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PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES SEEDLING OF FOUR *BROMUS INERMIS* ECOTYPES TO DROUGHT STRESS IN GERMINATOR AND GREENHOUSE CONDITION

Melika Hashemi*

Young Researchers and Elite club, Karaj Branch, Islamic Azad University, Karaj, IRAN

ABSTRACT

The effect of drought stress was studied on seed characteristics including: percent of germination, seedling length (mm/plant), root/shoot length ratio, seedling weight (g), seedling dry/fresh weight ratio (g/dfw) and seed vigor index (VI) in four ecotypes of Bromus inermis (Alborz 303, Mazandaran 3151, Firozkuh 3966 and Esfahan 200060) in germinator and greenhouse condition. In greenhouse experiment, three more physiological characteristics as chlorophyll, carbohydrates and proline contents were measured. 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. With regard to results, for all seed characteristics Esfahan (200060) ecotype was superior compared to other ecotypes. Increasing osmotic stress decreased the chlorophyll content and increased praline and carbohydrate content. The phenotypic correlation between measurements of two conditions was determined. The estimates were significant for most traits. Results of probit analysis of LD50 and LD90 the same trend were obtained.

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KEY WORDS

Physiological, Morphological, Drought stress, Bromus inermis.

***Corresponding author:** **Email:** mohitezistsabz@gmail.com; **Tel.:** +98 214462280

INTRODUCTION

Bromus inermis Boiss is one of important perennial grass species belong to *Bromus inermis*. 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. *Bromus inermis* grows in areas with 750 m to 2900 m altitude [1]. 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. *Bromus inermis* has an important role in grassland productivity in Iran. Little breeding work has been done on this species especially under Iran climatic conditions. Water deficit is one of the most important factors affecting plant physiology and development [2, 3]. Among the different abiotic stresses, drought is by far the most complex and devastating on a global scale [4, 5, 6]. The sustainable and economically viable solution for increasing crop productivity in arid and semiarid zones is genetic improvement [7, 8, 9]. It is often discussed and emphasized that crop genetic improvement lies in exploiting the gene pools of the wild relatives of the crop plant [10, 2, 11, 12]. We anticipate 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 [13, 14, 10, 15, 16].

Drought is one of the major causes for crop loss worldwide, reducing average yields with 50% and over [17]. 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 [18, 19, 20, 21]. The strategies developed by plants against water potential decrease depend on drought-inducing factors such as water retention kinetics, low temperature or high salinity [6, 22]. 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 [23, 8, 9, 15, 22].

Responses to drought are multiple and interconnected. It is well established that drought stress impairs numerous metabolic and physiological processes in plants [24]. It leads to growth reduction, reduction in the content of chlorophyll pigments and water, and changes in fluorescence parameters [13, 25, 11, 26, 27, 12]. Rangelands are areas unsuitable for cultivation, but provide forage for animals [28, 5]. Rangeland degradation is often manifested by decreases in plant yields. The dispersal of seeds through oversowing 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 [20, 29]. In addition to grazing by domestic livestock, rainfall patterns are the most important factors influencing rangeland condition [30, 5, 26]. 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 [21]. Grasses with network root systems bind soil better than woody species with taproot systems [4, 1, 10]. The objective of this study was to identify Physiological and morphological responses of four *Bromus inermis* ecotypes 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 2013. Four *Bromus inermis* ecotypes: Alborz 303, Mazandaran 3151, Firozkuh 3966 and Esfahan 200060 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 vigour index measures seedling performance, relating together the germination percentage and growth of seedlings produced after a given time [31, 22]. 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

15 days by using the field capacity (FC) method in greenhouse condition. the seeds of ecotypes were provided from gene bank 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.

When vegetative growth was completed, at the end of each treatment, the seedling length (the distance from soil surface to upper end of the longest leaves) of the *Bromus inermis* cultivars was measured (mm/plant). The fresh (g/FW) and dry (g/DW) biomass of the seedling was also determined. The

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carbohydrates content of drought stressed and irrigated (control) plants was determined using the method of Antron (1992). Carbohydrates were extracted from leaf samples (20 mg DW) according to Weimberg (1987) with minor modifications. The absorbance of the sample extract was Spectrophotometrically determined at 535 nm. The proline concentration was determined as (μmol g/DW) using a standard curve.

