Root Traits and Transpiration Efficiency in Fenugreek Ecotypes Grown under Different Water Treatments

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ABSTRACT

Fifteen endemic ecotypes of the fenugreek were evaluated under well-watered and water deficit stressed conditions in the lysimetric system. Results of analysis of variance revealed that there was significant genotypic diversity for all of the traits, except root branch number. Also, ecotype × water treatment interaction was significant for all traits, except days to ripening, canopy temperature, and grain weight. Based on structural equation modeling, predictors in the causal diagram could explain 59%, 29%, 65%, and 51% of the total variation of dependent traits consisting root length, stem diameter, shoot dry weight, and percent assimilate partitioned to grain, respectively. These dependent traits had high broad sense heritability and explained 100% variation of grain weight. Transpiration efficiency had a positive effect on grain weight through stem diameter, root length, shoot dry weight, and percent assimilate partitioned to grain. Results of membership function value of drought tolerance index and heat map clustering revealed that Jahrom ecotype was a highly drought susceptible ecotype, and Yazd and India were drought susceptible ecotypes. Also, Tiranchi and Shiraz were identified as drought tolerant ecotypes. Overall, under water stress conditions, the drought tolerant ecotypes had deeper roots than the other ones. Therefore, these ecotypes might be considered as donor parents in fenugreek breeding programs.

Keywords: Assimilate partitioning, Drought stress, Drought tolerance, Structural equation modeling.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the Leguminosae family and is used as a medicine or in stews, in Iran. This crop is native to an area ranging from Iran to northern India and is planted in China, India, Iran, Egypt, Ethiopia, Greece, Morocco, Ukraine, Turkey, etc. (Acharya *et al.*, 2006). The fenugreek leaves and seeds are used in different countries for various purposes including medicinal (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial, etc.) and food making (Rizvi *et al.*, 2013).

Drought is a main abiotic factor that restricts agricultural plant production. Plants are subjected to drought stress when water supply to roots becomes difficult or when the transpiration rate becomes greater than the ability of plants to absorb water from the soil. These two conditions often occur in arid and semi-arid regions such as Iran (Ghobadi *et al.*, 2013). Therefore, identification of genotypes that have high performance root system to use limited available water is important to improve crop productivity.

Plant breeders are rarely interested in a single trait selection. Therefore, they need to examine the relationships between various traits such as the relationship between seed

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yield and other traits (Leal-Bertioli *et al*., 2012). Structural equation modeling (SEM) is best suited for studies with the goal to understand the basic processes or mechanisms governing relationships between traits (Lamb *et al.,* 2011). Chen *et al*. (2012) used the membership function value of drought tolerance (MFVD) index to select drought-tolerant wheat genotypes. MFVD is a multivariate index in which multiple traits are used to calculate its value.

The root systems are always referred to as the "hidden half" of crops (Waisel *et al*., 2002) and a recent study highlighted the importance of roots in crops and mentioned that "roots are the key to the second green revolution" in agriculture (Gewin, 2010). The root structure, especially deeper roots, had a significant effect on the stable grain yield under drought condition (Feng *et al*., 2017). The inherent root architecture of a plant determines its ability to extract soil water resources in water-limited areas. An adaptive root growth response to an identical level of water stress development can improve drought tolerance by increasing water uptake from deeper soil layers (Ober *et al*., 2014).

Transpiration is a part of evapotranspiration which is the combined process of evaporation from soil and plant surfaces and transpiration from plants (Kirnak *et al*., 2001). Recently, a lysimetric system was used to evaluate transpiration efficiency (TE) by preventing water evaporation from the soil surface (Ratnakumar and Vadez, 2011). The TE was defined as the total dry mass produced per unit of water transpired. The TE is an important characteristic of plants, especially when water supplies are becoming scarcer in many regions (Haefele *et al*., 2009). Genotypes that show high TE always have better performance under water-deficit conditions (Jyostna-Devi *et al*., 2009).

Endemic ecotypes of plants are repositories of the world's crop variation, and their germplasms represent a large potential for sources of abiotic stress tolerance. Future crop improvement depends on the genetic diversity of traditional varieties and related wild species to tolerate many biotic and abiotic stresses

(Torres *et al*., 2013). Few studies have reported water deficit stress impact on fenugreek, like an assessment of drought tolerance in Iranian fenugreek ecotypes only for grain yield and dry biomass (Ahari *et al*., 2009). However, we are not aware of any published research on the genetic diversity of fenugreek for drought tolerance through physiological and root traits. Therefore, the present study was carried out to evaluate the genetic diversity in Iranian fenugreek ecotypes for root traits and transpiration efficiency to identify the traits related to the drought tolerance.

MATERIALS AND METHODS

Plant Materials and Experimental Site

Fifteen ecotypes of fenugreek were collected from different regions of Iran and were characterized (Table 1). A glasshouse experiment was done in the year 2016 at the Agricultural Faculty of Tarbiat Modares University (51° 09´ E; 35° 44´ N; altitude 1265 m), Iran.

