



RESEARCH PAPER

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Evaluation of phenotypic variability, genetic parameters, heritability and genetic gain in bread wheat genotypes under rainfed conditions

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Abstract

The present investigation was carried out to estimate the genetic variability of agro-physiological characters using biometrical genetic techniques in bread wheat genotypes under rainfed condition. The results of variance analysis (ANOVA) showed significant differences for stress yield (Ys), relative water content (RWC), evapotranspiration efficiency (ETE) and water use efficiency (WUE) under rainfed conditions indicating the presence of a considerable genotypic variation and possible selection of drought tolerant genotypes under rainfed conditions. Comparison of means showed that the genotype D-79-15 (G8) had the highest, WUE, relative chlorophyll content (RCC), stomatal conductance (SC), the ratio of chlorophyll a/ chlorophyll b (a/b) and Ys. Cluster analysis based on grain yield and physiological criteria classified the genotypes in three groups. Group 1 (drought tolerance) consisted of genotypes G3, G4 and G8, group 2 (semi-resistance) included genotypes G7 and group 3 (drought sensitive) discriminated genotypes G1, G2, G5 and G6. High heritability and genetic gain was observed for Ys, RWC, ETE, WUE and a/b. The results of phenotypic and genetic correlation coefficient (Table 4) displayed that RCC, ETE, SC and a/b contributed significantly towards Ys. High coheritability was observed between relative water loss (RWL) and Ys (5.042), RWL and WUE (5.317). Positive coheritability estimate was observed between Ys and all other physiological traits suggesting that selection of either of the characters would simultaneously affect the others positively.

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Introduction

Wheat (*Triticumaestivum* L.) is a crop of global significance. It is grown in diversified environmental conditions. Wheat is the most important grain and a staple food for more than one third of the world's population (Rashid *et al.*, 2013).

Drought is one of the main constraints to rainfed wheat production in the Mediterranean region, where wheat is extensively grown. Achieving genetic improvement in yield in these environments has been recognized as a difficult challenge for plant breeders, while progress in yield gains has been much higher in favourable environments (Richards *et al.*, 2002; Villegas *et al.*, 2007; Farshadfar *et al.*, 2012).

Genetic diversity of plants determines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced food production (Khodadadi *et al.*, 2011).

Wheat varieties being cultivated in world have good genetic potential for yield which is yet needed to be explored because of the enormous population pressure. The success of a breeding programme depends on the presence of genetic variation in the material at hand. Choice of the most effective breeding procedures depends to a large extent on knowledge of the genetic systems controlling the characters to be selected.

To formulate an efficient breeding program for developing new varieties, it is essential to understand the mode of inheritance (Morad, 2012). Primarily; biological variation presented in the plant population is of three types, viz., phenotypic, genotypic and environmental. Greater the genetic variation, greater the chances for bringing about sustainable improvement through selection. The heritability of a character described the extent to which is transmitted from one generation to the next. Heritability estimates are determined as the extent of phenotype which is determined by genetic make up or genotypic response is called heritability in broad sense. It is further added that heritability does not depend only

upon genetic factors but also environmental circumstances to which an individual is subjected (Falconer, 1970). Thus the knowledge of heritability for the particular character helps the plant breeder in predicting the behavior of the succeeding generations. Higher the heritability, the simpler the selection process and the greater the response, to selection (Khaliq *et al.*, 2009). Several researchers (Malik *et al.*, 1988; ALwawiet *et al.*, 2010) have emphasized the utility of the estimates of heritability and genetic advance in the prediction of response of quantitative characters to selection in wheat. Heritability alone is not very useful but this statistic along with genetic advance is valuable (Johnson *et al.*, 1955).

Genetic gain (GG) is usually estimated in field crops as the collective contribution of several breeding programmes. For the individual breeder, however, the genetic gain realized within a single programme is critical (Khalil *et al.*, 1995). Knowledge of the changes associated with advances in crop productivity is essential for understanding yield-limiting factors and developing strategies for future improvement (Donmez *et al.*, 2001).

A study of genetic variability with the help of suitable parameters such as genotypic coefficient of variation and heritability are thoroughly necessary to start an efficient breeding program. Although direct selection for various traits could be misleading, indirect selection via related traits with high heritability might be more impressive than direct selection (Toker and Cigirgan, 2004). Targeted opt of physiological traits that limit yield and have a high heritability may be more impressive than direct selection for yield (Sayaret *et al.*, 2007).

The objectives of the present investigation were to estimate (i) the genetic parameters for the study of genetic diversity in some agro-physiological traits, and (ii) to study the correlation between the traits studied under rain-fed conditions.

Materials and methods

Plant genetic materials and experimental layout

Eight genotypes of bread wheat (*Triticumaestivum*L.) including: Bahar (G1), Pishtaz (G2), Vrinak (G3), Yavaros (G4), S-80-18 (G5), Crasalborz (G6), Santor (G7) and D-79-15 (G8) were assessed in a randomized complete block design with three replications under and water stress conditions in the experimental greenhouse of the College of Agriculture, Razi University, Kermanshah, Iran (47° 9' N, 34° 21' E and 1319m above sea level). The seed samples were planted in the plastic pots with 15 cm diameter and 20 cm height and filled with 3kg soil containing sand and animal fertilizer, as 1: 1: 1. In the 3 leaves stage, there were 5 bushes in each pot. The pots were kept in the planting capacity area through regular watering (irrigation), the damp of the pots were maintained about 40 percent of the farm capacity in the stress environment.

Measurement of characters

At harvest time, stress yield (Ys) was measured from 5 spikes per pot. The following physiological characters were also measured in the stress condition.