For proline content, the method of Bates et al. (1973) was used to determine praline content. Proline was extract 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 praline concentration was determined as (μmol g/DW) using a standard curve.
The experiments were performed in a randomized block design with four replicates. Differences among the treatments as well as between the cultivars were tested using the SAS statistical program. Statistical variance analysis was performed using ANOVA and compared with least significant differences (LSD).

RESULTS

Germination percentage

The results of variance analysis indicate the meaningful effect of drought stress ($P \leq 0.01$) on germination in germinator and greenhouse conditions (**Table 3** and **6**). Increasing tolerance against dryness decreased the germination percentage in ecotypes significantly (Table 4 and 7). The comparison of average germination characteristics for different ecotypes, indicate that the seeds of ecotypes (3966) and (200060) have the better quantity for germination indicator in two germinator and greenhouse conditions. Generally the ecotype (3151) has lesser value in point of germination percentage among studied genotypes and has meaning difference with other ecotypes (**Table 5** and **8)**. The comparison of average germination of drought different treatments in both environments in every genotype indicated that the maximum germination was in control drought stress and the minimum germination percentage was in the treatment -0.9 MPa in the germinator and was 0.75 FC in the greenhouse (**Figure A and B**).

The Seedling length and the proportion of the root length to the shoot

The results of variance analysis in the germinator conditions indicated the meaningful effect of drought stress ($P \leq 0.05$) on the seedling length and the meaningless effect on the R/S length ratio (**Table 3**). The increase of tolerance against the dryness in the plants increased the seedling length in cultivars in both germinator and greenhouse condition while the proportion of R/S length ratio decreased (**Table 4 and 7- Figures C, D, E and F**). The results of variance analysis in the greenhouse conditions indicate the meaningful effect of water stress ($P \leq 0.01$) on the seedling length and R/S length ratio (**Table 6**). The comparison of average seedling length in germinator conditions indicator that the seeds of ecotypes (303) and (3151) have the better quantity while the seeds of ecotypes (3966) and (200060) have the highest level of proportion of R/S length ratio (Table 8).

The seed vigor index

The results of variance analysis in both germinator and greenhouse conditions indicate the meaningful effect of drought stress on the seed vigor index (**Table 3 and 6**). Overall the increase of osmosis stress decreased the seed vigor index in the studied cultivars. The average seed vigor was decreased in the control treatment with the indicator 44.71 in treatment -0.9 MPa in the germinator (**Table 4**). Also, the average seed vigor with the indicator 577 decreased in the control treatment (FC) to indicator 120 in treatment 0.75 FC in the greenhouse conditions (**Table 7**). The comparison of seed vigor index for different genotypes indicates that the ecotype (200060) has the highest level of seed vigor index in both germinator and greenhouse conditions (**Table 5 and 8**).

Dry weight and seedling dry/fresh weight ratio

The results of variance analysis indicate the meaningful effect of drought stress ($P \leq 0.01$) on the average dry weight in the germinator and greenhouse conditions (**Table 2 and 6**). The increase of water stress decreased the average dry weight significantly (**Table 4 and 7**). The maximum average of dry weight occurred in the control dryness treatment and it's minimum level was in the dryness treatment -0.9 MPa in the germinator and was 0.75 FC in the greenhouse (Figure I and J). The results of variance analysis in

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the germinator conditions indicate the meaningful effect of dryness tension ($P \leq 0.01$) on the D/F weight ratio in germinator and the meaningful effect ($P \leq 0.01$) in the greenhouse condition (Table 3 and 6). The comparison average of D/F weight ratio in the germinator conditions indicates that the seeds of ecotypes (3151) and (200060) have the better quantity in the greenhouse (**Table 5 and 8**). According to the average of D/F weight ratio in different water potentials, it was observed that the maximum of these amounts in the germinator was -0.9 MPa in the dryness treatment and was 0.75 FC in the greenhouse and increasing by increase stress (**Figure K and L**).