Preparation of Lysimeters and Growth Condition

The plants were grown in lysimeters of PVC cylinders (20 cm diameter, 100 cm height), which had a mixture of sandy loam (3:2 v/v basis) and well-crushed compost (3:1 v/v basis). Two layers of plastic mesh (1×1 mm) were used as end plate of cylinders allowing water drainage. The initial weights of filled cylinders were measured. The cylinders were kept on mesh platforms. Buckets were then attached to the bottom of cylinders and the junction points between each bucket and cylinder were sealed using cellophane to stop evaporation of drainage water from the buckets (Khodadadi *et al*., 2016b). Plants were grown in a glasshouse with 14 h photoperiod, the mean irradiance of 250 μmol

Table 1. Analysis of variance of studied traits in Iranian fenugreek ecotypes under different water treatments.

S.O.V[p]	df	DTF[a]	DTR[b]	SD[c]	CT[d]	SDW[e]	RDW[f]	RTSR[g]	BRN[h]	RL[i]	LRWC[j]	GW[k]	TE[l]	PAPG[m]	PAPS[n]	PAPR[o]
WT[p]	1	739.6**	2423.2**	6.2**	216.2**	12.1*	0.083*	11.5ns	16.04ns	728.1**	18303.3**	51.7**	1.45**	5173.01**	4013.6**	73.46**
Replication (WT)	4	5.9	91.4	0.037	4.3	1.3	0.002	9.9	1.3	13.8	5.6	2.5	0.022	403.8	358.2	2.82
Eco[q]	14	472.5**	196.2**	0.41**	9.8*	3.9**	0.095**	77.9**	17.4ns	765.4**	537.04**	5.9**	0.33**	564.3**	591.8**	16.12**
WT × Eco	14	22.3**	14.1ns	0.12**	2.5ns	2.1*	0.012**	14.1**	12.3**	145.6**	199.4**	0.13ns	0.08**	58.6*	55.37**	4.42**
Error	56	5.5	12.4	0.031	2.2	0.09	0.001	1.2	0.83	6.8	4.4	0.14	0.01	29.7	26.2	0.49

[a] Days to flowering, [b] Days to ripening, [c] Stem diameter, [d] Canopy temperature, [e] Shoot dry weight, [f] Root dry weight, [g] Root to shoot ratio, [h] Branch root number, [i] Root length, [j] Leaf relative water content, [k] Grain weight, [l] Transpiration efficiency, [m] Percent assimilate partitioned to grain, [n] Percent assimilate partitioned to shoot, [o] Percent assimilate partitioned to root, [p] Water treatment, [q] Ecotype. ns, *, and ** Indicate statistical non-significant, significant at 0.05 and 0.01 probability level.

m^{-2} s^{-1}, 22–31°C mean temperature (T), and 30–55% relative air humidity (RH).

Sowing and Crop Management

Prior to planting, all the cylinders received 2 L of water to bring the soil profile up to field capacity. Seeds were planted at a density of three seeds per cylinder and later on thinned to one plant per cylinder. To stop evaporation from the soil surface (Ratnakumar and Vadez, 2011), the soil surface of the cylinders was covered with aluminum foil. A split plot experiment based on completely randomized design (CRD) with three replications was used in the glasshouse experiment. A set of ecotypes were subjected to both well-watered (WW) and water deficit stressed (WDS) treatments. WW plants were kept in soil maintained at field capacity moisture for the entire experiment. WDS plants were irrigated similarly to WW plants up to stem elongation and from stem elongation to the flowering stage were kept at 50% of field capacity, after then watering was stopped.

The amount of water (I) used for the irrigation of each cylinder was recorded during the experiment. For soil fertility improvement, the third, fourth, and fifth irrigations were done with 500 mL of fertilizer solution (2 g.L^{-1}) (Green line NPK-20:20:20, Germany). The cylinders were immediately weighed after plants were harvested from above the soil surface. Subsequently, the amount of transpired water (TW) was calculated for each ecotype by the following Equation 1, which is shown in Figure 1.

$$TW = TWU - (FWC - PWC) - DW \quad (1)$$

Where, TWU, FWC, PWC and DW are total water (I) used, final and primary weight (g) of cylinder, and drainage water (I), respectively.

Trait Measurements

Measured traits included days to flowering (DTF; d), days to ripening (DTR; d), stem diameter (SD; mm), canopy temperature
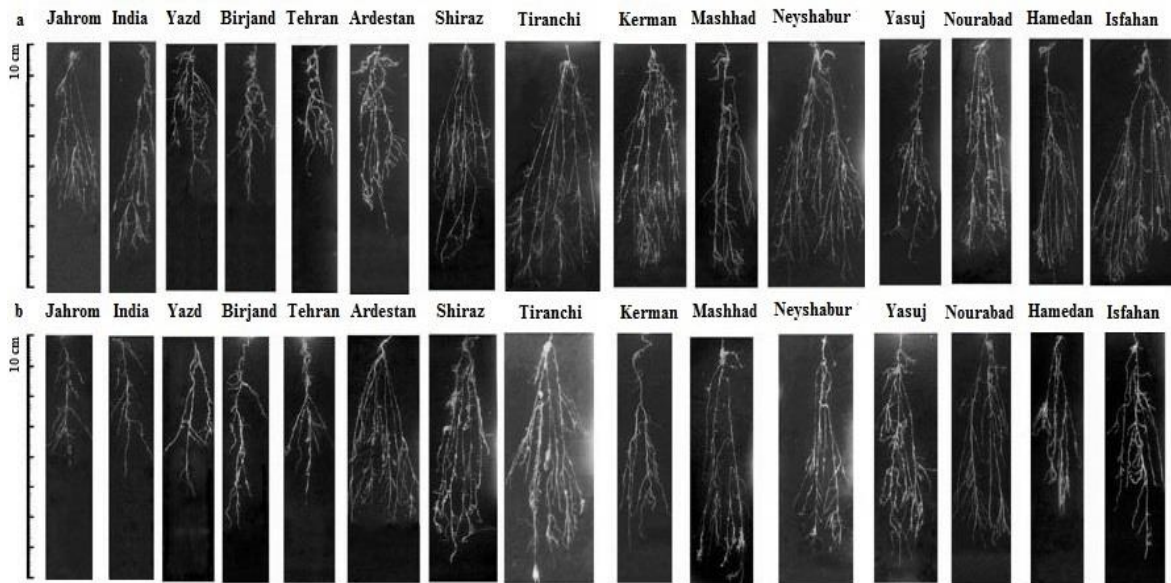
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Figure 1. Root shape of fenugreek ecotypes evaluated in lysimetric system. The root shape of ecotypes under well watered (a) and under water deficit stress (b).

