

Application of secondary traits in barley for identification of drought tolerant genotypes in multi-environment trials

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Abstract

Discriminant function analysis (DFA) is a biometric method that can be used by breeders to distinguish between drought tolerant genotypes. Drought stress is one of the major limiting factors that reduce crop production in semi-arid and arid regions around the world. A multi-environment trial was conducted on barley to investigate various phenological and morphological characteristics under both drought stress and irrigated conditions. Ten indigenous barley genotypes were evaluated in four cropping seasons and in two locations of Iran. Genetic variation was observed in all traits. The traits observed in all genotypes were significantly different. The average broad sense heritability predicted for secondary traits (0.88) was significantly more than grain yield (0.62). The results could significantly discriminate between low and high yield genotypes under drought stress by describing eight secondary traits including biomass, spike weight, grain numbers per main spike, grain numbers per plant, awn length, days to flowering, grain filling period and potential yield. High yield genotypes were selected by discriminant analysis (Eq. (1)). The discriminant score (DS) could explain 67% of grain yield variations and had a significant correlation ($r=0.82^{**}$) with the average of grain yield examined under drought stress over four years. Consequently, integrated selection can be used as a reliable approach to future breeding programs. Results of DFA indicated that the most important traits, in order of appearance, are awn length, grain filling period, spike weight, and grain numbers per main spike. The results demonstrated that secondary traits could be considered as proper criteria to improve the genetic gain of grain yield and to select tolerant cultivars for environments that are susceptible to drought.

Keywords: Barley; Discriminant function; Droughts stress; Secondary traits.

Abbreviations: DFA_Discriminant function analysis; DSI_Discriminant score index; GY_grain yield; BIO_biomass; HI_harvest index; HGW_hundred grain weight; SW_spike weight; GNP_grains number plant; GNS_grains number main spike; TN_tiller number; FTN_fertile tiller number; PH_plant height; SL_spike length; AL_awn length; DF_day to flowering; DR_day to ripening; GFP_grain filling period

Introduction

Barley (*Hordeum vulgare* L.) is among the oldest cereal crops in the world. Because of its nutritional value, barley is attracting considerable attention in Asia and northern Africa (Baik and Ullrich, 2008). Stress is defined as an altered physiological condition caused by biotic or abiotic factors that tend to disrupt the equilibrium in living organism. Drought stress is an important abiotic factor that can suppress the efficiency of photosynthesis by reducing leaf expansion; thereby, causing premature leaf senescence and lower food production (Wahid and Rasul, 2005). Approximately, 15 million km² of the land surface is covered by cropland (Ramankutty et al., 2008), of which 16% is predicted to be managed by irrigation (Siebert et al., 2005). In many parts of the world, including the western parts of Asia and Iran, plants frequently encounter drought stress due to the irregular distribution of rainfall. Tolerance to abiotic stresses including drought stress is highly complex

due to the existence of intricate interactions between stress factors and various molecular, biochemical and physiological phenomena, all of which can affect plant growth and development (Razmjoo et al., 2008). Consequently, the use of traditional breeding efforts and modern genetic approaches would improve the crops' tolerance to drought (Xiong et al., 2006). Understanding molecular, morpho-anatomical, and physiological mechanisms underlying plant responses to drought is of great importance and constitutes fundamental steps to breeding high yielding crops under water stress conditions (Martínez et al., 2007; Nam et al., 2001; Reddy et al., 2004; Zhao et al., 2008). Therefore, understanding the relationships between yield and yield components may help breeders identify key traits that are involved in crop yield under temporal drought stress conditions. Screening different genotypes under drought stress conditions is one of the main options for exploring

genetic variation to improve stress tolerant cultivars (Haddadin, 2015). There are considerable interests in discovering the mechanism of drought tolerance and the breeding of novel drought resistant cultivars (Zhao et al., 2010). It has been reported that agronomic traits such as grain yield and its components are the major selection criteria for breeding drought tolerance in barley (Hossain et al., 2012; Niazi-Fard et al., 2012). Edward and Wright (2008) reported that yield components like grain number and grain size decreased under pre-anthesis drought stress treatment in wheat. In barley, reduced number of tillers, spikes, grains per plant, and individual grain weight was shown to be causes of reduction of grain yield under drought stress (Duffus and Cochrane, 1993). There are three approaches proposed to be successful in the improvement of yield under drought stress conditions: 1) Breeding for yield under non stress conditions (i.e. yield potential) assuming that it will provide a yield advantage under stress condition. Cattivelli et al. (2008) reported that selection for high yield in non-stress conditions has, to a certain extent, indirectly improved yield in many water-limiting conditions. However, further progress should be undertaken to identify and improve key traits that can potentially reduce the gap between yield potential and actual yield under environments prone to drought. Indirect selection for yield potential and average yield under non-stress conditions may be of less importance in giving reliable results for the selection of drought-tolerant genotypes (Abdolshahi et al., 2013). 2) Breeding for maximum yield in environments prone to drought. Conventional breeding methods for cereals have been based on empirical selection for yield (Saxena and O'Toole, 2002). Due to annual fluctuations in environments that are susceptible to drought, this approach may not be successful because of the quantitative nature and low heritability estimate of grain yield, and higher genotype \times environment interaction (Babu et al., 2003). 3) Breeding for drought tolerance by using secondary traits. In grain crops that grain yield is a final purpose of production, other traits (including phenologic, morphologic and physiologic) that influence grain yield significantly are called secondary traits. Secondary traits are plant characteristics that are associated with crop yield under stress which can provide additional information for breeders to identify key stages to apply selection (Fischer, 2003). This approach offers the best option to improve grain yield under drought stress conditions, as the heritability of grain yield often decreases, whereas the heritability of secondary traits has been reported to remain constant (Bänziger, 2000; Bolanos and Edmeades, 1996). Several studies have shown the successful application of secondary traits for the genetic improvement of wheat under drought stress (Chen et al., 2012; Condon et al., 2004; Reynolds et al., 2009; Richards, 2006). In addition, these traits could be easily measured and selected concurrently in breeding programs. Genetic analysis of root system architecture, leaf water potential, panicle water potential, osmotic adjustment and relative water content are examples of efforts to measure secondary traits (Jongdee et al., 2002). A secondary trait would be suitable if it shows genetic relationship with grain yield under drought, with higher heritability, and these would be reproducible or feasible measures while having no association with yield loss under ideal conditions (Edmeades et al., 2001). Abdolshahi et al. (2015) reported the relationships between secondary

