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Original article

Drought resistance of bermudagrass accessions collected from Eastern Mediterranean

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Summary

Water shortages and climate change have brought renewed attention and urgency to develop turfgrasses with improved drought resistance. Hence, new sources of drought-resistant germplasm would aid breeding to develop turfgrasses for water-limited environments. This study aimed to determine drought resistance of 95 *Cynodon dactylon* (L.) Pers. (bermudagrass) accessions collected from the Mediterranean region of Turkey. The objectives were to (1) evaluate drought resistance of the accessions in field experiments with sandy soil and compare their performance to commercial cultivars, and (2) investigate the relationship between the ploidy levels and drought resistance traits. The accessions, belonging to five ploidy levels, and five commercial cultivars were established in the field and evaluated for drought resistance for two years. The accessions were classified into four drought resistance response groups, using drought resistance descriptors of leaf firing (LF) and turfgrass quality during dry-down (DQ) periods of 45 days in both growing seasons. The most drought-resistant group did not include any of the commercial cultivars and exhibited the lowest LF, highest DQ during drought stress, and recovered faster than the other groups. There were 10 days difference to reach 50% green cover during dry-down and recovery periods between the most resistant and susceptible groups. Increased ploidy level did not directly relate to enhanced drought resistance or faster recovery. The most drought-resistant group possessed accessions from all ploidy levels. The results support the existence of accessions with superior drought resistance that offer genetic resources for bermudagrass improvement suitable for water limiting environments.

Keywords
Cynodon dactylon, Days50, percent leaf firing, ploidy, turfgrass, turfgrass recovery

Significance of this study

What is already known on this subject?

- Genetic variation in *Cynodon dactylon* (common bermudagrass) is inadequately sampled and there is existing potential to add genetic variation to the germplasm collection. The bermudagrass evolutionary center of diversity extends from West Pakistan to Turkey. However, there is limited information available for drought resistance of bermudagrass accessions originating from this region.

What are the new findings?

- Wild bermudagrass ecotypes exhibited superior drought resistance than the commercial cultivars. Increased ploidy level did not directly relate to enhanced drought resistance or faster recovery from drought stress.

What is the expected impact on horticulture?

- The use of drought-resistant turfgrass cultivars is the best way to reduce irrigation requirement and enhance water conservation in urban landscapes. The accessions within the highest drought-resistant group could potentially be used as genetic resources in bermudagrass breeding programs designed to develop cultivars with enhanced drought resistance traits.

Introduction

The water demand for agriculture could double by 2050, whereas the availability of fresh water is predicted to drop by 50%, owing to climate change (Gupta et al., 2020), which will increase the severity and longevity of drought incidents (Trnka et al., 2011). Drought stress is considered as the main environmental factor limiting growth and development of turfgrasses (Yu et al., 2013; Zhang et al., 2019). For improving turfgrass performance in water-limiting environments, utilization of drought tolerant turfgrasses is an important approach. Therefore, breeding studies have focused on development of warm-season turfgrass cultivars with improved drought resistance (Zhang et al., 2017; Zhou et al., 2013a). However, the progress of breeding to improve drought tolerance in turfgrass can be restricted by the lack of drought-tolerant germplasm (Jiang, 2007). Therefore, the best strategy is to identify accessions with drought resistance traits and develop cultivars having improved drought resistance. Bermudagrass (*Cynodon* spp.) is considered to have superior drought resistance to most other warm-season turfgrass species (Carrow, 1995, 1996; Qian and Fry, 1997; Severmutlu et al., 2011; Zhang et al., 2019).

Common bermudagrass [*C. dactylon* (L.) Pers.] is a long-lived perennial sod-forming warm-season grass species used

for turf, pasture, forage, soil stabilization, and remediation of contaminated soils (Burton, 1947; Taliaferro, 2003). It is the most widely distributed and genetically variable species in the genus *Cynodon* (L.) Rich., containing eight species and ten varieties (Harlan et al., 1970) with a base chromosome number of X = 9 (Forbes and Burton, 1963; Wu et al., 2006). The *C. dactylon* is the most popular and prevalent form in nature (Forbes and Burton, 1963; Gulsen et al., 2009). The bermudagrass evolutionary center of diversity extends from West Pakistan to Turkey (Harlan and de Wet, 1969; Gulsen et al., 2009). All known ploidy levels (diploid to hexaploidy) are found throughout eastern Mediterranean region, having a sub-tropical climate (Gulsen et al., 2009). However, information is lacking for drought resistance of bermudagrass accessions originating from this region. Wild bermudagrass ecotypes exhibited better drought resistance than the commercial cultivars, and these accessions might be an excellent genetic source for drought resistance (Hays et al., 1991; Etemadi et al., 2005; Zhou et al., 2009; 2013a, b). Of the various regions, the Mediterranean climatic zone of Southwestern Australia had the highest frequency of drought-resistant bermudagrass ecotypes (Zhou et al, 2013b). Similarly, a Texas study, the genotypes developed from sub-tropical environments had superior drought resistance compared with those selected from temperate environments (Beard and Sifers, 1997). Taliaferro (2003) reported that genetic variation in *Cynodon*, particularly *C. dactylon* var. *dactylon*, is inadequately sampled and there is existing potential to add genetic variation to the germplasm collection. Therefore, the genetic variation identified in *Cynodon* accessions collected from Mediterranean region could have important potential for improvements in bermudagrass drought resistance.

Polyploidy is known to enhance plant fitness in stressful environments (Godfree et al., 2017), and they may occupy harsher environments relative to diploids (Grant, 1981; Levin, 1983). Polyploids often have enhanced drought resistance compared to their diploid relatives in wild plants and crops (Nassar, 1992, 2006; Pustovoitova et al., 1996; Maherali et al., 2009; Manzaneda et al., 2012; Akinroluyo et al., 2020). These findings are in contrast to some reports indicating that diploids were more resistant to abiotic stresses (Helgadottir et al., 2018), or that the differences were not significant among ploidy levels (Kemesyte et al., 2017). To our knowledge, there are no studies that associate drought resistance with different ploidy levels in bermudagrass. Hence, evaluation of the eastern Mediterranean accessions, including all ploidy series from diploids to hexaploids (Gulsen et al., 2009), for drought resistance under field conditions can be readily justified. The objectives of this study were to (1) evaluate drought resistance of 95 bermudagrass accessions collected from the eastern Mediterranean and compare their performance to commercial cultivars in sandy soil in field experiments, and (2) investigate the relationship between the ploidy levels and drought resistance traits of the accessions.

