 25% of field capacity.

Comparing the means of fresh shoot weight (table 1) indicates that fresh shoot weight is higher in 100% of field capacity (without stress). Highest fresh shoot weight (45.33) obtained in 100% of field capacity by the treatment Azotobacter 2 MPa for 2 days, and had significant difference compared with the control. The minimum fresh shoot weight was related to drought stress at 25% of field capacity. Shoot fresh weight (11.66) was the lowest in Azotobacter treatment of 5.0 MPa for 4 days. For this treatment difference was not statistically significant compared with the control.

Comparing the means of root length (table 1) indicated that the impacts are higher in 100% of field capacity (without stress). The highest root length (41.72 cm) observed in 100% of field capacity by Azospirillium treatment 1.5 MPa for 4 days. This treatment caused statistically significant difference compared with the control and other treatments in different levels of drought stress. The minimum root length was obtained in the drought stress with 50% of field capacity. The treatment Azotobacter 0.5MPa for 2 days created lowest root length (25.35) at drought stress with 50 % of field capacity. There was no significant difference compared with the control.

DISCUSSIONS

Plants are constantly exposed to abiotic stresses, among which drought is a major limiting factor for growth and crop production because it can elicit various biochemical and physiological reactions (Glick, 2004). Abiotic stress tolerance in PGPR has been studied to provide a biological understanding of the adaptation and survival of rhizobacteria under stress conditions (Arkhipova *et al*., 2007; Creus *et al*., 1998). Priming is an important mechanism of various induced resistance phenomena in plants (JM Beckers and Conrath, 2007).

Seed priming has been shown to advance germination and emergence rate for many agricultural plant species (e.g. Brocklehurst *et al*., 1984; Helsel *et al*., 1986; Alvarado *et al*., 1987; Evans and Pill, 1989; Bradford *et al*., 1990; Khan *et al*., 1992; Suzuki and Obayashi, 1994; Yamamoto *et al*., 1997). Studies of rangeland species are more limited. There is little knowledge about the response of microorganisms when released at water absorption conditions by seeds(Okon & Labandera-Gonzalez, 1994). In this study, root length improved in culture conditions the pot of F. arundinacea. Most of the compounds of bio-priming treatments effectively and significantly increased in root length. Root length can be considered as a factor affecting for improvement yield in drought stress conditions. In drought stress conditions, increasing the length and extent of the root will be able to guarantee plant survival. Azotobacter and Azospirillum bacteria caused the root length improved in the application drought stress conditions. Combination of priming with plant growth promoting bacteria (PGPR) showed that the root length of F. arundinacea increased drought stress conditions. In this species, treatment Azospirillum 1.5 Mpa for 4 days, compared with the other bio- prime treatments and decreasing trend that occured as a result of drought stress was appropriate treatment. Ahmad *et al*., (2005) pointed out that Azotobacter by secretion of indole acetic acid increased the root length. Many of the studies reported effect of inoculation with bacteria on the growth root growth is increasing root length such as increase the number root, dry root weight and increase cell division in the root meristem (Arsac *et al.,* 1990º Levanony & Bashan, 1989). In this study, the most dry and fresh weight of the roots in the F. arundinacea was obtained by inoculation Azotobacter treatment. Fulchieri *et al.,* (1993) reported that Azotobacter, by the production of gibberellin, caused the development root and its weight.

Bashan, (1986) and Kucey, (1988) in their study pointed out that root biomass of plants inoculated with Azosprillum is less compared with plants inoculated with Azotobacter. Although the researchers reported that increasing indices of stem growth with Azosprillum inoculation is more than Azotobacte.

Weight producted biomass from forage plants is an important index to improve the performance especially under stress conditions. In fact, the increase in weight per unit area of forage is considered as one of the symptoms management quality. Reduce application chemical fertilizers are an important indicator in the sustainable management, it obtained by biological fertilizers replacing. Interest in the use of biological methods instead of chemicals to fertilize the soil, and improve plant resistance against pathogens is currently growing.

The results showed that the bio-priming treatments significantly increased biomass production. Based on the results the Azotobacter in additive effects was better than Azosprillum in the F. arundinacea.

