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Effect of Trinexapac-Ethyl Growth Inhibitor and Drought Stress on Some Morpho-Physiological Traits of Wheatgrass (*Agropyron cristatum* L.)

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ABSTRACT

The wheatgrass (*Agropyron cristatum* L.), is a plant with potential source of turf in arid and semi-arid regions. It can be also cultivated in order to prevent the soil erosion by coverage of earth's surface. Drought stress is one of the most important factors that influence growth and productivity of plants in arid and semi-arid condition. In order to evaluate the effect of trinexapac ethyl concentrations (0, 0.25 and 0.5 kg/h) and drought stress (25, 50 and 75% of field capacity) treatments on some wheatgrass morphological traits, a pot experiment was conducted as factorial in completely randomized block designs with four replications in research greenhouses of Islamic Azad University, Isfahan (Khorasgan) Branch during 2015. The results showed that the maximum plant height and the lowest amount of proline obtained in 75% drought stress treatment. The lowest fresh weight of the aerial parts and the relative water content (RWC) were observed in 25% drought stress treatment. The highest dry weight of the shoots was measured in control, 0.25 kg/h trinexapac-ethyl and 75% drought stress treatments. The highest proline content and the lowest height of the plants were observed in 25% drought treatment with 0.5 kg/h of trinexapac-ethyl. In general, the research results indicated that under drought stress condition, application of trinexapac-ethyl by increasing the amount of proline and relative water content reduced stress damage and increased resistance to drought stress in the wheatgrass.

Keywords: *Agropyron cristatum* L, Proline, Relative water content, Plant growth regulator.

INTRODUCTION

The wheatgrass (*Agropyron cristatum* L.), comprises over 150 species, which 19 species are found in Iran. This type of lawn is compatible with different soil types and its establishment in the soil is easy. Wheatgrass is very resistant to drought stress, adapts to different environmental conditions (Sheikh-Mohammadi *et al.*, 2015; Turgeon, 1999) and introduced as a plant with potential source of turf for arid and semi-arid regions (Bayat *et al.*, 2016). Various species of wheatgrass are found in most rangelands of Iran and are considered as rangeland plants. Some species of wheatgrass are cultivated in order to prevent the soil erosion by coverage of earth's surface with roots (Sadeghi *et al.*, 2014).

Drought is one of the most important factors limiting the production of successful agricultural products around the world (Mahajan and Tuteja, 2005). Water scarcity and rapid decline in water resources are increasingly becoming the most important issue in many parts of the world, specifically in dry and semi-arid regions of the world. Actually, drought stress is a condition in which the cells and tissues are in a position in which no complete inflammation is observed (Rashidi and Yadegari, 2014). The range of effects of drought stress varies from a slight decrease in water potential to permanent withering and dryness of the plant. When the moisture content in the root zone is significantly reduced, the plant does not have water absorption ability, the plant is under drought stress condition. In drought condition, osmotic stress and disordering the ionic balance, can lead to disruption of the plant's activities, and finally, growth decline (Chai *et al.*, 2010; Bian and Jiang, 2009). Environmental stresses such as drought affect germination, growth and performance of plants (Altahawa *et al.*, 2013; Abedi and Pakniyat, 2010). Specific traits such as roots system features, the relative water content of the leaves and the osmoregulation ability affect the plant's function directly or indirectly during the drought stress conditions (Abbasi *et al*., 2014).

Today, plant growth regulators can play an important role in the management of the grass, among which we can refer to trinexapac-ethyl as the growth inhibitors. Trinexapac-ethyl, with the formula $C_{11}H_{12}O_5$, is one of the growth regulators in the management of lawns. Trinexapac-ethyl has great help in management of sports fields such as golf and football (Etemadi *et al.*, 2015). Under salinity stress, trinexapac-ethyl increases the resistance of the *Poa pratensis* lawn and increases the antioxidant capacity (Stir, 2006; Beasley & Baranham, 2005). Application of trinexapac-ethyl significantly reduced shoot growth in creeping bentgrass (*Agrostis stolonifera* L.) and hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. Cynodon transvaalensis Burtt-Davy] (Wherley and Sinclain, 2009). This growth regulator reduces cellular elongation in green tissue by preventing gibberellin biosynthesis. Trinexapac-ethyl is a cyclohexanedione that prevents the conversion of GA_{2O} to GA_1 by 3- beta hydroxylase and such as paclobutrazol inhibits the oxidation of ent-kauran to ent-kauronic acid, which is the primary step in the synthesis of Gibberellin (Heckman *et al.*, 2001; Lickfeldt *et al.*, 2001).