Materials and methods

Plant materials, establishment and maintenance of plots

The 95 bermudagrass accessions and five commercially available bermudagrass cultivars; vegetatively established 'Tifway' (*C. transvaalensis* Burt-Davy × *C. dactylon*), and seed established 'Blackjack', 'Contessa' (SWI-1045), 'NuMex Sahara', and 'Riviera' were evaluated for drought resistance in field experiments conducted in Mersin, Turkey during 2007–2009. These accessions were described in detail by Gulsen et al. (2009). The 95 accessions, collected from the Mediterranean coastal region of Turkey, were comprised of 66 tetraploids ($2n = 4x = 36$), 17 pentaploids ($2n = 5x = 45$), six hexaploids ($2n = 6x = 54$), three triploids ($2n = 3x = 27$) and three diploids ($2n = 2x = 18$).

Greenhouse grown ramets of each accession, 'Tifway', and selected representative plants established from seed for the seeded cultivars were transplanted to field plots at the Alata Agricultural Research Station, Erdemli, Mersin (lat. 36°37'N, long. 34°42'E) on 9 May 2007. The experimental design was a randomized complete block with three replications. Plot size was 1.0 m × 1.0 m with 0.5 m spacing between plots. Each plot was established by planting five, 5.0-cm-diameter plugs that were evenly distributed, one in the center and four towards the corners, in each plot. The soil type was a Typic Xerofluvent sandy soil (86% sand) with a 1.2% organic matter, EC of 0.17 dS m^{-1}, pH 8.3, P level of 28 mg kg^{-1}, and K level of 102 mg kg^{-1}. Starter fertilizer (15N-6.6P-2.5K) was incorporated in the upper 25 mm of the planting bed at 5 g Nm^{-2}. Nitrogen (5 g m^{-2}) was applied in June, July and August of 2007 to promote plot establishment. In 2008 and 2009, fertilizations in June and July were skipped due to drought treatments, and turf received 5 g N m^{-2} (33N–0P–0K) and 5 g K m^{-2} (0N-0P-43K) in May, August, and September. Plots were hand-weeded and space between plots were maintained by periodic application of glyphosate herbicide [isopropylamine salt of *N*-(phosphonomethyl) glycine] to control cross contamination. Turf was mowed weekly at 50 mm with clippings removed. Irrigation was provided three times daily for 2 weeks at 7 mm to enhance establishment. Subsequently, turf was irrigated to prevent visual turfgrass wilt symptoms.

Assessment of drought resistance

At the beginning of drought stress treatments, all the plots were fully covered with grasses and had uniform density. The irrigation was withheld for 45 d from 15 June to 1 August in 2008 and 2009. This study time was chosen because it coincided with the lowest average rainfall and highest average soil temperatures in Mersin, Turkey (June–August). The total rainfall in this period was 1.3 mm and 0.2 mm in 2008 and 2009, respectively. The average maximum and minimum temperatures were 37.2°C and 20.6°C, and 32.6°C and 21.9°C, in 2008 and 2009, respectively. The relative humidity ranged from 55% to 59%, and wind speed were between 10.4 km h^{-1} and 11.6 km h^{-1} during the two months of the study. At the beginning of drought stress treatment plots were watered to near field capacity as determined by the gravimetric method. Data were collected during June to July and post-drought stress periods (August to November) in both years. Percentage turfgrass leaf firing (LF) and turfgrass quality during drought stress (DQ) were recorded every three to four days for 45 days. To assess post turfgrass drought responses, the treatment plots were irrigated to near field capacity followed by the previously described irrigation. Percent turfgrass recovery (R) and turfgrass quality during recovery (RQ) were recorded every three to four days for 55 days. The LF was visually measured based on a 0–100% scale where 0% being no turfgrass leaf firing and 100% being completely dormant and brown turfgrass plants. The R was visually assessed using a 0–100% scale, with 0% being no green turfgrass vegetation and 100% green vegetation for the entire plot. Turfgrass quality as an integral of color, uniformity and density was rated visually on the scale of 1 (the worst; completely dormant and brown

turf) to 9 (the best) where 6 being acceptable turf quality (Morris and Shearman, 2008). Soil water content was determined gravimetrically, as a composite sample from three randomly chosen plots for each block, at initiation, and at the end of drought stress period (45th day) to determine spatial water content at 0–20, 20–40, and 40–60 cm in depths. Soil samples were collected using soil probe 2.5 cm^2 in diameter and oven dried at 72°C for 48 h. Volumetric moisture content (VMC) in the top 20 cm of soil from selected plots was measured using time domain reflectometry (Spectrum Technologies, East Plainfield, IL, U.S.A.).

Measurement of morphological characters

In order to evaluate the relationship between drought resistance and morphological characteristics of the accessions, stolon number (STN) and length (STL); internode length (STIL) and diameter (STD); leaf blade length (LL), width (LW), and pubescence (PUB); and shoot density (density) were measured. The STN per plot by counting all stolons growing out of each plug in each plot, STL by measuring the four longest stolons from each plug, STIL and STD of the third internode from the terminal tip of four longest stolons, and LL and LW by measuring on the third leaf from tip to down from five shoots, density by counting shoots on two 100-mm-diameter plugs selected from varying locations in each treatment plot, PUB by assessing presence of pubescence on either side of the third leaf on five shoots were measured in the field. The measurements were made on the plots established in parallel for morphological characterization at the same location and with the same plant material. Stolon, leaf and shoot characteristics were assessed 6 weeks, 4 and 6 months after establishment, respectively for two years.

Statistical analyses

Using Proc nlin (SAS Institute, Cary, NC, U.S.A.), scatter plots of the leaf firing (LF) data versus days after water withheld (DAWW) indicated a strong nonlinear relationship in every plot, and fit very well to a sigmoid variable slope model:

$$GC = \frac{k}{1 + e^{a+bt}} \quad \text{(Eq. 1)}$$

where t was DAWW, a and b were parameters of the curve, and k was maximum green cover (GC) (100% GC = 0% LF) at 0 DAWW. For each experimental unit, the sigmoid variable slope model was significantly fit to the change of GC during drought stress and the coefficient of determination (R^2) was higher than 90% in each case. Using the equations 2 and 3, Days50 (days required to reach 50% LF) for each accession was calculated (Tipton, 1984).

$$\frac{1}{2}k = \frac{k}{1 + e^{a+b\times(\text{Days}_{50})}} \quad \text{(Eq. 2)}$$

$$\text{Days}_{50} = -\frac{a}{b} \quad \text{(Eq. 3)}$$

DQ was assessed at the same time as LF. As for the LF vs. DAWW, the regression between DQ and DAWW and R vs. Days After Recovery Initiated (DARI) of every plot also fit a sigmoid model with R^2 > 90%. Then, DQ_{50} (the time when quality was 50% of the maximum at 0 DAWW) and the R_{50} (the time when GC reached 50% in the plot) were calculated using the same method for deriving LF_{50}.