(CT; °C), shoot dry weight (SDW; g), root dry weight (RDW; g), root to shoot ratio (RTSR; %), branch root number(BRN), root length (RL; cm), grain weight (GW; g), transpiration efficiency (TE; g.L^{-1}), percent assimilate partitioned to grain (PAPG; %), percent assimilate partitioned to shoot (PAPS; %), percent assimilate partitioned to root (PAPR; %), and leaf relative water content (LRWC; %).

According to Vadez *et al*. (2011) description, transpiration efficiency was calculated as the ratio of total biomass to the sum of transpired water for each of cylinders across the experiment. To unravel TE roles in water deficit tolerance, it was partitioned into three parts of percent assimilate partitioned (PAP) to root, PAP to shoot, and PAP to grain according to Eq. 2, 3, and 4 proposed by Khodadadi *et al*. (2017).

$$PAPR = \frac{TE_R}{TE_{TB}} \times 100 \qquad (2)$$

$$PAPS = \frac{TE_S}{TE_{TB}} \times 100 \qquad (3)$$

$$PAPG = \frac{TE_G}{TE_{TB}} \times 100 \qquad (4)$$

Where, TE_{TB}, TE_R, TE_S, and TE_G are transpiration efficiency based on total biomass, root mass, shoot mass, and grain mass, respectively. Canopy temperature was measured by portable telatemp model AG-42 infrared thermometer when drought stress increased to maximum at grain filling stage. To measure the leaf relative water content, sampling of leaves of each plant was randomly done. Leaf sampling was done during the grain filling stage when water deficit stress appeared and in the morning after the dew had dried. Fresh weights of leaf samples were taken, then, leaves were floated in distilled water in a petri dish for 12 h to determine the turgid weight and, finally, the leaves were dried at 70 °C for 48 h. Leaf relative water content was calculated according to Equation 5.

$$LRWC(\%) = \frac{fresh\ weight - dry\ weight}{turgid\ weight - dry\ weight} \times 100 \qquad (5)$$

Statistical Analysis

At first, the validity all hypothesis of analysis of variance were checked for data set of each

measured trait. A split-plot design with the following linear statistical model was used to analyze the glasshouse experiment data:

$$y_{ijk} = \mu + \beta_j + (\tau\beta)_{ij} + \gamma_k + (\beta\gamma)_{jk} + \varepsilon_{ijk}$$

(6)

Where, μ, τ_i, β_j, γ_k, $(\tau\beta)_{ij}$ and ε_{ijk} are overall mean, replication, water treatment effect, ecotype effect, main-plot error, and sub-plot error, respectively.

To construct the causal diagram of direct and indirect effects of explanatory variables on GW, sequential stepwise multiple regression was performed to organize the predictor variables into the first and second order paths on the basis of their respective contributions to total variation in grain weight and minimal co-linearity. To quantify the contribution of genotype × irrigation regime interaction effect and exclude or minimize the error effect (Khodadadi *et al*., 2016a), ReML estimated genotypic correlation coefficients were partitioned into direct and indirect effects based on the proposed causal diagram generated using AMOS ver. 20 (Arbuckle, 2011) software. The membership function value of drought tolerance (MFVD) was calculated according to the formula proposed by Chen *et al*. (2012). Genotypic correlation coefficients were calculated according to the formula proposed by Holland (2006) using SAS ver. 9.1 software (SAS, 2003). Analysis of variance and other common statistical analysis were done using SPSS ver. 22 (SPSS, 2013). Heat map cluster analysis was done using MetaboAnalyst (Xia *et al.* 2015) software.

RESULTS

Analysis of Variance

The results of ANOVA revealed that all traits were significantly affected by water deficit stress, but root to shoot ratio and root branch number were constant (Table 1). Genotypic differences were significant ($P \leq$ 0.01 and $P \leq$ 0.05) for all traits except for the root branch number. The genotype × water treatment interaction was observed significant for all traits, except for days to ripening, canopy temperature, and grain weight. The significant genotype × water treatment interaction reveals different response of genotypes in different water treatments for these traits. Root structure and size of the fenugreek ecotypes are shown in Figure 1, which reveals high genetic diversity in structure and size of root system between ecotypes.