Under drought stress, trinexapac-ethyl increased content of soluble sugar content and proline considerably and improved drought tolerance in wheatgrass (Etamadi *et al.*, 2015) and reduces the height of aerial parts in 4 to 6 weeks after treatment (Pessarakli, 2008).

The main objective of the present study was to evaluate the effects of trinexapac-ethyl growth inhibitor on some morphological characteristics of wheatgrass under drought stress conditions.

MATERIALS AND METHODS

The present study was carried out in four replications at the Greenhouse condition of Islamic Azad University, Isfahan, Iran, in 2015. Greenhouse day temperatures was 21 to 26 °C, with night temperatures somewhat lower (16 to 21°C). Relative humidity was set at 70 percent under natural light with average of 800 μmol m^{-2} s^{-1}. Study was done as a factorial experiment based on completely randomized design. The first factor was drought stress in three levels (25, 50 and 75% of field capacity) and the second factor was trinexapac-ethyl consist of three levels (0, 0.25 and 0.5 Kg/ha). Seeds of wheatgrass were purchased from local market in Isfahan, Iran. Culture medium consists of mixture of equal ratios of garden soil, cow manure (with the average total N content of 3%) and sand. The pH, Ec and field capacity of the studied soil were 7.5, 5.2 and 48%, respectively.

In each pot (with 18.5 cm diameter and 21 cm length with volume of 3.5 liter), 30 g/m^2 of seed were planted and covered with 1 cm of cow manure. Then, the pots were irrigated immediately and irrigation was carried out repeatedly 2-3 times a day until the seeds germinated. After sufficient growth (height of 4 cm) of grass, a tensiometer (model 2725, Soil moisture, USA) was used to apply drought treatments (25, 50 and 75% of field capacity). In current study, growth period was taken 2 month and trinexapac-ethyl was sprayed twice (once per month) by a hand sprayer during late afternoon. The volume of the spraying was maintains just to cover completely the plant foliage until drip.

Evaluated characteristics

In order to measure the fresh weight of the aerial parts (stem, node, internode, axillary bud, petiole, leaf and apical bud), the wheatgrass was collected after topping from 4 cm above ground by a scissor. Then removing the plants from the rinsing the mud with the water pressure and removed of the excess moisture and then immediately weighed by a digital scale with a precision of 0.0001. To measure the fresh and dry weight of the plant samples, the plants at first were weighted to determine fresh weight and then were placed in an oven at a temperature of 65°C for 48 hours and then weighed by the balance (Wherley and Sinclain, 2009).

The proline content of fresh weight was measured by spectrophotometer (Spectronic Instruments, Rochester, NY) by absorbing light at a wavelength of 520 nm (Bates *et al.*, 1973).

The relative water content (RWC) of the leaf was calculated as percentages. For this purpose fresh leaves samples were collected from all pots, weighted [fresh weight (FW)], and placed in a petri dish filled with distillated deionized water for 24 hours. After removing surface water on the leaves by tissue paper, the leaves were weighted [turgor weight (TW)] and then were dried at 80 °C for 48 hours and weighted [dry weight (DW)]. Leaf RWC was calculated according to the following formula (Barrs and Weatherley, 1962).

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Statistical Analysis

This research project was conducted in a factorial experiment based on completely randomized design with four replications. The obtained data were analyzed by SAS software (Ver. 9.2) and means were compared using Duncan's Multivariate Range Test at P≤ 0.05.

RESULTS AND DISCUSSION

Height of the plant

The analysis of variance in this study showed that the effect of drought and trinexapac-ethyl growth inhibitor on plant height was significant at the 1% level and the interaction between drought and trinexapac-ethyl on plant height was significant at the level 5% (Table 1). In current study, the highest plant height (34.55 cm) was observed in the treatment with 75% of the field capacity without the use of trinexapac-ethyl. Meanwhile, the lowest plant height (4 cm) was observed in the treatment of 25% field capacity with 0.5 kg/ha trinexapac-ethyl (Figure 1).

Table 1. Analysis of variance of drought and Trinexapac-ethyl on plant characteristics of wheatgrass

		Mean squares				
Source of Variation	**Degree of Freedom**	**Plant High**	**Fresh Weight (aerial part)**	**Dry Weight (aerial part)**	**Relative Water Content**	**Proline**
Replication	3	2.00 ns	1.69*	0.05 ns	51.35*	50314 ns
drought	2	34.55**	6.52**	4.20**	1122.50**	8361624**
Trinexapac-ethyl	2	13.06**	0.71 ns	0.39*	58.62*	1372600**
drought × Trinexapac-ethyl	4	4.58*	0.11 ns	0.17 ns	5.07 ns	185879**
Error	24	1.29	0.52	0.10	15.38	42704
Cv (%)	-	13.39	19.34	17.78	14.80	9.50

*, *: Significant at 1% and 5%, respectively; ns: not significant.