A cluster analysis of observations was undertaken for LF_{50} and DQ_{50} using ward linkage method (Hair et al., 2010). K-Means clustering algorithm were performed using built-in kmeans function of R suite and similarities were measured by squared Euclidean Distance to classify all the accessions into groups (R Core Team, 2020). Plots were generated using "plotly" library of the same program. After that, group was considered as a factor in further analysis to provide means

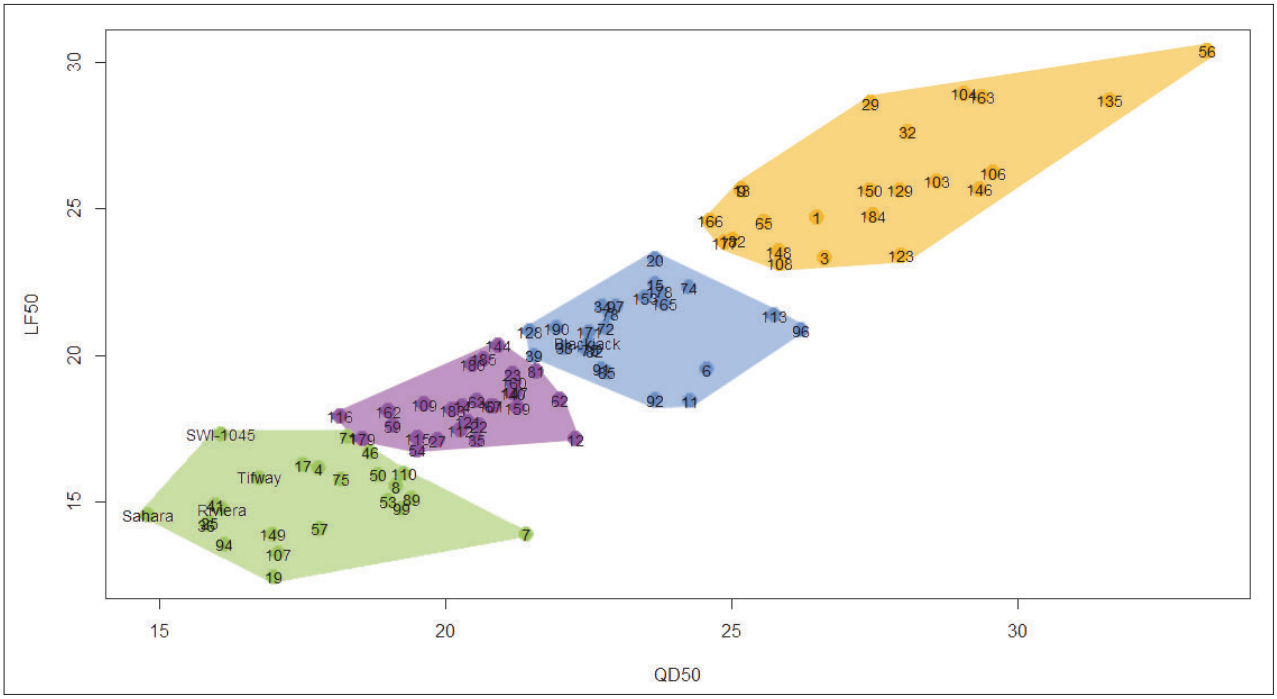


Figure 1. Clustering of 95 bermudagrass accessions and five commercially available cultivars; 'Tifway', 'Blackjack', 'Contessa' (SWI-1045), 'NuMex Sahara', and 'Riviera' based on LF_{50} (days required to reach 50% leaf firing) and DQ_{50} (days required to lose 50% of its initial quality under drought stress) in a field experiment conducted at Alata Horticultural Research Institute, Erdemli, Mersin, Turkey.

Table 1. Drought resistance characteristics of four groups and commercially available cultivars of bermudagrass measured from the field experiment conducted at the Alata Horticultural Research Institute, Erdemli, Mersin, Turkey.

Number of accessions							Means		
Group†	2×‡	3×	4×	5×	6×	Total	LF_{50}	DQ_{50}	R_{50}
1	1	1	9	10	2	23	25.80a*	27.52a	10.38a
2	2	1	17	4	1	36	20.84b	23.27b	13.91b
3	0	1	22	2	3	21	18.23c	20.43c	16.09c
4	0	1	22	1	0	13	15.06d	17.67d	17.89c
Total	3	4	70	17	6	100			

Commercial cultivars		Means		
		LF_{50}	DQ_{50}	R_{50}
4	Contessa	17.33ab	16.13a	13.13a
2	Blackjack	20.37b	22.53b	13.40a
4	Riviera	14.77a	16.13a	16.33ab
4	Sahara	14.57a	14.83a	18.23ab
4	Tifway	15.80a	16.77a	19.47b

† Groups were formed by applying cluster analysis to 95 genotypes based on LF_{50} (days required to reach 50% leaf firing) and DQ_{50} (days required to lose 50% of its initial quality under drought stress), R_{50} implies days required to reach 50% green cover after recovery (irrigation) initiated.
‡ 2×, 3×, 4×, 5×, and 6× denote ploidy levels from diploids to hexaploids.
* Within each column, means followed by the same letter are not significantly different based on Tukey's HSD test ($p < 0.05$).

for the different drought-resistant groups using GLM in SPSS 26.0 software (SPSS, Chicago, IL, U.S.A.). Similarly, the ploidy levels were taken as a factor to analyze effect of ploidy on LF_{50}, DQ_{50} and R_{50}. For measurements of LF, DQ, R, and RQ conducted in different time periods for the same experimental units, repeated measures analysis was applied using SPSS. Path coefficient analysis to predict direct and indirect effects of stolon and leaf characteristics on LF_{50}, DQ_{50} and R_{50} was performed using the SPSS. The causal relationships for the path coefficient analysis involved the eight morphological characters as predictor variables and LF_{50}, R_{50} and DQ_{50} as the response variable.

