Root and physiological characteristics associated with drought tolerance in Iranian tall fescue

Fatemeh Pirnajmedin · Mohammad Mahdi Majidi · Mahdi Gheysari

Abstract Research on crop response to deficit irrigation is important to reduce agricultural water use in areas where water is a limited resource. The objective of this study was to evaluate changes in physiological and root traits under mild and intense drought stress in tall fescue. It also sought to find associations between these changes and field drought tolerance. A total of 24 tall fescue genotypes were selected from a wide polycross population and assessed for field drought tolerance during 2011–2012 in the field. The genotypes were classified as tolerant, moderately tolerant, and susceptible based on drought stress tolerance index (STI), tolerance index (TOL), and yield reduction (YR). In 2013, 24 genotypes were clonally propagated and planted in polyvinylchloride (PVC) tubes under, three levels of moisture regimes. Root characteristics were investigated at 0–30 and 30–60 cm depths of soil. Also 11 physiological traits, dry forage yield, STI, TOL, and YR were recorded. At the 30–60 cm depths of soil, the root length increased by 5.95 and 7.30 % under mild and intense stress, respectively. Under mild stress, root area and root volume were positively correlated with STI. Consequences of drought stress, manifested as declined relative water content and chlorophyll, could be associated with a decrease in the activity of antioxidant enzymes. Some tall fescue genotypes had extensive root systems, high photosynthetic capacity, and less YR in the field. These genotypes may adapt to drought through drought avoidance and drought tolerance mechanisms. The application of principle component analysis for screening suitable genotypes was also discussed.

Keywords Fescue · Root · Selection index · Water stress

F. Pirnajmedin · M. M. Majidi (✉)
Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, 84156-8311 Isfahan, Iran
e-mail: majidi@cc.iut.ac.ir; mahdimajidi@yahoo.com

F. Pirnajmedin
e-mail: f.pirnajmedin@yahoo.com

M. Gheysari
Department of Water Engineering, College of Agriculture, Isfahan University of Technology, 84156-8311 Isfahan, Iran
e-mail: gheysari@cc.iut.ac.ir

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is an outcrossing allohexaploid (2n = 6x = 42) forage grass. The higher drought tolerance of this species compared to other cool-season grasses such as *Lolium perenne* (perennial ryegrass) and *Poa pratensis* (Kentucky bluegrass) makes it particularly appropriate for arid and semi-arid regions (Sheffer et al. 1987; Gazanchian et al. 2006). On the other hand, its

potential for summer dormancy is assumed to be linked with higher persistence over long dry summers (Norton et al. 2006). The plant has wide distribution with great genetic diversity in Iran and mainly grows in natural rangelands of the central, western, and northern regions of the country. In regions with lower precipitation, it is found along irrigation ditches and farm levees.

Drought is one of the most important abiotic stresses restraining the survival and growth of plants in arid and semi-arid areas of the world. About one-third of the world's cultivated land is in semi-arid and arid regions and this includes Iran (Atlin and Frey 1990; Blum 2011). Plants respond and adapt to drought stress with a variety of escape, avoidance, and tolerance mechanisms, all of which serve to improve the efficiency of water uptake, use, or loss (Wang and Huang 2004). Drought tolerance characteristics include root penetration into deeper portions of the soil profile and osmoregulation in plant tissues at low water potentials (Bonos et al. 2004). Some studies have indicated total root length to influence water and nutrient uptake. Meanwhile, water and nutrient uptake during drought may be more important criterion to drought avoidance (Huang et al. 1997). Therefore, improving drought avoidance and tolerance is a major objective in plant breeding programs for dryland agriculture (Kirigwi et al. 2004). Huang and Gao (2000) introduced enhanced root growth and water uptake in lower soil depths and maintenance of root activity as root physiological and morphological characteristics associated with drought avoidance in tall fescue cultivars. Bonos et al. (2004) suggested selection for deeper root production in tall fescue to be important for drought tolerance. Other researchers found a high positive correlation between root growth and drought tolerance (Ekanayake et al. 1985; Chloupek et al. 1999). Karcher et al. (2008) proposed selecting germplasms based on high root to shoot ratio in the greenhouse as a feasible method for improving drought tolerance of turf grass in the field.

Physiological factors also involve in drought stress injury and should thus be considered for describing drought tolerance in screening studies (Jiang and Huang 2001). Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals are produced under stress conditions (Meloni et al. 2003). These compounds can seriously disrupt normal metabolism through oxidative damage to lipids,

proteins, and nucleic acids (Rout and Shaw 2001). Antioxidative enzymes are critical components of ROS scavenging systems. Superoxide dismutase (SOD) is a major scavenger of superoxide and its enzymatic activity results in H_2O_2 which is then scavenged by catalase (CAT) and a variety of peroxidases (POX) (Cakmak and Horst 1991; Alscher et al. 2002). Evaluating the effects of environmental stresses on the antioxidant system in the apoplast has highlighted the significance of this space in plant response to abiotic stresses (Vanacker et al. 1998). Jiang and Huang (2001) reported reduced activity of ascorbate peroxidase (APX) in two cool-season turf grasses under drought and heat stress and justified the reduction by decreased CAT activity.

Drought stress damages photosynthetic apparatus and diminishes chlorophyll content (Fu and Huang 2001). Rong Hua et al. (2006) concluded that chlorophyll content could be considered as a reliable indicator in screening genotypes for drought tolerance. Among the solutes, water soluble carbohydrates (WSC), inorganic ions, and proline are associated with osmotic adjustment during drought and other environmental stresses in annual and perennial grasses (Bajji et al. 2001). Although plant species can differ considerably in their ability to accumulate proline upon stress, there is no clear relationship between this ability and stress tolerance among species (Maggio et al. 2002). Keles and Oncel (2004) found high relative water content (RWC) to be closely related to drought tolerance. Efforts are currently directed to access new, affordable, and reliable indices to help in the selection of drought tolerant genotypes (Hura et al. 2007). While drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress, values are confounded with differential yield potential of genotypes (Blum 2011). Therefore, several selection indices have been recommended on the basis of yield under stress and non-stress conditions in order to identify drought tolerant genotypes. Instances are stress tolerance index (STI) introduced by Fernandez (1992), which can be used to identify genotypes that produce high yields under both stress and non-stress conditions, and tolerance index (TOL) introduced by Rosielle and Hamblin (1981) based on differences in yields measured under non-stress and stress conditions. Using drought tolerance and susceptibility indices, Ebrahimiyan et al. (2012) assessed drought tolerance of 75 tall fescue genotypes

in three different flowering sets. Nevertheless, little information is still available about the relationship between these indices and root and physiological traits. The present study hence aimed to evaluate the response of physiological and root characteristics of tall fescue genotypes under drought stress conditions and to determine the relationship between these traits and drought and susceptibility indices under field drought conditions.

Materials and methods

Field experiment

Field experiment was conducted at the Research Farm of Isfahan University of Technology (located at Lavark, Iran, 32°30′N, 51°20′E, 1,630 m asl) during 2011–2012. The area had a clay loam soil (pH 7.5) with the average bulk density of 1.48 g/cm^3 in the top 60-cm layer of the soil surface. The mean annual precipitation and temperature were 140 mm and 15 °C, respectively. It also had dry summers with usually no rain from late May to mid-October.

After developing a broad-based population from polycross of a set of 25 parental tall fescue genotypes (Majidi et al. 2009), 24 (eight early-, eight mid-, and eight late-season flowering) genotypes were selected within the progenies based on drought tolerance and susceptibility indices (Ebrahimiyan et al. 2012). The genotypes were clonally propagated in a greenhouse and then transferred to the field in March 2011. A randomized complete block design with three replications was then used to evaluate the genotypes under control and water stress conditions (supplied when respectively 50 ± 5 % and 90 ± 5 % of the total available water was depleted from the root-zone) (Allen et al. 1998). The irrigation intervals during the growing season and between the two irrigation treatments were variable depending on weather conditions. In the early summers, the grass was cut from 5 cm above the ground at 50 % flowering stage and the weight of herbage was recorded for all plants. The three selection indices including STI (Fernandez 1992), TOL (Rosielle and Hambline 1981), and yield reduction ratio (YR) were calculated based on the dry forage yield (DFY) under control and water stress conditions (Table 1) according to the following formulae:

$$STI = (Ys \times Yp)/(Ymp)^2 \qquad (1)$$

$$TOL = Yp - Ys \qquad (2)$$

$$YR = (Yp - Ys)/Yp \qquad (3)$$

where Ys and Yp are the yield of the ith genotype under stress and normal conditions, respectively. Ymp is the mean yield of all genotypes in control condition.

