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Drought Stress Mitigation of Bermudagrass Grown in Farmyard Manure Media with Coated Seed

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A B S T R A C T

Bermudagrass is one of the most commonly used warm-season turfgrasses worldwide. In this study, the tolerance to drought stress with uncoated seeds and seeds coated with biological preparations containing *Trichoderma harzianum*, Bacillus subtilis, and *Bacillus megaterium* in bermudagrass were assessed separately in soil media and farmyard manure respectively. Seeds of Gobi (registered cultivar) were used as plant material. Seeds of Gobi were planted in 2 different growing media, uncoated grass seeds were sown in the A (A = garden soil + river sand + peat) group growing medium and coated seeds were sown in the B (B = garden soil + river sand + farmyard manure) group growing medium. As the coating material, which is a new generation seed coating preparation containing *Trichoderma harzianum*, *Bacillus subtilis* and *Bacillus megaterium*, was preferred. Io create drought stress [I0 (0), I1 (25%), I2 (50%) and I3 (75%)], 4 doses of irrigation regimes were determined. The traits of clipping yield, shoot dry weight, root dry weight, leaf burning and turfgrass quality were all affected by levels of drought stress. However, farmyard manure and seed coating mitigated the adverse effects of drought stress.

1. Introduction

Bermudagrass (*Cynodon species*) is one of the most commonly used turfgrasses in tropical and subtropical regions (Arslan and Cakmakci, 2004; Karimi et al., 2018). Bermudagrass species form a very dense, strong and thick turfgrass layer (Juraimi, 2001) with narrow width leaf, which makes the grass surface vary between thin, very thin or medium-textured. The color of the leaves ranges from very light green to dark green, and growth occurs entirely horizontally via stolons and rhizomes (Ihtisham et al., 2018). This specie produces a vigorous, low-growing turfgrass stand with high density and tolerances to both traffic and drought stress (Xiong et al., 2007).

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Furthermore, bermudagrass is a turf species that is ideally suited for golf courses, sports fields, parks, and recreational places in hot, dry, or tropical regions. As known, urban green areas have grown in popularity over the previous decade (Ihtisham et al., 2020).

Drought is defined as a prolonged absence of precipitation that causes a significant decrease in soil water content and plant growth (Janmohammadi et al., 2008). Drought directly affects the growth and development of the plant, resulting in decreased yield potentials (Tiryaki, 2016; An and Liang, 2012). It is estimated that drought has affected an approximately 25-30% area used for agricultural purposes.

In agricultural terms, drought is related to the amount of water that the plant can absorb with its roots from the field during the growth period rather than the total amount of precipitation throughout the year (Blum, 1989; Kalefetoglu and Ekmekci, 2005). Plants that experience water deficiency during their growth period suffer from stunted growth and yield losses (Tuberosa, 2012; Turner et al., 2014). Furthermore, drought is one of the abiotic stress factors that have the significant impact on plant growth and development. (Farooq et al., 2009). Drought is becoming more severe in Türkiye as the temperature rises and precipitation decreases. Therefore, it is necessary to take measures to reduce the effects of drought on agriculture (Ozturk, 2015).

In the fight against such stress factors, the development of resistant/tolerant species and varieties is seen as the most permanent and practical solution (Samancioglu and Yildirim, 2015). However, since these methods and applications are expensive and time-consuming, so more practical and economic solutions are being studied. Nowadays, seeds are coated with several active substances to give tolerance to plants grown under stress conditions (Kaufman, 1991). Furthermore, the coating approach promotes plant growth and development, and it also includes materials that are resistant to various diseases and pests (Taylor et al., 1998). For this purpose, the transfer of rhizobacteria group bacteria to seeds for biocontrol purposes is one of the appropriate techniques (Deaker et al., 2004; Junges et al., 2013; Vavrina and McGovern, 1990).

Farmyard manure is an organic material mainly produced from animal excreta, other than in the case of green manure, which is mainly composed of plant sources, and it may be utilized as an organic source of nutrients in the soil (Wu and Ma 2015). These are comparatively inexpensive and eco-friendly inputs (Dauda et al. 2008). Farmyard manure also aids in improving soil's physical characteristics and enhances the chemical characteristics of the soil by strengthening soil organic nitrogen, carbon, potassium, and phosphorous content in the soil (Bayu et al., 2006a). Chahal et al. (2020) reported that it is evident that adding farmyard manure to the soil increases soil health and plant development.

The aim of this study is to examine the growth of uncoated seeds in A media and coated seeds in B media to observe whether applying farmyard manure and biological seed coating preparations containing *Trichoderma harzianum*, *Bacillus subtilis* and *Bacillus megaterium* results in mitigating the effects of drought stress in bermudagrass.

2. Materials and Methods

The trials were performed in plastic pots with a height of 22 cm and a diameter of 20 cm at the Akdeniz University of Agriculture Faculty Research Area. The trials were conducted in a fully open field environment between May 2021 and September 2021. During the trial (May-September) minimum, maximum and average temperatures with relative humidity values of the trial area in the province of Antalya were recorded and, are given in Table 1.