Relationship between Traits, Direct and Indirect Effects of Variables on the GW

The ReML estimated genotypic and phenotypic coefficients of correlation between GW and TE with other traits in WW and WDS conditions are presented in Table 2. All traits had significant genotypic and phenotypic relationship with GW, except days to flowering, days to ripening, root branch number, and percent assimilate partitioned to root. There was a significant positive correlation between TE and percent assimilate partitioned to grain, shoot dry weight, stem diameter, root length, leaf relative water content, root dry weight and GW; also, TE had a significant negative correlation with canopy temperature (Table 2).

The direct and indirect effects of explanatory traits on four endogenous traits are presented in Table 3. The causal diagram of structural equation modeling based on genotypic correlation coefficients and genotypic variance estimates obtained from combined analysis of WW and WDS conditions are shown in Figure 2. According to the causal diagram, 100%, 59%, 29%, 65% and 51% of the total variation in grain weight, root length, stem diameter, shoot dry weight and percent assimilate partitioned to grain were explained by their explanatory traits, respectively. The greatest standardized (0.751) and unstandardized (0.064) direct and positive effects on the GW was confirmed by percent assimilate

Table 2. ReML estimated genotypic (below diagonal) and phenotypic (above diagonal) coefficients of correlation and its standard error (in parenthesis) between investigated traits measured on Iranian fenugreek ecotypes under different water treatments (well watered and water deficit stress) conditions.

	GW[a]	TE[b]	CT[c]	PAPS[d]	PAPG[e]	PAPR[f]	SDW[g]	SD[h]	RL[i]	LRWC[j]	DTF[k]	DTR[l]	BRN[m]	RDW[n]	RTSR[o]
GW	1	0.77** (0.08)	-0.43** (0.12)	-0.83** (0.06)	0.85** (0.05)	-0.09 (0.21)	0.32 (0.18)	0.45** (0.15)	0.38* (0.19)	0.72** (0.09)	0.18 (0.23)	0.28 (0.19)	0.23 (0.17)	0.57** (0.15)	0.40* (0.19)
TE	1.03** (0.09)	1	-0.52** (0.10)	-0.54 (0.37)	0.57** (0.13)	-0.14 (0.20)	0.74** (0.08)	0.63** (0.10)	0.53** (0.14)	0.70** (0.10)	0.39* (0.18)	0.43** (0.16)	0.47** (0.13)	0.66** (0.11)	0.21 (0.20)
CT	-0.99** (0.17)	-0.88** (0.13)	1	0.30* (0.13)	-0.30* (0.13)	0.04 (0.20)	-0.44** (0.11)	-0.40** (0.12)	-0.37** (0.12)	-0.48** (0.10)	-0.31* (0.14)	-0.25 (0.14)	-0.33** (0.12)	-0.30** (0.06)	-0.18 (0.14)
PAPS	-0.94** (0.05)	-0.76 (0.67)	0.71** (0.23)	1	-0.98** (0.01)	-0.12 (0.19)	0.05 (0.19)	-0.39* (0.15)	-0.24 (0.20)	-0.72** (0.08)	0.04 (0.23)	-0.13 (0.20)	-0.21 (0.17)	-0.51** (0.15)	-0.64** (0.12)
PAPG	0.97** (0.04)	0.78** (0.17)	-0.74** (0.22)	-0.99** (0.01)	1	-0.05 (0.20)	-0.02 (0.19)	0.38* (0.16)	0.21 (0.20)	0.73** (0.08)	0.01 (0.22)	0.16 (0.20)	0.18 (0.17)	0.41* (0.17)	0.51** (0.15)
PAPR	-0.02 (0.32)	0.06 (0.38)	0.04 (0.41)	-0.27 (0.32)	0.12 (0.34)	1	-0.17 (0.19)	0.04 (0.19)	0.22 (0.19)	0.02 (0.20)	-0.43 (0.28)	-0.18 (0.19)	0.18 (0.17)	0.61** (0.14)	0.82** (0.15)
SDW	0.60 (0.28)	0.76** (0.17)	-0.50 (0.34)	-0.18 (0.38)	0.21 (0.38)	-0.15 (0.42)	1	0.61** (0.19)	0.54** (0.13)	0.32 (0.17)	0.47** (0.15)	0.48** (0.14)	0.50** (0.13)	0.49** (0.15)	-0.15 (0.20)
SD	0.80** (0.18)	0.81** (0.14)	-0.37 (0.35)	-0.56 (0.25)	0.56* (0.25)	0.08 (0.39)	0.79** (0.19)	1	0.69** (0.09)	0.56** (0.12)	0.13 (0.20)	0.33 (0.17)	0.56** (0.11)	0.56** (0.13)	0.21 (0.18)
RL	0.51* (0.23)	0.79** (0.19)	-0.61** (0.25)	-0.37 (0.28)	0.35 (0.29)	0.26 (0.34)	0.82** (0.22)	0.91** (0.11)	1	0.40* (0.17)	0.07 (0.23)	0.25 (0.20)	0.46** (0.14)	0.62** (0.07)	0.35 (0.19)
LRWC	1.11** (0.16)	0.71** (0.19)	-0.69** (0.24)	-0.99** (0.11)	0.98** (0.10)	0.26 (0.42)	0.17 (0.46)	0.55 (0.28)	0.52 (0.30)	1	0.16 (0.21)	0.35* (0.17)	0.44** (0.15)	0.56** (0.09)	0.41* (0.17)
DTF	0.23 (0.26)	0.46 (0.26)	-0.52 (0.27)	0.07 (0.29)	-0.01 (0.29)	-0.43 (0.28)	0.72** (0.24)	0.12 (0.32)	0.15 (0.30)	0.19 (0.34)	1	0.70** (0.10)	0.20 (0.18)	0.13 (44.6)	-0.18 (0.22)
DTR	0.38 (0.24)	0.46 (0.25)	-0.41 (0.29)	-0.03 (0.31)	0.21 (0.28)	-0.21 (0.31)	0.59* (0.25)	0.28 (0.30)	0.23 (0.29)	0.32 (0.31)	0.90** (0.07)	1	0.27 (0.16)	0.24 (0.20)	-0.07 (0.21)
RN	0.67 (0.56)	0.67 (0.46)	0.02 (0.78)	-0.03 (0.54)	-0.02 (0.56)	0.27 (0.62)	1.14** (0.63)	0.69 (0.39)	0.91 (0.55)	-0.08 (0.82)	0.34 (0.51)	0.14 (0.51)	1	0.51** (0.13)	0.23 (0.17)
RDW	0.74** (0.15)	0.83** (0.14)	-0.69** (0.25)	-0.72** (0.17)	0.65** (0.20)	0.61** (0.21)	0.58* (0.27)	0.77** (0.18)	0.80** (0.14)	0.78** (0.17)	0.07 (33.11)	0.27 (0.28)	0.97 (0.53)	1	0.75** (0.10)
RTSR	0.53* (0.23)	0.53 (0.30)	-0.53 (0.32)	-0.75** (0.15)	0.66** (0.20)	0.82** (0.11)	0.07 (0.41)	0.41 (0.32)	0.46 (0.27)	0.77** (0.27)	-0.29 (0.28)	0.03 (0.31)	0.28 (0.54)	0.85** (0.15)	1

