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Effect of Drought Stress and Paclobutrazol- Treated Seeds on Physiological Response of *Festuca arundinacea* L. Master and *Lolium perenne* L. Barrage

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ABSTRACT

To study interaction between drought stress and pachlobutrazol as treated seeds before planting, seeds of *Festuca arundinacea* L. Master and *Lolium perenne* L. Barrage were soaked with 0, 20, 30 and 40 mg.L^{-1} paclobutrazol for 24 h on shaker during imbibitions stage of seed germination. Treated seeds were sowed in the pots. After 102 days, when the seedlings well established, drought stress was applied in 50% field capacity (FC), 25% FC and well-watered. Interaction effect of Paclobutrazol × drought stress × cultivar was significant on relative water content (RWC). Drought stress increased electrolyte leakage whereas the highest concentration of paclobutrazol reduced EL. The highest total chlorophyll content was observed in Master at well-watered however at 25% FC in Barrage the lowest chlorophyll content is resulted. Paclobutrazol 40 mgL^{-1} was the highest total chlorophyll content. *Festuca arundinacea* L. Master at 25% FC Was the highest proline content. All paclobutrazol concentrations in *Festuca arundinacea* L. Master resulted higher proline content. Paclobutrazol 20 mgL^{-1} at 25% FC had higher shoot dry weight. The highest root dry weight resulted in Barrage at well-watered with 30 mgL^{-1} paclobutrazol. Root length was the highest in well-watered and lower in 25% FC.

Key Words: Tall Fescue; growth retardant; drought stress; chlorophyll content; proline content; turfgrass

INTRODUCTION

High vegetative growth of turfgrasses has been resulted in studying the chemical mowing agents. Such compounds are of interest because of their potential to reduce labor, fuel, and equipment costs for turfgrass maintenance (Elkins, 1982; Johnson and Faulkner, 1985; Taylor, 1985). Decreasing water availability has become more of a problem for turf management as a result of low precipitation and an increasing demand for water from agriculture, industry and homes. The destructive impact of drought may grow as the climate changes. Therefore, water issues and drought tolerance is a long-term concern in turfgrass management and will impact billions of dollars of turfgrass/environmental horticultural industries on into the future (Huang, 2008). Drought stress is one of the most detrimental factors limiting turfgrass growth. There exists large variability in drought resistance among turfgrass species and cultivars (Huang, 2008).

The use of the plant growth regulators (PGRs) as ‘‘chemical mowing agents’’ was envisioned many years ago because of the tremendous economic benefits (Davis and Curry, 1991) and additional potential benefits including: improved color, fewer clipping, deeper roots, fewer seedheads, less time spent in trimming (Johnson, 1992). The potential disadvantages are leaf burn, reduced turf recuperative ability, increased weeds, and increased disease incidence (Feltcher et al., 2000). These chemicals decrease detrimental effects of mechanical mowing such as reduced root growth, increased number of ports of entry for disease organisms, increased water loss, and decreased water absorption (Watschke, 1986). Application of PGRs directly to seeds may circumvent some of these limitations for bedding plant production (Pasian and Bennett, 2001). The systemic properties of paclobutrazol and other triazoles have been shown to allow the application of growth retardants to seeds with either minimal or no effect on seed germination (Pasian and Bennett, 2001). Triazoles inhibit monooxygenases, which oxidase in three steps ent-kaurene to ent-kaurenoic acid, an early reaction in GA biosynthesis (Hedden and Kamiya, 1997; Rademacher, 2000).The primary action of triazole-type growth regulators consists of lowering plant content of GA through inhibition of GA biosynthesis (Rademacher, 2000). Drought stress suppresses turfgrass growth and causes deterioration of turf quality. Proper application of certain plant growth regulators can enhance plant stress tolerance (Schmidt and Zhang, 1997a; Schott and Walter, 1991; Van Staden et al., 1994). When water resources become limited, the turf- grass manager is restricted in irrigating turfgrass and acceptable quality turf must be maintained with less water. Turfgrass managers have at their disposal the ability to alter many different cultural practices which may reduce the amount of water lost through turfgrass evaporated transpiration. The aim of this study is to investigate effects of drought stress on paclobutrazol treated seeds of tall fescue and perennial ryegrass.