Pot experiment

The experiment involved two factors (moisture levels and genotypes) with three replications arranged in a completely randomized design. A total of 24 genotypes were clonally propagated and planted in polyvinylchloride (PVC) tubes (60 × 16 cm) filled with a mixture of coarse river sand and silt loam soil (1:2 v/v) collected from the field in 2013. The plants were grown from March to July 2013 under natural condition (daily temperatures of 18–28 °C and photoperiod of 14 h). The time of irrigation in control treatment was determined based on soil water depletion in the root zone with maximum allowable depletion equal to 0.5 (Allen et al. 1998) as follow:

$$\theta_{irrig} = \theta_{FC} - (\theta_{FC} - \theta_{WP}) \times 0.5 \qquad (4)$$

where θ_{irrig} is the soil water content at irrigation time, θ_{FC} is the volumetric soil water content at field capacity (%), and θ_{WP} is volumetric soil water content at wilting point (%).

The reference crop evapotranspiration was calculated based on the United Nation's Food and Agriculture Organization (FAO)-56 Penman–Monteith equation (Allen et al. 1998). The evapotranspiration of grass in the container condition was thus computed as follows:

$$ET_{act} = ET_0 \times K_{MC} \qquad (5)$$

where ET_0 and ET_{act} are grass evapotranspiration under the field and container conditions (mm day^{-1}), respectively. K_{MC} is the microclimate coefficient and was assumed to be 1.2 (Gheysari et al. 2009). When $\sum ET_{act}$ reached to irrigation depth (D_{irrig}), it was the irrigation time for all irrigation levels. At this time, the control level received D_{irrig} and other irrigation levels received a proportion of the water applied to the control level (50 and 75 % for intense and mild stresses, respectively):

Table 1 Information on tall fescue genotypes assessed for drought tolerance in field during 2009–2012

Genotype code	Origin	Flowering status	2009–2010			2011–2012			Drought tolerance
			TOL	STI	YR	TOL	STI	YR	
1	Iran, Yazdabad	Medium	72.07	0.85	52.23	41.58	0.71	45.80	Susceptible
3	Iran, Yasuj	Medium	−10.65	0.03	−69.30	−2.75	0.07	−13.58	Tolerant
11	Hungary, unknown	Medium	41.06	0.47	43.62	17.41	1.46	16.56	Tolerant
17	Iran, Fozve	Medium	47.50	0.46	48.67	−36.62	0.59	−80.49	Tolerant
19	Iran, Shahrood	Medium	15.07	0.17	29.10	−14.83	0.11	−72.50	Tolerant
21	Iran, Fozve	Medium	105.33	2.09	50.02	51.08	2.89	31.36	Moderately tolerant
23	Poland, unknown	Medium	22.19	0.63	23.69	−66.5	0.35	−271.42	Tolerant
25	Iran, Shahrood	Medium	45.17	0.88	37.00	10.66	4.02	6.48	Tolerant
1	Iran, Yazdabad	Early	72.58	0.41	60.07	2.33	1.26	2.34	Susceptible
2	Iran, Yasuj	Early	62.23	0.24	63.88	−1.58	0.08	−6.41	Susceptible
3	Iran, Yasuj	Early	55.41	0.73	41.77	46.66	1.85	32.31	Moderately tolerant
4	Iran, Mobarake	Early	85.18	0.47	63.55	44.95	0.36	56.16	Susceptible
9	Iran, Fozve	Early	72.16	0.48	57.46	0.33	0.58	0.49	Susceptible
10	USA, New Jersy	Early	68.38	1.32	39.24	141.18	0.59	83.87	Susceptible
14	Hungary, Csesznek	Early	39.97	0.12	60.66	11.04	0.16	26.47	Tolerant
16	Iran, Fozve	Early	32.44	0.40	34.83	−11.58	0.80	−15.94	Moderately tolerant
2	Iran, Yasuj	Late	16.48	0.55	18.34	−31.83	0.12	−100.31	Tolerant
3	Iran, Yasuj	Late	189.37	1.94	69.22	162.20	2.32	56.28	Susceptible
6	Iran, Daran	Late	39.51	0.31	47.08	−46.75	0.08	−230.86	Moderately tolerant
7	Iran, Daran	Late	12	0.16	23.49	4.79	0.07	13.31	Moderately tolerant
12	Hungary, unknown	Late	−2.47	0.11	−7.00	−23.12	0.09	−80.43	Moderately tolerant
15	Iran, Yazdabad	Late	47.03	0.40	48.57	141	0.35	81.34	Moderately tolerant
20	Iran, Fozve	Late	59.18	0.48	53.30	49.83	0.25	53.87	Moderately tolerant
25	Iran, Shahrood	Late	53.62	0.80	41.83	258.64	1.46	78.83	Moderately tolerant
LSD			13.01	0.39	34.42	14.58	0.15	26.91	

$$D_{irrig} = (\theta_{FC} - \theta_{WP}) \times 0.5 \times D \qquad (6)$$

where D is the soil column depth (cm).

Measurements

During the 60-day treatment period, various physiological characteristics of shoot were measured. Leaf water status was determined by estimating the RWC according to Ritchi's et al. (1990) method.

Spectrophotometry was used to measure total chlorophyll (Tchl), chlorophyll a, chlorophyll b, and carotenoids (Arnon 1949). Moreover, proline content (Pro) was determined based on the method described by Bates (Bates et al. 1973). WSC content was assessed according to Dubois et al. (1956) method. For enzyme extracts and assays, 0.1 g of leaves were sampled and frozen in liquid nitrogen. They were subsequently ground in 1 ml solution containing 50 Mm phosphate buffer (pH 7.8), 1 %

Polyvinylpolypyrrolidone (w/v), 2 Mm Ethylenedia-minetetraacetic acid (EDTA), 0.2 % Triton X-100, 50 Mm Tris hydrochloride (Tris–HCL), and 2 Mm Dithiothreitol (DTT). The homogenate was centrifuged at 14,000 rounds per minute (rpm) for 30 min and the supernatant was collected and used for enzyme assays. Catalase (CAT) activity was calculated as the reduction of the absorbance at 240 nm for 2 min following the decomposition of H_2O_2 (Change and Maehly 1955). Moreover, APX activity was measured as the decrease in absorbance at 290 nm for 2 min (Nakano and Asada 1981). The activity of peroxidase (POX) was assessed as the increase of absorbance at 470 nm for 2 min (Herzog and Fahimi 1973). Enzyme activities were expressed on the basis of per unit protein weight. Protein content was determined by using bovine serum albumin as the standard Bradford (1976).

The grass was cut manually from 5 cm above the PVC tube and each plant's weight of herbage was recorded after drying at 72 °C for 48 h. The three selection indices (STI, TOL, and YR) were calculated. At the end of the 60-day dry down period, all plants in each container were harvested and the shoots were separated from the roots. The soil column was sliced into two layers (0–30 and 30–60 cm). The roots in each layer were washed free of soil and root wet weight (RWW) was measured immediately. The characteristics of all roots such as root length (RL) and root area (RA) were measured by a computer scanner and GiA Roots software (Galkovskyi et al. 2012). Root volume (RV) was measured as recommended by Archimedes. Root dry weight (RDW) was obtained after roots were dried in an oven at 85 °C for 48 h. Finally, the root to shoot ratio (R/S) was calculated.

Statistical analysis

Data were tested for normality by Kolmogorove Smirnove test and was subjected to analysis of variance (ANOVA) using SAS (2001) to determine difference among treatments and genotypes for each variable. Treatment means were separated using the LSD test ($P < 0.05$). The correlation coefficients between traits were calculated using proc CORR of SAS. Principle component analysis (PCA) was performed based on correlation matrix to reduce the multiple dimensions of data space (Johanson and Wichern 2007) using SAS and the biplot was drawn using Stat Graphics software.

Results

Evaluation of drought tolerance in the field

Results from the analysis of variance showed a significant difference in DFY between the water stress and control environments ($P < 0.01$). The effects of genotype and genotype × environment interaction were also significant for all measured traits (Data not shown). Moreover, water stress reduced DFY in both years. After a hierarchical cluster analysis based on forage yield, STI, TOL, and YR, from the 24 tall fescue genotypes, eight were classified as tolerant, nine as moderately tolerant, and seven as susceptible (Table 1).