morpho-physiological traits and grain yield in bread wheat genotypes under drought stress conditions by identification of a discriminant function explaining 76% of grain yield variation. The photosynthetic organs of the spike in cereals (e.g. lemma, palea, awn, and glumes) are essential sources of carbohydrate accumulation in developing grains. In barley, carbohydrates derived from the lemma, palea, and awn account for approximately 76% of the total grain dry weight (Duffus and Cochrane, 1993). Many spike features are capable of acquiring adaptation to drought stress such as xeromorphic anatomy (Araus et al., 1993), osmotic adjustment (Tambussi et al., 2007), water use efficiency (Blum, 1985), and delayed senescence (Tambussi et al., 2007). Blum (1986) emphasized the concept that long awns, especially the high photosynthetic area of awn ears, are an important criterion for the selection process to improve wheat production under drought conditions. Moreover, the efficiency of long awns is due to their high ratio between carbon exchange and transpiration rate (Blum, 1985; Hosseini et al., 2012). Araghi and Assad (1998) reported that canopy temperature, stomatal resistance and rate of water loss are important indicators of drought resistance in wheat. Foulkes et al. (2007) stated that the green flag-leaf area duration is strongly associated with grain yield under drought, and suggests the importance of selecting this trait in breeding high-yielding wheat in rain-fed environments where terminal drought is a serious problem. Recently, breeding efforts have focused on early flowering and a longer grain-filling period (Villegas et al., 2010).

A type of selection that is based on multiple traits is an important option in breeding programs to improve grain yield under drought stress conditions. Discriminant Function Analysis (DFA, hereafter) is an appropriate technique to achieve this goal, in comparison with multiple regression. DFA provides an equation that gives maximum separation of high and low yield genotypes, whereas multiple regression offers an equation that connects grain yield and secondary traits (Abdolshahi et al., 2015). The objective of the present research was to evaluate several phono-morphological traits related to the potential of plant yield and drought tolerance, and to apply all effective secondary traits concurrently in a function with the aim of segregating genotypes into categories of low and high grain yield by using the discriminant function analysis as a highly efficient biometric technique under drought stress conditions.

Results

Combined variance analysis

Results of combined variance analysis indicated that genotype (G) and genotype \times environment (G \times E) effects were significant in terms of all the traits considered under both irrigated and stress conditions ($P \leq 0.01$; Table 1). This reflects genetic variation and different reactions of genotypes to different environments.

Genetic parameters analysis

The highest values of phenotypic and genotypic coefficients of variation belonged to the number of grains per plant and spike weight, whereas plant height and 'days to ripening' had the lowest values. Comparison of genetic (0.89) and

genetic × environment (4.15) variance demonstrated the magnitude of genetic variation by environmental interaction that resulted in moderate heritability (0.62) for grain yield (Table 2). The highest and lowest variances of interaction effect (genotype × environment) were attributed to the number of grains per plant and 100-grains weight, respectively. The highest broad sense heritability ($h^2=0.95$) was for grains number per main spike and spike weight and the lowest ($h^2=0.1$) was obtained for plant height. The highest and lowest values of expected response to selection (R%) were obtained for the number of grains per plant (R=79.5) and 100-grains weight (R=0.17), respectively (Table 2). Among the 15 traits, just eight of them could significantly separate the studied genotypes into two categories of high and low yield groups (Table 3).

Discriminant function analysis

DFA has been utilized, as a comprehensive criterion, in order to discriminate the genotypes related to both groups (i.e. high and low yield) and also to select high yield genotypes (Sharma, 1996). Discriminant function's formula was fitted, according to standardized data of eight effective secondary traits as follows:

$$DS = 0.558 \text{ GFP} + 0.585 \text{ AL} + 0.551 \text{ SW} + 0.457 \text{ GNS} + 0.4 \text{ YP} + 0.119 \text{ DF} + 0.004 \text{ BIO} - 0.192 \text{ GNP} \quad (1)$$

Where DS is the discriminant score, GFP is grain filling period, AL is awn length, SW is spike weight, GNS is grain number per main spike, YP is potential yield, DF is day to flowering, BIO is biomass and, GNP is grain number per plant. By using DFA, these eight traits were regarded as important ones that could be considered to improve grain yield under drought stress conditions and were employed concurrently to evaluate DS. The average of broad sense heritability predicted for secondary traits (0.88) was 26% more than the general heritability of yield (0.62). Because of high broad sense heritability under drought stress conditions, secondary traits could be considered besides yield by breeders. The equation of discriminant function (Eq. (1)) shows that awn length (0.585 coefficient), grain filling period (0.558 coefficient), spike weight (0.551 coefficient) and grain number per main spike (0.457) are respectively the most crucial secondary traits for discriminating high and low yield genotypes in barley under drought stress conditions. According to fitted formula of discriminant function, DS was calculated for the genotypes studied under drought stress conditions, based on standardized data of eight secondary traits entered into the model. Genotypes with positive DS (higher than zero) have settled in the group 1, while those with negative DS (lower than zero) appear in group 2; i.e., cutoff value equals zero (Table 4). There is an indication that the biometric technique of discriminant function could discriminate genotypes, relating to the groups 1 (high yield genotypes) and 2 (low yield genotypes) with correct classification rate of 100% during years 2011-2012 and 2013-2014 (Table 4). The 'Rihane' genotype (-1.29) which belonged to group 1 settled in group 2 in 2012-2013 because of its negative DS. Furthermore, the 'Makuyi' genotype (0.99) which was a member of the group 2 was later arranged in the group 1, due to its positive DS, in the

years 2014-2015. Therefore, the discriminant function technique's accuracy was found to be 90% (9/10=90%) in the second and fourth years. The highest rate of DS belonged to Zarjo (2.91) and Kavir (2.42) genotypes, and the lowest was for Strin (-2.34) and Nimruz (-2.22) genotypes, respectively.