In addition, principal component analysis (PCA) was used to transform a number of observed variables to a smaller number of artificial variables (principal components) to account for most of the variance in the observed variables. Therefore, attributes (LF_{50}, DQ_{50}, GC_{50}, Density, STN, STL and STD) having significant genotypic variation were used for PCA in the study using XLSTAT 2016.02 version (Addinsoft, Paris, France). Pearson correlation coefficients of LF_{50}, DQ_{50} and R_{50} were calculated with SPSS.

Results

Soil water content by weight at 0–60 cm soil depth was 11.1%, measured just before the drought stress exposure and declined to 3.0% by the 45th day of the study. The VMC value measured with TDR was 16.7 when plots were fully watered to field capacity before initiation of the drought stress study, compared with dry soil's value of 4.8 after 45 days of drought stress.

The cluster analysis using dry-down period data of LF_{50} and DQ_{50} classified the accessions into four distinct groups (G) with G1 = 23, G2 = 24, G3 = 28, and G4 = 20 accessions, respectively (Figures 1 and S1). Groups 1–4 represent rankings for drought resistance from highest to lowest and the difference between groups was significant (Table 1). For example, the G1 accessions reached to LF_{50} 10 days later than G4 under drought (Table 1). None of the commercially available cultivars were in G1 (Table 1; Figure 1). They were in either G2 ('Blackjack') or G4 ('Contessa', 'Riviera', 'NuMex Sahara', 'Tifway'). Compared to the most drought-resistant accessions, LF_{50} values among the cultivars was 5 to 11 days earlier (Table 1). As for turfgrass quality under drought stress, there were 10 days difference between the most resistant and most susceptible groups to lose 50% of their initial quality. This difference was about 8 days among the cultivars, and up to 13 days between the resistant group and the cultivars

Table 2. Pearson correlations (r) among traits measured in a field experiment conducted at the Alata Horticultural Research Institute, Erdemli, Mersin, Turkey, using 95 bermudagrass accessions.

	LF50†	R50	DQ50
R50	-.698**		
DQ50	.931**	-.681**	
Ploidy	.244*	-.067	.262**

† Traits include LF_{50} (days required to reach 50% leaf firing) and DQ_{50} (days required to lose 50% of its initial quality under drought stress), R_{50} (days required to reach 50% green cover after irrigation initiated), and Ploidy (2× to 6×).
*, ** indicates significant correlation at the $P = 0.05$ or 0.01, respectively.

Table 3. Drought resistance characteristics of five ploidy groups of bermudagrass accessions measured from the field experiment conducted at the Alata Horticultural Research Institute, Erdemli, Mersin, Turkey.

Ploidy†	LF_{50}‡	DQ_{50}	R_{50}
2×	22.02ab*	23.89ab	10.30a
3×	19.34a	21.54a	15.25b
4×	18.76a	21.10a	15.47b
5×	23.50b	25.52b	11.88ab
6×	21.65ab	23.44ab	14.94b

† 2×, 3×, 4×, 5×, and 6× denote ploidy levels from diploids to hexaploids.
‡ LF_{50} (days required to reach 50% leaf firing) and DQ_{50} (days required to lose 50% of its initial quality under drought stress), R_{50} (days required to reach 50% green cover after irrigation initiated).
* Within each column, means followed by the same letter are not significantly different based on Tukey's HSD test ($p < 0.05$).