Root and physiological assessment in pot experiment

Analysis of variance indicated that the effect of moisture was highly significant for all root traits at both depths. Significant differences were found among the genotypes for all root traits in both depths. The interaction of moisture environment and genotype was also significant for the measured traits (Table 2).

Comparisons with the control treatment revealed the RL at the 0–30 cm depth of the root zone decreased by 11.86 and 41.65 % under mild and intense drought stresses respectively. Meanwhile, under the same conditions, the roots were significantly longer than the control at the 30–60 cm depth (Table 3). On the other hand, at the 0–30 cm depth, intense drought stress caused significant reduction in the RA compared to the control and mild drought stress conditions. However, no significant difference in root zones at the 30–60 cm depths was found between intense drought stress and control conditions. Moreover, at the 0–30 cm depth, the RV and root dry matter under control condition were higher than the values obtained under two levels of drought stress. Nevertheless, no such significant differences were detected at the 30–60 cm depth between intense drought stress and control conditions. Higher drought stress was associated with increased root to shoot ratio (by about 7.69 and 54.16 % at the 0–30 cm and 30–60 cm depths of root, respectively) (Table 3).

Mild and intense drought stress conditions significantly reduced DFY by 15.56 and 50.31 %, respectively. Similarly, RWC decreased by 28.09 and

Table 2 Analysis of variance for root traits in 24 tall fescue genotypes under moisture environments (control, mild stress and intense stress) under pot experiment in 2013

Source of variation	df	Mean square						
		Root length	Root area	Root volume	Root wet weight	Root dry weight	Root dry matter percent	Ratio of root/shoot
Depth 0–30 (cm)								
Environment	2	192,774,553.2[**]	1,277,700.60[**]	407.57[**]	672.31[**]	14.89[**]	53.05[**]	0.08[**]
Genotype	23	17,800,660.8[**]	116,493.45[**]	40.26[**]	33.41[**]	0.46[**]	21.22[**]	0.06[**]
Environment × genotype	46	3,850,766.4[**]	23,090.47[**]	15.44[**]	16.47[**]	0.12[**]	33.30[**]	0.03[**]
Error	144	208,604	2,041.41	2.37	2.64	0.04	0.35	0.001
Coefficient of variation		7.29	8.00	13.59	15.83	15.34	4.23	8.67
Depth 30–60(cm)								
Environment	2	4,115,326.5[**]	57,426.74[**]	38.98[**]	200.43[**]	1.78[**]	63.55[**]	0.34[**]
Genotype	23	34,391,537.7[**]	236,914.64[**]	28.64[**]	71.63[**]	0.72[**]	32.44[**]	0.07[**]
Environment × genotype	46	13,227,517.3[**]	44,903.24[**]	16.06[**]	19.18[**]	0.20[**]	30.81[**]	0.02[**]
Error	144	88,573	3,121.78	1.01	0.50	0.01	0.48	0.0008
Coefficient of variation		4.62	9.63	10.53	7.58	10.53	6.01	9.63

[**] Significant at $P < 0.01$

Table 3 Means of root traits in 24 tall fescue genotypes under three levels of moisture environments (control, mild stress and intense stress) under pot experiment in 2013

Moisture environments	Root length (cm)	Root area (cm^2)	Root volume (cm^3)	Root wet weight (g)	Root dry weigh (g)	Root dry matter percent	Ratio of root/ shoot
Depth 0–30 (cm)							
Control	7,623.78[a]	648.63[a]	14.00[a]	13.26[a]	1.85[a]	14.98[a]	0.39[b]
Mild stress	6,719.23[b]	634.21[a]	10.54[b]	10.37[b]	1.44[b]	14.05[b]	0.36[c]
Intense stress	4,447.79[c]	411.03[b]	9.44[c]	7.15[c]	0.94[c]	13.26[c]	0.42[a]
Depth 30–60 (cm)							
Control	6,157.36[b]	555.33[b]	9.96[a]	9.78[b]	1.13[a]	12.04[a]	0.24[c]
Mild stress	6,523.84[a]	610.60[a]	8.71[b]	10.86[a]	1.12[a]	10.48[b]	0.28[b]
Intense stress	6,606.58[a]	572.91[b]	10.01[a]	7.58[c]	0.85[b]	12.17[a]	0.37[a]

Mean followed by the same letter in each column are not significantly different according LSD test (probability level of 5 %)

37.24 % under mild and intense drought stress levels, respectively. Intense drought stress significantly increased not only carotenoids (Car), chlorophyll a/b (Chl*a/b*), chlorophyll a (Chl*a*), proline (Pro), and WSC) contents, but also peroxidase (POX) activity. Total chlorophyll content (Tchl), catalase (CAT) and APX activities decreased under intense drought stress conditions compared to control condition (Table 4).

Correlation coefficients between different traits with TOL and STI were calculated in the pot experiment and are presented in Table 5. Under both mild and intense drought stress conditions, DFY was

highly and positively correlated with STI but negatively correlated with TOL and YR. Total RA (TRA) and total root volume (TRV) were positively correlated with STI only under mild stress condition. Moreover, STI had a moderately negative correlation with root to shoot ratio only under intense drought stress condition. TOL and YR were negatively correlated with TRL only under mild stress condition (Table 5).

Under control condition, chlorophyll a/b and chlorophyll b were significantly correlated with all root characteristics. Meanwhile, RWC was correlated with

Table 4 Means of physiological traits and dry forage yield in 24 tall fescue genotypes under three levels of moisture environments (control, mild stress and intense stress) under pot experiment in 2013

Moisture environments	Dry forage yield (g)	RWC	Carotenoids (mg/g leaf)	Proline (μmol/g leaf)	Chla (mg/g leaf)	Chlb (mg/g leaf)	Total chlorophyll (mg/g leaf)	Ratio of Chl a/b (mg/g leaf)	WSC (mg/ml)	CAT (μ mol min^{-1} mg^{-1} protein)	APX (μ mol min^{-1} mg^{-1} protein)	POX (μ mol min^{-1} mg^{-1} protein)
Control	4.69[a]	54.61[a]	9.57[c]	0.3[c]	1.11[c]	0.87[a]	1.98[a]	1.27[c]	137.85[b]	1.07[a]	0.67[a]	1.37[c]
Mild stress	3.96[b]	39.27[b]	10.48[b]	0.35[b]	1.31[b]	0.65[b]	1.96[a]	2.01[b]	164.94[a]	1.08[a]	0.61[a]	2.25[b]
Intense stress	2.33[c]	34.27[c]	11.52[a]	0.5[a]	1.40[a]	0.49[c]	1.86[b]	2.58[a]	156.02[a]	0.68[b]	0.48[b]	2.48[a]

Mean followed by the same letter in each column are not significantly different according to LSD test (probability level of 5 %)

CAT, POX, and TRV (Data not shown). Under mild stress conditions, TRL, TRA, TRV, total root wet weight (TRWW), and total root dry weight (TRDW) were positively correlated with DFY. However, no significant correlations were detected between photosynthetic pigments (Chla, Chlb, Tchl, Chla/b and Carotenoids) and DFY (Table 6). While all photosynthetic pigments expect chlorophyll a/b had negative correlations with CAT under mild stress condition, no such correlations were observed under intense drought stress. RWC was significantly correlated with chlorophyll b, Tchl, chlorophyll a/b, and proline under mild stress condition. At the same time, proline and APX were significantly correlated with TRL and total root dry matter (TRDM), respectively. Under mild stress condition, TRA, TRWW, TRDW and root to shoot ratio (R/S) were positively correlated with POX. However, similar correlations did not exist under intense drought stress. RWC was significantly correlated with CAT, APX, and POX under intense drought stress. Chlorophyll a, water soluble carbohydrate (WSC), and TRWW had positive correlations with proline under intense stress condition. Under the same condition, CAT and chlorophyll a/b were positively correlated with TRDM and root to shoot ratio, respectively (Table 6).

