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Morphological and phonological development of *Festuca ovina* and *Festuca arundinaceae* to cold temperature regarding GDD

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Key words: *Festuca ovina*, *Festuca arundinaceae*, Cold treatment, GDD, Seedling growth.

Abstract

Festuca genus has a high value for grazing and forage conservation concerning the livestock. This research was conducted in laboratory and greenhouse and outdoor of Research Institute of Forest and Rangeland in Tehran, Iran. For outdoor experiment, half of pots of greenhouse were transferred outdoor until their flowering and growth stages and GDD were recorded. In the flowering stage, morphological characteristics including stem number, stem length, peduncle length, panicle length, plants‟ fresh and dry weight of each ecotype were recorded. The collected data of each experiment were separately analyzed using factorial experiment based on completely randomized design. The results of laboratory experiment showed the significant effects of chilling (4 °C) treatment on germination percent, speed of germination in both species and vigor index for *F.ovina*. Regarding *F.ovina* in vegetative stage, the mean values of all of traits were higher in control treatment than those of cold treatment. Similarly, in outdoor experiment, the mean values of reproductive traits in the control treatment were higher than those for cold treatment concerning both species. *F.ovina* had higher mean values for all of reproductive traits as compared to *F.arundinaceae*. Results also showed that GDD of plants which were subjected to cold treatment were lower than those for control treatment for both vegetative and flowering stages. It has been concluded that cold treatment reduced flowering dates in reproductive stage.

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Introduction

The genus of Festuca as native grass has been extended in Europe, Asia, North America and northern Africa (Robert *et al.*, 2013). The genus has 300 species worldwide growing in the tropical areas of the world (Sehat Niaki, 1995). Eighteen perennial and annual species of Poa genus grow in Iran (Mozafarian, 2007). Both *Festuca ovina* and *Festuca arundinaceae* naturally grow in Zagros and Alborz mountains‟ rangelands in the west and north of Iran.

They grow in the areas with the altitude of 750 to 2900 m and are used for grazing the sheep and goats (Rechinger, 1970). Festuca spp. is often used with regard to the growing conditions that are not ideal for other crops and a grass crop is needed. Its resistance and fast growth make it high-yielding forage and allow it to withstand greater grazing pressure in comparison to the other grass species of cool season. Another use of Festuca spp. is to control the erosion. It has the early growth in spring and is of good quality for animal productivity and good adaptability in severe conditions in all over the country. In recent years, higher grazing pressure and unpalatable weed invasion have led to increase the soil erosion and consequently decrease the accessions of these species. Therefore, the cultivation of those areas by new improved grass varieties is the most economical and possible tool of recovery. Extension of the grass-cultivated area and change of sowing time due to global warming or climate changes are of great concerns in the arid and semiarid regions where precipitation is insufficient for plant germination and growth. Most Festuca species grow in the altitude of 2000 m where the weather is cold. Therefore, for the extension of its cultivation in plain plateau that is warmer than higher altitude, it is necessary to study their reactions to the cold treatments.

GDD is the means of establishing a cumulative measurement of heat units during a specific period. Heat units are best and simply determined by looking directly at temperature. Temperature is considered as the main factor that influences the rates of plant growth although others such as moisture level, day length and light qualities also have secondary effects (Holen and Dexter, 1996). The concept of GDD has been further integrated into crop breeding where GDD is used to note the differences in cultivars (University of Massachusetts Extension, 2005) and heading times of grasses (Griffith and Nelson, 2000). If seed set, leaf stage development, flowering and similar phonological traits can be followed by GDD, the extension of growing area will be noticed. Griffith and Nelson (2000) suggested that management practices such as cultivation can be timed on the basis of an accumulated GDD time scale.

This time scale is yearly much more consistent in predicting plant development rather than the use of calendar date. Similarly, cocksfoot (*Dactylis glomerata* L.) was related to the accumulated temperature and suggested that this relationship could be used to predict spring growth. Aamlid (1992) compared the vegetative growth of *Poa pratensis* at temperatures of 21/12, 21/6, 12/12 and 12/6°C for four days/nights and at photoperiods of 12, 16, 20 and 24 h and found higher tillering during short days and at wide temperature range. Gunn and Farurr (2002) have studied the impacts of over 4°C temperature on leaf area, dry mater, root and shoot length and carbohydrate contents of *Poa annua* and showed that all of these traits were increased at temperature over than 4°C.

Pannangpetch and Bean (1984) showed that the germination of cocksfoot population has been increased by pre-chilling treatment. Keeping in view the feed production importance of this grass, the present study mainly aims to design an experiment on the impacts of pre-cool temperature on seedling morphological and phonological traits and Growth Degree Days (GDD) of two species including *Festuca ovina* and *Festuca arundinaceae*.