The use of plant growth promoting bacteria leads to several natural ways growth including non-symbiotic nitrogen fixation (Boddey & Do bereiner, 1988), increase the solubility of phosphorus (Reyes *et al.,* 2002), Production of phytohormones (Bent *et al*., 2001), and production of various compounds (e.g., antibiotics and lytic enzymes) with anti-pathogenic properties (Romero *et al.,* 2007). Despite the decrease in fresh and dry weight of shoot with increased drought stress, decreasing trend in seeds treated with Azosprillum and Azotobacter was significantly lower compared with the control. This could be considered as useful way to increase biomass production in F. arundinacea. These results are consistent with findings of Nanda *et al*., (1995) in the increasing fresh and dry weight of corn seed inoculation with bacteria, Azotobacter and Azospirillum, Youssef *et al*., (2004) in the increasing fresh and dry weight of *Salvia officinalis* seed inoculation with bacteria, Azotobacter and Azospirillum and the research results Chabot *et al*., (1993) and Zahir *et al*., (2000), which reported inoculated Azosprillum increased corn shoot dry weight. Increasing the germination and emergence is known as the most important capabilities of priming (Heydecker & Coolbaer, 1977). These two traits are the most important parameters in determining the seedling vigor (Alizadeh & Jafari, 2006).

In this study, the combination of osmotic priming with beneficial microorganisms caused increasing in the emergence vigor percentage of F. arundinacea. Osmotic seed priming as one of the most effective methods to increase the percentage emergence of grass is confirmed (Hardegree *et al.*, 2002). Therefore, the convergence of these methods of plant growth promoting bacteria can enhance the beneficial effects. The significant increase of the percentage emergence (about 13.5 %) was observed for F.

a*rundinacea by* the treatment 5.0 MPa 2 days Azotobacter compared with control. The results showed that decreasing trend of the percentage emergence by treatment Azotobacter half MPa for 2 days the stress level zero (100% of field capacity) to the highest levels of stress, i.e. 25 % of field capacity was only 5%. So the significant difference was not found between the levels of stress. Emergence percentage in the control treatment showed 10% decrees of the stress level zero (100% of field capacity) to the highest level of stress(25 % of field capacity). This was about twice related the reduction made in the treatment of Azotobacter 0.5MPa for 2 days. So decreasing trend in the emergence percentage affected by stress in the seeds that were treated with bio-priming was much lower than the control.

CONCLUSION

The results showed that plant growth promoting bacteria with the technology of osmotic priming of seeds can be increased yield of forage F. arundinacea species under glasshouse conditions. Bio-priming of seeds with *Azosprillum* and *Azotobacter* were significantly increased the growth compared with control treatment. Azotobacter showed relatively higher performance than in Azospirillum. Accordingly, there can be of Azotobacter a greater ability to coexist with F. a*rundinacea species.* Bio-priming treatments improved the yield. However, in the case of F. arundinacea the treatment of Azotobacter 0.5 and 2 MPa for 2 days could be considered as superior treatments as other treatments used in this study.