Principle component analysis (PCA) revealed that the first and second components explained more than 52 and 49 % of the variation in mild and intense stress, respectively (Table 7). Under mild stress condition, principle component 1 (PC1) had higher correlation with chlorophyll a, chlorophyll b, Tchl, chlorophyll a/b, Tchl/carotenoids, and carotenoids. As higher values of these characteristics showed higher photosynthetic capacity, PC1 was named "photosynthetic capacity'' under mild stress condition. Principle component 2 (PC2) had higher correlation with DFY, TRL, TRA, TRV, TRWW, TRDW, and root to shoot ratio. Since higher values of these characteristics indicated greater root production capacity and thus yield productivity of the genotypes, PC2 was called "potential of root production''. To classify the genotypes based on PCA, the biplot of PC1 and PC2 was constructed (Fig. 1). As a result, genotypes 2L, 23M, 21M, and 14E were found to have high photosynthetic capacity (high PC1) and high potential to root and yield production (high PC2) under mild drought stress. Under intense drought stress, PC1 had negative correlations with chlorophyll a, chlorophyll

Table 5 Correlation coefficients of different traits with TOL, STI and reduction in mild stress and intense stress condition under pot experiment in 2013

Traits	Mild stress			Intense stress		
	TOL	STI	YR	TOL	STI	YR
Wet forage yield (g)	−0.56**	0.55**	−0.59**	−0.23ns	0.35ns	−0.34ns
Dry forage yield (g)	−0.82**	0.79**	−0.86**	−0.77**	0.84**	−0.91**
Dry matter percent	−0.30ns	0.18ns	−0.29ns	−0.54**	0.66**	−0.62**
Crown diameter (cm)	0.19ns	−0.25ns	0.19ns	0.21ns	−0.04ns	0.13ns
Plant height (cm)	−0.03ns	0.005ns	−0.04ns	−0.32ns	0.28ns	−0.32ns
Number of stems per plant	0.04ns	−0.08ns	0.04ns	−0.11ns	0.53**	−0.20ns
Total root length (cm)	−0.49**	0.30ns	−0.48**	0.03ns	−0.007ns	−0.003ns
Total root area (cm^2)	−0.24ns	0.43**	−0.27ns	0.13ns	−0.08ns	0.09ns
Total root volume (cm^3)	−0.26ns	0.47**	−0.28ns	0.19ns	−0.09ns	0.17ns
Total root wet weight (g)	−0.27ns	0.38ns	−0.28ns	0.05ns	−0.05ns	0.06ns
Total root dry weight (g)	−0.31ns	0.15ns	−0.29ns	0.08ns	−0.13ns	0.08ns
Total root dry matter percent	−0.05ns	−0.22ns	−0.007ns	−0.27ns	0.12ns	−0.29ns
Ratio of root/shoot	−0.17ns	−0.09ns	−0.14ns	0.31ns	−0.58**	0.43*
Relative water content	0.07ns	−0.18ns	0.09ns	−0.16ns	−0.005ns	−0.15ns
Chl$a$ content (mg/g leaf)	−0.32ns	0.15ns	−0.32ns	−0.05ns	0.19ns	−0.02ns
Chl$b$ content (mg/g leaf)	−0.27ns	0.12ns	−0.25ns	−0.04ns	0.23ns	−0.03ns
Cartenoeid content (mg/g leaf)	−0.40*	0.21ns	−0.38ns	−0.12ns	0.19ns	−0.11ns
Total chlorophyll (mg/g leaf)	−0.31ns	0.14ns	−0.29ns	−0.05ns	0.21ns	−0.03ns
Ratio of Chl$a$/Chl$b$	0.18ns	−0.08ns	0.17ns	0.08ns	−0.20ns	0.07ns
Ratio of TChl/Car	−0.03ns	−0.05ns	−0.03ns	0.05ns	0.18ns	0.08ns
Proline content (µmol/g leaf)	−0.36ns	0.21ns	−0.38ns	0.19ns	0.19ns	0.14ns
Water soluble carbohydrates (mg/ml)	−0.24ns	0.13ns	−0.23ns	0.11ns	0.03ns	0.03ns
CAT content (µmol min^{-1} mg^{-1} protein)	0.26ns	−0.26ns	0.29ns	−0.31ns	0.23ns	−0.36ns
APX content (µmol min^{-1} mg^{-1} protein)	−0.22ns	−0.12ns	−0.19ns	0.001ns	0.04ns	−0.007ns
POX content (µmol min^{-1} mg^{-1} protein)	−0.22ns	0.22ns	−0.22ns	0.02ns	0.17ns	−0.01ns

ns non-significant

* Significant at 5 % level of probability

** Significant at 1 % level of probability

b, Tchl, Tchl/carotenoids, and carotenoids and a positive correlation with chlorophyll a/b. Therefore, selection based on high PC1 values can lead to sensitive genotypes with low photosynthetic capacity. On the other hand, PC2 was positively correlated with TRL, TRA, TRV, TRWW, TRDW, and root to shoot ratio. Thus, genotypes with high PC2 are suitable for intense stress conditions. According to the biplot analysis of PC1 and PC2 (Fig. 2), genotypes 2L, 17M, 14E, and 15L had low PC1 and high PC2 and were hence identified as preferable genotypes for intense stress condition.

Cluster analysis generally confirmed the results of PCA. Under mild drought stress in the pot experiment, the genotypes were classified into four separate clusters (Fig. 3). The first group included seven genotypes with moderate-high photosynthetic capacity and low potential of root production. The second group consisted of seven genotypes with low-moderate photosynthetic capacity and moderate-high potential of root production. The third group comprised four genotypes (2L, 23M, 21M and 14E) with moderate-high photosynthetic capacity and moderate-high potential of root production. They were nearly in

Table 6 Correlation coefficients of different traits under mild stress (above diagonal) and intense stress (below diagonal) conditions under pot experiment in 2013

	DFY	RWC	chla	chlb	car	Tchl	Chla/b	Pro	WSC
DFY	1	−0.08 ns	0.22 ns	0.15 ns	0.29 ns	0.20 ns	−0.05 ns	0.31 ns	0.19 ns
RWC	0.09ns	1	−0.23*	−0.35**	−0.22*	−0.28**	0.45**	−0.28**	−0.18 ns
Cha	0.07ns	−0.22*	1	0.93***	0.95***	0.99***	−0.62***	0.02 ns	0.12 ns
Chb	0.08ns	−0.16 ns	0.79***	1	0.90***	0.95***	−0.85***	0.03 ns	0.14 ns
car	0.14ns	−0.12 ns	0.93***	0.81***	1	0.95***	−0.65***	0.06 ns	0.24*
Tchl	0.07ns	−0.20 n.s.	0.95***	0.93***	0.92***	1	−0.72***	0.02 ns	0.13 ns
Chl a/b	−0.09ns	0.15 ns	−0.48***	−0.88***	−0.57***	−0.70***	1	−0.01 ns	−0.11 ns
Pro	−0.008ns	−0.06 ns	0.36**	0.06 ns	0.27**	0.24*	0.14 ns	1	0.02 ns
WSC	0.001ns	−0.02 ns	−0.06 ns	−0.16 ns	−0.16 ns	−0.11 ns	0.23*	0.39**	1
CAT	0.35ns	0.35**	0.05·s	0.17 ns	0.14 ns	0.11 ns	−0.20 ns	0.05 ns	0.11 ns
APX	0.01ns	0.33**	−0.01 ns	−0.04 ns	0.12 ns	−0.02 s	0.02 ns	0.12 ns	−0.21 ns
POX	0.08ns	−0.34**	0.24*	0.19·s	0.09 ns	0.23*	−0.07 ns	0.05 ns	−0.11 ns
TRL	0.06ns	−0.12 ns	0.12 ns	0.04 ns	0.20 ns	0.09 ns	0.007 ns	0.22 ns	0.01 ns
TRA	−0.02ns	−0.07 ns	0.03 ns	−0.06 ns	0.10 ns	−0.01 ns	0.12 ns	0.25*	0.0008 ns
TRV	−0.08ns	−0.06 ns	0.09 ns	−0.01 ns	0.15 ns	0.04 ns	0.11 ns	0.30 ns	−0.007 ns
TRWW	−0.01ns	−0.07 ns	0.22*	0.09 ns	0.26*	0.17 ns	0.02 ns	0.30**	−0.09 ns
TRDW	−0.03ns	−0.02 ns	0.03 ns	−0.08 ns	0.08 ns	−0.01 ns	0.17 ns	0.19 ns	0.009 ns
TRDM	0.24ns	0.27 ns	−0.23*	−0.21 ns	−0.25*	−0.23*	0.20 ns	−0.20 ns	0.18 n.s.
R/S	−0.52**	0.03 ns	−0.05 ns	−0.20 ns	−0.03 ns	−0.12 ns	0.28**	0.11 ns	0.02 ns