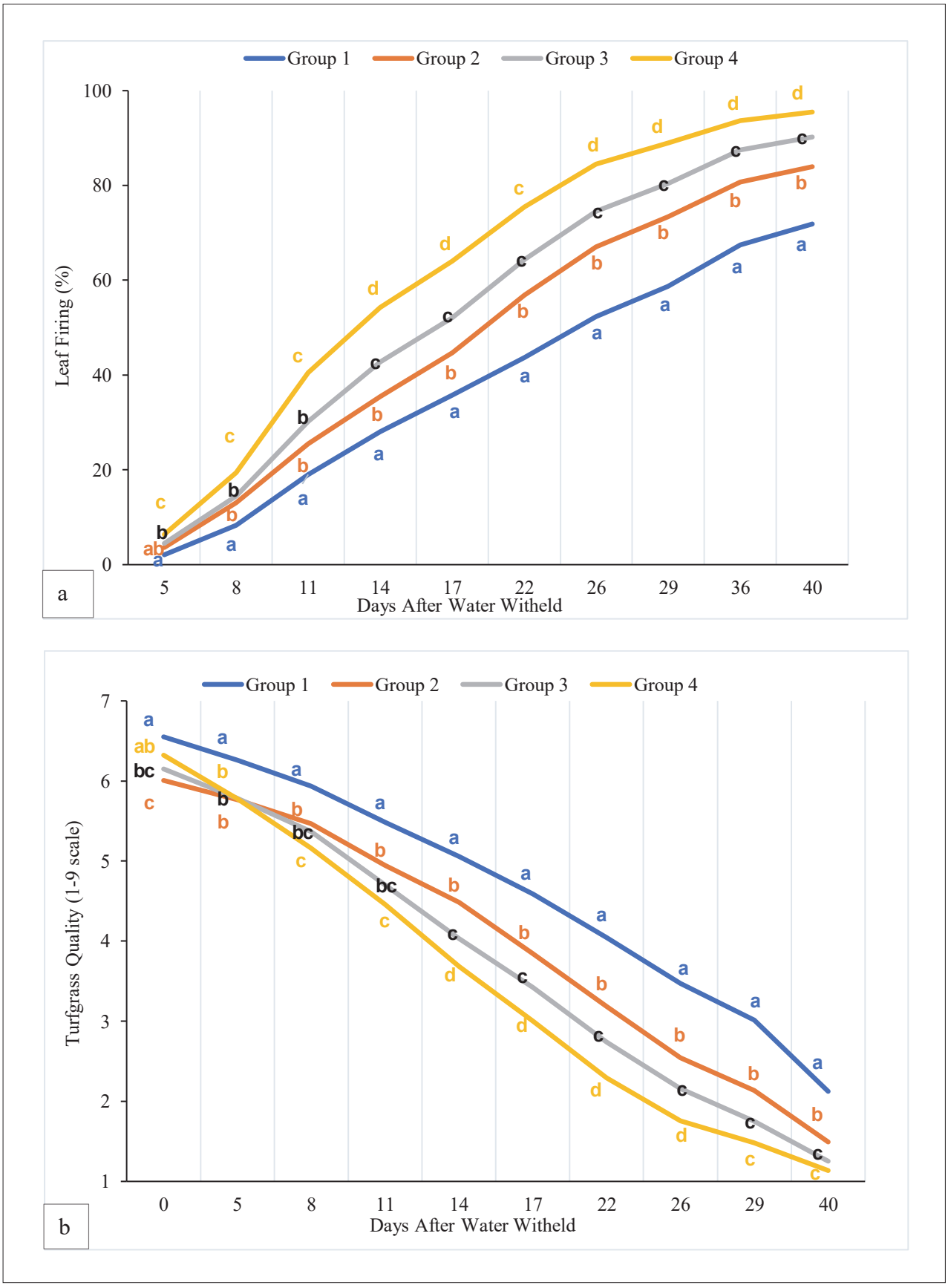


Figure 2. (a) Leaf firing and (b) Turf quality of four groups of bermudagrass accessions grown under field conditions at Alata Horticultural Research Institute, Erdemli, Mersin, Turkey. Groups were formed by applying cluster analysis to 95 accessions and 5 cultivars ('Tifway', 'Blackjack', 'Contessa', 'NuMex Sahara', 'Riviera') based on LF_{50} and DQ_{50} where these characters indicated the days required for each experimental units to decline to 50% of the maximum green cover and turf quality, respectively. Group means were the average of LF or TQ of all the genotypes from each group. Within one time period, means followed by the same letter are not significantly different based on Tukey's HSD test ($p < 0.05$).

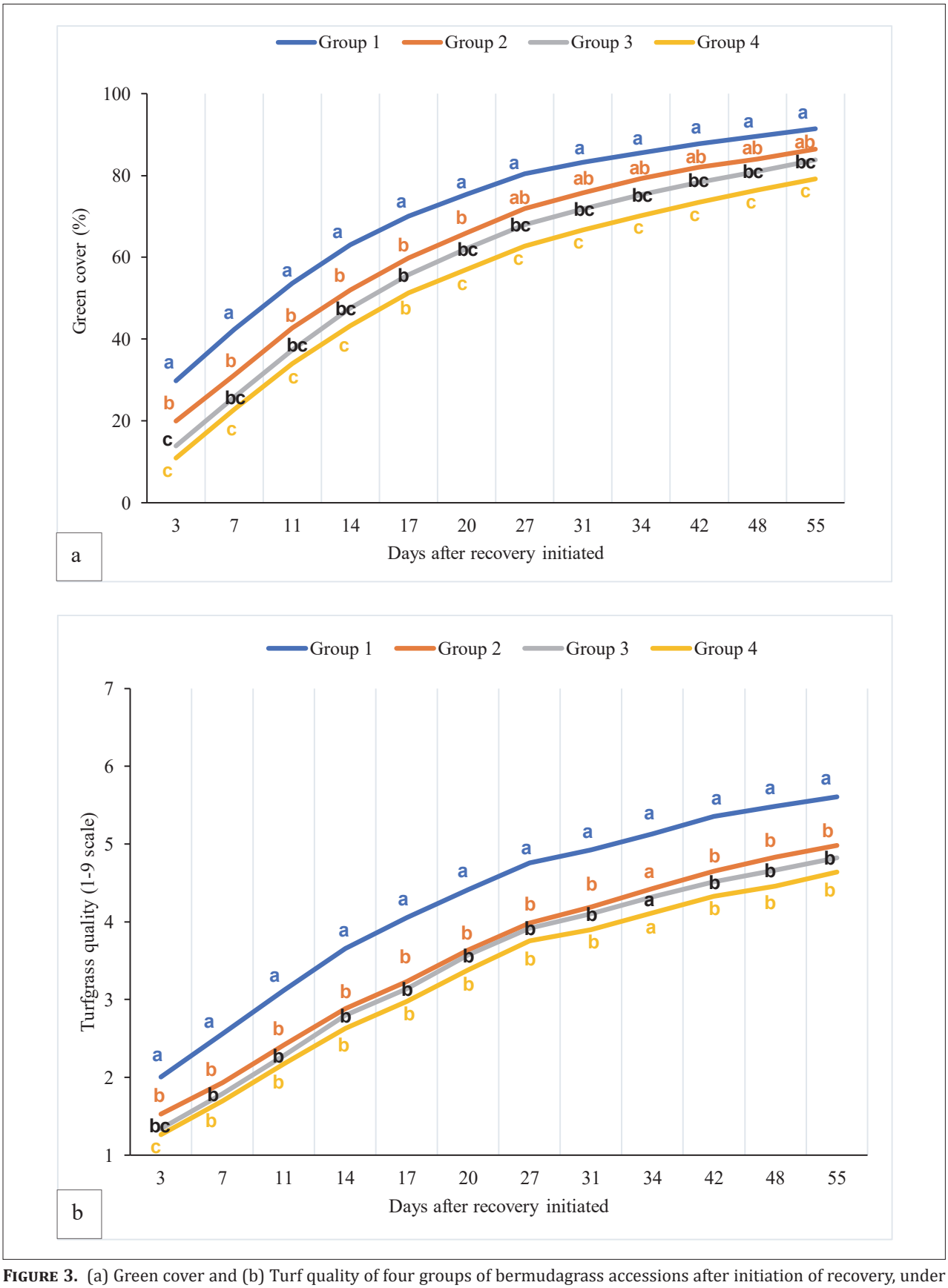


Figure 3. (a) Green cover and (b) Turf quality of four groups of bermudagrass accessions after initiation of recovery, under field conditions at Alata Horticultural Research Institute, Erdemli, Mersin, Turkey. Groups were formed by applying cluster analysis to 95 accessions and 5 commercially available cultivars ('Tifway', 'Blackjack', 'Contessa' (SWI-1045), 'NuMex Sahara', 'Riviera') based on LF_{50} and DQ_{50} where these characters indicated the days required for each experimental units to decline to 50% of the maximum green cover and turf quality, respectively. Group means were the average of green cover or turfgrass quality of all the genotypes from each group. Within one time period, means followed by the same letter are not significantly different based on Tukey's HSD test ($p < 0.05$).