Proline content, carbohydrate and chlorophyll

The drought stress in this study had the meaningful effect ($P \leq 0.01$) on the proline, carbohydrate and chlorophyll content (**Table 6**). Increasing osmosis stress decreased the chlorophyll content and increased praline and carbohydrate content (**Table 7**). The comparison of average level of these three factors (Proline, carbohydrate and chlorophyll content) for different cultivars indicates that the seeds of genotype (3966) have the highest chlorophyll content and the seeds of genotype (200060) have the highest proline and carbohydrate content (**Figure M, N and O).**

The relationship between germinator and greenhouse for all traits is important. In terms of time, and economy, evaluation in germination is easier than greenhouse and filled. In other words, the evaluation based on greenhouse in depending on the correlation between characters in both environments. The phenotypic correlation between measurements of two conditions is summarised in **Table 1**. The estimates were significant for most traits. Overall, the data showed that results based on germinator were strong indicators of greenhouse performance.

Results of probit analysis for determination of lethal dose concentration (LD50) showed the ecotypes (200060) and (3151) with average values of 11.90 and 5.01 had higher and lower toleration to drought stress. For LD90 the same trend were obtained (**Table 2 and Figure 1**).

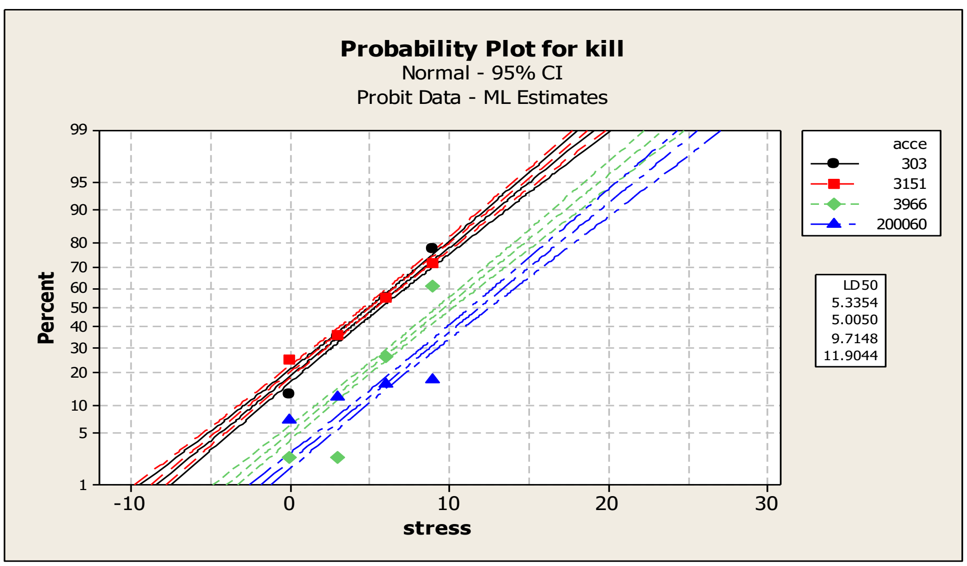

Fig 1. Probit analysis for determination of lethal dose concentration of PEG6000 for stopping seed germination in four ecotypes of *Bromus inermis*

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Table1. Phenotypic correlation between germinator and glasshouse for seed germination traits

TRAITS	GER %	SHOOT L.	ROOT L.	SEEDLING	VIGOR	Root/Sh	Fresh W.	Dry W.	Dry/Fresh
CORRELATION	0.99^{**}	0.88^{**}	0.81^{**}	0.90^{**}	0.96^{**}	0.01^{ns}	0.83^{**}	0.32^{ns}	0.06^{ns}

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Table2. Probit analysis for determination of lethal dose concentration of PEG6000 for stopping 50% (LD50) and 90% (LD90) of seed germination in four ecotypes of *Bromus inermis*

Ecotypes	LD_{50}	SE	LD_{90}	SE
303	5.34	0.20	12.90	0.34
3151	5.01	0.20	12.57	0.33
3966	9.71	0.26	17.28	0.45
200060	11.90	0.32	19.47	0.52