	CAT	APX	POX	TRL	TRA	TRV	TRWW	TRDW	TRDM	R/S
DFY	−0.23ns	0.01 ns	0.31 ns	0.49**	0.49**	0.48**	0.49**	0.36*	−0.14 ns	0.12 ns
RWC	0.23*	−0.05 ns	−0.17 ns	−0.13 ns	−0.09 ns	−0.06 ns	−0.15 ns	−0.08 ns	0.08 ns	−0.17 ns
Cha	−0.39**	0.11 ns	0.04 ns	0.008 ns	−0.009 ns	−0.15 ns	0.01 ns	0.04 ns	−0.09 ns	0.12 ns
Chb	−0.41**	0.20 ns	0.06 ns	0.009 ns	−0.08 ns	−0.11 ns	−0.02 ns	−0.007 ns	−0.05 ns	−0.13 ns
car	−0.39**	0.10 ns	0.001 ns	0.02 ns	−0.01 ns	−0.15 ns	0.01 ns	−0.08 ns	−0.14 ns	−0.19 ns
Tchl	−0.41**	0.15 ns	0.05 ns	0.009 ns	−0.03 ns	−0.14 ns	−0.001 ns	0.02 ns	0.08 ns	−0.13 ns
Chl a/b	0.43***	−0.32**	−0.09 ns	0.01 ns	0.15 ns	0.05 ns	0.07 ns	0.08 ns	−0.01 ns	0.13 ns
Pro	−0.16ns	−0.001 ns	−0.08 ns	0.41***	0.11 ns	0.08 ns	0.11 ns	0.22*	0.20 ns	0.25*
WSC	−0.07ns	0.18 ns	−0.08 ns	0.08 ns	−0.18 ns	0.02 ns	0.03 ns	0.14 ns	−0.21 ns	−0.19 ns
CAT	1	−0.37**	−0.28**	0.12 ns	0.009 ns	0.23*	0.17 ns	0.05 ns	−0.04 ns	0.13 ns
APX	0.03ns	1	0.18 ns	0.12 ns	0.09 ns	0.03 ns	−0.06 ns	0.15 ns	0.38**	0.22*
POX	−0.05ns	0.01 ns	1	0.23*	0.53***	0.21 ns	0.43***	0.46***	0.12 ns	0.42***
TRL	0.14ns	0.004 ns	−0.08 ns	1	0.64***	0.59***	0.58***	0.63***	0.23*	0.63***
TRA	0.13ns	−0.06 ns	−0.14 ns	0.86***	1	0.56***	0.66***	0.70***	0.17 ns	0.64***
TRV	0.03ns	−0.03 ns	−0.16 ns	0.84***	0.92***	1	0.41***	0.44***	0.09 ns	0.46***
TRWW	−0.11ns	−0.011 ns	−0.04 ns	0.79***	0.79***	0.82***	1	0.69***	−0.22*	0.54***
TRDW	−0.08ns	−0.13 ns	−0.12 ns	0.73***	0.81***	0.82***	0.88***	1	0.39***	0.82***
TRDM	0.34**	−0.17 ns	0.007 ns	−0.23*	−0.07 ns	−0.12 ns	−0.31**	−0.04 ns	1	0.48***
R/S	−0.24*	−0.05 ns	−0.27*	0.41***	0.53***	0.57***	0.60***	0.72***	−0.02 ns	1

DFY Dry forage yield, *RWC* relative water content, *Chla* chlorophyll a content, *Chlb* chlorophyll b content, *Car* carotenoids contents, *Tchl* total chlorophyll, *Chl-a/b* ratio of Chla/Chlb, *Pro* proline content, *WSC* water soluble carbohydrates, *CAT* catalase activity, *APX* ascorbate peroxidase activity, *POX* peroxidase activity, *TRL* total root length, *TRA* total root area, *TRV* total root volume, *TRWW* total root wet weight, *TRDW* total root dry weight, *TRDM* total root dry matter percent, *R/S* ratio of root to shoot

ns: non-significant, *: Significant at 5 % level of probability and **: Significant at 1 % level of probability

Table 7 Principal component loadings for the traits measured on 24 tall fescue genotypes under two levels of drought stress (mild and intense stress) under pot experiment in 2013

Characters	Mild stress		Intense stress	
	PC1	PC2	PC1	PC2
Dry forage yield (g)	0.19	0.23	−0.07	−0.11
Total root length (cm)	0.11	0.33	−0.05	0.36
Total root area (cm^2)	0.09	0.35	0.001	0.39
Total root volume (cm^3)	0.02	0.32	−0.03	0.40
Total root wet weight (g)	0.10	0.32	−0.09	0.39
Total root dry weight (g)	0.11	0.35	0.01	0.39
Total root dry matter percent	−0.008	0.11	0.14	−0.11
Ratio of root/shoot	0.03	0.33	0.09	0.33
Relative water content	−0.21	−0.004	0.12	−0.04
Chl$a$ (mg/g leaf)	0.35	−0.13	−0.38	0.03
Chla b (mg/g leaf)	0.36	−0.15	−0.39	−0.04
Cartenoeid content (mg/g leaf)	0.34	−0.14	−0.37	0.06
Total chlorophyll (mg/g leaf)	0.36	−0.14	−0.41	0.001
Ratio of Chl a/b	−0.31	0.15	0.33	0.08
Ratio of Tchl/car	0.27	−0.09	−0.36	−0.06
Proline content (μmol/g laef)	0.06	0.13	−0.12	0.15
Water soluble carbohydrates (mg/ml)	0.10	−0.05	0.10	−0.004
CAT content (μ mol min^{-1} mg^{-1} protein)	−0.24	0.09	−0.05	−0.04
APX content (μ mol min^{-1} mg^{-1} protein)	0.12	0.02	0.001	−0.01
POX content (μ mol min^{-1} mg^{-1} protein)	0.16	0.19	−0.13	−0.08
TOL	−0.21	−0.15	0.04	0.12
STI	0.14	0.15	−0.13	−0.12
Eigenvalue	6.12	5.37	5.67	5.28
Percent of variation	28.24	24.44	25.80	24.04
Cumulative percentage	28.24	52.68	25.80	49.84

vicinity of STI, DF, POX, and proline on the biplot (Fig. 1). The rest of the genotypes, which had low photosynthetic capacity and low-moderate potential of root production, were clustered into the fourth group.

Under intense drought stress, the genotypes were again categorized into four distinct clusters (Fig. 4). The first group contained seven genotypes with low photosynthetic capacity and low potential of root production. The second group included eight genotypes with high photosynthetic capacity and low-moderate potential of root production. The third group consisted of four genotypes with low photosynthetic capacity and low potential of root production. The remaining genotypes (2L, 17M, 14E, 15L and 7L), which had moderate-high photosynthetic capacity and moderate-high potential of root production (nearly adjacent to all root traits and proline on the biplot) were allocated to the fourth cluster (Fig. 2).

Discussion

Drought tolerance mechanisms depend on extensive root systems and physiological functions such as osmoregulation in plant tissues and protection against oxidative damage. The present study evaluated a set of diverse genotypes of tall fescue in terms of field drought tolerance (based on drought tolerance and susceptibility indices) and the response of root and physiological traits. Significant differences between all traits of the selected genotypes indicated large genetic variation in this germplasm. This variation can be used for selecting drought tolerant genotypes.

The pattern of root distribution within a soil profile differs based on crop species, soil type, moisture, fertility, and other environmental variables. In our study, while drought stress conditions caused significant increases in RL at the 30–60 cm depths of soil, such a change was not observed at the 0–30 cm depths of the root zone. In other words, in well-watered conditions, tall fescue species spread their roots on the surface to use less energy for water uptake (Sheffer et al. 1987). Hung and Gao (2000) reported that in some tall fescue genotypes, increasing drought stress was associated with decreased RL at the 0–20 cm and 20–40 cm depths of the root zone. The same conditions, however, increased RL at the 40–60 cm depths. Increased RL at deeper soil layers during drought stress has also been detected in other species and is thus considered as an important adaptation mechanism to improve the efficiency of plant water uptake (Molyneux and Davies 1983; Gallardo et al. 1996).