Materials and methods

Seed materials

This research was conducted on two species of *Festuca ovina* and *Festuca arundinaceae* in the laboratory and greenhouse in Research Institute of Forest and Rangeland in Tehran, Iran during 2012-2013.

Laboratory experiment

In laboratory, four replicates (25 seeds per replicate) of sterilized seeds were placed in petri dishes on double Whatman papers (TP). For protection against molds, the water was used to moisten the seed samples and substrata contained 0.002% of Benomil fungicide. The seed samples were moist chilled at 4 °C for two weeks as compared with control treatment. Samples of both treatments (chilling and control) were transferred into a germinator at 20±4°C with 1000 lux light for 15 days. After seedling growth (15 days), the vegetative traits including germination percentage, germination speed, vigor index, root length, shoot length and fresh and dry weight of seedling were measured.

Percentage and speed of germination were recorded for 3, 6, 9, 12 and 15 days. In accordance with Maguire (1962), the speed of germination was calculated by the following equation 1:

$$G.S = \frac{\sum n}{\sum n(n \times DN)} \times 100$$

Where

n= number of seeds germinated in mentioned days.

DN= number of days after sowing corresponding to n.

G.S= speed of germination and the highest one is the fastest speed.

After the growth of seedlings for 15 days, the lengths of roots and shoots of 10 randomly-selected seedlings from each replicate were measured. The vigor index measures the seedling performance and relates the germination percentage and growth of seedlings produced after a given time (Abdul-Baki and Anderson, 1973). It is calculated by following equation 2:

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

Where

VI = Vigour index

%Gr = Final germination percentage

MSH = Mean seedling height.

Greenhouse experiment (vegetative growth evaluation)

For greenhouse experiment, the seed samples were sown on six pots (Size: 22cm) with the fluctuation temperatures of 20±5°C for a day and 5-12°C per night using light illumination about 6000-10000 lux and randomized block design.

For cold treatment and vernalization, the pots of 30thday growth were subjected to the temperature of 4°C for two weeks. The control groups were not treated by cold weather. After cold treatments, all of the pots were placed in normal conditions of greenhouse in order to complete their vegetative growth. After 45 days, in the half of pots, such vegetative traits as germination percentage, shoot length, root length, root/shoot length ratio, seedling length, vigor index, seedling fresh weight, seedling dry weight and dry/fresh weight ratio were measured in three replications.

Outdoor experiment for calculating of GDD

This experiment was initially started in greenhouse when the vegetative growth of cold and control treatments was completed and half of pots were transferred outdoors to continue their normal growth. For three remaining pots, the flowering stage and morphological characteristics including plant height, peduncle length and panicle length and number of panicle of each species were recorded. Then, the cut and their fresh weights were measured. Samples were placed into an oven (80°C) for 48 h. Their dry weights were also measured.

GDD was calculated using the equation postulated by Frank *et al.* (1993) as follows equation 3:

$$GDD = \frac{T_{\max} + T_{\min}}{2} - T_{base}$$

Where

GDD= Growth degree days

Tmax= Maximum daily temperature

Tmin= Minimum daily temperature
Tbase= base temperature set at 4 °C

Most of cool season‟s grass cultivars require a vernalization period of cold temperature (4°C or lower) before starting a period of long day (13 hours) for the floral initiation in spring (Ehlke and Undersander, 1990).

Statistical analysis
Data of each experiment were separately analyzed using GLM single-variable method (SAS, 2004). A factorial analysis based on completely randomized design was conducted for each experiment. The cold and species effects were tested by ANOVA and treatment means were compared by Duncan method.

Laboratory experiment
Mean comparisons of species in the laboratory showed that the overall means of germination percent, speed of germination and vigor index for *F.ovina* were significantly higher than *F. arundinaceae*. In contrast, seedling fresh and dry weight values were higher for *F. arundinaceae* than those for *F. ovina* (Table 1). Data presented in (Table 1), have proved that there were significant effects of pre chilling (4°C) treatment on increasing the germination percent, speed of germination in both species and vigor index for *F. ovina*, but there was no significant effect of cold treatment on other traits. For some characteristics such as shoot and root length, seedling length and dry/fresh weight ratio in *F. ovina*, the higher values were obtained in control treatment (Table 1).

Results

Table 1. Effects of pre moist chilling (4°C) treatment for two weeks on seed germination characteristics of two Festuca species in laboratory conditions.