[a] Grain weight, [b] Transpiration efficiency, [c] Canopy temperature, [d] Percent assimilate partitioned to shoot, [e] Percent assimilate partitioned to root, [f] Percent assimilate partitioned to grain, [g] Shoot dry weight, [h] Stem diameter, [i] Root length, [j] Leaf relative water content, [k] Days to flowering, [l] Days to ripening, [m] Branch root number, [n] Root dry weight, [g] Root to shoot ratio.

Table 3. Direct, indirect and total path coefficients of explanatory variables on GW, PAPG, SDW, SD and RL endogenous variables in Iranian fenugreek ecotypes under different water treatments conditions.

|	Mode of action	Standardized	Unstandardized	Standardized total	Unstandardized total
PAPS→PAPG	Direct effect on PAPG	-0.563	-0.548	-0.563	-0.548
DTF →SDW	Direct effect on SDW	0.471	0.043	0.471	0.043
TE → PAPG	Direct effect on PAPG	0.440	19.650	0.440	19.650
TE → SDW	Direct effect on SDW	0.265	0.791	0.522	1.556
TE → SD	Direct effect on SD	0.542	0.776	0.542	0.776
TE → RL	Direct effect on RL	0.351	15.672	0.621	27.740
PAPR → RL	Direct effect on RL	0.177	1.09	0.177	1.09
SD → SDW	Direct effect on SDW	0.473	0.985	0.473	0.985
SD → RL	Direct effect on RL	0.498	15.55	0.498	15.55
PAPG →GW	Direct effect on GW	0.751	0.064	0.751	0.064
SDW → GW	Direct effect on GW	0.283	0.364	0.283	0.364
SD → GW	Direct effect on GW	0.325	0.872	0.423	1.134
RL → GW	Direct effect on GW	-0.072	-0.006	-0.072	-0.006
PAPS → GW	Indirect effect via PAPG	-0.422	-0.035	-0.422	-0.035
DTF → GW	Indirect effect via SDW	0.133	0.016	0.133	0.016
TE → GW	Indirect effect via PAPG	-	-	0.33	1.258
TE → GW	Indirect effect via SDW	-	-	0.146	0.567
TE → GW	Indirect effect via SD	-	-	0.227	0.888
TE → GW	Indirect effect via RL	-	-	0.043	-1.664