Table 1. Meteorological data of Antalya province, May-September 2021

Months	Ave. temp. (ºC)	Min. temp. (ºC)	Max. temp. (ºC)	Relative humidity (%)
May	22.93	16.34	28.75	64.44
June	25.45	19.35	31.32	61.11
July	31.28	24.95	37.68	46.07
August	31.13	24.66	37.48	47.71
September	26.08	19.96	31.95	56.85

For trial sieved garden soil was used. Pots were filled with 2 different growing media A and B mixed in a 4:1:1 volumetric ratio for coated and uncoated seeds respectively.

A media= garden soil + river sand + peat (4:1:1), used uncoated seeds

B media= garden soil + river sand + farmyard manure (4:1:1), used coated seeds

This prepared growing medium mixture was analyzed in laboratories and some of its chemical and physical properties are given in Table 2.

Bermudagrass-Gobi variety was used in this experiment. Uncoated grass seeds were sown in the A group growing medium, while coated seeds were sown in the B group growing medium. As the coating material, the commercial product called Panoramix, which is a new generation seed coating preparation containing *Trichoderma harzianum*, *Bacillus subtilis* and *Bacillus megaterium*, was preferred. Panoramix was obtained from Koppert Biological Systems. The coating preparation was applied to bermudagrass seeds at a rate of 4 L/1000 kg of seeds.

Table 2. The chemical and physical properties of the soil and sand mixture used in the plastic pots

A Media			B Media		
Characteristics	Determined amount	Evaluation	Characteristics	Determined amount	Evaluation
pH	7.8	Light alkali	pH	8.0	Light alkali
EC, µS/cm	222	Low	EC, µS/cm	597	Medium
Lime, %	29.4	Too limy	Lime, %	18.0	Too limy
Organic material, %	10.5	Low	Organic material, %	11.9	Low
Total nitrogen, %	0.336	Low	Total nitrogen, %	0.728	Enough
Phosphorus, ppm	0.8	Low	Phosphorus, ppm	1.45	Low
Potassium, ppm	34.32	Low	Potassium, ppm	498.5	Too much
Calcium, ppm	520.7	Enough	Calcium, ppm	658.7	Enough
Magnessium, ppm	27.0	Enough	Magnessium, ppm	50.25	Enough
Iron, ppm	1.72	Enough	Iron, ppm	7.66	Too much
Manganese, ppm	0.77	Low	Manganese, ppm	0.86	Low
Zinc, ppm	0.56	Enough	Zinc, ppm	0.63	Enough
Copper, ppm	1.14	Enough	Copper, ppm	1.46	Enough

Grass seeds were sown in the prepared pots with the seed number adjusted to cover the pot surface on May 25, 2021 and grown until they completely covered the surface of the pot. From the sowing till the end of the experiment, all pots were fertilized once every two weeks with NPK (15.15.15) compound fertilizer at a rate of 5 gr/m^2/month. The plants were reaped from a height of 4 cm once every 2 weeks. The grass completely covered the surface of the pots as well as the drought practices also started on 20th August and ended on September 30.

To create drought stress [I0 (0), I1 (25%), I2 (50%) and I3 (75%)] 4 doses of irrigation regimes were determined. Control pots (I0) were not treated with water during the trial period. As a result of the weighing, the amount of water lost in the pots was determined and water was given in appropriate amounts for the selected irrigation regime.

The experiment design was a level factorial design with 4 replicates for each stress level, with divided parcels consisting of 2 growing media and 4 drought stress levels forming the main parcels of varieties of turfgrass and the sub-parcels of the drought stress levels.

During the experiment, leaf burning rate (0-100% scale), turfgrass quality (1-9 scale), Clipping yield per pot (g), root dry weight (g) and shoot dry weight (g) values were measured.

The leaf burning (drying) rate of the grass leaves in each pot was determined using a 0–100% scale. On this scale, 0% indicates that there is no burning in the leaves, and 100% indicates that all the leaves in the pot are completely burned (Uddin et al., 2009).

Grass quality was evaluated every 15 days using a visual 1-9 quality scoring scale. On this scale, 1 = very bad with completely dead/yellow grass texture, 6.0 = minimum acceptable grass quality, 9.0 = ideal shoot density, texture, green color and homogeneity with excellent quality (Alshammary et al., 2004). Grass clipping was performed every two weeks throughout the trial at a considerable height, leaving 2 cm of grass on the plant., and the shot dry weight was recorded after drying for two days at 75°C.

The date was collected during the field trial were statistically analyzed by ANOVA and Duncan's multiple range test for comparisons were determined by SAS 9.3 (SAS Institute, 2011).

