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The effect of environmental characteristics and metabolism in response to water stress in three *Festuca* species

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Abstract

In order to determine the reaction of three *Festuca* species to drought stress, two separate experiment were conducted in both germinator and greenhouse conditions using factorial experiment based of completely randomized design with three replications in 2010 in Tehran, Iran. The drought treatments were four levels of osmotic potential (0, -0.3, -0.6 and -0.9 MPa) in germinator and four levels of osmotic potential (FC, 25% FC, 50% FC and 75% FC) in greenhouse that were made by poly ethylene glycol (PEG 6000) solution and field capacity method, respectively. Data were collected and analyzed for germination percent, root length, shoot length, seedling length, root/shoot length ratio (RSR), seedling weight, seedling dry/fresh weight ratio (DFR) and seed vigor index. In greenhouse, chlorophyll, carbohydrates and proline contents were also measured. The results showed significant differences among species, droughts levels and species by drought interaction effects for the most of traits in both conditions. However, the relationship between germinator and greenhouse, quantified using correlation was high. It was concluded that evaluation under germinator conditions is desirable for selection purposes. In both conditions all of seedling traits except RSR, DFR were increased and proline and carbohydrate content were increased by increasing osmotic potential. The results showed that the *F.ovina* species had higher values for the most of seedling attributes than other species. Results of probit analysis of LD₅₀ and LD₉₀ showed the same trend.

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Introduction

Festuca is one of important perennial grass species belong to is belong to the genus of *Festuca*, subgenus of *Festucae* and a family of *Poaceae*. It naturally grows in Zagros and Alborz mountains rangelands in the west and north of Iran. It is being used for grazing and hay production and consumed by livestock. *Festuca ovina* grows in areas with 750 m to 2900 m altitude (Ebrahimzadeh 2010 cited from Rechinger, 1970). It has early growth in spring and good quality for animal productivity and good adaptability in vast range of sever conditions in all over the country. In recent years, higher grazing pressure and unpalatable weed invasion had led to increasing soil erosion and consequently decreasing population of this species. Therefore, re-vegetating of those areas by new improved grass varieties is the most economical and possible means of recovery.

Festuca genus has an important role in grassland productivity in Iran. Little breeding work has been done on this species especially under Iran climatic conditions (Moghaddam 1998).

Under water deficiency conditions, the rate of water loss from transpiration exceeds the rate of water absorption by the roots, and plants undergo water stress. Water stress can vary from a small decrease in water potential to the lethal limit of desiccation (Liberato *et al.* 2007). Among the different abiotic stresses, drought is by far the most complex and devastating on a global scale (Pennisi, 2008). Although range plants have mechanisms that help reduce damage from water stress, water deficiency conditions lasting a month cause plants to experience water stress severe enough to reduce herbage production. (Larcher, 2003, Nevo *et al.*, 2002). It anticipates a growing interest in wild relatives of crops and landraces in an attempt to identify superior alleles among these that the domestication bottleneck and modern agriculture have left behind (Tanksley and McCouch, 1997; Grandillo *et al.*, 2007, Lippman *et al.*, 2007; Feuillet *et al.*, 2008).

Drought is one of the major causes for crop loss worldwide, reducing average yields with 50% and over. In addition, water stressed plants could be more sensitive to other biotic or abiotic stresses such as pathogen attack, chilling or air pollution, which limits plant productivity. Plant stress resistance can be studied at molecular, cellular or physiological levels (Bohnert *et al.*, 1995, McCue and Hanson, 1990; Wang *et al.*, 2003). In water stress, plant height and herbage biomass accumulation are reduced. Leaf senescence increases and as a result, nutritional quality of forage decreases. The rate of sexual reproduction is diminished as a result of a decrease in seed stalk numbers and height and a reduction in numbers of seeds in the seed heads. Rate of vegetative reproduction is reduced because the number of axillary buds and the number of secondary tillers decrease. (Zareh chahouki, 2009; Tavili, 2007). In order to respond to drought, plants have developed the capability to rapidly perceive stressful factors and trigger the accumulation of a large number of newly synthesized mRNAs and polypeptides (Ingram and Bartels, 1996; Urao *et al.*, 1999; Urao *et al.*, 2000). Responses to drought are multiple and interconnected. It leads to growth reduction, reduction in the content of chlorophyll pigments and water, and changes in fluorescence parameters (Lu and Zhang, 1999; Lima *et al.*, 2002; Colom and Vazzana, 2003; Souza *et al.*, 2004; Zlatev and Yordanov, 2004; Ekmekci *et al.*, 2005; Mohsenzadeh *et al.*, 2006; Rong-hua *et al.*, 2006; Nayyar and Gupta, 2006; Yang *et al.*, 2006). Rangelands are areas unsuitable for cultivation, but provide forage for animals (Miller, 1997).