Canonical correlation (CR^2) of discriminant function

Squared canonical correlation (CR^2) of discriminant function (Eq. (1)) was estimated by investigating DSs relationships and yield under drought stress equals 0.67 (Fig. 1). These results show that discriminant function variables could justify 0.67% of variability between high and low yield genotypes in the current research. The DS had a positive and significant correlation with yield under drought stress and normal conditions for all years (Table 5). These consequences confirm the integrated selection criterion efficiency.

Investigating of the effective secondary traits revealed that grain number per plant with 79.09% has the most rate of expected response to selection (R%) (Table 2). This trait had a positive and significant correlation with grain yield under drought stress during 2011-2012 ($r=0.65$, $p<0.05$), 2012-2013 ($r=0.67$, $p<0.05$), 2013-2014 ($r=0.71$, $p<0.05$) and, 2014-2015 ($r=0.81$, $p<0.01$) (Table 5). This trait, associated with the highest genotypic and phenotypic coefficients of variability (GCV=23.71 and PCV=24.91) (Table 2), entered the equation of discriminant function (Eq. (1)). Relatively high genetic advance could be expected in response to selection regarding a number of grains per main spike (13.88%), as one of the presented traits in discriminant function (Eq. (1)) with a high factor coefficient (0.457) and the highest broad sense heritability (0.95) (Table 2). This had a positive and significant correlation with yield under drought stress ($r=0.67$, $p<0.05$) (Table 5).

Genotypic and phenotypic correlation

Genotypic and phenotypic coefficients of correlation showed that the correlation of grain number per plant with yield ($r=0.92$, $p<0.01$), biomass ($r=0.92$, $p<0.01$), spike weight ($r=0.88$, $p<0.01$), grain number per main spike ($r=0.86$, $p<0.01$) and awn length ($r=0.67$, $p<0.05$) were positive and significant (Table 6). Awn length was the most important effective secondary trait with the highest coefficient factor 0.585 (Eq. (1)) and a broad sense heritability of 0.95, which had a positive and significant genotypic correlation with yield ($r=0.79$, $p<0.01$), biomass ($r=0.64$, $p<0.01$), harvest index ($r=0.77$, $p<0.01$), spike weight ($r=0.68$, $p<0.05$), grain number per plant ($r=0.67$, $p<0.05$) and grain filling period ($r=0.72$, $p<0.05$) (Table 6).

Discussion

In recent studies, it has been observed that the significant G×E effect on effective secondary traits indicates that various genotypes react to different environments. Similar reports are available regarding barley (Chand et al., 2008; Soluki et al., 2008) and wheat (Shah et al., 2009). The highest

Table 1. Analysis of variance (ANOVA) for traits of 10 barley genotypes under drought stress and well-watered conditions in four years.

Source of variation	df	Mean Square						
		GY	BIO	HI	HGW	SW	GNP	GNS
Environment (E)	7	188.19**	1276.05**	760.34**	4.77**	2.46**	218464**	785.72**
Error 1	16	0.69	3.38	47.67	0.09	0.06	220.76	20.40
Genotype (G)	9	34.29**	155.92**	440.32**	0.95**	10.24**	94292**	2599.21**
G×E	63	12.87**	22.40**	182.33**	0.36**	0.56**	8869.70**	122.39**
Error 2	144	0.43	1.88	40.00	0.09	0.08	512.13	17.18
CV (%)	-	10.29	9.89	13.26	6.79	10.85	8.99	7.82

*and **: significant at 0.05 and 0.01 probability levels. GY: grain yield; BIO: biomass; HI: harvest index; HGW: hundred grain weight; SW: spike weight; GNP: grains number plant; GNS: grains number main spike

Table 1. Continue.

Source of variation	df	Mean Square							
		TN	FTN	PH	SL	AL	DF	DR	GFP
Environment (E)	7	370.03**	311.67**	5267.80**	29.14**	12.45**	27979**	29089**	230.02**
Error 1	16	2.35	2.62	27.44	0.42	0.98	3.76	2.82	6.26
Genotype (G)	9	34.77**	27.13**	172.67**	14.44**	36.56**	220.84**	211.08**	54.74**
G×E	63	11.51**	7.08**	155.97**	2.40**	5.03**	28.53**	20.96**	16.64**
Error 2	144	1.51	1.37	23.45	0.41	0.66	2.66	4.08	3.36
CV (%)	-	11.69	14.77	6.77	8.40	6.14	0.92	0.97	5.99

*and **: significant at 0.05 and 0.01 probability levels. TN: tiller number; FTN: fertile tiller number; PH: plant height; SL: spike length; AL: awn length; DF: day to flowering; DR: day to ripening; GFP: grain filling period

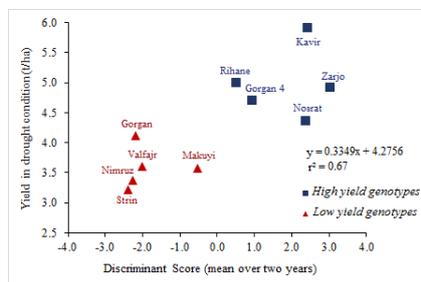


Fig 1. Association of discriminant score and grain yield in drought stress condition

Table 2. Mean and genetic parameters for various characters of barley genotypes across four years.