In the current study, intense drought stress was accompanied by reduced RA compared to mild drought stress. Moreover, intense drought stress significantly increased RV, root dry matter percent, and root to shoot ratio at the 30–60 cm depths of soil. The mentioned increments enhance the availability of root system distribution at deeper soil layers during drought stress and hence contribute to better water absorption (Serraj et al. 2004; Farre and Faci 2009). Increased root to shoot ratio during drought stress has also been introduced as a drought avoidance mechanism (Guo et al. 2002; Bonos et al. 2004; Karcher et al. 2007).

Similar to the findings of Pand and Singh (Pande and Singh 1981), we observed decreased RDW under intense drought stress (Table 3). However, mild stress significantly increased root RDW. In fact, higher drought stress reduced net assimilation by the roots and consequently root weight (Khalid 2006). Nevertheless, since root hairs, which have high absorption potential, constitute a small percentage of the total root weight, root weight is not an important factor in determining root activity (Farre and Faci 2009).

In the present study, intense drought stress decreased DFY, RWC, chlorophyll b, Tchl, CAT, and APX. Meanwhile, mild drought stress only decreased DFY, RWC, and chlorophyll b (Table 4). On the other hand, intense and mild drought stress conditions increased chlorophyll a/b, carotenoids, proline, POX, WSC and chlorophyll a levels (Table 4). Chlorophyll content is a major determinant of photosynthetic capacity under intense stress, i.e. higher chlorophyll content and stability have been reported to be associated with drought tolerance. Thus, selecting genotypes based on increased or stable chlorophyll content may prevent yield loss under drought stress. Although reduced chlorophyll content has been suggested as a reaction to drought stress, similar to our findings, some studies have documented elevated chlorophyll content under mild drought stress (Garcia-Valenzuela et al. 2005; Ebrahimiyan et al. 2013). This increment might be the result of slower cellular growth relative to chlorophyll synthesis (Garcia-Valenzuela et al. 2005). Consistent with our findings, Ebrahimiyan et al. (2013) reported increased chlorophyll a/b which can be justified by faster damage to chlorophyll b compared to chlorophyll a under intense stress condition. While chlorophyll b is found exclusively in photosynthetic antennas,

chlorophyll a exists in both the reaction centers of photosystems I and II and photosynthetic antennas (Lichtenthaler and Buschmann 2001). Several researchers have reported reduced DFY under drought stress which is evidently due to decreased water potential and the consequent decline in net assimilation by the leaves (Sarker et al. 1999; Merewitz et al. 2010). Karcher et al. (2007) concluded that the reduction in DFY under drought stress is a drought avoidance mechanism in most turf grasses.

The effects of stresses on enzyme activity depend on crop species and type, duration, and intensity of drought stress. Decreased CAT and APX activities under drought stress have been observed in cool-season turf grasses (Smirnoff 1993; Castillo 1996; Jiang and Huang 2001). Generally, the oxidative injury induced by intense drought stress is characterized by the reduction in antioxidant enzymes and increase in lipid peroxidation. Carotenoids can act as a non-enzymatic antioxidant. They have multiple roles, e.g. light harvesting and protection from oxidative damage caused by drought, in developing drought tolerance (Simkin et al. 2008; Jaleel et al. 2009). Higher carotenoid contents under intense drought stress could be attributed to increased proline, POX, and WSC. The latter is in turn caused by the inhibition of growth and hydrolysis of complex carbohydrates (such as starch) through lowering the water potential (Moradshahi et al. 2004). The accumulation of compatible solutes such as proline and WSC is a common adaptation mechanism acquired by annual and perennial grasses under water deficit stress (Bajji et al. 2001). These compounds also improve plant stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions (Rudoplh et al. 1986).

The pot experiment in the current study showed a positive correlation between DFY and STI. In contrast, TOL and YR were negatively correlated with DFY under mild and intense stress conditions. This difference can indicate that each index demonstrates specific biological responses to drought. According to the results of correlations between different traits and TOL, STI, and YR in the pot experiment, under mild stress, selection based on higher TRL, TRA, and TRV will lead to genotypes with higher yield, yield stability, and drought tolerance. However, a moderately negative relationship was found between root to shoot ratio and STI under intense stress. Naturally with
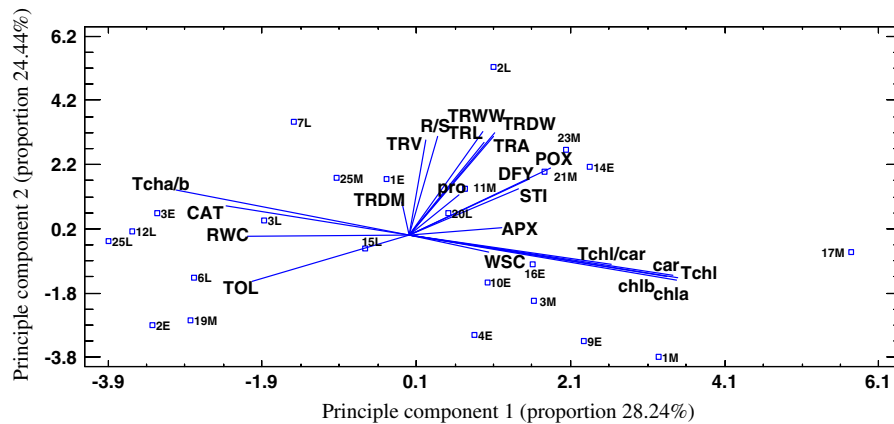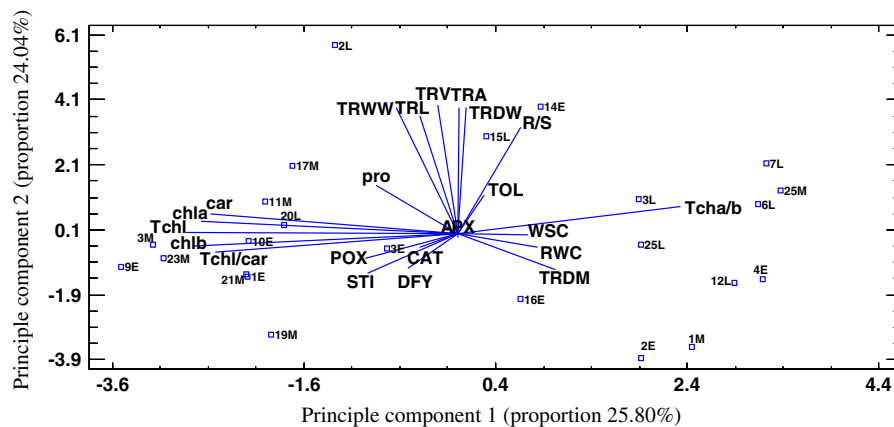
Fig. 1 The bipolt display of root and physiological traits, stress tolerance indices and tall fescue genotypes yield levels under mild stress condition (*DFY* dry forage yield, *RWC* relative water content, *Chla* chlorophyll a content, *Chlb* chlorophyll b content, *Car* carotenoids contents, *Tchl* total chlorophyll, *Tchl*/Car ratio of Tchl/Car, *Chl-a/b* ratio of Chla/Chlb, *Pro* proline content, *WSC* water soluble carbohydrates, *CAT* catalase activity, *APX* ascorbate peroxidase activity, *POX* peroxidase activity, *TRL* total root length, *TRA* total root area, *TRV* total root volume, *TRWW* total root wet weight, *TRDW* total root dry weight, *TRDM* total root dry matter, *R/S* root to shoot ratio, *STI* stress tolerance index, *TOL* tolerance index). Definition origin of the genotypes can be seen in Table 1

Fig. 2 The bipolt display of root and physiological traits, stress tolerance indices and tall fescue genotypes yield levels under intense stress condition (*DFY* dry forage yield, *RWC* relative water content, *Chla* chlorophyll a content, *Chlb* chlorophyll b content, *Car* carotenoids contents, *Tchl* total chlorophyll, *Tchl*/Car ratio of Tchl/Car, *Chl-a/b* ratio of Chla/Chlb, *Pro* proline content, *WSC* water soluble carbohydrates, *CAT* catalase activity, *APX* ascorbate peroxidase activity, *POX* peroxidase activity, *TRL* total root length, *TRA* total root area, *TRV* total root volume, *TRWW* total root wet weight, *TRDW* total root dry weight, *TRDM* total root dry matter, *R/S* root to shoot ratio, *STI* stress tolerance index, *TOL* tolerance index). Definition origin of the genotypes can be seen in Table 1

augmentation of the drought stress, the shoot production reduce more than root production therefore root to shoot ratio increase (Kmoch et al. 1957). This mechanism can be especially beneficial for surviving from intense drought stress conditions. Hence, as suggested by Karcher et al. (2008) and Bonos et al. (2004), root to shoot ratio can be used as a selection criterion for drought tolerance under intense stress.