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MATERIALS AND METHODS

Tall fescue (*Festuca arundinacea* L. Master) and perennial ryegrass (*Lolium perenne* L. Barrage) seeds from local market were soaked in paclobutrazol solution at concentrations of 20, 30 and 40 $mg{\cdot}L^{-1}$ for 24 hr in a flask on a shaker device (Model 75, Burrell Co., Pittsburgh, PA, USA). After soaking, seeds were sown in 70 Percent clay loam soil with 30 Percent sand (7:3, V/V). Pots were transferred in research greenhouse of Ferdowsi University of Mashhad. Pots were irrigated thoroughly before sowing of seeds so that soil mixture was completely wet. After soil reach to field capacity (FC) stage, seeds were sown in pots. After 102 days, when seedlings well established, drought stress was applied as 50%FC, 25%FC and well-watered. At the end of experiment, electrolyte leakage (EL) was measured as an assessment of permeability. This procedure was based on Lutts et al. (Lutts et al., 1995). Relative water content (RWC) was based on Weatherley (Weatherley, 1950 and 1951). Total chlorophyll was measured by Arnon (Arnon, 1949). Proline was determined by Bates et al. (Bates et al., 1973) and at the end, fresh and dry weight of shoot and root and length of root were measured in all treatment. This research was carried out using factorial experiment based on a completely randomized design with 4 replications by 96 pots and treatments were applied in Jan 2008. Statistical analysis was done with MSTATC software. Means compared with Duncan's multiple range tests (DMRT) at 5 % level.

RESULTS AND DISCUSSION

Electrolyte leakage (EL)

Drought stress increased EL (Table.2) whereas the highest concentration of paclobutrazol reduced EL, (Fig.3). The highest EL is manifested in 25% FC (Table.2). Interaction of paclobutrazol, drought stress and cultivars had no significant effect on electrolyte leakage. Paclobutrazol, drought stress, paclobutrazol × drought stress and cultivar × drought stress had significant effect on electrolyte leakage. Paclobutrazol at 0, 20, 30 mgL^{-1} in combination with 25% FC had highest EL but paclobutrazol at 40 mgL^{-1} and 25% FC showed decrease in EL (Table.4). *Festuca arundinacea* L. Master in 25% FC is depicted the highest EL. Two cultivars in well-watered had the lowest EL (Table.3). In a study by Abraham et al. (2004) EL was affected by drought stress in low, moderate, and high drought resistance group of Texas bluegrass, Kentucky bluegrass, and their hybrids. EL increased compared with the initial non stress level for all of three groups of genotypes. The low resistance group had significantly higher EL than the high and moderate group (Abraham et al., 2004).

In fact, the resistant genotypes exhibited better membrane stability than susceptible ones under severe drought stress, as demonstrated by the lower EL (Jiang and Huang, 2001).

Relative water content (RWC)

Drought stress decreased RWC (Table.2). Interaction of drought stress and cultivar had significant effect on RWC. Appling 50% FC in *Festuca arundinacea* L. Master was manifested the highest RWC as like as well-watered however the lowest RWC in *Festuca arundinacea* L. Master and 25% FC was observed (Table.3). Paclobutrazol 30 mgL^{-1} reversed drought stress 50% FC to result the highest RWC (Table.4). At 0, 30, 40 mgL^{-1} paclobutrazol in Barrage and 20, 30 mgL^{-1} paclobutrazol in Master, RWC was in the highest level (Table.5).

Interaction of Paclobutrazol ×drought stress × cultivar treatment on RWC was significant. At 50% FC in combination with paclobutrazol 20 mgL^{-1} in *Festuca arundinacea* L. Master and 50% FC with paclobutrazol 30 mgL^{-1} in *Lolium perenne* L. Barrage had higher RWC. As it resulted, lower RWC is observed in interaction of 25% FC with paclobutrazol 20 mgL^{-1} and without paclobutrazol in *Festuca arundinacea* L. Master and it has the same result with 20 mgL^{-1} paclobutrazol in combination with 25% drought stress in *Lolium perenne* L. Barrage (Table.1).