Rangeland degradation is often manifested by decreases in plant yields. The dispersal of seeds through over sowing is an important strategy to actively restore vegetation in degraded areas as their seed banks are usually depleted of viable seed. One of the greatest challenges in restoration ecology is to sow a seed type or cultivar that has the capacity to produce abundant biomass and cover in a short period of time (Van den Berg, 2002). In addition to

grazing by domestic livestock, rainfall patterns are the most important factors influencing rangeland condition (Hoffman and Ashwell, 2001). Drought, extreme temperatures, increased soil salinity, soil crusting or sand covering, as well as pathogens and herbivores all adversely affect the germination and growth of seedling. Grasses, with strong development of underground organs, tend to have efficient adaptive mechanisms to cope with drought, fire and herbivory and provide superior protection against soil erosion than most woody shrub and tree species (Imevbore, 2003). Iran is one of the countries that majority of its extent is located in arid and semi-arid regions. 64% of Iran (100 million hectares) is covered by dry lands, and their area is increasing (Jafari and Firouzabadi 2001). Only 35% of the whole area of Iran has more than 250 mm of annual rainfall (Kuchaki, 2008). Therefore, in Iranian rangelands, water is one of the most important factors that limits plant growth and causes the failure of range improvement plans. The objective of this study was to identify Physiological and morphological responses of three *Festuca* species to drought stress in germinator and greenhouse.

Materials and methods

The study was conducted in the germinator and greenhouse. For each trail a factorial experiment based on completely randomized design was conducted in Gene Bank division in Research Institute of Forests and Rangelands, Tehran, Iran, in November 2009. Three *Festuca* species: *F.ovina*, *F.arundinacea* and *F.pratensis* were used in this study.

Germinator Experiment

Water stress treatment was applied during 15 days by adding PEG 6000 (50 gL⁻¹) (Fluka, Buchs, France) to the watering solution in germinator condition, In germinator, For each accession 100 pure seeds were sterilized with 70% ethyl alcohol for five minutes and washed with distilled water. Four replicates (25 seeds per replicate) of sterilized seed were placed in Petri dishes on double Whatman papers (TP). For protection against molds, the water used to moisten

the seed samples and substrata contained 0.002% Benomil fungicide. The samples were immediately transferred into a germinator at (20±4°C) with 1000 Lux light for 15 days.

After growth of seedlings for 15 days, the length of roots and shoots of 10 randomly-selected seedlings from each replicate were measured. The vigor index measures seedling performance, relating together the germination percentage and growth of seedlings produced after a given time (Abdul-Baki and Anderson, 1973). It was calculated by following equation:

$$Vi = \frac{\%Gr \times MSH}{100}$$

Where:

VI = vigour index

%Gr. = final germination percentage

MSH = mean seedling height

Greenhouse Experiment

The seeds of the same ecotypes were sown on pots with fluctuation temperatures 20±5°C during day and (5-12) C° during night of greenhouse. In order to vegetative growth development, the pots were irrigated normally for three weeks. Water stress treatment was applied using four levels of osmotic potential (FC, 25% FC, 50% FC and 75% FC) for three weeks by using the field capacity (FC) method (Panda *et al.*, 2004). At the end of each treatment, the seedling were counted to estimate germination percent. The root length and shoot length (the distance from soil surface to upper end of the longest leaves) was measured (mm/plant). The fresh (g/FW) and dry (g/DW) biomass of the seedling and vigor index was also determined.

The carbohydrates content of drought stressed and irrigated (control) plants were determined using the method of Irigoyen *et al.* (1992). Carbohydrates were extracted from leaf samples (20 mg DW) according to Weimberg (1987) with minor modifications. The absorbance of the sample extract was Spectropho-

tometrically determined at 535 nm. The proline concentration was determined as ($\mu\text{mol g/DW}$) using a standard curve.

For proline content, the method of Bates *et al.* (1973) was used to determine proline content. Proline was extracted from leaf samples (20 mg DW) according to Weimberg (1987) with minor modifications. The absorbance of the sample extract was spectrophotometrically determined at 520 nm. The proline concentration was determined as ($\mu\text{mol g/DW}$) using a standard curve.

The factorial experiments based on completely randomized design were analysed using ANOVA. Differences among the treatments as well as the cultivars and their interaction effects were tested using the DMRT method. The relationship between germinator and greenhouse, quantified using phenotypic correlations. Probit analysis was used to determine the lethal dose concentration of PEG6000 for stopping 50% (LD50) and 90% (LD90) of seed germination in three *Festuca* species. All statistical analyses were conducted by SAS9 (SAS Inst. 2004).