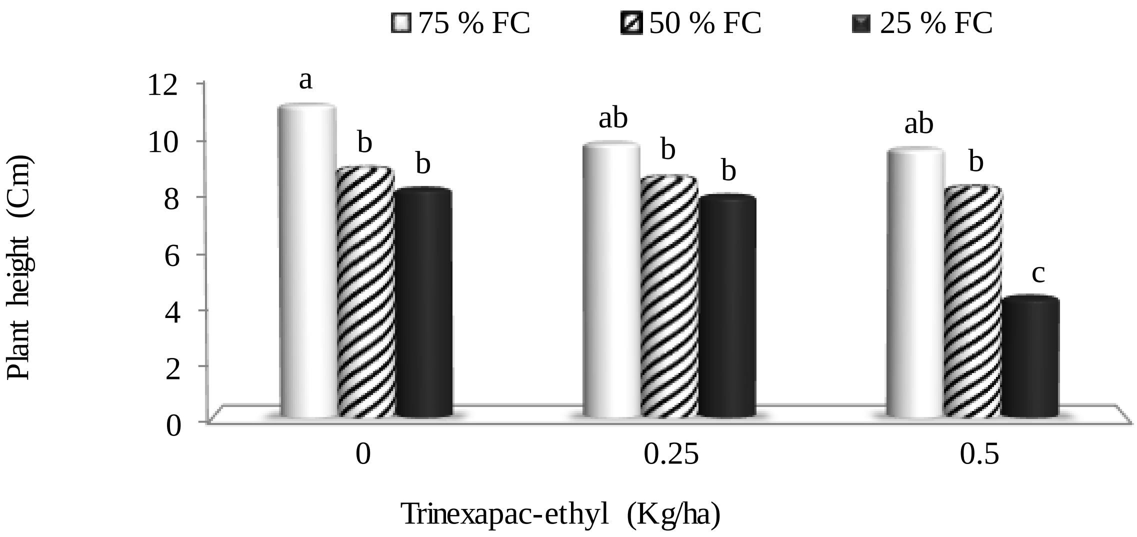

Figure1. The mean comparison of drought and trinexapac-ethyl effect on the plant height

A reason for the reduction of plant height under the influence of the trinexapac-ethyl regulator is that it is generally used to reduce height and frequency of topping (Lickfeldt *et al*., 2001). Since trinexapac-ethyl involved in the synthesis of gibberellic acid and prevents the conversion of GA_{2O} to GA_1, it also inhibits the activity of the enzyme 3 beta-hydroxylase, thereby reducing height of the plant (King *et al.,* 1997). It also increases photochemical activity and has no negative effect on the fresh and dry weight of roots (Fagerness and Yelverton, 2001; Zhang and Sehmidt, 2000). On the other hand, trinexapac-ethyl, that being absorbed in leaves and crowns, prevents cell elongation through reduction of the production of gibberellic acid, and continuously decreases the number of toppings, increases the visual quality, improves the growth characteristics and, as a result, resistance to environmental stress (Rohallahi and Kafi, 2011).

Fresh and dry weight of the aerial part

The results of analysis of variance showed that the effect of drought treatment at 1% level on fresh and dry weight of aerial part was significant (Table 1). Trinexapac-ethyl had significant effect (5%) on dry weight of aerial part. The results of mean comparison of drought treatment revealed that the highest fresh weight of aerial part in treatments with 75 and 50% of the field capacity was observed with 4.42 g and 3.83 g respectively, which did not show any significant difference. The lowest fresh weight of aerial part was obtained in the treatment of 25% of the field capacity with 2.96 g (Table 2).

The results of mean comparison under effect of drought condition indicated that the highest dry weight of the aerial part (2.34 g) was obtained in 75% of the field capacity. Meanwhile, in the treatment of 25% of the field capacity, it was observed with a minimum of

1.16 g (Table 2). Also, the dry weight of the aerial part did not show any significant difference in control and 0.25 kg/ha of trinexapac-ethyl (1.96 g and 1.76 g, respectively). The lowest dry weight of the aerial part (1.60 g) observed in 0.5 kg/ha treatment (Table 2).