Table 1. The effect of paclobutrazol and drought stress on relative water content and root dry weight of *Festuca arundinacea L. Master* and *Lolium perenne L. Barrage*.

Treatment			Relative water content	Root dry weight
PP_{333}(mg/L)	Cultivar	Drought stress		
	Master	Well-watered	84.92ab	56.45 bcd
0		50%FC	78.01bcd	33.65 efg
		25%FC	45.09g	19.63 g
		Well-watered	84.72ab	61.5 bcd
20		50%FC	95.51a	20.35 g
		25%FC	49.51g	22.85 g
		Well-watered	84.60ab	65.11 bc
30		50%FC	87.09ab	22.39 g
		25%FC	50.49g	26.26 efg
		Well-watered	80.35bcd	59.99 bcd
40		50%FC	78.14bcd	21.10 g
		25%FC	52.93fg	16.56 g
	Barrage	Well-watered	83.38abc	55.81 bcd
0		50%FC	77.40bcd	43.56 def
		25%FC	74.21bcde	27.55 efg
		Well-watered	75.01bcde	46.35 cde
20		50%FC	63.74ef	28.55 efg
		25%FC	49.74g	22.84 g
		Well-watered	70.69cde	112.4 a
30		50%FC	95.35a	34.60 efg
		25%FC	67.16de	27.00 efg
		Well-watered	81.99abc	72.79 b
40		50%FC	87.60ab	29.05 efg
		25%FC	54.09fg	25.55 fg

†Mean followed by the same letter is not significantly different at 5% level of probability using DMRT.

Table 2. The effect of drought stress on electrolyte leakage, relative water content, total chlorophyll, proline content, shoot dry weight (g), root dry weight (g) and root length (cm) of studied turfgrass cultivars.

Drought stress	Electrolyte leakage	Relative water content	Total chlorophyll content	Proline content	Shoot dry weight	Root dry weight	Root length
Well-watered	11.57 c†	80.71 a	23.80 a	2.308 c	9.364 a	29.16 a	49.47 a
50%FC	33.99 b	82.86 a	21.39 b	4.489 b	8.929 a	23.53 a	45.59 b
25%FC	83.62 a	55.40 b	16.53 c	13.80 a	0.261 b	2.164 b	0.67 c

†Mean followed by the same letter is not significantly different at 5% level of probability using DMRT.

Table 3. The effect of cultivar and drought stress on electrolyte leakage, relative water content, total chlorophyll, proline content and shoot dry weight (g) of studied turfgrass cultivars.

Cultivar	Drought stress	Electrolyte leakage	Relative water content	Total chlorophyll content	Proline content	Shoot dry weight
	Well-watered	10.03 e†	83.65 ab	25.18 a	2.589 d	9.364 a
Master	**50%FC**	36.83 c	84.69 a	22.27 b	5.872 c	8.929 a
	25%FC	88.86 a	49.50 d	19.42 c	16.54 a	0.426 b
	Well-watered	13.12 e	77.77 b	22.42 b	2.026 d	9.329 a
Barrage	**50%FC**	31.15 d	81.02 ab	20.50 bc	3.106 d	9.588 a
	25%FC	78.39 b	61.30 c	13.64 d	11.07 b	0.261 b

†Mean followed by the same letter is not significantly different at 5% level of probability using DMRT.

Table.4. The effect of paclobutrazol and drought stress on electrolyte leakage, relative water content, shoot dry weight (g), root dry weight (g) of studied turfgrass cultivars.