Traits	Mean	GCV	PCV	σ^2_g	$\sigma^2_{g \times e}$	σ^2_{res}	h^2_{bs}	R%
Grain Yield	6.35	14.88	18.83	0.89	4.15	0.43	0.62	1.05
Biomass	13.86	17.02	18.39	5.56	6.84	1.88	0.86	3.06
Harvest Index	47.69	6.88	8.98	10.75	47.45	40.00	0.59	3.51
100-Grain Weight	4.47	3.53	4.47	0.02	0.09	0.09	0.62	0.17
Spike Weight	2.67	23.80	24.47	0.40	0.16	0.08	0.95	0.86
Grains Number/ Plant	251.61	23.71	24.91	3559.30	2785.86	512.13	0.91	79.50
Grains Number/ Spike	52.97	19.18	19.64	103.20	35.07	17.18	0.95	13.88
Tiller Number	10.52	13.24	16.19	1.94	3.33	1.51	0.67	1.59
Fertile Tiller Number	7.93	16.30	18.97	1.67	1.90	1.37	0.74	1.56
Plant Height	71.50	1.17	3.75	0.70	44.17	23.45	0.10	0.36
Spike Length	7.58	9.35	10.24	0.50	0.67	0.41	0.83	0.91
Awn Length	13.21	8.68	9.34	1.31	1.46	0.66	0.86	1.49
Days to Flowering	176.85	1.60	1.72	8.01	8.62	2.66	0.87	3.70
Days to Ripening	207.72	1.35	1.43	7.92	5.63	4.08	0.90	3.74
Grain Filling Period	30.57	4.12	4.94	1.59	4.43	3.36	0.70	1.47

GCV and PCV: Genotypic and Phenotypic coefficient of variability; σ^2_g , $\sigma^2_{g \times e}$ and σ^2_{res} : Genetic, Genetic × Environmental and Error variance; h^2_{bs} : Heritability in broad sense; R%; expected response to selection (percentage of mean)

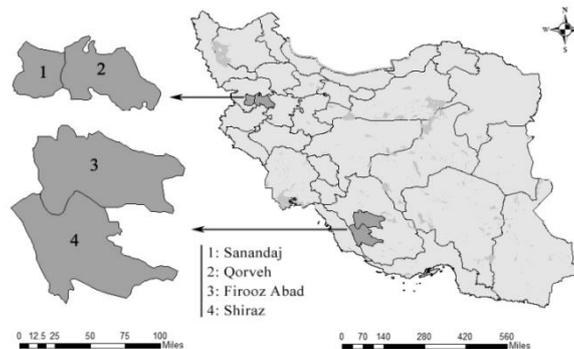


Fig 2. Location of experimental sites on the Iran map.

Table 3. Mean of high yield (group 1) and low yield genotypes (group 2), and t-test for secondary traits that could significantly separate two groups.

Trait	Mean of high yield genotypes (group 1)	Mean of low yield genotypes (group 2)	t- test
Biomass	9.89	7.68	3.51**
Spoke weight	2.93	1.99	5.03**
Grain number plant	207.84	154.74	4.16**
Grain number spike	59.29	40.55	4.34**
Awn length	13.85	11.69	5.56**
Days to flowering	172.85	177.55	2.46*
Grain filling period	30.22	27.32	4.67**
Yield potential	9.79	7.06	2.80*

*and **: significant at 0.05 and 0.01 probability levels.

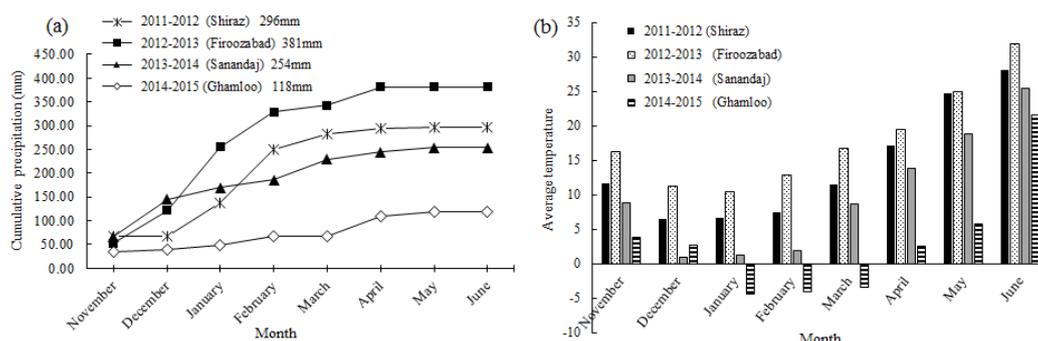


Fig 3. Cumulative precipitation (a) and Temperature (b) from sowing (November) to harvesting (June) in four years.

Table 4. Discriminant score and classification for two groups of high and low yield genotypes under drought stress condition in 2011-2012, 2012-2013, 2013-2014, 2014-2015 and mean of traits over four years.