Results

Germinator Experiment

The results of analysis of variance in germinator, showed significant differences among species, drought stress and species by drought interaction effects for all of the traits (Table 1). All of the traits except RSR and DFR, were decreased by increasing osmotic potential (Table 2). The maximum values of the traits were obtained for the control treatment and its minimum level was in the drought treatment -0.9 MPa in the germinator. For germination percent, there was no difference between control and -0.3 MPa and for other traits, this treatment was ranked as the second. The traits tend to drop sharply by increasing osmotic potential to 0.6 MPa and 0.9 MPa (Table 2). The average values of both RSR and DFR were significantly decreased by increasing osmotic potential and the highest values were obtained for 0.9

MPa (Table 2).

The comparisons among three species in germinator are shown in Table 3. Results showed that, *F.ovina* species with an average value of 79.25 had a higher germination percent than the other two species.

The species \times drought interaction effects for all traits are shown in Fig 1. The comparison among species for every level of drought stress indicated that *F.ovina* species were stable by increasing osmotic potential. Suggesting that it was more resistant to drought than other species (data are not shown). Using probit analysis, LD50 was estimated as -0.99 MPa osmotic potential for *F.ovina* species. The results indicated that *F.ovina* was more resistant than other species for drought stress and it could be used for cultivation in moderate rainy rangelands with 300 mm annual precipitation (Table 4).

Greenhouse experiment

The results of analysis of variance in greenhouse, showed significant differences among drought stress and species by drought interaction effects for germination percent, root length, shoot length, seedling length, vigor index, RSR, seedling fresh weight, seedling dry weight DFR, chlorophyll index, proline and non-structural carbohydrates (Table 5). By increasing osmotic potential, all germination traits except RSR, DFR were decreased, in contrast proline and carbohydrates content were increased by drought stress (Table 6).

For germination traits, the higher values were obtained for the control treatment and their minimum level was obtained in the drought treatment of 75% FC. For germination% there was no difference between control and 25% FC and for other traits the 25% FC treatment was ranked as the second. The traits tend to drop sharply by increasing osmotic potential to 50% FC and 75% FC (Table 5). The average values of RSR and DFR, proline and carbohydrates content were significantly increased by increasing osmotic potential and the highest values were obtained for 75% FC (Table 5).

In comparisons among three species in greenhouse (Table 7), results showed that, *F.ovina* species had higher value for germination%, seedling length and vigor index. In comparison among species for every levels of drought stress indicated that *F.ovina* species were stable for germination percent by increasing osmotic potential. The declines of seedling length and vigor index in *F.ovina* species was less as compared to other species by the results indicated that both species of *F.ovina* and *F.pratensis* were more resistance than for drought stress and they could be used for cultivation in moderate rainy rangelands (data are not shown).

Relationship between germinator and greenhouse

The relationship between germinator and greenhouse for all traits is important. Since, selection in germinator could be more efficient than that based on germinator and field depending on the correlation between characters. The phenotypic correlation between two environments were estimated. The estimates were high and significant for germination percent ($r=0.99^{**}$), root length($r=0.76^{**}$), shoot length ($r=0.98^{**}$), seedling length ($r=0.91^{**}$), vigor index($r=0.97^{**}$), RSR($r=0.75^{**}$) and seedling fresh weight($r=0.75^{**}$) indicated that results based on germinator were strong indicators of greenhouse and probably field experiment (Table 8).

Table 1. Analysis of variance of germination properties of *Festuca* species in germinator condition.

Source of Variance	DF	Germination %	Shoot L. (mm)	Root L. (mm)	RSR (mm)	Seedling L. (mm)	Vigor index	FW (g)	DW (g)	DFR (g)
Drought(D)	3	16556 **	26469 **	27157 **	0.7655 *	106937 **	127659 **	0.077 **	0.00004 ns	0.0979 **
Species(S)	2	1868 **	125 *	5916 **	5.3066 **	4660 **	2730 **	0.0041 *	0.0008 **	0.0727 **
E × D	6	260 ns	241 ns	399 **	0.7122 **	1147 *	1275 *	0.0005 ns	0.00005 ns	0.0062 ns
Error	132	334	142	129	0.215	417	603	0.0013	0.0001	0.0044
CV %		24.8	23.2	21.8	41.04	19.7	28.5	23.4	35.4	33.5

*, ** = Means of squares are significant at 5%, 1%, respectively.

Table 2. Effect of drought stress on seed germination properties of *Festuca* species in germinator condition.