Species	Treat-ment	Germi-nation (%)	Germi-nation Speed	Shoot Length (mm)	Root Length (mm)	Root/ Shoot Ratio	Seed-ling Length (mm)	Vigor Index	Fresh Weight (mg)	Dry Weight (mg)	Dry/ Fresh Weight Ratio
F. arundinaceae	Control	55.33 d	5.22 d	33.10b	39.48a	1.19a	72.58 b	40.22c	62.00b	4.00 a	0.070 ab
	Cold	62.88 c	5.64 c	35.12a	40.10a	1.14a	75.22 a	47.25b	87.22a	4.66 a	0.045 b
F. ovina	Control	81.55 b	8.56 b	35.80a	39.66a	1.10a	75.47 a	61.75a	33.66c	1.44 c	0.096 a
	Cold	85.33 a	9.04 a	33.75b	37.47b	1.11a	71.23 b	60.94a	33.66c	2.22 b	0.055 b

Means of each columns followed by the same letters had no significant differences (P<0.05) based on DMRT method.

Vegetative growth evaluation in greenhouse
Mean comparisons of species in greenhouse showed that the overall means of germination percent, root/shoot length ratio and seedling length for *F. ovina* were significantly lower than those for *F. arundinaceae*. In contrast, the overall mean values of root and shoot length, vigor index and fresh and dry weights of seedling were higher for *F. ovina* than *F. arundinaceae* (Table 2). The effects of cold treatment were significant on shoot length, vigor index and seedling weight in both species and the lower values were obtained in cold treatment (Table 2).

Reproductive traits' evaluation
Effects of cold treatment on reproductive traits showed significant effects of high temperature (control) on plant fresh and dry weights (Table 3). Comparing the species, the results showed significant differences between two species for all the reproductive traits. *F. arundinaceae* with the average value of 8.1 for the reproductive stem had higher

values than *F. ovina* with 3.2 for stems. For other traits as stem length, panicle length, spikelet number, peduncle length, fresh weight and dry weight, the higher values were attributed to *F. ovina* (Table 4).

Table 2. Impacts of cold treatment (30th day seedling) on seedling characteristics of two Festuca species in greenhouse conditions.

Species	Treat-ment	Germi-nation (%)	Shoot Length (mm)	Root Length (mm)	Root/ Shoot Ratio	Seedling Length (mm)	Vigor Index	Fresh Weight (mg)	Dry Weight (mg)	Dry/ Fresh Weight Ratio
F. arundinaceae	Control	72.00 a	37.42c	104.4a	2.80b	141.8 a	101.9 a	8390 b	4040 b	0.48 ab
	Cold	61.33 b	28.15d	92.17a	3.29a	120.3 b	74.16ab	2926 c	967.8 c	0.33 b
F. ovina	Control	50.66 c	65.36a	95.88a	1.46c	161.2 a	82.57a	9622 a	5472 a	0.56 a
	Cold	44.44 c	54.71b	82.51b	1.52c	137.2 a	62.99b	8525 b	4525 b	0.52 a

Means of each columns followed by the same letters had no significant differences (P<0.05) based on DMRT method.

Table 3. Impacts of cold treatment on phonological traits of 30 day seedling in reproductive stage of *Festuca arundinaceae* and *Festuca ovina* as compared with control treatment.

Treatment	Number of Reproductive Stem	Stem Length (cm)	Panicle Length (cm)	Spikelet Number	Peduncle Length (cm)	Fresh Weight (g)	Dry Weight (g)
Control	6.34 a	32.98 a	6.51 a	17.04 a	5.72 a	21.36 a	11.26 a
Cold	6.16 a	32.24 a	6.32 a	17.20 a	5.41 a	13.049 b	6.30 b

Means of each columns followed by the same letters had no significant differences (P<0.05) based on DMRT method.

Table 4. Comparing phonological traits in reproductive stage of *Festuca arundinaceae* and *Festuca ovina*.

Species	Number of Reproductive Stem	Stem Length (cm)	Panicle Length (cm)	Spikelet Number	Peduncle Length (cm)	Fresh Weight (g)	Dry Weight (g)
F. arundinaceae	8.10 a	30.44 b	4.74 b	16.00 b	4.65 b	15.53 b	7.12 b
F. ovina	3.20 b	36.84 a	9.70 a	20.02 a	7.04 a	23.75 a	13.96 a

Means of each columns followed by the same letters had no significant differences (P<0.05) based on DMRT method.

There was no significant species for cold treatment‟s interaction and effects concerning the reproductive traits; however, in *F. arundinaceae*, the effects of cold treatment were significant for number of spikelet and plant dry and fresh weights. For *F. arundinaceae*, the higher and lower spikelet numbers with the average values of 16.53 and 14.85 were obtained for the cold and control treatments, respectively. In contrast, in both species, higher values of dry and fresh biomass weights were obtained in control treatment (Table 5).