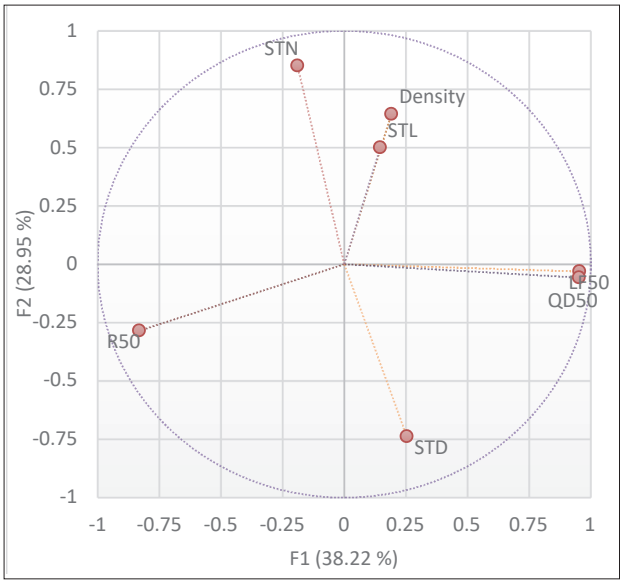


Figure 4. Plot of eigen vectors for seven traits, collected from 95 bermudagrass accessions and 5 cultivars ('Tifway', 'Blackjack', 'Contessa', 'NuMex Sahara', 'Riviera'), measured in field study grown in a sandy soil in Alata Horticultural Research Institute, Erdemli, Mersin, Turkey. Traits included LF_{50}, DQ_{50}, R_{50}, density (shoot), STN (stolon number), STL (stolon length), STD (stolon diameter). Eigen vector plots can be interpreted as follows: (1) trait vectors with a narrow angle between them indicates positive correlation exist between the traits, the narrower the angle the greater the correlation; (2) trait vectors perpendicular to each other indicates no correlation between the traits; (3) trait vectors in opposing directions indicates a negative correlation exists between the traits.

(Table 1). The pace of recovery following irrigation differed among the groups. The G1 reached to R_{50} (50% GC) one week earlier than G4 in the recovery period (Table 1). The R_{50} values of the cultivars were 3 ('Blackjack' and 'Contessa') and 9 days ('Tifway') later than the most drought-resistant group.

The change of LF and DQ with time of each group had a good fit to the sigmoid variable slope model. Repeated measure analysis revealed that group variation was significant, and G1 exhibited significantly less LF and higher quality from other groups, beginning on the 5th day, through the dry-down period (Figure 2a–b). From five to 22 days after water was withheld, the difference in %LF between G1 and G4 was about two-fold (Figure 2a).

For those traits such as green turfgrass cover and turfgrass quality measured at a 3–4-day interval during recovery period, group variation was also significant (Figure 3a–b). G1 recovered faster than the other three groups as indicated by higher percent of turfgrass green cover, especially after the first three weeks, and turfgrass quality throughout the evaluation dates (Figure 3a–b). For example, accessions in G1 reached 54% green turfgrass cover, compared to 34% for the G4, at 11 days after resuming irrigation. The LF_{50} and DQ_{50} evaluated during the dry-down period were significantly correlated to each other ($r = 0.93$; $P < 0.01$) and both were also correlated to R_{50} ($r = -0.70$ and -0.68; $P < 0.01$) (Table 2).

When the ploidy level was considered as a factor in analysis for the same variables (LF_{50}, DQ_{50}, R_{50}) at the genotypic level, ploidy based separation was not evident (Table 3). Interestingly, the most drought-resistant group included 23 accessions in total from all ploidy levels, involving 13% of tetraploids and 59% of pentaploids (Table 1). Pearson correlation coefficients were significant between ploidy level and LF_{50}, and DQ_{50}, but not with R_{50} (Table 2).

Correlations were partitioned into direct and indirect effects through path coefficient analysis (Table 4). Density, STN, STL, and STD had significant directs effects on LF_{50}, DQ_{50} and R_{50}. The magnitudes of these direct effects on LF_{50} and DQ_{50} were similar, an indication that it explains a true relationship. Principal component analysis utilized LF_{50}, DQ_{50}, R_{50}, density, STL, STN, and STD at genotypic level. Two principal components were extracted and plotted together where the first three components explained 83% of the total variation (Figure 4; Table S1). The first (PC1) and the second (PC2) components explained 38.2% and 29% of the variance, respectively (Figure 4). The relatively large loads in PC1 are LF_{50}, DQ_{50}, and R_{50}, and in PC2 are STN, STD, and density, while it is STL for PC3 (Table S1). These results yielded high positive correlations between LF_{50} and DQ_{50}, and density and STL (Figure 4). In addition, LF_{50} and DQ_{50} measured during dry-down were negatively correlated with R_{50} (Figure 4). The density, STL, and STD were positively correlated to LF_{50} and DQ_{50}.

Table 4. Phenotypic path coefficients showing direct and indirect effects of morphological components on drought resistance traits of LF_{50}, R_{50} and DQ_{50} using 95 bermudagrass accessions grown at Alata Horticultural Research Institute, Erdemli, Mersin, Turkey.

	Indirect effects†								Direct effects‡		
	Density	STN	STL	STIL	STD	LW	LL	PUB	LF50	R50	DQ50
Density		.176*	-.192*	.005	-.195	-.154	-.048	-.094	**.480***	**-.394***	**.371***
STN	.281*		.656*	-.222	-.381*	-.413*	.178	.103	**-.434***	**.283***	**-.406***
STL	-.230*	.494*		.414*	-.108	.190	-.141	-.019	**.388***	**-.382***	**.371***
STIL	.005	-.142	.351*		-.159	-.120	.369*	-.206	**-.112**	**.043**	**-.090**
STD	-.198	-.243*	.091	-.159		.101	.001	-.035	**.253***	**-.069**	**.314***
LW	-.213	-.358*	.219*	-.163	.137		.521*	.184	**.106**	**-.024**	**.084**
LL	-.051	.120	-.126	.389*	.001	.404*		-.103	**.004**	**.121**	**.037**
PUB	-.070	.048	-.012	-.151	-.026	.100	-.072		**.116**	**.090**	**.076**

† Indirect effects of morphological components include: Density (shoot density), STN (number of stolon), STL (stolon length), STIL (stolon internode length), STD (stolon internode diameter), LW (leaf blade width), LL (leaf blade length), PUB (leaf pubescence).

‡ Direct effects include: LF_{50} (days required to reach 50% leaf firing) and DQ_{50} (days required to lose 50% of its initial quality under drought stress), R_{50} (days required to reach 50% green cover after irrigation initiated).