TE → GW	Indirect effect via SD and RL	-	-	0.019	-0.072
TE → GW	Indirect effect via SD and SDW	-	-	0.071	0.278
PAPR →GW	Indirect effect via RL	-0.013	-0.007	-0.013	-0.007
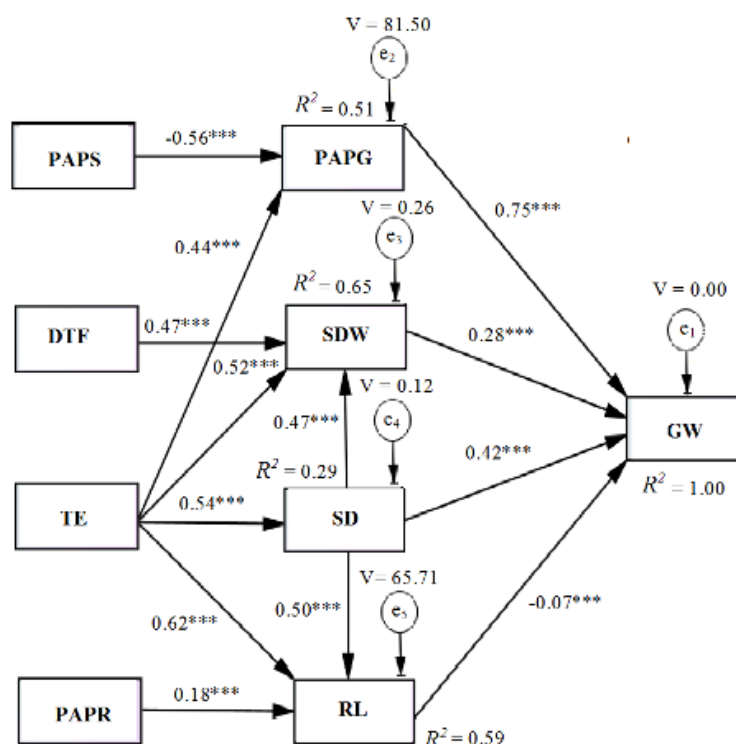
Figure 2. Fitted observed variable model. Standardized total (direct + indirect) path coefficients of all significant (*** significant at 0.001 level of probability) paths are displayed. Coefficient of determination (R^2) value and error variance (V) of the four endogenous variables are displayed. Other symbols are defined under Table 1.
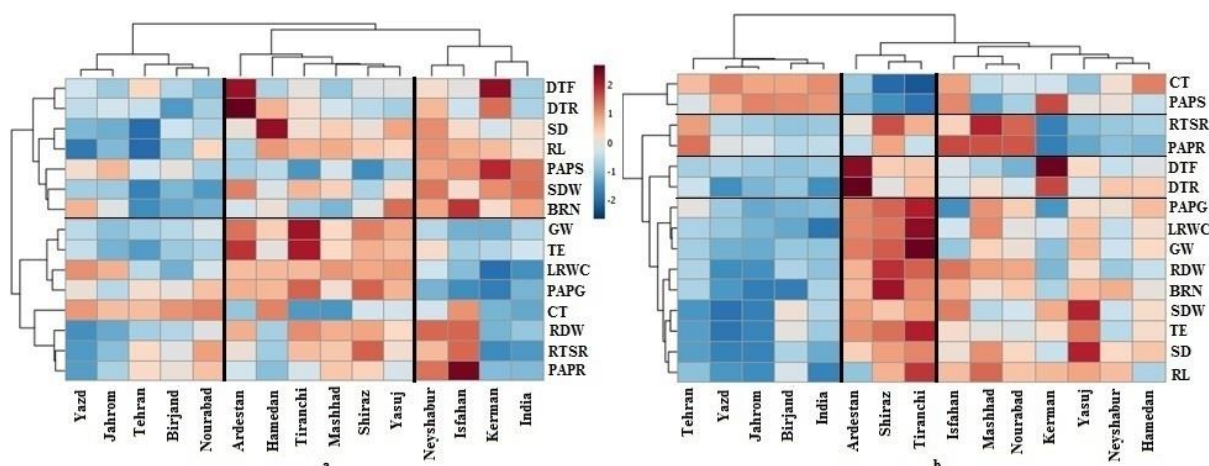
Figure 3. Heat map and clustering of traits' changes and of studied ecotypes under well watered(a) and under water deficit stress(b). Each row represents a trait and each column represents an ecotypes. other symbols are defined under Table 1.

partitioned to grain. The greatest standardized (0.33) indirect and positive effects on GW were conferred by TE via percent assimilate partitioned to grain.

Identification of Drought Tolerant Ecotypes

Results of grouping ecotypes based on

MFVD values are presented in Table 4. The fenugreek ecotypes were divided into four groups viz. drought tolerant (Mashhad, Tiranchi, and Shiraz), drought susceptible (Yazd and India), highly drought susceptible (Jahrom) and relatively drought tolerant (other ecotypes).

The results based on heat map clustering of ecotypes in WW (Figure 3-a) revealed that fenugreek ecotypes belonged to three

Table 4. The membership function value of drought tolerance (MFVD) and classification of Iranian fenugreek ecotypes at different levels of drought tolerance (underlined value indicates class of ecotype).