Trinexapac-ethyl inhibits aerial lengthening by Gibberellin synthesis, so it reduces dry and wet weights (Rademacher, 2000). Johnson (1997) investigated the effect of trinexapac-ethyl on the Tifway bermuda grass and observed that the fresh and dry weight of the topped portion was reduced by 28 to 75% by using trinexapac-ethyl. In addition, Huang and Gao (2000) also reported that the fresh and dry weight of the aerial part decreased under drought stress conditions.

Relative water content

The results of variance analysis indicated that the effect of drought at 1% level and the effect of trinexapac-ethyl at the level of 5 kg/ha had significant effect on the relative water content the relative water content in wheatgrass (Table 1). Also, the means comparison of drought effect showed that the highest relative water content (37%) was observed in 75% of the field capacity. In treatment with 25% of field capacity the relative water content was less than other treatments (Table 2). Also, the results of the comparison of the means effect of trinexapac-ethyl on the relative water content indicated that the highest effect (29%) was obtained with 0.5 kg/ha of trinexapac-ethyl and no significant difference was observed between control treatments and 0.25 kg/ha of trinexapac-ethyl (Table 2).

The relative water content of the plant is one of the most important factors in determination of the balance between the water supply to the leaf and the speed of transpiration. So, it is considered as an important indicator in choosing the drought tolerant plants (Blum and Ebercon, 1981). Water stresses in protoplasts that sensitive to dehydration cause damage to membrane systems. So, the continuity of the membranes of the plastids, mitochondria, nuclei, dictyosomes and cell membranes is reduced. Permeability of membranes to salts soluble in the low-potential water confirms the theory of membrane degradation during water stress (Oliver, 1991).

In this study, by increasing the concentration of trinexapac-ethyl, the relative water content of the leaf increased. Trinexapac-ethyl increased the relative water content of the Kentucky bluegrass, which is consistent with resulted of present study (Xu and Huang, 2011). Also, by increasing the concentration of trinexapac-ethyl in *Festuca arundinacea* cultivar Rebel increasing the leaf relative water content observed (Sheikh-Mohammadi *et al.*, 2015). Trinexapac-ethyl inhibits aerial lengthening by Gibberellin synthesis, so it reduces dry and wet weights (Rademacher, 2000). Johnson (1997) investigated the effect of trinexapac-ethyl on the Tifway bermuda grass and observed that the fresh and dry weight of the topped portion was reduced by 28 to 75% by using trinexapac-ethyl. In addition, Huang and Gao (2000) also reported that the fresh and dry weight of the aerial part decreased under drought stress conditions.

Table 2. Effect of drought stress and Trinexapac-ethyl on some traits of wheatgrass

treatments	levels	Fresh weight of the aerial part (g)	Dry weight of the aerial part (g)	Relative water content (%)
Trinexapac-ethyl	Control	3.97[a†]	1.96[a]	25.50[b]
	0.25	3.77[a]	1.76[ab]	24.96[b]
	0.5	3.49[a]	1.60[b]	29.03[a]
Drought stress	25	2.96[b]	1.16[c]	17.50[c]
	50	3.83[a]	1.82[b]	25.26[b]
	75	4.42[a]	2.34[a]	36.72[a]

[†]Values followed by the same letter whiten columns were not significantly different at 5% level (DMRT).

Proline content

The results of variance analysis indicated that the effect of drought treatment, trinexapac-ethyl and the interaction effects of drought and trinexapac-ethyl on plant proline content were significant at 1% level (Table 1). According to the results, the highest amount of proline (3726 µg/kg FW) was observed in 25% of field capacity and 0.5 kg/ha of trinexapac-ethyl which showed a significant difference with other treatments. Also, the lowest amount of proline (1336 µg/kg FW), was observed in 75% of the field capacity without using the trinexapac-ethyl, which did not show significant difference with the treatments of 75% field capacity with 0.5 and 0.25kg/ ha of trinexapac-ethyl (Figure 2).