REFERENCES

1. Ahmad, F., Ahmad, I., Khan, M.S., 2005. Indole acetic acid production by the indigenous isolate of *Azotobacter* and *Fluorescent Pseudomonas* in the presence and absence of Tryptophan. Turkish Journal of Biology, 29,29-34
2. Alizadeh, M.A., Jafari, A.A., 2006. Seed and seedling responses of ecotypes of *Bromus*, *Agropyron* and *Medicago* to *Fusarium solani* and *F. oxysporum*. Journal of New Seeds. 8, 71-81.
3. Alvarado AD, Bradford KJ, Hewitt JD. 1987. Osmotic priming of tomato seeds: e€ects on germination, ®eld emergence, seedling growth and fruit yield. Journal of the American Society of Horticultural Science 112: 427±432.
4. Arkhipova, T. N., Prinsen, E.,Veselov, S. U., Martinenko, E. V., Melentiev, A. I. and Kudoyarova, G. R., 2007. Cytokinin producing bacteria enhance plant growth in drying soil. Plant Soil292,305"315.
5. Arsac, J.F., Lamothe, C., Mulard, D. and Fages, J., 1990. Growth enhancement of maize through *Azospirilum lipoferum* inculcation: effect of plant genotype and bacterial concentration. Agronomie, 10:649-654.
6. Bashan, Y., Holguin, G., 1997a. Azospirillum-plant relationships: environmental and physiological advances (1990-1996). Canadian Journal of Microbiology, 43, 103-121.
7. Barnes R.F. and Baylor J.E. 1995. Forages in a changing world. In: Barnes R.F., Miller D.A. and Nelson C.J. _eds_, Forages, pp. 3–13. Iowa State University Press, Ames, Iowa, USA.
8. Basra, A.S., Ullah, E., Warraich ,E.A., Cheema, M.A., Afzal, I.,2003. Effect of storage on growth and yield of primed canola(*Brassica napus*) seeds. International J. Food Agric. Biol. 2, 117-120
9. Bent, E., Tuzun, S., Chanway, C.P. and Eneback, S., 2001. Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. Canadian Journal of Microbiology, 47, 793–800.
10. Boddey, R.M. and Do bereiner, J., 1988. Nitrogen fixation associated with grasses and cereals: Recent results and perspectives for future research. Plant and Soil, 108, 53–65.
11. Bradford KJ, Steiner JJ, Trawatha SE. 1990. Seed priming in¯uence on germination and emergence of pepper seed lots. Crop Science 30: 718±721.
12. Brocklehurst .,P.A., Dearman ,J, Drew., R.L.K.,1984. E€cts of osmotic priming on seed germination and seedling growth in leek. Scientia Horticulturae 24,201±210.
13. Bruggink, G.T., Ooms, J.J., Vander Toorn, P., 1999. Induction of longevity in primed seeds. Seed Sci. Res. 9, 49-53
14. Cantliffe, D.J., 1991. Benzyladenine in the priming solution reduces thermodormancy of lettuce seeds. Horticulture Technology, 1,95–97.
15. Capron, I., Corbineua, F., Dacher, F., Job,C., Come,D., Job ,D., 2000. Sugar beet seed priming: Effects of priming conditions on germination, solubilization of 11-s globulin and accumulation of LEA proteins. Seed Sci. Res. 10,243-254
16. Chiu, K.Y., Chen ,C.L., Sung, J.M., 2002. Effect of priming temperature on storability of primed *sh-2* sweet corn seed. Crop Sci. 42, 1996-2003