3. Results and Discussion

Drought stress significantly influenced the growth and development of plants and grass quality in Bermudagrass (Table 3). However, the effect of drought stress exhibited distinct results in both the growth medium and the biological seed coating applications (A and B media) (Table 3). In Bermudagrass grown clipping yield levels under drought stress conditions ranged between 2.31 g and 3.17 g. When the drought stress severity increased, the clipping yield decreased. However, as observed in Table 3, the clipping yield was significantly affected by the growth medium. Shoot dry weight varied between 5.14 g and 9.56 g, whereas root dry weight ranged between 4.06 and

6.36 g. Drought stress negatively affected shoot and root growth in Bermudagrass. When the leaf burning rate, an essential quality attribute of grass plants, is evaluated, it has been observed that drought stress induces a leaf burn rate of 96.67 % (Table 3). Turfgrass quality, which is the most important indicator of general plant development in turf areas, was also considerably affected by drought stress, and drought at I0 level had the lowest (1.00) degree, while it had the highest value (8.50) in I3 application. When Table 3 is thoroughly evaluated, it has been seen that the growth medium (GM) treatments (A and B) likewise cause statistically significant variations in terms of the evaluated parameters (except for root dry weight). In addition, the GM*D interaction was also revealed to be important for shoot dry weight, leaf burning, and turfgrass quality.

Table 3. Mean comparison of main effects drought stress levels in Bermudagrass

Drought stress	Clipping yield (g)	Shoot dry weight (g)	Root dry weight (g)	Leaf burning (%)	Turfgrass quality (1-9)
I0	2.55 AB[x]	5.74 B	4.45 A	96.67 A	1.00 D
I1	2.31 B	5.14 B	4.06 A	77.17 B	4.50 C
I2	2.88 AB	9.56 A	6.32 A	39.00 C	6.00 B
I3	3.17 A	8.91 A	6.36 A	7.33 D	8.50 A
Growing media (GM)	***	***	Ns	***	***
Drought (D)	*	**	Ns	***	***
GM*D	ns	**	Ns	**	*

[X]: Means with different letter(s) in each trait is significantly at 5% probability level according to Duncan's multiple range test. *, **, ***, ns: represent significant at 5%, 1%, 0.1% and non-significant, respectively.

Drought resistance mechanisms of turfgrass species fall into the categories of drought avoidance (or desiccation avoidance), drought tolerance, and drought escape (Pornaro et al., 2020).

In this study, the effect of drought stress on the Gobi Bermudagrass variety was studied. As multiple studies (Aydinsakir et al., 2014; Baldwin et al., 2006; Zhou et al., 2013; Carrow and Duncan, 2003) reported, drought stress severely affected the turf growth and quality and increased the rate of leaf burn in two different growing conditions were determined. Etemadi et al. (2005) revealed that considerable variations exist among the accessions regarding their drought resistance. Also, a selection of drought resistance among accessions may be achieved based on the total root length. Also, leaf burning provides a practical evaluation of total turfgrass drought resistance (Carrow and Duncan, 2003).

Aydinsakir et al. (2014) in their study to assess the responses of Seaspray and TifBlair grass varieties to water restriction, observed that drought stress severely damaged the quality of the grass by causing leaf burns. From this point of view, it has been discovered that the delivery of water up to 75 % of the evaporation gives both water savings and continuity in grass quality. Despite the negative effects of drought stress, plants with additional farmyard manure and biological seed coating preparation treatments (Application B) were less affected by drought stress (Table 3).

Similarly, it is mentioned by Arslan and Citak (2016) that farmyard manure techniques assist in the continuation of growth and development by mitigating the negative effects of existing stress such as salinity and drought on plants. Again, Bicakci et al., (2020) reported that biological seed coating increases germination and development by minimizing the effects of drought stress in the alfalfa plant. Sheaffer et al. (1988) revealed that seeds coated with chemical and biological solutions are particularly beneficial in providing healthy seedling development and better plant establishment under harsh environmental conditions. Essentially, the most prominent characteristic of the coating approach is to promote to plant development under varied stress conditions (Taylor et al., 1998). In other words, seed applications are important in terms of revealing the genetic and physiological potentials of plants. For this reason, several biological products are transferred to seeds by the coating approach an effectively applied as seed coating before sowing (Junges et al., 2013).

Farmyard manure is readily available in the crop-livestock farming systems and it has the potential for use in the fertilization programs of all crops to reduce dependence on inorganic fertilizers while maintaining good soil health (Bayu et al., 2006b). Similarly, Du et al. (2020) report that farmyard manure treatment has a lot of positive effects on soil properties as well as plant growth, development, and yield.

Parallel to the gradual increase / proliferation of grass areas, it also causes an increase in the amount of water used in these areas, often even the city's main water supply is used for this purpose (Sahin and Kara, 2005). For this reason, lawn managers show great interest in water conservation, research ways to reduce water consumption or prefer grass species and varieties that consume less water (Bastug and Buyuktas, 2003).

4. Conclusion

The gradual increase in grass areas also increases the amount of clean water used for these areas. It is very important to use existing water resources more effectively under the threat of global climate change. In this study, the response of Bermudagrass under drought stress conditions was investigated. It has been seen that the effects of drought stress would be tolerated to some extent by farmyard manure and seed coating application. The results of this research showed that it is possible to maintain the quality of Bermudagrass under drought stress conditions with limited applications.

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