Year	Genotype	Group 1- High yield genotypes					Group 2- Low yield genotypes				
		Rihane	Kavir	Nosrat	Zarjo	Gorgan 4	Gorgan	Nimruz	Valfajr	Makuyi	Strin
2011-2012	Discriminant score	1.33	0.53	1.37	2.21	0.76	-1.66	-0.27	-0.65	-0.80	-2.83
	Classification	1	1	1	1	1	2	2	2	2	2
2012-2013	Discriminant score	-1.29	2.51	1.80	2.56	0.91	-1.06	-2.54	-1.89	-0.67	-0.33
	Classification	2	1	1	1	1	2	2	2	2	2
2013-2014	Discriminant score	0.85	3.10	2.31	2.23	0.33	-2.07	-1.89	-1.94	-1.23	-1.70
	Classification	1	1	1	1	1	2	2	2	2	2
2014-2015	Discriminant score	1.15	1.26	1.91	2.23	0.65	-2.22	-2.04	-0.86	0.99	-3.06
	Classification	1	1	1	1	1	2	2	2	1	2
Mean over four years	Discriminant score	0.58	2.42	2.35	2.91	0.97	-2.12	-2.22	-1.98	-0.56	-2.34
	Classification	1	1	1	1	1	2	2	2	2	2

Discriminant scores are calculated based on standardized data, genotypes with higher discriminant score than zero belong to group 1 and lower than zero belong to group 2 (i.e. cutoff value = 0.00)

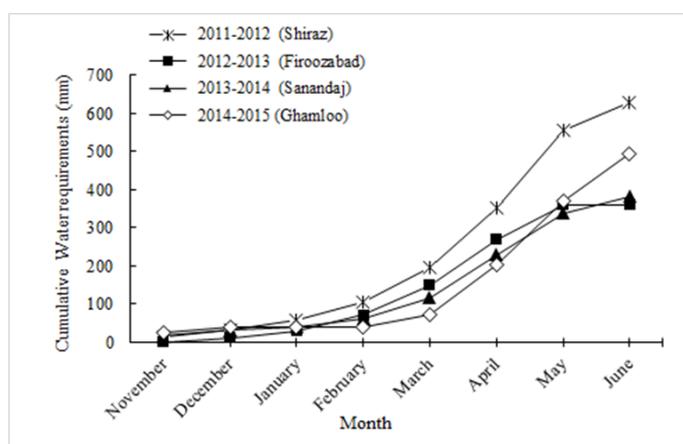


Fig 4. Cumulative Water requirements (mm) from sowing (November) to harvesting (June) in four years.

Table 5. Correlation coefficients of secondary traits and discriminant score with grain yield under drought stress and well-watered conditions in four years (data of secondary traits are mean of four years).

Grain Yield	Biomass	Spike weight	Grain number plant	Grain number spike	Awn length	Days flowering	Grain filling period	Discriminant score
Grain yield in drought stress condition								
2011-2012	0.70*	0.57	0.65*	0.20	0.79*	-0.41	0.71*	0.62*
2012-2013	0.42	0.68*	0.67*	0.70*	0.65*	-0.56	0.65*	0.72*
2013-2014	0.66*	0.66*	0.71*	0.48	0.75*	-0.77**	0.80**	0.73*
2014-2015	0.95**	0.59	0.81**	0.27	0.80**	-0.43	0.37	0.63*
Average	0.81**	0.73*	0.82**	0.67*	0.89**	-0.58	0.76*	0.82**
Grain yield in well-watered condition								
2011-2012	0.74*	0.65*	0.61*	0.69*	0.29	-0.29	0.31	0.74*
2012-2013	0.56	0.59	0.73*	0.47	0.2	-0.39	0.26	0.53
2013-2014	0.91**	0.78**	0.95**	0.68*	0.50	-0.32	0.24	0.67*
2014-2015	0.86**	0.83**	0.98**	0.72*	0.67*	-0.12	0.20	0.90**
Average	0.89**	0.83**	0.91**	0.73*	0.73*	-0.28	0.27	0.80**

*and **: significant at 0.05 and 0.01 probability levels

Table 6. Genotypic and phenotypic correlation coefficients of different traits (the upper and lower values indicate genotypic and phenotypic correlation coefficients, respectively).

		GY	BIO	HI	HGW	SW	GNP	GNS	TN	FTN	PH	SL	AL	DF	DR
BIO	G	0.95**													
	P	0.90													
HI	G	0.57	0.24												
	P	0.56	0.21												
HGW	G	0.06	0.14	-0.18											
	P	0.10	0.11	-0.21											
SW	G	0.86**	0.78**	0.55	-0.07										
	P	0.82	0.76	0.49	-0.28										
GNP	G	0.92**	0.92**	0.42	-0.31	0.88**									
	P	0.89	0.88	0.40	-0.25	0.84									
GNS	G	0.83**	0.76**	0.46	-0.61*	0.98**	0.86**								
	P	0.82	0.75	0.44	-0.48	0.96	0.85								
TN	G	-0.05	0.17	-0.24	0.46	-0.20	0.03	-0.18							
	P	-0.05	0.16	-0.24	0.48	-0.26	0.02	-0.19							
FTN	G	0.11	0.25	0.02	0.51	-0.13	0.20	-0.09	0.94**						
	P	0.12	0.24	0.01	0.52	-0.17	0.19	-0.10	0.94						
PH	G	0.56	0.76**	0.07	0.06	0.69*	0.53	0.55	-0.05	-0.10					
	P	0.54	0.66	0.12	0.05	0.45	0.48	0.50	-0.04	-0.08					
SL	G	-0.42	-0.23	-0.45	0.29	-0.26	-0.32	-0.20	0.25	0.29	0.38				
	P	-0.46	-0.29	-0.56	0.30	-0.40	-0.38	-0.28	0.22	0.27	0.18				
AL	G	0.79**	0.64**	0.77**	0.15	0.68*	0.67*	0.48	-0.06	0.16	0.42	-0.42			
	P	0.81	0.59	0.73	0.03	0.64	0.63	0.43	-0.09	0.14	0.35	-0.57			
DF	G	-0.46	-0.34	-0.21	-0.49	0.53	0.07	0.38	-0.20	-0.27	-0.12	0.45	-0.58		
	P	-0.46	-0.32	-0.23	-0.53	0.21	0.08	0.34	-0.16	-0.23	-0.10	0.10	-0.62*		
DR	G	-0.30	-0.17	-0.38	-0.40	0.90**	0.33	0.71*	-0.32	-0.33	-0.02	0.38	-0.22	0.91**	
	P	-0.24	-0.09	-0.34	-0.43	0.43	0.28	0.56	-0.23	-0.26	-0.01	-0.02	-0.28	0.94	
GFP	G	0.60*	0.40	0.44	0.26	0.75**	0.64*	0.62*	-0.35	-0.13	0.20	-0.34	0.72*	-0.55	-0.28
	P	0.56	0.36	0.41	0.30	0.54	0.57	0.58	-0.27	-0.10	0.11	-0.44	0.61	-0.06	-0.16