17. Chabot, R., Antoun, H., Cescas, M. P., 1993. Stimulation of the growth of maize and lettuce by inorganic phosphorus- solubilizing microorganisms. Canadian Journal of Microbiology, 39, 941-947
18. Creus, C. M., Sueldo, R. J., Barassi, C. A., 1998. Water relations in Azospirillum-inoculated wheat seedlings under osmotic stress. Can. J. Bot. 76,238–244.
19. Dearman ,J., Brocklehust ,P.A., Drew .,R.L.K., 1987. Effect of osmotic priming and aging on the germination and emergence of carrot and leek seed. Annu. Appl. Biol. 111, 717-722
20. Enebak, S.A., Wei,G, Kloepper., J.W .,1997. Effects of plant growthpromoting
21. rhizobacteria on loblolly and slash pine seedlings. For Sci 44,139–144
22. Evans, T.A., Pill ,W.G., 1989. Emergence and seedling growth from osmotically primed or pregerminated seeds of asparagus(Asparagus o€ cinalis L.). Journal of Horticultural Science 64, 275±282.
23. Farooq, M., Basra S.M.A., Wahid, A.,2006. Priming of field sown rice seed enhances germination, seedling established, allometry and yield. Plant Growth Regul. 49, 285-294
24. Giri ,G.S., Schillinger .,W.F.,2003. Seed priming winter wheat for germination, emergence and yield. Crop Sci. 43, 2135-2141
25. Glick, B.R.,Liu ,C., Ghosh ,S., Dumbroff, E.B.,1997. The effect of the plant growth promoting rhizobacteriaum Pseudomonas putida GR 12–2 on the development of canola seedlings subjected to various stresses. Soil Biol Biochem 29,1233–1239
26. Glick, B. R., 2004. Bacterial ACC deaminase and the alleviation of plant stress. Adv. Appl. Microbiol. 56,291"312
27. Fulchieri, M., Lucangeli, C. and Bottini, R., 1993. Inoculation with Azospirillum affects growth and gibberellin status of corn seedling roots. Plant Cell Physiology, 34,1305-1309.
28. Hannaway, D., Franden, S., Copper, J., 1999. Tall Fescue (*Festuca arundinacea* Scherb), reported from Oregon State University, USA. PP. 20
29. Hardegree, S.P., Jones, T.A., Van Vactor, S.S., 2002. Variability in Thermal response of Primed and Non-primed Seeds of Squirreltail [*Elymus elymoides* (Raf.) Swezey and *Elymus multisetus* (J. G. Smith) M. E. Jones]. Annals of Botany, 89, 311-319.
30. Hardegree, S.P., Jones, T.A.,Van Vactor, S.S., 2002. Variability in Thermal response of Primed and Non-primed Seeds of Squirreltail [*Elymus elymoides* (Raf.) Swezey and *Elymus multisetus* (J. G. Smith) M. E. Jones]. Annals of Botany, 89, 311-319.
31. Hartz TK, Caprile J. 1995. Germination of sh2 sweet corn following seed disinfestations, solid-matrix priming and microbial seed treatment. Hort. Sci. 30: 1400-1402
32. Harris, D., Joshi,A., Khan, P.A., Gothkar, P., Sodhi, P.S., 1999. Onfarm seed priming in semi-arid agriculture development and evaluation in maize, rice and chickpea in India using participatory methods. Exp. Agric. 35,15-29.
33. Harris, D., Rashid ,A., Hollington, P.A., Jasi ,L., Riches, C., 2002. Prospects of improving maize yields with “on-farm seed priming”. In NP Rajbhandari, JJ Ranson, K Adhikari, AFE Palmer, eds, Sustainable maize production systems for Nepal. NARC and CIMMYT, Kathmandu, Nepal. pp180-185
34. Helsel, D.G., Helsel ,Z.R., Minor, H.C., 1986. Field studies on osmocondi- tioning soybeans. Field Crops Research 14,291±297.
35. Heydecker, W., Coolbaer, P., 1977. Seed treatments for improved performance survey and attempted prognosis. Seed Science and Technology, 5, 353–425.
36. Heydecker, W., Higgins, J., Gulliver, R.L., 1973. Accelerated germination by osmotic seed treatment. Nature. 246, 42-46
37. Jauhar P.P. 1993. Cytogenetics of the *Festuca-Lolium* complex: relevance to breeding. Springer-Verlag, Berlin; New York. 255 pp.
38. JM Beckers, G., Conrath, U.,2007. Priming for stress resistance: from the lab to the field. Current Opinion in Plant Biology.2007, 10,425–431
39. Kao, A.L., Chang, T.Y., Chang, S.H., Su, J.C., Yang, C.C., 2005. Characterization of a novel *Arabidopsis* protein family At MAPR homologous to 25-Dx/IZAg/Hpr6.6 proteins. Bot. Bull.,Academy of Sinica 46, 107-118
40. Kasim, W.A.,Osman, M.E., Omar, M.N., El-Daim IAA, Bejai, S., Meijer, J., 2013. Control of drought stress in wheat using plant-growthpromoting bacteria. J Plant Growth Regul 32,122–130.
41. Khan, A.A., Maguire, J.D., Abawi, G.S,, Ilyas, S., 1992. Matriconditioning of vegetable seeds to improve stand establishment in early ®eld plantings. Journal of the American Society for Horticultural Science 117,41±47.
42. Kucey, R.M.N., 1988. Alteration of size of wheat root system and nitrogen fixation by associative nitrogen- fixation bacteria measured under field conditions. Canadian Journal of Microbiology, 34, 735-739.
43. Lee, S.S., Kim, J.H., 2000. Total sugars, and amylase activity, and germination after priming of normal and aged rice seeds. Korean J. Crop