Paclobutrazol (mgL^{-1})	Drought stress	Electrolyte leakage	Relative water content	Shoot dry weight	Root dry weight
	Well-watered	10.66 e†	84.15 ab	8.698 bcd	56.13 bc
0	**50%FC**	37.71 c	77.71 b	10.57 ab	38.61 cd
	25%FC	87.91 a	59.65 c	5.056 f	23.59 d
	Well-watered	13.43 e	79.86 b	11.36 a	53.97 bc
20	**50%FC**	34.31 c	79.63 b	8.166 cd	24.45 d
	25%FC	88.67 a	49.62 d	6.424 def	22.85 d
	Well-watered	10.85 e	77.64 b	7.552 cd	88.75 a
30	**50%FC**	37.59 c	91.22 a	9.595 abc	28.49 d
	25%FC	88.16 a	58.82 c	7.315 cde	26.63 d
	Well-watered	11.35 e	81.17 b	5.191 ef	66.39 b
40	**50%FC**	26.36 d	82.87 ab	9.128 abc	25.08 d
	25%FC	69.75 b	53.51 cd	8.342 bcd	21.05 d

†Mean followed by the same letter is not significantly different at 5% level of probability using DMRT.

Table 5. The effect of cultivar and paclobutrazol on relative water content, proline content, root dry weight (g) of studied turfgrass cultivars.

Cultivar	Paclobutrazol (mgL^{-1})	Relative water content	Proline content	Root dry weight
	0	69.34 b†	7.988 a	36.57 b
Master	**20**	76.58 a	7.772 a	34.93 b
	30	74.06 a	8.684 a	37.92 b
	40	70.47 b	8.887 a	32.55 b
	0	78.33 a	5.227 b	42.31 ab
Barrage	**20**	62.83 c	6.166 b	32.58 b
	30	77.73 a	4.840 b	58.00 a
	40	74.56 a	5.370 b	42.46 ab

†Mean followed by the same letter is not significantly different at 5% level of probability using DMRT.

Abraham et al., (2004) reported that RWC was affected by drought stress in low, moderate, and high drought resistance group of Texas bluegrass, Kentucky bluegrass, and their hybrids during the first test. They indicated that the high resistance group controlled the leaf water status better than the low and moderate groups. Leaf RWC started to decrease below the control level at 18 day under drought alone. The high RWC of resistant genotypes was probably the result of their better ability for water uptake at low soil water potential (Volaire et al., 1998). Effect of paclobutrazol-treated Triticale plants during water stress on relative water content was studied and resulted to increase RWC in this plant (Berova and Zlater, 2003). It is well documented that a critical component of the dehydration tolerance for grasses is cell membrane stability (Crowe et al., 1987; Volaire and Lelievre, 2001).

Total chlorophyll content

Drought stress reduced total chlorophyll content (Table. 2). Paclobutrazol 40 mgL^{-1} had higher total chlorophyll content (Fig. 2). Total chlorophyll content was higher in *Festuca arundinacea* L. Master compare to *Lolium perenne* L. Barrage (Fig.4). Interaction of drought stress and cultivars had significant effect on total chlorophyll content. The highest total chlorophyll content was observed in *Festuca arundinacea* L. Master at well-watered whereas at 25% FC in *Lolium perenne* L. Barrage the lowest chlorophyll content was resulted (Table.3).

As it reported by Jiang and Huang, effect of drought stress on tall fescue and Kentucky bluegrass resulted reduction of chlorophyll content (Jiang and Huang, 2001). But drought stress caused a reduction in total chlorophyll content in *Sesamum indicum* plants (Abraham et al., 2008).

Paclobutrazol alone and in combination with drought increased the total chlorophyll (Abraham et al., 2008). Sebastian et al. (2002) reported enhanced chlorophyll synthesis in *Dianthus* treated with paclobutrazol. The higher chlorophyll content in triazole treated radish may be related to the influence of triazole on endogenous cytokinin levels. It has been proposed that triazoles stimulate cytokinin synthesis that enhances chloroplast differentiation, chlorophyll biosynthesis and prevents chlorophyll degradation (Fletcher et al., 2000).