*and **: significant at 0.05 and 0.01 probability levels. GY: grain yield; BIO: biomass; HI: harvest index HGW: hundred grain weight; SW: spike weight; GNP: grains number plant; GNS: grains number main spike; TN: tiller number; FTN: fertile tiller number; PH: plant height; SL: spike length; AL: awn length; DF: day to flowering; DR: day to ripening; GFP: grain filling period

Table 7. List and characteristics of the 10 barley genotypes and soil physic-chemical properties in four experimental sites.

Cultivars	Spike type	Origin	Year of release	Classification by climate	Drought tolerant/susceptible
Gorgan	2	Sweden	-----	Moderate	Susceptible (Arshadi et al., 2016)
Rihane	6	ICARDA	1993	Moderate	Moderate (Arshadi et al., 2016; Nazari and Pakniyat, 2010)
Kavir	6	USA	1979	Moderate	Tolerant (Arshadi et al., 2016; Nazari and Pakniyat, 2010)
Nosrat	6	Iran	2008	Moderate	Tolerant (Sadeghi-Shoae et al., 2014; Saeidi et al., 2013)
Nimruz	2	CIMMYT	1997	Warm	Susceptible (Zare, 2012)
Valfajr	6	Egypt	1985	Moderate	Susceptible (Nazari and Pakniyat, 2010)
Makuyi	6	Italy	1990	Cold	Susceptible (Nazari and Pakniyat, 2010)
Zarjo	6	Iran	1949	Cold	Tolerant (Sadeghi-Shoae et al., 2014)
Gorgan 4	2	Sweden	1962	Moderate	Moderate (Arshadi et al., 2016; Saeidi et al., 2013)
Strin	2	-----	-----	-----	Susceptible (Arshadi et al., 2016)

Location	Soil texture	Soil particles (%)			EC (ds.m ⁻¹)	PH	O.C (%)	Total N (%)	Phosphorus (ppm)	Potassium (ppm)
		Clay	Silt	loam						
Shiraz	Silt	26	44	30	0.625	7.8	1.24	0.128	31	480
Firoozabad	Silt loam	20	44	36	0.610	7.19	2.18	0.201	35	540
Sanandaj	Clay loam	42	20	38	0.868	7.42	0.78	0.078	9.35	253
Ghamloo	Clay silt	44	36	20	0.925	7.75	1.07	0.107	9.2	205

Table 8. Analysis of variance and expected mean of squares.

Source of variation	Degree of freedom	Mean of squares	Expected mean of squares
Environment	(e-1)		
Error 1	e(r-1)		
Genotype (G)	(g-1)	M ₁	$\sigma_{res}^2 + r\sigma_{g \times e}^2 + re\sigma_g^2$
G*E	(g-1)(e-1)	M ₂	$\sigma_{res}^2 + r\sigma_{g \times e}^2$
Error 2	e(g-1)(e-1)	M ₃	σ_{res}^2

and lowest variance of G×E belonged to the number of grains per plant and spike characteristics (e.g. spike weight, spike length, awn length and 100-grain weight), respectively. The traits with low G×E variance are stable in different environments. The genotypic coefficients of variability (GCV) indicated differences in the genetic variation of the traits being studied. The variation could be deemed high, while in other cases it may be low. When the variation of traits is high, the accuracy of selection is high too (Falconer, 1989). For spike weight, the grain number per main spike had 0.95 of heritability and the awn length had 0.86 of heritability, which were high rates, and variation was deemed mostly of genetic origin. High heritability allows effective selection for these traits (which are less affected by the environment) under drought stress (Maniee et al., 2009). Araus et al. (1993) argued that barley has many characteristics that appear to be adaptations to drought stress, including xeromorphic anatomy. Blum (1986) claims that long awns of wheat are a logical criterion to improve yield in hot and dry conditions. The average of broad sense heritability predicted for the secondary traits that were entered into discriminant function (0.88) was 26% more than the one for grain yield (0.62). This result illustrates the high importance of these traits in contributing to drought stress tolerance in genotypes. Also, a low heritability of yield, compared to the secondary traits, implies that environmental factors have a major role in the phenotypic variation of yield. Consequently, selecting a favorable genotype directly based on grain yield would not be effective. Abdolshahi et al. (2015) reported that the heritability of yield decreased, while the heritability of secondary traits would be

approximately constant under drought stress. High heritability of secondary traits and also the high genotypic correlation with grain yield under drought stress strongly suggests that secondary traits are a better criterion to improve yield. Additionally, a slow pace of advancement in selecting improved yield has made breeders focus on secondary traits (Landjeva et al., 2008). The effective secondary traits including biomass, spike weight, grain numbers per main spike, grain numbers per plant, awn length, days to flowering, grain filling period and potential yield were used concurrently as integrated criterion in the discriminant function to investigate high yield genotypes, and all traits that entered Eq. (1) had positive and significant correlation with grain yield under drought stress, except for days to flowering (Table 5). These results illustrated the importance of these traits in finding higher yield genotypes in breeding programs under drought stress. DFA is a better technique in comparison with multiple-regression to improve yield (Farshadfar, 2012). DFA with a correction classification rate of 100% was known as a powerful multi-variate method to discriminate between genotypes relating to group 1 (high yield) and 2 (low yield) in barley. Awn length, grain filling period, spike weight and grain numbers per main spike were known as the most valuable secondary traits with high breeding value for improving drought tolerance in barley when cultivated in environments that are prone to drought, which is due to the high coefficients (Eq. (1)), high heritability (Table 2) and positive significant correlations with yield (Table 5). Therefore, selecting is based on these traits and can improve the genetic gain of yield under drought stress. Researchers have previously

assessed the importance of these traits to confirm their role in improving yield (Aminzadeh, 2010; Blum, 2005; Hossain et al., 2012; Niazi-Fard et al., 2012; Samarah, 2005).