Drought Treatment	Germination %	Shoot L. (mm)	Root L. (mm)	RSR (mm)	Seedling L. (mm)	Vigor index	FW (g)	DW (g)	DFR (g)
Control	92.67 a	79.94 a	83.06 a	1.34 a	163 a	151.46 a	0.221 a	0.031 a	0.253 a
-0.3 MPa	88.78 a	66.64 b	65.78 b	1.12 b	132.42 b	118.29 b	0.157 b	0.030 a	0.229 a
-0.6 MPa	66.22 b	39.18 c	36.32 c	1.04 b	75.50 c	53.21 c	0.125 c	0.029 a	0.177 b
-0.9 MPa	46.55 c	19.62 d	22.63 d	1.03 b	42.25 d	20.92 d	0.118 c	0.028 a	0.135 c

Means of the columns with the same letter had no significant differences based on DMRT ($P \leq 0.05$).

Table 3. Means comparison of seed germination characteristics in three *Festuca* species.

Ecotypes Name	Germination %	Shoot L. (mm)	Root L. (mm)	RSR (mm)	Seedling L. (mm)	Vigor index	FW (g)	DW (g)	DFR (g)
<i>F.arundinacea</i>	68.94 b	52.36 a	50.82 b	1.04 b	103.18 b	79.33 b	0.16 a	0.02 b	0.17 c
<i>F.pratensis</i>	69.25 b	47.95 a	77.03 a	1.90 a	124.98 a	95.64 a	0.13 b	0.03 a	0.27 a
<i>F.ovina</i>	79.25 a	51.18 a	46.81 b	1.03 b	97.99 b	90.19 ab	0.15 a	0.03 a	0.21 b

Means of the columns with the same letter had no significant differences based on DMRT ($P \leq 0.05$).

Table 4. Probit analysis for determination of lethal dose concentration of PEG6000 for stopping 50% (LD₅₀) and 90% (LD₉₀) of seed germination in three *Festuca* species.

Species	LD ₅₀		LD ₉₀	
	PEG lethal dose concentration	SE	PEG lethal dose concentration	SE
<i>F.arundinacea</i>	7.57	0.10	14.27	0.21
<i>F.pratensis</i>	7.60	0.19	14.30	0.26
<i>F.ovina</i>	9.75	0.13	16.45	0.24

Table 5. Analysis of variance of germination properties of *Festuca* species in greenhouse condition.

Source of variance	DF	Germ %	Root L. (mm)	Shoot L. (mm)	Seedling L. (mm)	Vigo Index	RSR (mm)	FW (g)	DW (g)	DFR (g)	Chlorophyll index	Proline μmol g/DW	Carbohydrates μmol g/DW
Drought (D)	3	16556 **	99651 **	727280 **	1359843 **	2103857 **	0.89 **	1315 **	74.53 **	0.07 **	7982 **	0.15 **	0.03 *
Species (S)	2	1868 **	5002 *	11081 *	29054 **	135557 **	0.11 ns	78.8 **	9.11 **	0.01 ns	7.31 ns	0.01 ns	0.03 ns
D x E	6	260 ns	749 ns	2112 ns	3073 ns	14070 *	0.1 *	16.13 ns	3.24 **	0.01 *	37.65 ns	0.003 ns	0.002 ns
Error	132	334	1429	3029	3673	6484	0.04	7.78	0.68	0.007	24.30	0.008	0.01
CV %		24.85	21.60	17.88	12.55	20.97	31.44	33.74	40.20	31.62	16.13	5.74	30.59

*, ** = Means of squares are significant at 5%, 1%, respectively.

Table 6. Effect of drought stress on seed germination properties of *Festuca* species in greenhouse condition.

Drought	Germination %	Root L. (mm)	Shoot L. (mm)	Seedling L. (mm)	Vigo index	RSR (mm)	FW (g)	DW (g)	DFR (g)	Chlorophyll index	Proline μmol g/DW	Carbohydrates μmol g/DW
Control	92.6 a	239 a	465 a	704 a	654 a	0.53 c	15.4 a	3.82 a	0.25 b	42.3 a	1.45 c	0.32 b
25% FC	88.7 a	190 b	378 b	568 b	506 b	0.51 c	10.8 b	2.57 b	0.23 b	41.8 a	1.53 b	0.36 ab
50% FC	66.2 b	153 c	242 c	396 c	258 c	0.66 b	4.82 c	1.19 c	0.27 b	27.6 b	1.58 b	0.40 ab
75% FC	46.5 c	116 d	144 d	261 d	116 d	0.85 a	1.96 d	0.62 d	0.33 a	10.7 c	1.67 a	0.43 a

Means of the columns with the same letter had no significant differences based on DMRT (P≤0.05).