Growth Degree Days (GDD)

Growth Degree Day (GDD) of species was estimated during three phonological stages as tillering, flowering and ripening. At tillering and flowering stages, *F. arundinaceae* had higher GDD in both cold and control treatments (Fig. 1). In contrast, at ripening stage, higher GDD was obtained for *F. ovina*. During all the phonological stages, GDD of cold treatment was lower than control treatment (Fig. 1).

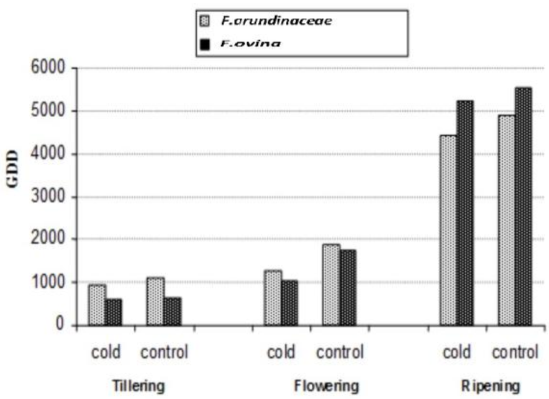

Fig. 1. Growth degree days of *Festuca arundinaceae* and *Festuca ovina* regarding the effects of cold treatment on 30 day seedlings in three stages including tillering, flowering and ripening.

Table 5. Interaction of cold treatment on phonological traits of 30 day seedling in reproductive stage of *Festuca arundinaceae* and *Festuca ovina* as compared with control treatment.

Species	Treatment	Number of Reproductive Stem	Stem Length (cm)	Panicle Length (cm)	Spikelet Number	Peduncle Length (cm)	Fresh Weight (g)	Dry Weight (g)
F. arundinaceae	Control	8.87 a	30.00 b	4.38 b	14.85 c	4.72 b	18.51b	9.34 b
	Cold	7.16 a	30.47 b	4.89 b	16.53 b	4.51 b	13.00 c	5.52 c
F. ovina	Control	3.00 b	38.50 a	10.25 a	20.80 a	7.26 a	33.53 a	19.48 a
	Cold	3.40 b	35.18 ab	9.15 a	19.24 a	6.82 a	13.97 c	8.43 b

Means of each columns followed by the same letters had no significant differences (P<0.05) based on DMRT method.

Discussion

The results of laboratory experiment showed significant positive effects of pre chilling (4°C) treatments on germination percent, speed of germination in both species and vigor index for *F. ovina*, but there was no significant effect of cold treatments on other seedling traits.

Alizadeh and Jafari (2010 and 2011) in cocksfoot found the pre-cool effects on the same traits. Cold treatment is one important factor for breaking dormancy and seed priming for germination.

Similarly, Pannangpetch and Bean (1984) found that the germination percentage of cocksfoot population was increased by pre-chilling treatment. Results of greenhouse experiment for *F.ovina* showed that the mean values of these traits except root/shoot length ratio were higher in control treatment than those for cold treatment. The laboratory results and showed that both species grow rapidly in control treatment.

Gunn and Farurr (2002) reached the same conclusion. By studying the impacts of over 4°C temperature on vegetative growth of *Poa annua*, they found that all the traits were increased in a temperature over than 4°C. In the outdoor experiment, the results showed that the mean values of fresh and dry weights of both species were higher in the control treatment than those for the cold one. *F. ovina* had higher mean values for all the reproductive traits except reproductive stem in comparison with *F.arundinaceae*.

As (Fig. 1), has already shown, Growth Degree Day (GDD) of two species was calculated in three stages including tillerring, flowering and ripening. During tillerring, the impacts of cold treatment on GDD were not significant. It means that in early stage of seedling growth, GDD has no effects on vegetation stage. However, for flowering stage, both species had lower GDD than control treatment (Fig. 1). The reason is that for panicle emergence date, both species tend to early heading as compared to control treatment and it means that the lower temperature was necessary for vernalization. Considering the effects of cold treatment, GDD of flowering stage was lower than control treatment (Fig. 1) and it means the processing of phenology had been affected by cold treatment as compared with control one.

Calculating Growth Degree Day (GDD) during phenology is an important factor for the prediction of optimum time of sowing, flowering, harvesting and grazing. Results showed that in both species, those plants which were subjected to cold treatment had lower GDD values than those for control treatment for both vegetative and flowering stages. It was then concluded that cold treatment reduced flowering dates in reproductive stage. This result indicated that *F. arundinaceae* as a cold season forage grass for going to flowering stage needs winter coldness in comparison with *Festuca ovina* and it must be sown in autumn. The latter species had no reaction to cold treatment for reproductive traits; however, Aamlid (1992) in *Festuca ovina* found higher tillering for short days along with wide temperature range.

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