In this study, a negative relationship was observed between chlorophyll a/b and APX activity under mild stress, i.e. lower chlorophyll a/b after mild stress can cause a relative increase in APX activity. Meanwhile, the positive correlations of RWC with CAT and APX indicate that decreased RWC during intense stress was associated with loss of chlorophyll and lipid peroxidation which in turn triggered oxidative injury. Similar
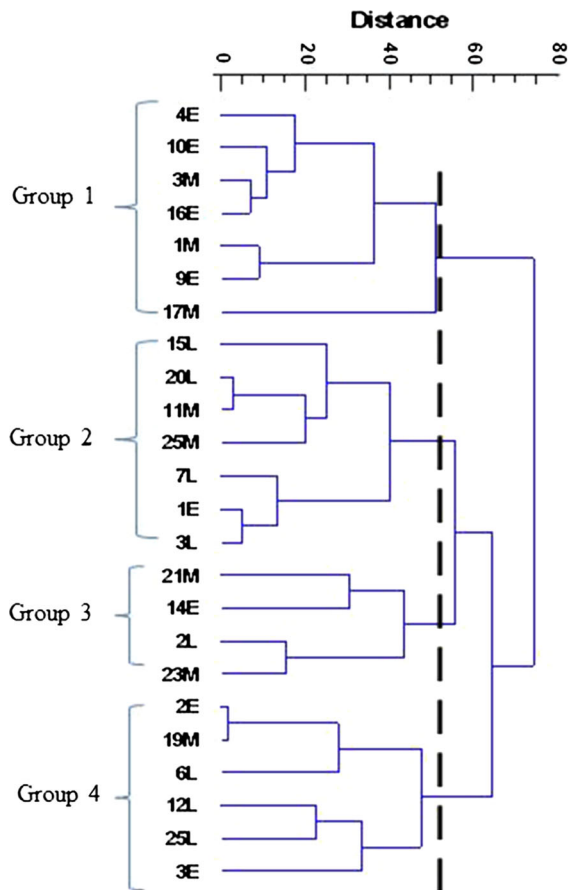
Fig. 3 Dendrogram of 24 tall fescue genotypes evaluated under mild drought stress using Ward clustering method. Definitio origin of the genotypes can be seen in Table 1

Fig. 4 Dendrogram of 24 tall fescue genotypes evaluated under intense drought stress using Ward clustering method. Definition origin of the genotypes can be seen in Table 1

findings were also reported by Jiang and Huang (2001). We also discovered a positive relationship between WSC and proline under intense drought stress. In other words, proline and WSC are linked with osmotic adjustment and drought tolerance (Bajji et al. 2001). Under mild stress, the positive correlations between proline and TRL on the one hand, and between POX and TRA, TRWW, TRDW, and root to shoot ratio, on the other, confirmed the significance of these physiological traits in determining root traits under drought stress condition and selection criteria for drought tolerant genotypes.

Wide distribution of genotypes on the biplot of PCA, which was confirmed by cluster analysis, indicated that tolerant genotypes had extensive root systems and high photosynthetic capacity and could thus adapt to drought through drought avoidance
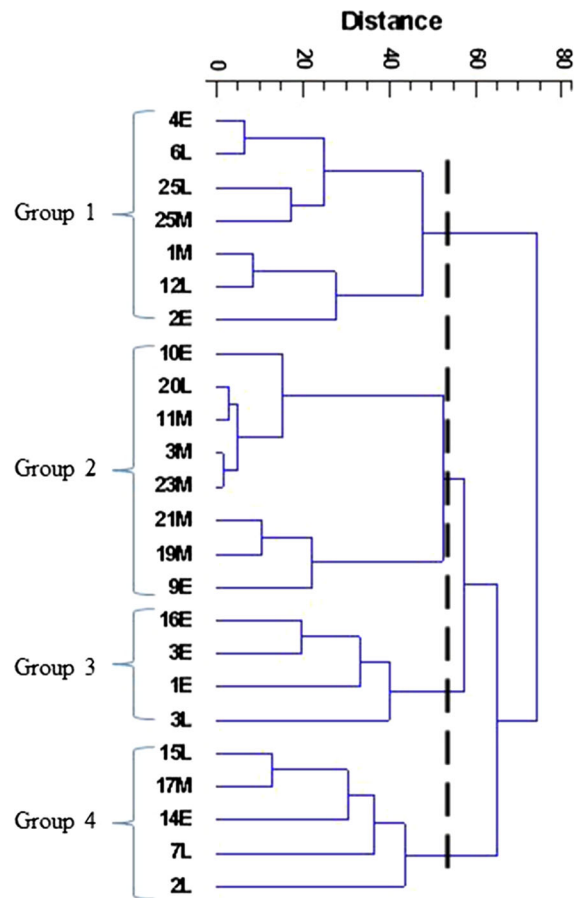
and tolerance mechanisms. Moderately tolerant genotypes usually had one of the drought tolerance mechanisms such as highly extensive root systems or proper physiological and metabolic functions. Susceptible genotypes had less extensive root systems and photosynthetic capacity. We failed to establish a complete relationship between genotype classifications based on drought tolerance in the field and pot experiments which is evidently due to difference on moisture regimes, and other environmental variables. While some genotypes such as 2L and 14E were tolerant, 1M, 4E, 2E, and 10E were susceptible. Others, e.g. 7L and 20L, were moderately tolerant both in the field and pot experiments. Apparently, the selected genotypes had high genetic variation in terms of DFY, root system, and physiological traits in response to drought stress.

In conclusion, the results of this study suggested that soil moisture stress could greatly influence root characteristics and physiological functions and thus affect plant growth and biomass production. This effect is highly dependent on drought stress intensity. Large genetic variation between the selected genotypes in all measured traits highlighted the fact that selection of tall fescue genotypes based on simultaneous increments in photosynthetic capacity, forage yield, and root production under drought stress would lead to improved drought tolerance. Although root to shoot ratio might act as part of a survival mechanism under intense drought stress, this trait had no significant relationship with drought tolerance in forage tall fescue. Generally, field screening of genotypes for drought tolerance and susceptibility indices is useful for characterizing genotypes with different levels of yield reduction under drought stress. However, combining the field results with root and physiological responses could be more beneficial to describing genetic variation and distinguish superior genotypes under stress conditions. The results of PCA and cluster analysis indicated the superiority of some genotypes (2L and 14E) under drought stress conditions and can be used in development of synthetic varieties. Biplots may also be employed for identification of contrasting genotypes when planning to develop mapping populations for genome studies of drought tolerance in tall fescue.

References

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration: guidelines for computing crop requirements. FAO Irrig Drain pap 56:41–51

Alscher RG, Erturk N, Heath LS (2002) Role of super oxide dismutase (SOD) in controlling oxidative stress in plant. J Exp Bot 153:1331–1341

Arnon DI (1949) Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24:1–15

Atlin GN, Frey KJ (1990) Selection oat for yield in low productivity environments. Crop Sci 30:556–561

Bajji M, Lutts S, Kinet JM (2001) Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (*Triticum durum* Desf.) cultivars performing differently in arid conditions. Plant Sci 160:669–681

Bates LS, Waldren RP, Teare LD (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Blum A (2011) Plant breeding for water limited environments. Springer, New York

Bonos SA, Rush D, Hignight K, Meyer WA (2004) Selection for deep root production in tall fescue and perennial ryegrass. Crop Sci 44:1770–1775

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein- dye binding. Anal Biochem 72:248–254

Cakmak I, Horst W (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glysine max* L.). Plant Physiol 83:463–468

Castillo FJ (1996) Antioxidant protection in the inducible CAM plant *Sedum album* L. following the imposition of severe water stress and recovery. Oecologia 107:469–477

Change B, Maehly AC (1955) Assay of catalases and peroxidase. Meth Enzymol 2:764–775

Chloupek O, Skacel M, Ehrenbergerova J (1999) Effect of divergent selection for root size in field-grown alfalfa. Can J Plant Sci 79:93–95