* Denotes significance at $P < 0.05$.

Discussion

The 45-day-long dry-down period in a sandy soil and arid conditions provided severe dehydration stress in both growing seasons studied. The 3% (or 4.8 VMC by TDR) water measured at the experimental site at the end of dry-down period was likely at or below the permanent wilting point.

Leaf firing was characterized by, referring to necrosis starting at leaf tips and margins and progressing down the leaf in response to tissue water stress caused by progressive drought conditions. The LF variable is a good tool to assess turfgrass drought resistance (Carrow, 1996; Beard and Sifers 1997; Lu et al., 2009). Similar to previous studies (Steinke et al., 2011; Zhou et al., 2013a, b, 2014; Zhang et al., 2019), Days50 values were calculated for LF and DQ, and used as the criteria to evaluate the level of drought resistance among bermudagrass accessions in the study. The 95 accessions were divided into four groups based on LF_{50} and DQ_{50}. Importantly, a number of accessions had superior drought resistance compared to the commercial cultivars. Similarly, previous studies reported that bermudagrass accessions from Iran and Australia also performed better than the standards under drought conditions (Etemadi et al., 2005; Zhou et al., 2009, 2013b). For example, the most drought-resistant accession 56 (4× ploidy) maintained its 50% GC (LF_{50}) over 30 days, 10 days (50%) longer than 'Blackjack', the most drought-resistant commercially available cultivar studied (Figure 1; Table 1). Moreover, the same accession recovered quickly and reached 50% GC (R_{50}) on the 6th day of the recovery period, one week earlier than 'Blackjack'. Therefore, utilization of the drought-resistant G1 accessions in breeding programs may offer a viable alternative for water conservation and for improving turfgrass performance in water-limiting areas. Zhou et al. (2014) also observed considerable genotypic variation for drought resistance among Australian bermudagrass ecotypes grown in a sandy soil, and among eastern Mediterranean accessions in this study where LF_{50} (i.e., GC_{50}) ranged from 12 to 30 days. Zhou et al. (2013b) found wild accessions of bermudagrasses collected from a Mediterranean climate were a valuable source of drought resistance. Gasith and Resh (1999) reasoned this as evolutionary adaptation of the biota to drought/recovery cycles experienced under Mediterranean ecosystems. The accessions with contrasting drought resistance could be used as materials to study mechanism of drought resistance.

Drought avoidance, tolerance, and escape are mechanisms used by turfgrasses during drought (Merewitz and Huang, 2013). In drought avoidance, the grass maintains water status by increasing water uptake through root system and/or reducing water loss through leaves (Levitt, 1980). The amount, depth, and spatial distribution of roots influence available water for the grass at the onset of drought (Carrow, 1996). Although the below-ground characteristics were not evaluated in this study, the drought resistance of accessions in G1 may be attributed to ability to extract soil water under drought stress conditions through development of deep and extensive roots (Carrow, 1996; Zhou et al., 2013b, 2014; Zhang et al., 2019) and the presence of extensive rhizomes (Zhou et al., 2014). Similarly, in a sandy soil, bermudagrass ecotypes originating from the Mediterranean climatic zone of Australia proved to have superior drought resistance correlated with the ability to extract more soil water at 30–170 cm and presence of highly rhizomatous structure (Zhou et al., 2014). The relatively low drought resistance of commercial cultivars under sandy soil shows the need to make concurrent selection for quality and high dehydration stress under severe conditions. Using some of the most promising accessions, phenotypic recurrent selection may be used to develop high drought-resistant cultivars suitable for dry environments.

Under prolonged drought stress, the above ground parts of bermudagrass die off, but the grass has the potential to resume growth from its rhizomes (Kim et al., 2009; Zhao et al., 2011). Turfgrass drought recovery is a measure of percent green turfgrass cover after drought conditions are relieved and is a component of drought resistance (Beard and Sifers, 1997). Recovery in turfgrass is crucial for plant survival, persistence, and function (Steinke et al., 2010; Volaire and Lelievre, 2001) and often involves the combination of the recovery of damaged plant tissue and growth of new tissue after drought-induced dormancy (Chai et al., 2010). Both Pearson correlation and PCA analysis indicated that accessions with low leaf firing ratings under drought stress demonstrated higher turfgrass quality values and improved post-drought stress shoot recovery than plants with high leaf firing ratings, suggesting potential of genetic mechanism involvement (Table 2; Figure 4). Shoot traits including dense canopy, growth habit (horizontal vs. vertical) may contribute to lower evapotranspiration rate, which would delay dehydration of the grass (Huang et al., 2014; Kim and Beard, 1988). Path analysis yielded similar direct effects of density, STN, STL, and STD on LD_{50}, DQ_{50} and R_{50}. In *Lolium perenne* L., higher tiller density also contributed to both a higher drought resistance and faster recovery after the drought (Barker et al., 1985; Tozer et al., 2017). Hence, selection for higher shoot density and STD, and longer STL would indirectly select for higher drought resistance, and faster recovery. The PC1 involved LF_{50}, DQ_{50}, and R_{50}, the PC2 resided STN, STD, and density in PCA analysis further showed the interdependency among the traits within each component.

Genetic consequence of polyploidization may contribute to polyploid advantage in extreme habitats (Parisod et al., 2010) with polyploids generally occupying drier areas (Maherali et al., 2009; Manzaneda et al., 2012) and exhibiting increased resistance (Nassar, 1992, 2006; Pustovoitova et al., 1996). In *Lolium multiflorum* ssp. *multiflorum*, induced tetraploids produced more dry matter, phenolic and antioxidant compounds, and conferred higher drought resistance than their diploid progenitor (Akinroluyo et al., 2020). The 24% and 26% correlation between ploidy and drought resistance traits (LF_{50}, DQ_{50}) indicated a low to medium level relationship that was lacking for recovery (R_{50}) in this study. The increase in genome size does not seem to linearly raise drought resistance of bermudagrass. Although, the majority of pentaploids belonged to the most drought-resistant group, it also included accessions from every other ploidy level. The hexaploid and tetraploid accessions with high drought resistance can be used to broaden genetic base of bermudagrass for superior drought resistance. This would be possible by either crossing hexaploids in G1 (accessions 129, 135) with diploid *C. transvaalensis* to generate tetraploid progenies (Sever Mutlu et al., 2014), or by utilizing tetraploids to breed for seeded types.