Table3. Analysis of variance of germination properties of *Bromus inermis* in germinator condition

Source of Variance	DF	Germination %	Shoot L.(mm)	Root L.(mm)	R/S (mm)	Seedling L. (mm)	Vigor index	FW (g)	DW (g)	D/F (g)
Drought(D)	3	6234 **	1823 **	9824 **	9309 **	0.1063 ns	38081 **	44840 **	0.0369 **	0.00003 ns
Ecotype(E)	3	4493 **	1492 **	376 *	122.6 ns	0.3259 *	895 *	7693 **	0.0148 **	0.0010 **
E × D	9	558 **	99.6 **	177 ns	421.7 **	0.2816 **	877.4 **	1032 **	0.0016 *	0.0001 **
Error	48	122.7	27.88	110.7	88.29	0.100	293.3	248.6	0.0006	0.00001
CV %		16.07	19.72	20.09	18.49	30.66	16.59	19.87	16.31	16.67

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

Table4. Effect of drought stress on seed germination properties of *Bromus inermis* in germinator condition

Drought Treatment	Germination %	Shoot L.(mm)	Root L. (mm)	R/S (mm)	Seedling L. (mm)	Vigor index	FW (g)	DW (g)	D/F (g)
Control	89 a	37.27 a	77.53 a	78.29 a	1.01 a	155.81 a	140.05 a	0.22 a	0.03 a
-0.3 MPa	79.75 b	33.39 b	66.01 b	61.69 b	1.01 a	127.71 b	103.40 b	0.17 b	0.02 a
-0.6 MPa	63 c	22.72 c	44.54 c	39.93 c	0.97 a	84.47 c	53.44 c	0.13 c	0.02 a
-0.9 MPa	44 d	13.68 d	21.36 d	23.36 d	1.15 a	44.71 d	20.41 d	0.12 c	0.03 a

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

Table5. Means comparison of seed germination characteristics in four *Bromus inermis* ecotypes

Ecotypes Name	Germination %	Shoot L. (mm)	Root L. (mm)	R/S (mm)	Seedling L. (mm)	Vigor index	FW (g)	DW (g)	D/F (g)
Alborz (303)	55.75 c	21.93 c	48.15 b	49.73 a	1.11 ab	97.88 b	62.77 b	0.15 bc	0.02 c
Mazandaran (3151)	54 c	16.03 d	48.99 b	47.63 a	1.20 a	96.63 b	58.30 b	0.16 b	0.03 a
Firozkuh (3966)	78 b	13.39 b	53.61 ab	51.84 a	0.93 b	105.54 ab	94.92 a	0.13 c	0.01 d
Esfahan (200060)	88 a	37.71 a	58.68 a	54.08 a	0.90 b	112.75 a	101.31 a	0.20 a	0.03 b

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

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Table6. Analysis of variance of germination properties of *Bromus inermis* in greenhouse condition

Source of Variance	DF	Germ %	Root L. (mm)	Shoot L. (mm)	Seedling L. (mm)	Vigo index	R/S (mm)	FW (g)	DW (g)	D/F (g)	Chlorophyll Index	Proline µmol g/DW	Carbohydrates µmol g/DW
Drought (D)	3	5837 **	48700 **	300037 **	585785 **	824177 **	0.17 **	636 **	35.53 **	0.07 **	3468 **	0.08 **	0.03 **
Ecotype (E)	3	4221 **	5646 **	26215 **	12734 **	48383 **	0.19 **	31.49 *	7.73 **	0.02 **	124 **	0.05 **	022 **
D x E	9	509 **	3353 **	6207 **	9930 **	6784 *	0.02 *	11.12 ns	2.98 **	0.01 **	43.63 **	0.0007 ns	0.002 **
Error	48	69.12	602	922	1953	3176	0.014	7.53	0.30	0.004	15.41	0.001	0.0003
CV %		10.35	14.71	10.10	9.45	16.33	19.69	34.55	26.88	23.66	12.89	2.51	5.45

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

Table7. Effect of drought stress on seed germination properties of *Bromus inermis* in greenhouse condition