Ecotype	U_i [a]	Highly tolerant $U_i \geq \overline{U} + 1.64 \times Sdu$	Tolerant $U_i \geq \overline{U} + Sdu$	Semi tolerant $U_i \geq \overline{U} - Sdu$	Susceptible $U_i \geq \overline{U} - 1.64 \times Sdu$	Highly susceptible $\overline{U} - 1.64 \times Sdu > U_i$
Kerman	0.618	-0.292	-0.202	<u>0.078</u>	0.167	0.167
Mashhad	0.836	-0.074	<u>0.016</u>	0.296	0.386	0.386
Yazd	0.509	-0.400	-0.311	-0.031	<u>0.059</u>	0.059
Birjand	0.609	-0.301	-0.211	<u>0.069</u>	0.158	0.158
Tehran	0.682	-0.228	-0.138	<u>0.142</u>	0.231	0.231
Neyshabur	0.582	-0.327	-0.238	<u>0.042</u>	0.132	0.132
Shiraz	0.864	-0.045	<u>0.044</u>	0.324	0.414	0.414
Yasuj	0.790	-0.120	-0.030	<u>0.250</u>	0.339	0.339
Nourabad	0.808	-0.102	-0.012	<u>0.268</u>	0.358	0.358
Hamedan	0.703	-0.206	-0.117	<u>0.163</u>	0.253	0.253
Isfahan	0.641	-0.269	-0.179	<u>0.101</u>	0.191	0.191
Ardestan	0.768	-0.141	-0.052	<u>0.228</u>	0.318	0.318
India	0.515	-0.394	-0.305	-0.025	<u>0.065</u>	0.065
Tiranchi	0.908	-0.002	<u>0.088</u>	0.368	0.458	0.458
Jahrom	0.425	-0.485	-0.395	-0.115	-0.026	<u>-0.026</u>

[a] Is the average value of the membership function of 12 traits for the ecotype (i) for drought tolerance, average value ($\overline{U}$) and standard deviation (SD).

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groups. The first group included Yazd, Jahrom, Tehran, Birjand, and Nourabad ecotypes that had low values for many of the traits. The second group included Ardestan, Hamedan, Tiranchi, Mashhad, Shiraz and Yasuj ecotypes. The Tiranchi ecotype showed the greatest value for TE, GW, and PAPG. The Ardestan ecotype showed the greatest value for DTR and DTF (Figure 3-a). The third group included Neyshabur, Isfahan, Kerman and India ecotypes, which exhibited high value for PAPS, SDW, RN, and RL, especially Isfahan ecotype showed high values for PAPR and RN (Figure 3-a).

The results of heat map clustering of ecotypes in WDS indicated that ecotypes were grouped into three main groups and two subgroups in the third group. The first group members were Tehran, Yazd, Jahrom, Birjand, and India ecotypes. The comparative picture of values of traits of WW and WDS members of this group showed that these ecotypes were drought susceptible as they had low values for productivity traits (GW, PAPG and TE) and LRWC, but had high values for CT and PAPS in WDS relative to WW (Figure 3-b). These ecotypes in WDS also had low value of root number, root length and root dry weight (Figure 1-b and Figure 3-b). The second group included Ardestan, Shiraz and Tiranchi ecotypes. These ecotypes exhibited highest values for productivity traits that were relatively high for many of traits except CT and PAPS. Shiraz and Tiranchi ecotypes developed the deepest and most hairy roots in WDS and these ecotypes had more hairy roots in WDS than those in WW (Figure 1-b and Figure 3-b). The third group included the ecotypes of Isfahan, Mashhad, Nourabad, Kerman, Yasuj, Neyshabur, and Hamedan, which mostly had moderate values for the measured traits.

DISCUSSION

The ANOVA showed the presence of significant differences among ecotypes for most of the studied traits, indicating high genetic diversity among the ecotypes that can be used as appropriate parents for breeding purposes. The significance of water treatment and water treatment × genotype effects for root and shoot dry weight suggests that morphological adaptation of plants to water deficit is ecotype dependent and plants reduce shoot to decrease the evaporative surface area that subsequently causes low water consumption (Guimarães *et al*., 2015).

The performance of ecotypes under water-stressed condition is a major criterion to select the most suitable ecotype(s) for use in the breeding program for the development of high yielding lines suitable to the particular stress condition (Fang *et al*., 2017). Therefore, it seems that the best strategy for breeding plan for drought tolerance is to evaluate yield and yield correlated traits and select ecotypes with high yield in the combination of both well watered and water deficit stress conditions (Khodadadi *et al*., 2016a). The genetic relationship between productivity traits (TE and GW) and other measured traits were evaluated in the present study to identify surrogate traits for the improvement of fenugreek productivity under water deficit stressed conditions.

Positive and significant genetic correlation between the root traits (root dry weight and root length) and GW in combined analysis of WW and WDS conditions (Table 2) showed that improvement in these traits could lead to gain in GW. Koolachart *et al*. (2013) noticed that the higher root length and root dry weight in peanut remained positively associated with grain yield under drought stress. Also, it was observed that root length was significantly associated with grain yield in screening of maize genotypes in glasshouse under water deficit condition (Ali *et al*., 2016). Similarly, Álvarez *et al*. (2011) determined that plants use the strategy of deeper roots establishment to improve their survival during seasonal drought stress. However, a high yield can be attained by using ecotypes which have high net assimilation rate and are able to maintain their growing potential even under less

favorable conditions prevailing at the end of the season (Suojala, 2000). Lopes and Reynolds (2010) found that drought tolerant genotypes of wheat chose alternative strategies depending upon the water availability at depth: if water was available at deeper layer of the soil, drought tolerant genotypes allocated more assimilate to deeper roots to uptake more water, resulting in high yield; while, where water was not available at deeper layer of the soil, allocation of more assimilate into grain was preferred.