Conclusion

Results of this study are especially relevant for environments that have Mediterranean-like subtropical climate and sandy soils where drought stress can develop in a few days. As the future climate is still reasonably unpredictable, particularly for the hot and dry Mediterranean areas, breeders need to access to a range of well-characterized accessions for breeding more resilient turfgrass cultivars. The results of a

two-year study for drought resistance demonstrated the existence of a large genetic variability for the trait among bermudagrass accessions. The commercially available cultivars studied were not in the most drought-resistant grouping, indicating the room for further improvement for the trait. The accessions identified in the most drought-resistant grouping of this study could potentially be used as genetic resources in bermudagrass breeding programs designed to develop cultivars with enhanced drought resistance traits. Increased ploidy level did not directly relate to enhanced drought resistance or faster recovery from drought stress for the accessions studied here.

Genotypes with contrasting drought resistance were identified and further studies are justified to improve our understanding of the mechanisms and traits involved. In particular, the effects of root and rhizome structure on drought resistance under prolonged stress and rapid recovery may be elucidated using selected accessions from this study.

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Conflict of interest

The authors declare no conflict of interest.

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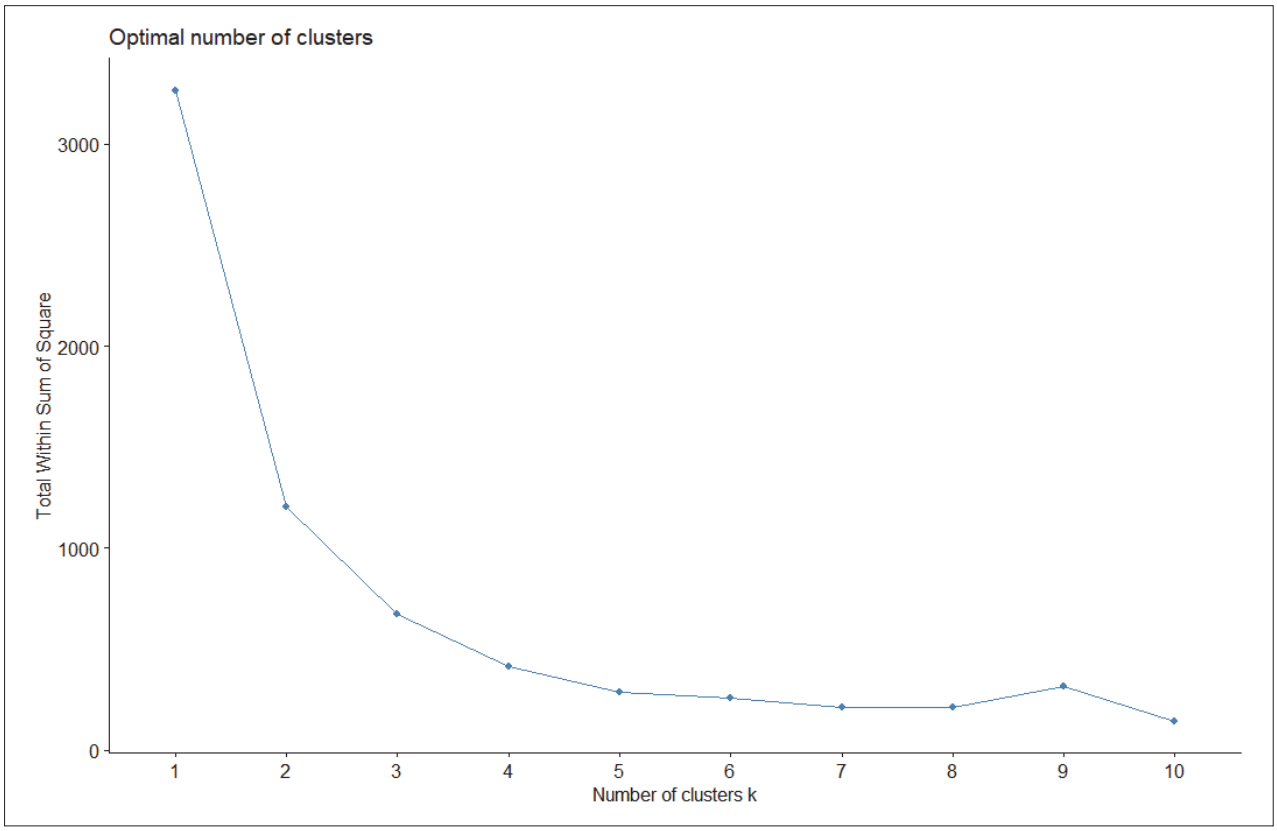
Supplemental Information

Supplemental Information – Table S1. Principle components (PCs) for drought resistance traits of 95 bermudagrass accessions grown at Alata Horticultural Research Institute, Erdemli, Mersin, Turkey

	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	2.676	2.026	1.131	0.506	0.320	0.274	0.066
Variability (%)	38.224	28.946	16.163	7.235	4.569	3.918	0.944
Cumulative %	38.224	67.171	83.334	90.569	95.138	99.056	100.000
	F1	F2	F3	F4	F5	F6	F7
LF50†	**0.913**‡	0.001	0.001	0.007	0.046	0.000	0.033
R50	**0.694**	0.082	0.000	0.011	0.195	0.019	0.000
DQ50	**0.910**	0.003	0.000	0.000	0.053	0.001	0.032
Density	0.037	**0.415**	0.367	0.120	0.001	0.059	0.000
STN	0.036	**0.726**	0.047	0.061	0.016	0.113	0.000
STL	0.021	0.251	**0.646**	0.000	0.001	0.081	0.000
STD	0.065	**0.548**	0.070	0.308	0.008	0.001	0.000

† Traits include: LF_{50} (days required to reach 50% leaf firing), R_{50} (days required to reach 50% green cover after irrigation initiated), DQ_{50} (days required to lose 50% of its initial quality under drought stress), Density (shoot density), STN (number of stolon), STL (stolon length), STD (stolon internode diameter).

‡ Values in bold correspond for each variable to the factor for which the squared cosine is the largest.



Supplemental Information – Figure S1, related to Figure 1. Optimum number of clusters for 95 bermudagrass accessions based on LF_{50} (days required to reach 50% leaf firing) and QD_{50} (days required to lose 50% of its initial quality under drought stress) of a field experiment conducted at Alata Horticultural Research Institute, Erdemli, Mersin, Turkey.