Drought	Germination %	Root L. (mm)	Shoot L. (mm)	Seedling L. (mm)	Vigo index	R/S (mm)	FW (g)	DW (g)	D/F (g)	Chlorophyll index	Proline µmol g/DW	Carbohydrates µmol g/DW
Control	83.5 a	237 a	455 a	692 a	577 a	0.55 b	15.54 a	3.93 a	0.26 b	43.05 a	1.45 d	0.28 d
25% FC	72 b	178 b	369 b	548 b	396 b	0.49 b	10.58 b	2.53 b	0.23 b	39.88 b	1.54 c	0.33 c
50% FC	70.4 c	141 c	225 c	366 c	257 c	0.66 a	3.87 c	1.07 c	0.28 b	28.37 c	1.58 b	0.36 b
75% FC	46 d	109 d	152 d	261 d	120 d	0.72 a	1.78 d	0.66 d	0.38 a	10.49 d	1.70 a	0.43 a

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

Table8. Means comparison of seed germination characteristics in four *Bromus inermis* ecotypes in greenhouse condition

Ecotypes Name	Germination %	Root L.(mm)	Shoot L.(mm)	Seedling L. (mm)	Vigo index	R/S (mm)	FW (g)	DW (g)	D/F (g)	Chlorophyll index	Proline µmolg/DW	Carbohydrates µmol g/DW
Alborz (303)	52 c	193 a	266 c	459 b	239 b	0.71 a	9.19 a	3.05 a	0.34 a	26.60 b	1.61 b	0.49 a
Mazandaran (3151)	50 c	149 b	358 a	508 a	254 b	0.45 c	6.60 b	1.48 c	0.24 c	30.17 a	1.47 d	0.21 b
Firozkuh (3966)	67 b	162 b	291 b	454 b	304 a	0.61 b	6.86 b	1.70 bc	0.28 cb	32.64 a	1.53 a	0.20 b
Esfahan (200060)	82 a	160 b	285 bc	446 b	366 a	0.64 ab	9.11 a	1.96 b	0.3 ab	32.37 a	1.66 c	0.50 a

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

DISCUSSION

Drought stress tolerant cultivars are the varieties which have no meaningful decrease in germination indicators against increasing the water deficiency level. The ecotypes (3966) and (200060) in both germinator and greenhouse conditions are considered as more tolerant varieties. In seeds (303) and (3151), increasing drought stress decreased the average percentage of germination, so it could be said that in the condition in which there is the possibility of water stress in the germination stages, because of sensitivity of these ecotypes, it is better use these seeds not to be used because these genotypes are sensitive to the drought stress.

Generally, the results showed that the drought stress had negative effect on the growth characteristics. The results obtained from other researches [32, 9, 33, 27] confirm this issue that increasing the water stress decreases the growth of plant organs because 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 [28]. One of the depressant factors photosynthesis in the severe drought stress is the reduction of chlorophyll content [16]. Some researches stated that the increase of dryness level in the plant decreased the chlorophyll