Generally, in crop improvement programs, achieving higher yields by improving TE through higher percent assimilate partitioned into grain (PAPG) requires an understanding of the extent of genetic variation present in the major determinant traits such as photosynthesis rate and CO_2 concentrations inside of leaf (Gilbert *et al.*, 2011). However, genetic variation in TE could probably be due to the genotype × environment (soil and climate characteristics) interaction (Jackson *et al.*, 2016). The fenugreek ecotypes used in this study had an appropriate genetic potential for response to water deficit. The relationship between TE and root length indicated that drought tolerant ecotypes (Tiranchi and Shiraz) tend to increase root length to reach deeper water and increase TE. The positive genetic correlation between TE and GW is remarkable as high TE is not appropriate unless it leads to high harvest index under drought stress (Blum, 2009). Siahpoosh and Dehghanian (2012) observed a positive correlation between water use efficiency and TE while Ratnakumar and Vadez (2011) noticed that root depth and root length density did not discriminate tolerant genotypes from sensitive genotypes and remain related poorly to net water extraction. The tolerance to drought was mostly explained by the capacity to maintain a high harvest index under drought in tolerant genotypes.

Nardino *et al.* (2016) reported that there was a positive genetic correlation between stem diameter and grain yield in maize.

Therefore, selection for stem diameter as surrogate trait can lead to crop yield improvement under drought stress. Stems can act as temporary storage organs for water-soluble carbohydrate and anytime that plant is faced with harsh environment this source (stem) of assimilate can be used for grain filling (Blum, 2011). Therefore, we can conclude that the drought-tolerant genotypes may choose alternative strategies such as increasing root length to uptake more water and/or increasing other features such as stem diameter to reserve carbohydrate for grain filling and/or allocating a higher percentage of total assimilate to grain than other organs.

The significant negative relationship between canopy temperature and GW and TE reveals that selection for low values of this trait might be useful to improve drought tolerant fenugreek ecotype. Sukumaran *et al.* (2015) and Zia *et al.* (2013) reported that there was a significant correlation between canopy temperature and grain yield under water stress condition in wheat and maize crops, respectively. Therefore, they used canopy temperature as a screening tool to identify drought tolerant spring wheat. Also, Pasban (2011) found that canopy temperature can be used as a selection index to identify drought tolerant safflower genotypes under water deficit stress.

CONCLUSION

The findings of the present study suggested that there were high genetic differences between fenugreek ecotypes in response to water deficit stress (WDS) and this genetic diversity potential can be used to improve fenugreek grain yield under WDS. A significant positive genetic correlation was observed between transpiration efficiency (TE) and percent assimilate partitioned to grain, shoot dry weight, stem diameter, root length, leaf relative water content, root dry weight and grain weight (GW), however, TE showed significant negative genetic correlation with canopy temperature. The

total variation of GW was explained by root length, stem diameter, shoot dry weight, and percent assimilate partitioned to grain in the causal diagram. Based on the positive or negative relationship between GW and correlated traits, plant breeders can select for higher or lower values of these traits to improve GW under WDS, respectively. Results of the membership function value of drought tolerance (MFVD) index and heat map clustering were similar, although the heat map analysis seemingly provided more clear separation between ecotypes. Mashhad, Tiranchi and Shiraz ecotypes were identified as drought tolerant. These ecotypes could probably be carriers of drought tolerance alleles and can be considered as donor parents in fenugreek breeding programs.

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صفات ریشه و کارایی تعرق در اکوتیپ های شنبلیله تحت شرایط رطوبتی مختلف

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چکیده

پانزده توده بومی شنبلیله در دو شرایط رطوبتی عدم تنش و تنش کمبود آب در سیستم لایسیمتر مورد ارزیابی قرار گرفتند. تجزیه واریانس نشان داد که تنوع ژنتیکی معنی‌داری بین اکوتیپ‌ها براساس همه صفات به جز برای صفت تعداد ریشه‌های جانبی وجود داشت. همچنین اثر متقابل اکوتیپ × محیط رطوبتی برای همه صفات به جز روز تا رسیدگی، دمای کانوپی و عملکرد دانه معنی‌دار بود. بر اساس مدلسازی معادلات ساختاری، متغیرهای پیش‌بینی کننده در نمودار علت و معلولی به ترتیب ۵۹، ۲۹، ۶۵ و ۵۴ درصد از کل تنوع صفات وابسته شامل طول ریشه، قطر ساقه، وزن خشک اندام هوایی و درصد کربن انتقال یافته به دانه را توجیه کردند. این صفات وابسته درونی وراثت‌پذیری عمومی بالایی داشتند و 100%کل تنوع عملکرد دانه را توجیه کردند. کارایی تعرق تاثیر مثبتی بر عملکرد دانه از طریق قطر ساقه، طول ریشه، وزن خشک اندام هوایی و درصد کربن انتقال یافته به دانه داشت. نتایج حاصل از مقدار تابع شاخص تحمل به خشکی و گروهبندی حرارتی نشان داد که توده جهرم به تنش خشکی حساسیت بالایی داشت و تودهای یزد و هندی حساس به خشکی بودند. همچنین تودهای تیرانچی و شیراز متحمل به خشکی بودند. به طور کلی تودهای متحمل به خشکی طول ریشه بلندتری نسبت به سایر تودها در شرایط تنش کمبود آب نشان دادند. بنابراین این تودها ممکن است به عنوان والدین تلاقی در برنامه های اصلاحی معرفی شوند.