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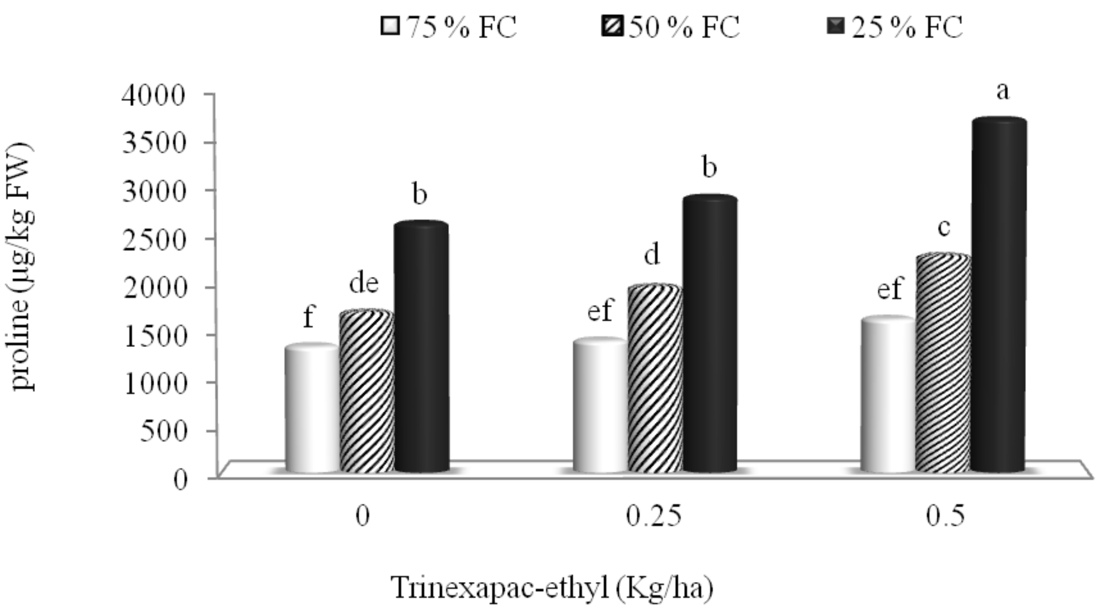

Figure 2. The various levels of drought and trinexapac-ethyl effect on the amount of proline

In current study, the effect of proline was also analyzed. Increasing resistance of plants to drought stress conditions under the influence of trinexapac-ethyl could be an increase in compatible solutions, including proline. In fact, the proline accumulation and chlorophyll fluorescence prevention are among the drought tolerance markers that make the plant adaptable to water scarcity conditions (Keyvan, 2010; Zhu and Gong, 2005). By sweeping hydroxyl radicals under oxidative stress conditions, proline prevents cell membrane deterioration and preserves the structure of proteins and enzymes as an osmolite (Blum, 2011; Talebi *et al.,* 2013). Changing the proline content in relation with drought stress helps maintain the water level in plant (Simova-Stoilova *et al.,* 2008). Increasing the concentration of this amino acid that helps with osmotic regulation can result from prevention of proline decomposition, reduction of protein synthesis or increase of protein breakdown. It can be assumed that growth inhibitors, including trinexapac-ethyl, are effective preventers of proline decomposition, reducers of proline content and adjusters of its biosynthesis under drought stress.

By metabolite accumulation such as proline, soluble carbohydrates and some ions, most plants adjust the osmotic balance and deal with environmental stresses such as drought in order to maintain their water level (Hosseinian-Khoshroo *et al.,* 2013). Also, trinexapac-ethyl improved the water relationships in the plant and increased relative water content of the leaves. The relationship between the relative water content of the leaves and the water potential of the tissues may be due to the increase of compatible soluble ingredients, glucose and the increase of proline (Amiri-Nasab *et al.,* 2013).

Furthermore, the amount of proline in the plant was increased to maintain osmotic balance, protect the membranes and macromolecules (Nayyar, 2003; Mahajan and Tuteja, 2005). The synthesis and accumulation of osmolites are different between plant species and different varieties of a species (Pinhero *et al.,* 2001). It can be argued that osmolites accumulate in cytosol can adjustment osmotic pressure. Also, this increasing trend of soluble sugars is consistent with proline (Jiquan *et al.,* 2000). The accumulation of proline in the plant under stress conditions can help maintain plant balance. The proline accumulation in the cells is accompanied by the prohibition of protein denaturation. Maintenance of the structure and activity of enzymes and also the membrane from ROS damage under the water scarcity conditions and also catabolism of proline by the dehydrogenase enzyme is stopped in this situation (Teulat *et al.,* 2006; Chaves *et al.,* 2002).

CONCLUSION

In general, trinexapac-ethyl growth regulators with increasing proline and relative water content of plants under drought stress conditions reduced stress damages and increased the drought stress resistance in wheatgrass. In addition, according to the current results, drought stress reduced the weight of plant tissue and the relative water content of the plant, which reduced the stem growth and fresh and dry weight of the plant organs. That is why we count it as one of the most important plant traits in response to drought stress. Therefore, this growth inhibitor can be used to increase the resistance of wheatgrass to drought stress and improve the growth characteristics of this plant.

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