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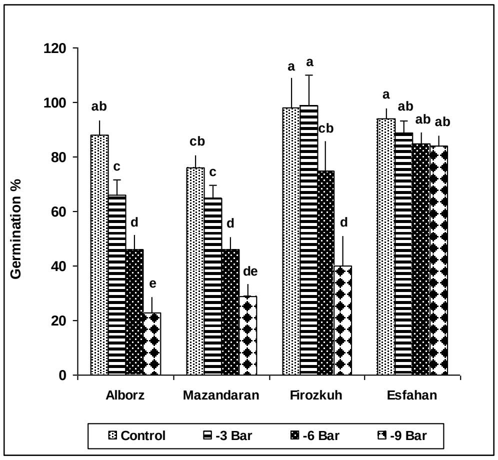
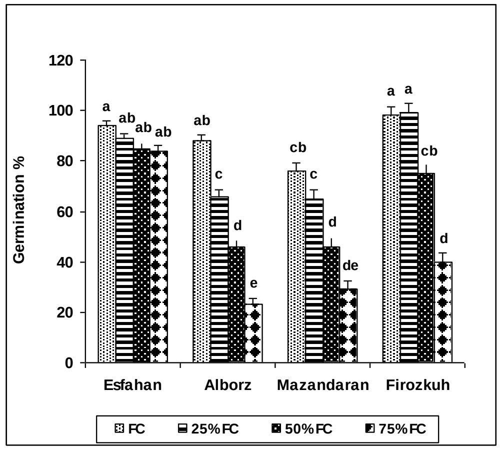
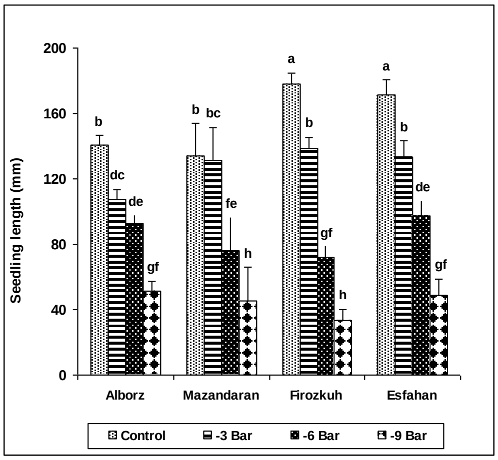


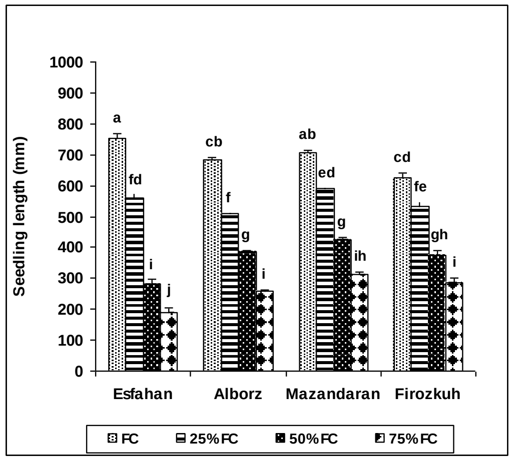
A- Germination% (Germinator)



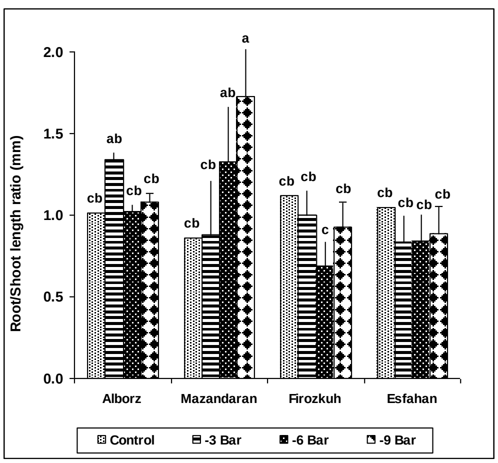
B- Germination% (Greenhouse)



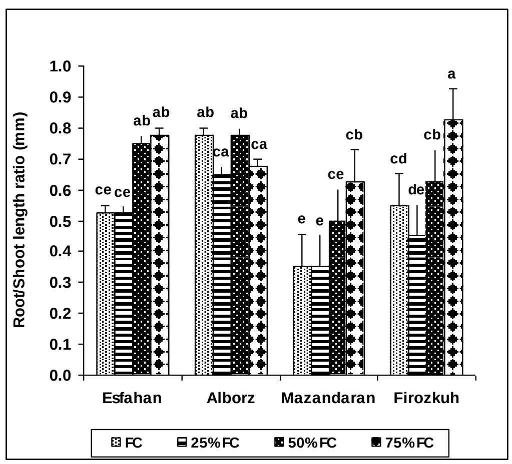
C-Seedling Length (Germinator)



D- Seedling Length (Greenhouse)



E-Root/Shoot Length ratio (Germinator)



F-Root/Shoot Length ratio (Greenhouse)

viscosity in the leaves and cause to decrease the photosynthesis in the plant [29, 25]. The proline and carbohydrate content increased in cultivars of *Bromus inermis* 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