Sci. 45, 108-111

44. Levanony, H., Bashan, Y., 1989. Enhancement of cell division in wheat root tips and growth of root elongation zone induced by *Azospirillum brasilens* Cd. Canadian Journal of Microbiology, 67,2213-2216.
45. Mayaka, S., Tirosh, T., Glick, B.R., 2004. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166,525–530.
46. Michel ,B.E., Kaufmann,M.R.,1973. The Osmotic Potential of Polyethylene Glycol 6000. Plant Physiol. 51, 914-916.
47. Murungu, F.S., Nyamugafata, P., Chiduza, C., Clark, L.J., Whalley, W.R., 2003. Effects of seed priming aggregate size and soil matric potential on emergence of cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.). Soil Tillage Res. 74, 161-168
48. Nanda, S.S., Swain, K.C., Panda, S.C., Mohanty, A.K. and Alim, M.A., 1995. Effect of nitrogen and biofertilizers in fodder rainfed upland conditions of Orisa. Current. Current Agricultural Research, 8:45-47.
49. Okon, Y., Labandera-Gonzalez, C.A., 1994. Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. Soil Biology and Soil Biochemistry, 26, 1551-1601.
50. Parera, C.A., Cantliffe, D.J., 1994. Pre-sowing seed priming. Hort. Rev. 16, 109-141
51. Reginato, R.J., 1993. Field quantification of crop water stress. Am. Soc. Agric. Eng. 26, 772-775
52. Reyes, I., Bernier, L., Antoun, H., 2002. Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of *Penicillium rugulosum*. Microbial Ecology, 44,39–48.
53. Romero, D., de Vicente, A., Rakotoaly, R.H., Dufour, S.E., Veening, J.W., Arrebola, E., Cazorla, F.M., Kuipers, O.P., Paquot, M. and Perez-Garc1´a, A., 2007. The iturin and fengycin families of lipopeptides are key factors in antagonism of Bacillus subtilis toward Podosphaera fusca. Molecular Plant-Microbe Interactions, 20,430–440.
54. Sharifi Tehrani, M., Mardi M., Sahebi, J., Catalán, P., Díaz-Pérez A., 2009. Genetic diversity and structure among Iranian tall fescue populations based on genomic-SSR and EST-SSR marker analysis. Plant Systematics and Evolution, 282, 57–70.
55. Shoebitz, M., Ribaudo ,C.M., Pardo ,M.A., Cantore ,M.L., Ciampi L, Curá, J.A .,2009). Plant growth promoting properties of a strain of *Enterobacterludwigii*isolated from *Loliumperenne*rhizosphere. Soil Biol. Biochem. 41(9),1768-1774.
56. Sleper, D.A., West, C.P., 1996. Tall Fescue In: ASA, CSSA, SSSA(eds) Cool-season forage grasses. Agronomy Monograph no. 34. ASA, CSSA, SSSA, Madison, Wis., pp 471–473
57. Sleper, D.A., 1985. Breeding tall fescue. J Plant Breed Rev 3,313–342
58. Malay, C.,Saha, Rouf Mian , John C. Zwonitzer Konstantin Chekhovskiy , Andrew A. Hopkins.,2005. An SSR- and AFLP-based genetic linkage map of tall fescue(Festuca arundinacea Schreb.). Theor Appl Genet , 110, 323–336
59. Sturz, A.V., Christie, B.R., Novak, J.,2000. Bacterial endophytes: potential role in developing sustainable system of crop production. Crit. Rev. Plant Sci. 19,1-30.
60. Taylor, A.G., Allen, P.S., Bennett, M.A., Bradford,K.J., Burris, J.S., Misra, M.K., 1998. Seed enhancements. Seed Sci. Res. 8, 254-256
61. Suzuki, H., Obayashi ,S., 1994. E€ects of seed treatments on the seedling emergence, growth and yield of spring-sown carrot. Journal of the Japanese Society for Horticultural Science 63, 73±79.
62. Timmusk, S., Wagner ,E.G.H.,1999. The plant growth-promoting rhizobacterium Paenibacillus polymyxa induces changes in Arabidopsis thalianan gene expression: a possible connection between biotic and abiotic stress responses. Mol Plant-Microb Interact 12,951–959
63. Windauer, L., Altuna, A., Benech-Arnold, R.,2007. Hydrotime analysis of *Lesquerella fendleri* seed germination responses to priming treatments. Ind. Crop Prod. 25,70-74
64. Zahir, A.Z., Abbas, S.A., Khalid, A. and Arshad, M., 2000. Substrate dependent microbially derived plant hormones for improving growth of maize seedling. Pakistan Journal of Biological Science, 3, 289-291.
65. Yamamoto, I., Turgeon ,A.J, Duich ,J.M., 1997. Field emergence of solid matrix seed primed turfgrasses. Crop Science 37, 220±225.
66. Youssef, A.A., Edris, A.E. and Gomaa, A.M., 2004. A comparative study between some plant growth regulators and certain growth hormones producing microorganisms on growth and essential oil composition of *Salvia officinalis* L. Plants. Annals of Agricultural Science, 49,299-311.