Proline content

Festuca arundinacea L. Master had higher proline content (Fig. 4). Drought stress increased proline content (Table. 2). *Festuca arundinacea* L. Master at 25% FC resulted higher proline content whereas at well-watered for *Festuca arundinacea* L. Master and *Lolium perenne* L. Barrage, lower proline content is shown (Table. 3). All paclobutrazol concentrations in *Festuca arundinacea* L. Master resulted higher proline content in contrast to *Lolium perenne* L. Barrage (Table. 5).

Batlang manifested that effects of paclobutrazol and drought stress (irrigation withheld for 11 or 16 days beginning 14 DAP) on proline content of greenhouse-grown maize seedlings was determined at 25 DAP. It resulted higher proline content in drought stress with and without paclobutrazol and it reduced to lower content in well-watered with and without paclobutrazol (Batlang, 2006). Under drought-stress conditions, paclobutrazol increased proline content in black locust (*Robinia pseudoacacia*) seedlings (Shen and Zeng, 1993). Drought stress increased proline accumulation in maize (Carceller et al., 1999; Ibarra-Cabalero et al., 1988; Voetberg and Sharp 1991). Proline in maize was important in osmotic adjustment and therefore drought tolerance (Batlang, 2006). Paclobutrazol did not significant effect on proline content. It should also be noted that PB did not significantly increase the proline content in this study probably due to species difference and perhaps the level of moisture stress at the time of sample (Batlang, 2006).

Shoot dry weight

Shoot dry weight was significantly higher in *Festuca arundinacea* L. Master than *Lolium perenne* L. Barrage (Fig. 4). Drought stress caused a reduction in shoot dry weight. Higher shoot dry weight is dedicated to well-watered in two cultivars. However there was no significant difference between two levels of drought stress (Table. 3). At well-watered with paclobutrazol 20 mgL^{-1}, higher shoot dry weight was observed and lower shoot dry weight was shown in 25% FC without paclobutrazol (Table. 4). Also it has been shown by Batlang that drought stress resulted lower shoot dry weight and also paclobutrazol at well-watered caused to increase shoot dry weight in Maize but paclobutrazol caused to decrease shoot dry weight in drought stress condition (Batlang, 2006).

Root dry weight

Paclobutrazol, drought stress, cultivar and paclobutrazol × drought stress × cultivar had significant effect on root dry weight. Root dry weight reduced with drought stress condition (Table. 2). At 30 mgL^{-1} paclobutrazol, higher root dry weight is resulted (Fig.1).The secondary characteristic changes observed in triazole treated plants include morphological changes such as, reduced shoot growth and increased root growth (Zhu et al., 2004). In another report, paclobutrazol retarded shoot growth, enhanced lateral root growth, produced darker green leaves, and resulted in a more extensive root system (Lin and Zhang, 1999). *Lolium perenne* L.Barrage had higher root dry weight (Fig.4). The highest root dry weight resulted in *Lolium perenne* L. Barrage at well-watered with 30 mgL^{-1} paclobutrazol (Table.1). At 30 mgL^{-1} paclobutrazole treatment on *Lolium perenne* L. Barrage showed higher root dry weight (Table.5). Well-watered condition with paclobutrazol 30 mgL^{-1} showed higher root dry weight (Table.4). In agreement to our results, Jiang and Huang showed that root dry weight in Kentucky bluegrass reduced with drought stress at 20-40 cm soil depth (Jiang and Huang, 2001). In Maize, drought stress is resulted a reduction in root dry weight. Paclobutrazol at drought stress caused to increase root dry weight in Maize (Batlang, 2006).

Root length

Drought stress had significant effect on root length. Root length was the highest in well-watered and lower in 25% FC (Table. 2). Carcellar et al. (1999) reported that, root growth is reduced during drought stress in maize seedlings.