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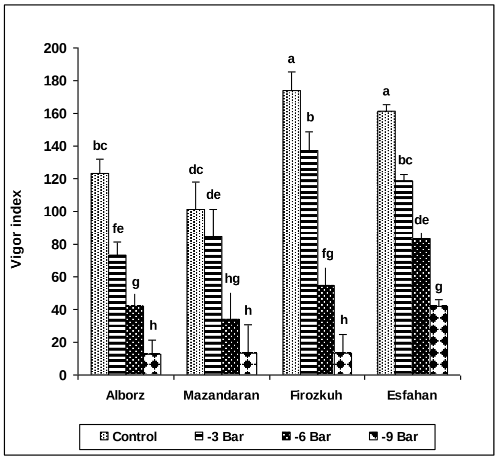
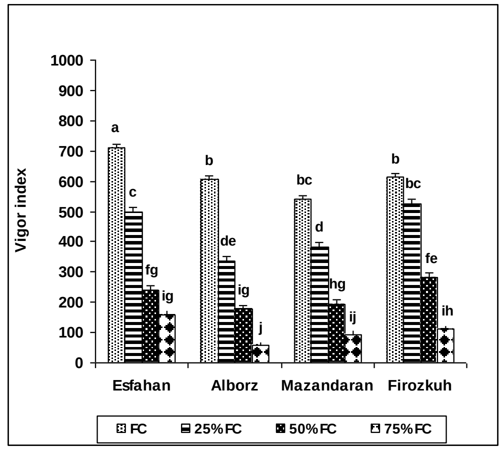
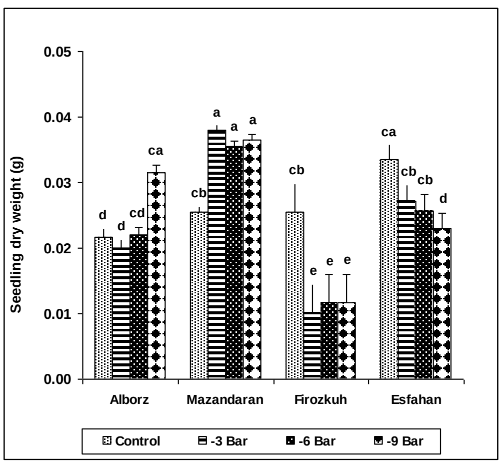
state and chaotropic, this increase cause the persistency during drought stress and makes a chance in order that adaptation solutions to be able to provide the conditions of agitation effects decrease. Comparison of (Germination percentage, Seedling Length and Root/Shoot Length ratio) four Br. inermis ecotypes under drought stress sown in germinator and greenhouse condition (Means of the columns with the same letter had no significant differences based on DMRT (P≤0.05).



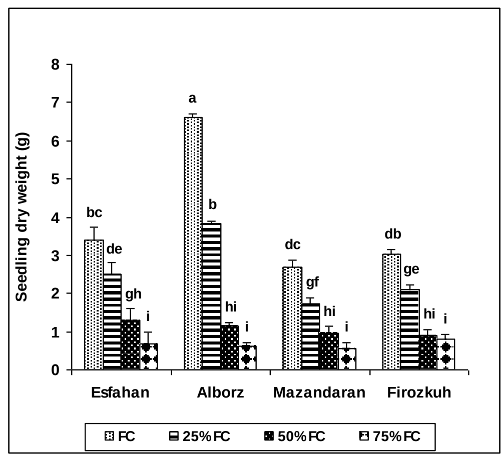
G-Vigor index (Germinator)



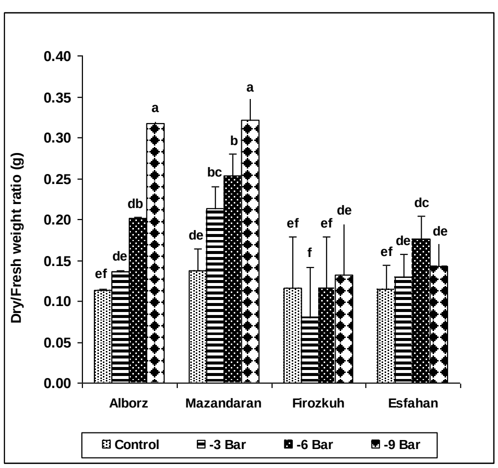
H- Vigor index (Greenhouse)