The awn is one of the closest photosynthesizing organs to the grain in barley, and therefore the grain yield would inevitably increase by its lengthening. The benefit of long awns is linked to their high ratio between carbon exchange and transpiration rate (Blum, 1985; Hosseini et al., 2012). Yasseen and Al-Omary (1994) reported that the occurrence of drought stress during grain filling period accelerates the leaves senescence and reduces the grain filling period and grain weight. The grain filling of cereals is a starch biosynthesis mechanism. It is believed that four enzymes, i.e. sucrose synthases, adenosine diphosphate-glucose-pyrophosphorylase, starch synthase, and a starch branching enzyme play crucial roles in the process (Taiz and Zeiger, 2006). Drought stress during the grain filling period in barley reduced 40% of the grain filling rate (Sánchez-Díaz et al., 2002) and also reduced the economic yield by abating individual grain weight (Jamieson et al., 1995) amount to 49-57 % (Samarah, 2005). This study was conducted in Iran, a country that is located on a global dry belt where drought stress frequently occurs at the end of the growing season. Therefore, the 'days to flowering' is an important criterion for the process of selection. 'Days to flowering' was considered as a crucial characteristic, and was one of the eight secondary traits that entered the discriminant function (Eq. (1)) which had a negative and significant correlation with yield under drought stress ($r = -0.77$, $p < 0.01$) (Table 5). Developing short-duration varieties has been an effective strategy for minimizing yield loss from terminal drought, as early maturity allows the crop to suffer a shorter period of stress (Kumar and Abbo, 2001). The integrated selection criterion (Eq. (1)) could justify 67% of grain yield variability under drought stress and had a positive significant correlation with yield in all irrigated and drought stressed environments. There was a positive and highly significant genotypic correlation of yield with grain numbers per plant ($r = 0.92$, $p < 0.001$), while grain numbers per main spike had negative and significant genotypic correlation with the 100-grain weight ($r = -0.61$, $p < 0.05$). In other words, an effort to boost yield by increasing grain numbers would be ineffective because of a reduction in individual grain weight. Grain yield had a positive and significant correlation with biomass yield and grain numbers per unit area under drought stress conditions (Aslani et al., 2012).

Materials and methods

Plant material

Ten Iranian and foreign agronomical barley genotypes, with different capabilities of tolerating drought (Arshadi et al., 2016) were planted under field conditions for 4 agronomical years (2011- 2015) in different geographical locations of Iran (Fig. 2). The selected genotypes for the current study were released from as early as 1949 to 2008. These cultivars vary in terms of spike type, cultivation climate and drought tolerance, as they are cultivated broadly in the diverse Iranian climate (Table 7).

Filed experiment

Experiments were conducted in four agricultural research stations in Fars and Kurdistan provinces, the southern and north-western parts of Iran, from 2011 to 2012 in Shiraz station (1540 m above sea level, at 37° and 29' N; 32° and 52' E), from 2012 to 2013 in Firozabad station (1327 m above sea level, at 35° and 28' N; 40° and 52' E), from 2013 to 2014 in Sanandaj station (1380 m above sea level, at 35° and 16' N; 47° and 1' E) and from 2014 to 2015 in Ghamloo station (1850 m above sea level, at 35° and 23' N; 46° and 41' E), respectively (Fig. 2). This experiment was conducted once each year, throughout the four-year duration, in the form of randomized complete block design with 3 replications under both rain-fed and irrigated conditions. Before planting, the soil was sampled from different surface layers of the fields (in depths 0 to 30 centimeters) and also were sieved by a 2mm sieve in order to be analyzed (Table 7). Planting was manually performed in early November. Each experimental unit included 6 rows, each row having 2 meters of length, and were spaced 25 centimeters apart. The plant density was 200 grains per m². Cultivation under rain-fed conditions were accomplished without irrigation. The irrigated plots were given 500 liters of water each time the irrigation was performed, and this amount was fixed in all sites. Weeding was manually controlled during the growth period. Distribution and quantity of precipitation and temperature were researched on a monthly basis for the years of the experiments (Fig. 3). Total amounts of precipitation for each agronomical year were 296, 381, 254, 118 ML, respectively. In order to reassure the occurrence of drought stress under rain-fed conditions, graphs relating to water requirement of barley and distribution of precipitation for different cultivated areas were drawn (Fig. 4).

Measurement of Traits

In barley, traits were measured based on the descriptor of International Institution of Plant Genetic Resource (IPGRI, 1994). Sampling was performed randomly by removing marginal effect from central units. Various traits were measured including grain yield, biomass, harvest index, 100-grain weight, spike weight, grain numbers per plant, grain numbers per main spike, tiller number, number of fertile tillers, plant height, awn length, spike length, days to flowering, days to ripening, grain filling period.

Statistical analysis

Statistical software of SAS_{9.4} was employed to test the hypothesis of variance analysis, the combined analysis (based on randomized complete block designs) and the correlation analysis among traits. Both rain-fed and irrigated conditions, from 2011 to 2015, were considered as 8 environments. Then, variance components such as genotypic variance (σ_g^2), genotype \times environment interaction ($\sigma_{g \times e}^2$) and residual variance (σ_{res}^2) were calculated for measured traits as follows (Fehr, 1987) (Table 8):

$$\text{Genotypic variance } (\sigma_g^2) = \frac{(M_1 - M_2)}{re} \quad (2)$$

$$\text{Genotype } \times \text{ Environment variance } (\sigma_{g \times e}^2) = \frac{(M_2 - M_3)}{r} \quad (3)$$

$$\text{Error variance } (\sigma_{\text{res}}^2) = M_3 \quad (4)$$

Where 'r' indicates the number of replications, and 'e' represents the number of environments.