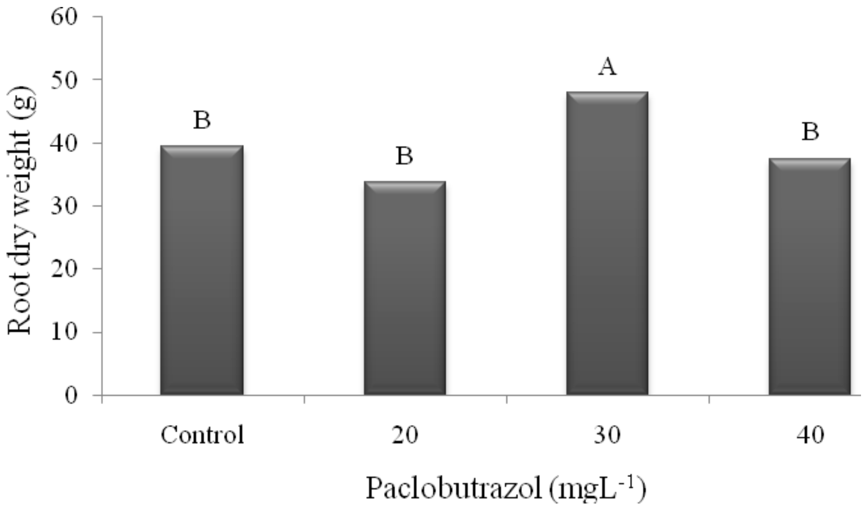

Figure 1. Effect of paclobutrazol on root dry weight of studied turfgrass cultivars. Columns with the same letters are not significantly different using DMRT at 5% probability level.

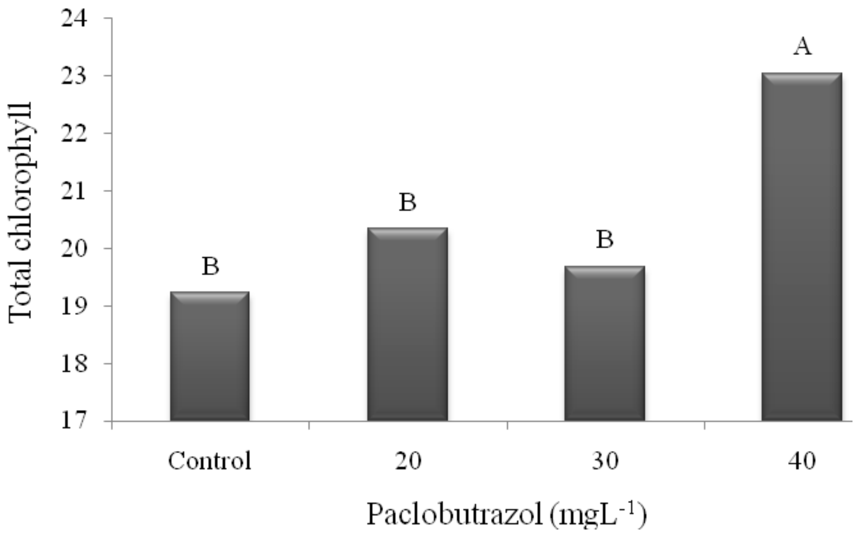

Figure 2. Effect of paclobutrazol on total chlorophyll of studied turfgrass cultivars. Columns with the same letters are not significantly different using DMRT at 5% probability level.