Table 7. Means comparison of seed germination characteristics in three *Festuca* species in greenhouse condition.

Ecotypes Name	Germination %	Root L. (mm)	Shoot L. (mm)	Seedling L. (mm)	Vigo index	RSR (mm)	FW (g)	DW (g)	DFR (g)	Chlorophyll index	Proline μmol g/DW	Carbohydrates μmol g/DW
<i>F.arundinacea</i>	68.9 b	166 a	300 ab	467 b	345 b	0.60 b	7.94b	2.05 b	0.29a	3.01 a	30.4 a	1.57 a
<i>F.pratensis</i>	69.2 b	170 a	285 b	455 b	345 b	0.73 a	11.2a	3.01 a	0.26a	3.06 a	29.8 a	1.50 a
<i>F.ovina</i>	79.2 a	184 a	320 a	504 a	432 a	0.65 ab	7.85b	1.81 b	0.26a	3.37 a	30.8 a	1.56 a

Means of the columns with the same letter had no significant differences based on DMRT (P≤0.05).

Table 8. Phenotypic correlation between germinator and glasshouse for seed germination traits

Traits	Ger %	Shoot L.	Root L.	Seedling	Vigor	RSR	Fresh W.	Dry W.	DFR
Correlation	0.99**	0.98**	0.76**	0.91**	0.97**	0.75**	0.75**	0.63*	-0.08 ^{ns}

Discussion

Drought stress tolerant cultivars are the varieties which have no meaningful decrease in germination indicators against increasing the water deficiency level. The *F.ovina* species in both germinator and greenhouse conditions is considered as more tolerant varieties. Generally, the results showed that the drought stress had negative effect on the germination percent and seedling growth characteristics. The dryness cause delay in germination characteristics. In the similar results Rice and Dyer (2001) working with *Aristida tectorum* showed that late emerging seeds has lower competitive ability than early emerging seeds.

The results showed that the drought stress had negative effect on the growth characteristics. The results obtained from other researches (Kaul and Shankar, 1988; Kim *et al.*, 1994) confirm this issue that increasing the water stress decreases the seedling growth. Drought stress cause increasing the salts viscosity in plant organs. So, by increasing the salts viscosity increase the osmosis stress of soil solution, so the amount of energy which the plant need to absorb the water from the soil will be increased, this action cause to increase the respiration and decrease the plant performance (Brason *et al.*, 1967). Rice and Dyer (2001) working with *Aristida tectorum* showed that late emerging seeds have lower competitive ability than early emerging seeds. The studies of Sharifi Kashan (2010) and Plauz (2003) showed that the growing up of water stress causes the decrease in shoot dried weight and increase in root dried weight and root:shoot ratio. The capacity of the root system for rapid and early development is an important factor in drought resistance. It is well known that increased efficiency of moisture absorption is primarily due to the development of an extensive root system. Also, it is a common feature among the arid and semi-arid environment perennials that these plants develop root systems that are larger than their shoots. This is an important factor in survival. The moisture collected by the extensive root system is drawn upon for the consumption of a reduced shoot.

The preponderance of the root system over the above-ground shoot system facilitates adjustment of the water balance of desert plants. One of the depressant factors photosynthesis in the severe drought stress is the reduction of chlorophyll content (Behero *et al.*, 2002). Some researches stated that the increase of dryness level in the plant decreased the chlorophyll viscosity in the leaves and cause to decrease the photosynthesis in the plant (Flaxas *et al.*, 1999, 2002 and 2004). The proline and carbohydrate content increased in cultivars of *Festuca ovina* species during the drought stress. This increase caused to establish the state of transparent phase (vitreous) in lost water protoplasm which can protect the membranes. Also increase of carbohydrate/ion ratio prevents the establishment of toxicity state and chaotropic, this increase cause the persistency during drought stress and makes a chance in order that adaptation solutions to be able to provide the conditions of agitation effects decrease.

All of seedling traits except RSR, DFR were increased and proline and carbohydrate content were increased by increasing osmotic potential. The results showed that the *F.ovina* species had higher values for the most of seedling attributes than other species. The phenotypic correlation estimates between two environments was high and significant for for the most of traits (Table 8) indicated that results based on germinator were strong indicators of greenhouse and probably field experiment. This result were in agreement with Pourhadian and Khajehpour (2010), that found percent of seeds emerging during the first 2-4 days of emergence is a valuable laboratory index for field emergence of wheat. In contrast Wang *et al.* (2004) were unable to predict field emergence using laboratory tests.

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