The genotypes and environments were all considered as random factors. Both genotypic and phenotypic variance and co-variance were estimated based on the Expected Value of Mean Squares (EMS) and Mean Products (MP) (Baker, 1986; Falconer, 1989; Miller et al., 1958). In order to calculate broad sense heritability of traits, genotypic variance was divided by phenotypic variance (Fehr, 1987) as follows:

$$h_{\text{bs}}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{g \times e}^2}{e} + \frac{\sigma_e^2}{re}} = \frac{\sigma_g^2}{\sigma_p^2} \quad (5)$$

Where σ_p^2 indicates phenotypic variance.

Heritability standard deviation was calculated based on the Pešek and Baker (1969) method, the Genotypic Coefficient of Variability (GCV) and Phenotypic Coefficient of Variability (PCV). It was calculated by using genotypic variance (σ_g^2), phenotypic variance (σ_p^2) and mean values of traits (\bar{x}) based on the following relationships:

$$\text{GCV} = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100 \quad (6)$$

$$\text{PCV} = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100 \quad (7)$$

Genotypic and phenotypic coefficients of correlation were calculated by using the genotypic and phenotypic variance and co-variance, via the formula introduced by (Miller et al., 1958):

$$r_p = \frac{\sigma_{p1,2}}{\sqrt{(\sigma_{p1}^2)(\sigma_{p2}^2)}} \quad (8)$$

$$r_g = \frac{\sigma_{g1,2}}{\sqrt{(\sigma_{g1}^2)(\sigma_{g2}^2)}} \quad (9)$$

Where r, σ , σ^2 , g, p, 1 and 2 indicate the coefficient of correlation, co-variance, variance, genotypic and phenotypic signs, the first traits and the second traits, respectively.

The standard error of genotypic correlation was calculated to test genotypic coefficient of correlation (Falconer, 1989) as follows:

$$\sigma(\gamma_g) = (1 - r_g) \sqrt{\frac{\sigma(h_x^2)\sigma(h_y^2)}{h_x^2 h_y^2}} \quad (10)$$

Where σ , h_x^2 , h_y^2 are respectively the standard error and heritability of the first and second traits. The h_x^2 and h_y^2 were calculated as follows:

$$h_x^2 = \frac{\sigma_{gx}^2}{\sigma_{px}^2} \quad (11)$$

$$h_y^2 = \frac{\sigma_{gy}^2}{\sigma_{py}^2} \quad (12)$$

Response to selection (R) was estimated based on genetic parameters as follows (Falconer and Mackay, 1996):

$$R = ih^2 \sqrt{\sigma_p^2} \quad (13)$$

Note that i is the intensity of selection, and therefore if 20% of the genotypes are selected in breeding programs, its value will be 1.4. Analyzing the discriminant function was performed by SPSS₂₁. Based on mean values of grain yield under drought stress condition during this 4-year research, 10 existing cultivars were classified into two groups: 5 high and 5 low grain yield genotypes (Abdolshahi et al., 2015). Among the 15 measured traits, biomass, spike weight, number of grains per plant, number of grains per spike, awn length, days to flowering, grain filling period and potential

yield were parameters that entered the discriminant function (Table 3) which could separate genotypes into two groups (Group1 and group 2) based on the T-test (Abdolshahi et al., 2015). The traits were standardized just before the discriminant function was analyzed as follows:

$$z_{ij} = \frac{x_{ij} - \bar{x}_i}{s_i} \quad (14)$$

Where Z_{ij} is the standard score for j^{th} genotype in i^{th} trait, and X_{ij} is a raw data for j^{th} genotype in i^{th} trait, \bar{x}_i is the average of i^{th} trait for all genotypes and S_i is standard deviation of the i^{th} trait.

Square Canonical correlation (CR^2) was estimated for discriminant (Sharma, 1996) as follows:

$$CR^2 = \frac{SS_b}{SS_t} \quad (15)$$

Where SS_b and SS_t are between groups and the total sum of square, respectively. CR^2 is the variety between groups, being explained by the discriminant function. CR^2 is a multiple regression such as R^2 . Discriminant function analysis was performed by using 5 high and 5 low yield genotypes under drought stress conditions, while variance analysis and other genetic parameters were calculated based on the 10 genotypes.

Conclusion

Results showed that secondary traits have higher heritability in comparison with yield under drought stress. Furthermore, these traits had high correlations with grain yield. Therefore, using these traits might be a proper alternative to inefficient approaches for the selection of genotypes with higher yields under drought stress. In the current research, the secondary traits displayed significant genotypic correlations with yield, high genetic variability and high broad sense heritability. The discriminant function technique was used as an integrated selection criterion to recognize high and low yield genotypes. By using eight effective secondary traits concurrently, discriminant scores (DSs) were calculated for all genotypes. This indicator could successfully discriminate between high and low yield genotypes under drought stress (Fig. 1). DS had a positive and significant correlation with yield under drought stress during the first ($r=0.62^*$), second ($r=0.72^*$), third ($r=0.73^*$) and fourth years ($r=0.63^*$), and there was an average of four years ($r=0.82^*$). Consequently, secondary traits such as awn length, grain filling period, spike weight and grain numbers per main spike could be employed in future breeding programs. Their feasibility is due to their cheap and simple measurement, genetic variability, high heritability, positive and significant correlations with yield under drought stress, and high coefficients in discriminant function (Eq. (1)). Cultivars of Zarjo and Kavir showed the highest values of DS and were recognized as the most drought tolerant genotypes. The Kavir cultivar was superior to Zarjo in environments that are prone to drought, due to its higher yield (Fig. 1).

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