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–358

Ebrahimiyan M, Majidi MM, Mirlohi A, Gheysari M (2012) Drought tolerance indices in a tall fescue population and its polycross progenies. Crop Pasture Sci 63:360–369

Ebrahimiyan M, Majidi MM, Mirlohi A, Noroozi A (2013) Physiological traits related to drought tolerance in tall fescue. Euphytica 190:401–414

Ekanayake IJ, Toole JCO, Garrity DP, Massajo TM (1985) Inheritance of root characteristics and their relation to drought tolerance in rice. Crop Sci 25:927–933

Farre L, Faci JM (2009) Deficit irrigation in maze for reducing agricultural water use in a mediterranean environment. Agric Water Manag 96:383–394

Fernandez GCJ (1992) Effective selection criteria for assessing plant stress tolerance. In: Kuo CC (ed), Proceedings of an international symposium on adaptation of food crops to temperature and water stress. AVRDC, Shanhua. 257–270

Fu J, Huang B (2001) Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environ Exp Bot 45:105–114

Galkovskyi T, Mileyko Y, Bucksch A, Moore B, Symonova O, Price CA, Topp CN, Iyer-Pascuzzi AS, Zurek PR, Fang S, Harer J, Benfey PN, Weitz JS (2012) GiA Roots: software for the high-throughput analysis of plant root system architecture. BMC Plant Biol 12:116

Gallardo M, Jackson LE, Thompson RB (1996) Shoot and root physiological responses to localized zones of soil moisture in cultivated and wild lettuce (*Lactuca* spp.). Plant Cell Environ 19:1169–1178

Garcia-Valenzuela X, Garcia-Moya E, Rascon-Cruz Q, Herrera-Estrella L, Aguado-Santacruz GA (2005) Chlorophyll accumulation is enhanced by osmotic stress in graminaceous chlorophyllic cells. Plant Physiol 162:650–661

Gazanchian A, Khoshkholghsima NA, Malboobi MA, Majidi-Heravan E (2006) Relationships between emergence and soil water content for perennial cool-season grasses native to Iran. Crop Sci 46:544–553

Gheysari M, Mirlatifi SM, Bannayan M, Homaee M, Hoogenboom G (2009) Interaction of water and nitrogen on maize grown for silage. Agric Water Manag 96:809–821

Guo G, Liu SHQ, An S, Ren X, Lin RN (2002) Effect of limited water supply on root growth and development of winter wheat and the characters of soil moisture use before planting. J Appl Meterol 13:621–626

Herzog V, Fahimi H (1973) Determination of the activity of peroxidase. Anal Biochem 55:554–562

Huang B, Gao H (2000) Root physiological characteristics association with drought resistance in tall fescue cultivars. Crop Sci 40:196–203

Huang B, Duncan RR, Carrow RN (1997) Drought resistance mechanisms of seven warm-season turfgrasses under surface soil drying: II. Root aspects. Crop Sci 37:1863–1869

Hura T, Grzesiak S, Hura K, Thiemtm E, Tokarz K, Wedzony M (2007) Physiological and biochemical tools useful in drought tolerance detection in genotypes of winter triticale: accumulation of Ferulic acid correlates with drought tolerance. Ann Bot 100:767–775

Jaleel CA, Manivannan P, Wahid A, Faros M, Al-juburi HJ, Somasundaram R, Panneerselvam R (2009) Drouhgt stress in plants: a review on morphological characteristics and pigments composition. Int J Agric Biol 11:100–105

Jiang Y, Huang B (2001) Drought and heat stress injury to two cool-season turfgrass in relation to antioxidant metabolism and lipid peroxidation. Crop Sci 41:436–442

Johanson RA, Wichern DW (2007) Applied multivariate statistical analysis. Prentice Hall Inter, Inc, New Jersey

Karcher D, Richardson M, Landreth J (2007) Drought tolerance of tall fescue and bluegrass cultivars. Ag Exp Stn Res Ser 557:17–20

Karcher DE, Richardson MD, Hignight K, Rush D (2008) Drought tolerance of tall fescue populations selected for high root/shoot ratios and summer survival. Crop Sci 48:771–777

Keles Y, Oncel I (2004) Growth and solute composition in two wheat species experiencing combined influence of stress conditions. J Plant Physiol 51:228–233

Khalid KHA (2006) Influence of water stress on growth, essential oil and chemical composition of herbs (Ocimum sp.). Agrophysics 20:289–296

Kirigwi FM, Van Ginkel M, Trethowan R, Sears RG, Rajaram S, Paulsen GM (2004) Evaluation of selection strategies for wheat adaptation across water regimes. Euphytica 135:361–371

Kmoch HG, Raming RE, Fox RL, Koelher FE (1957) Root development of winter wheat as influenced by soil moisture and nitrogen fertilization. Agron J 49:20–26

Lichtenthaler HK, Buschmann C (2001) Chlorophylls and Carotenoides: measurement and characterization by UV-VIS spectroscopy. John Wiley and Sons, Inc, New York

Maggio A, Miyazaki S, Veronese P, Fujita T, Ibeas JI, Damsz B, Narasimhan ML, Hasegawa PM, Joly RJ, Bressan RA (2002) Does proline accumulation play an active role in stress-induced growth reduction? Plant J 31:699–712

Majidi MM, Mirlohi A, Amini F (2009) Genetic variation, heritability and correlations of agro-morphological traits in tall fescue (Festuca arundinacea Schreb). Euphytica 167:323–331

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 49:69–76

Merewitz E, Meyer W, Bonos S, Huang BR (2010) Drought stress responses and recovery of Texas × Kentucky hybrids and Kentucky bluegrass genotypes in temperate climate conditions. Agron J 102:258–268

Molyneux DE, Davies WJ (1983) Rooting pattern and water relations of three pasture grasses growing in drying soil. Oecologia 58:220–224

Moradshahi A, Salehi Eskandari B, Kholdebarin B (2004) Some physiological responses of canola (Brassica napus L.) to water deficit stress under laboratory condition. Iran J Sci Technol Trans A 28:43–49

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Norton MR, Volaire F, Lelievre F (2006) Summer dormancy in Festuca arundinacea Schreb: the influence of season of sowing and a simulated mid-summer storm on two contrasting cultivars. Aust J Agric Res 57:1267–1277

Pande H, Singh JS (1981) Comparative biomass and water status of four range grasses growth under two soil water conditions. J Range Manag 34:480–484

Ritchi SW, Naguyen HT, Holiday AS (1990) Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. Crop Sci 30:105–111

Rong Hua L, Pei guo G, Baum M, Grando S, Ceccarelli S (2006) Evaluation of chlorophyll content and fluorescence parameter as indicator of drought tolerance in barley. Agric Sci Chin 5:751–757

Rosielle AA, Hambline J (1981) Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci 21:943–946

Rout NP, Shaw BP (2001) Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Sci 160:415–423

Rudoplh AS, Crowe JH, Crowe LM (1986) Effects of three stabilizing agents-proline, betaine, and trehalose on membrane phospholipids. Arch Biochem Biophys 245:134–143

Sarker AM, Rahman MS, Paul NK (1999) Effect of soil moisture on relative leaf water content chlorophyll, proline and sugar accumulation in wheat. J Agrono Crop Sci 183:225–229

SAS institute (2001) User's guide. Release 9.2 SAS Institute, Cary N. C. Nos SAS and SSSA, Madison, W. pp 225–293

Serraj R, Krishnamurthy L, Kashiwagi J, Kumar J, Chandra S, Crouch JH (2004) Variation in root traits of chickpea (Cicer arietinum L.) grown under terminal drought. Field Crops Res 88:115–127

Sheffer KM, Dunn JH, Minner DD (1987) Summer drought response and rooting depth of three cool-season turfgrasses. Hort Sci 22:296–297

Simkin AJ, Moreau H, Kuntz M, Pagny G, Lin C, Tanksley S, Mc Carthy J (2008) An investigation of carotenoid biosynthesis in Coffea canephora and Coffea arabic. J Plant Physiol 165:1087–1106

Smirnoff N (1993) The role of active oxygen in the responses of plants to water deficit and desiccation. New Phytol 125:27–58

Vanacker H, Carver TLW, Foyer CH (1998) Pathogen induced changes in the antioxidant status of the apoplast in barley leaves. Plant Physiol 117:1103–1114

Wang Z, Huang B (2004) Physiological recovery of kentuky bluegrass from simultaneous drought and heat stress. Crop Sci 44:1729–1736