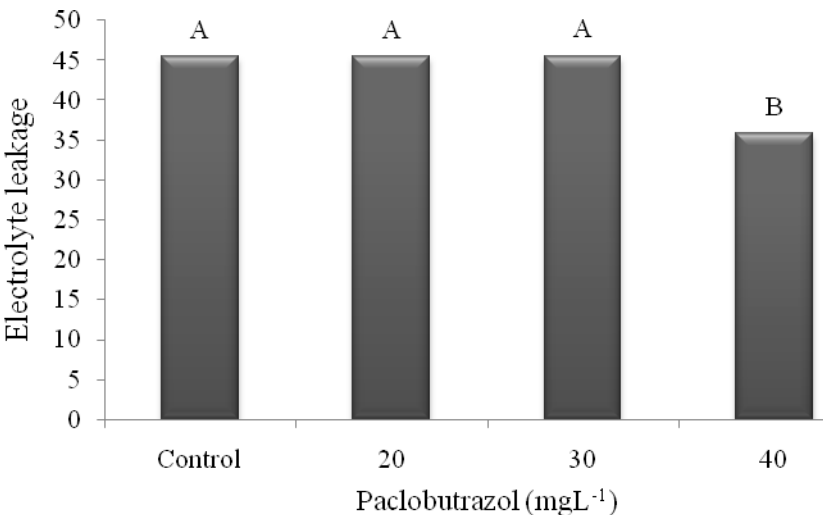

Figure 3. Effect of paclobutrazol on electrolyte leakage of studied turfgrass cultivars. Columns with the same letters are not significantly different using DMRT at 5% probability level.

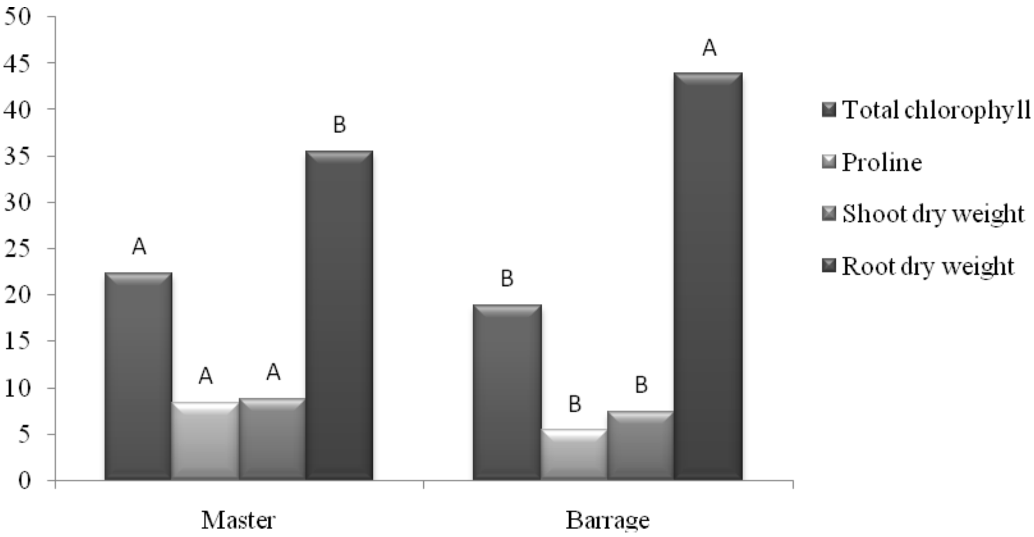

Figure 4. Effect of two cultivars on total chlorophyll, proline, shoot dry weight and root dry weight of studied turfgrass cultivars. Columns with the same letters are not significantly different using DMRT at 5% probability level.

CONCLUSIONS

Seed-treatments with triazoles may have some promise for altering turfgrass growth and response to drought such that the seedlings are more likely to survive. These findings suggest some good avenues of approach for continuing studies. As it showed in study, seed soaking of *Festuca arundinacea* L. Master with paclobutrazol resulted higher quality in some treatments but the results from this work also showed that seed soaking of turfgrass with paclobutrazol effect on plant physiological and morphological characters was short lived. Hence it can not manage turfgrass quality at drought stress condition in all physiological factors. But as it observed in this study, it depends on cultivar, paclobutrazol concentration usage. In regard with this experiment, paclobutazol at the highest concentration could reversed drought stress effects on electrolyte leakage. Total chlorophyll content and proline content was resulted higher level in *Festuca arundinacea* L. Master. In this study also at higher drought stress condition higher total chlorophyll content was detected in *Festuca arundinacea* L. Master.

Taking everything into account, seed soaking of turfgrass with paclobutrazol could affect growth and physiological traits of turfgrass cultivars during drought stress but it related to concentration of paclobutrazol and cultivar. However according to findings from other works it seems that factors such as method of application, other treatments of seeds before treatment with paclobutrazol and duration of application could alter the longevity of paclobutrazol (Batlang, 2006; Shahrokhi et al, 2008).

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