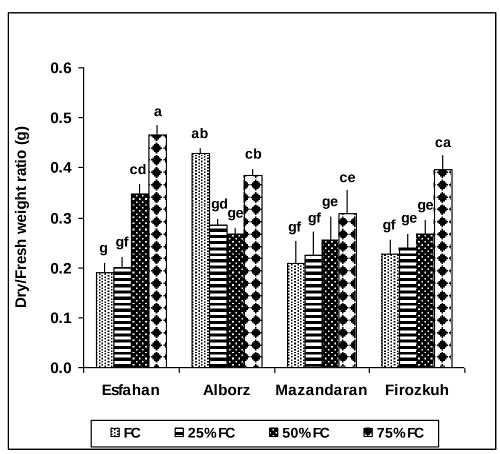
I-Seedling dry weight (Germinator)



J- Seedling dry weight (Greenhouse)



K-Dry/Fresh weight ratio (Germinator)



L- Dry/Fresh weight ratio (Greenhouse)

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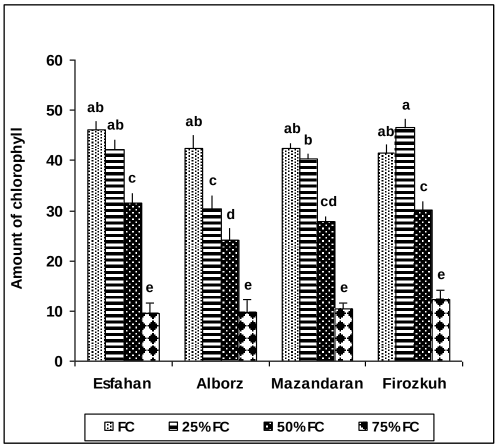
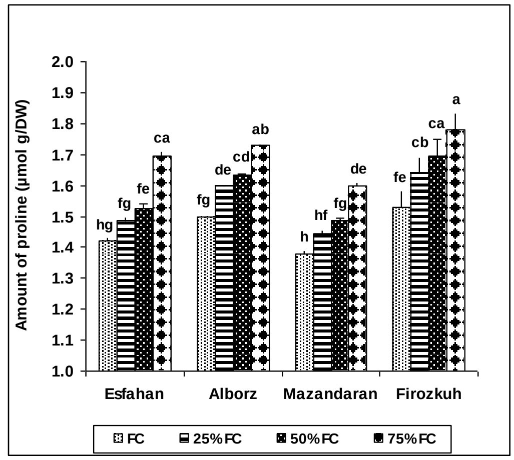
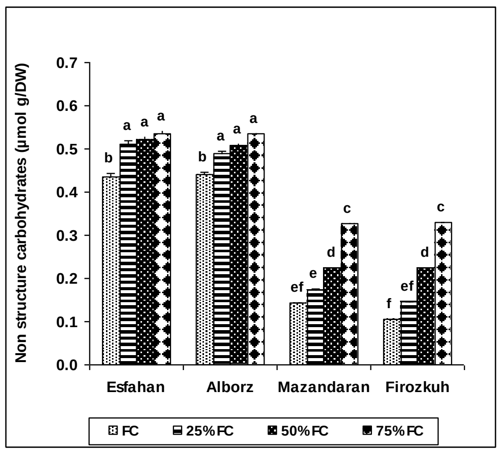
Comparison of (Vigor index, Seedling dry weight and Dry/Fresh weight ratio) four Br. inermis ecotypes under drought stress sown in germinator and greenhouse condition (Means of the columns with the same letter had no significant differences based on DMRT (P≤0.05).



M-Amount of chlorophyll (Greenhouse)



N- Amount of proline (Greenhouse)



O-Non structure carbohydrates (Greenhouse)

Comparison of (Amount of chlorophyll, Amount of proline and Non structure carbohydrates) four Br. inermis ecotypes under drought stress sown in greenhouse condition

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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