Relative water content (RWC)

Relative water content was determined according to Turner (1986), where the fresh leaves were taken from each genotype and each replication was weighed immediately after anthesis stage to record its fresh weight (FW). Then they were placed in distilled water for 4 h and weighed again to record turgid weight (TW). After that, they were subjected to oven for drying at 70°C for 24 h to record dry weight (DW). The RWC was calculated using the following equation:

$$\text{RWC (\%)} = ((\text{FW} - \text{DW}) / (\text{TW} - \text{DW})) \times 100$$

Relative water loss (RWL)

Five young fully expanded leaves were sampled for each of three replications at anthesis stage. The leaf samples were weighed (FW), wilted for 4 hour at 35°C, reweighed (W4h), and oven dried for 24 h at 72°C to obtain dry weight (DW). The RWL was calculated using the following formula (Gavuzzi *et al.*, 1997):

$$\text{RWL (\%)} = [(\text{FM} - \text{W4h}) / (\text{FW} - \text{DW})] \times 100$$

Water use efficiency (WUE) and evapotranspiration efficiency (ETE)

WUE was calculated by referring to Ehdaie and Waines (1993) according to total consumed water through wheat life circle. Three seeds from each line were sown in the greenhouse, two of which were eliminated 10 days after germination. One empty pot was used in each replication to calculate the amount of evaporation. The pots were irrigated with the measured amount of water. The run-off water in each pot was subtracted from the water applied to each pot. After 39 days, the dry matter (after drying at 70°C for 24 h) and the amount of water applied were used to calculate WUE using the following formula:

$$\text{ETE} = \text{TDM} / \text{TWU}$$

$$\text{WUE} = \text{GY} / \text{TWU}$$

Where, TDM = total dry mater; TWU = total water used and GY= grain yield.

Relative chlorophyll content (RCC)

The chlorophyll content in the flag leaf was determined using a chlorophyll meter (SPAD-502, Japan). Five flag leaves of each genotype grown in rainfed condition were measured after anthesis stage. Three measurements were accomplished randomly in the middle of the flag leaf for each plant, and the average.

Sample was used for the analysis

Chlorophyll a, b (Chl a, Chl b)

Chlorophylls a and b were measured by the method described by Horii *et al.* (2007) with a slight modification after anthesis stage. 3 ml of 99.5% methanol was added to the leaf tissue (50 mg) and incubated in dark for 2 h. The samples were homogenized and centrifuged at 10000 rpm for 10 min. Absorbance of the samples at 650 nm and 665 nm was measured by the UV spectrophotometer. Absolutemethanol (99.5%) was used as a blank. Chl a, Chl b and Chl T contents were calculated using the following equations:

$$\text{Chlorophyll a (\mu g/mL)} = 16.5 \times A_{665} - 8.3 \times A_{650}$$

$$\text{Chlorophyll b (\mu g/mL)} = 33.8 \times A_{650} - 12.5 \times A_{665}$$

Stomatal conductance (SD)

Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) was measured by Porometer- AP4 (Delta Devices, Cambridge, UK). Three random plants were selected in each pot for determining gaexchange parameters. All measurements were made on the portion of the flag leaf exposed to full sunlight, at about halfway along its length.

Excised leaf water retention (ELWR)

Excised leaf water retention was determined according to Farshadfar and Sutka (2002), where the youngest leaves before anthesis stage were collected and weighed (FW), left for 4 h, then wilted at 20°C and reweighed (WW4h). ELWR was calculated using the following formula:

$$\text{ELWR (\%)} = [1 - ((\text{FW} - \text{WW4h})/\text{FW})] \times 100$$

Statistical analysis

The analysis of variance and Duncan's Multiple Range Test (DMRT) were performed to test the significant difference between the means. The mean squares were used to estimate genotypic, phenotypic and environmental variance (σ^2_g , σ^2_p and σ^2_e) according to Johnson *et al.* (1955). The coefficient

of variation was calculated based on the formulas suggested by Burton (1952). The genotypic, phenotypic and environmental coefficient of variation (PCV, GCV and ECV) and heritability (h^2_{bs}) were recalculated as suggested by Singh and Choudhury (1999), genetic gain (GG) by Allard (1960) as well as correlation coefficient by Zaman *et al.* (1982).

Results and discussion

Analysis of variance and mean comparisons

The results of variance analysis (ANOVA) showed significant differences for Ys, RWC, ETE and WUE under rainfed conditions indicating the presence of a considerable genotypic variation and possible selection of drought tolerant genotypes under rainfed conditions (Table 1). No significant difference was found between the addition lines for a/b, RWL, RCC and ELWR, but as F-test in the analysis of variance can only detect large differences between the genotypes, therefore non-significance in the table of analysis of variance does not mean no significant difference between addition lines for these criteria, that is why mean comparisons classified these traits in different groups (Farshadfar *et al.*, 2013b).

Table 1. Analysis of variance for the characters investigated.

S.O.V	D.F	Ys	a/b	RWC	RWL	RCC	ELWR	SC	ETE	WUE
Replications	2	0.001 ^{ns}	0.002 ^{ns}	690.95 ^{**}	95.8 ^{ns}	3.12 ^{ns}	210.6 ^{ns}	1.82 ^{**}	1010.4 ^{ns}	103.05 ^{ns}
Genotypes	7	0.092 ^{**}	0.001 ^{ns}	88.7 ^{**}	211.75 ^{ns}	4.80 ^{ns}	150.5 ^{ns}	0.001 ^{ns}	13812.5 ^{**}	6732.4 ^{**}
Error	14	0.001	0.001	5.68	222.2	1.98	130.95	0.004	935.3	50.21
CV%	-	3.53	4.06	5.06	24.34	4.14	25.85	14.91	6.74	3.35

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

Table 2. Mean comparisons of agro-physiological traits.

Codes	Ys	WUE	ETE	RCC	RWL	RWC	SC	a/b	ELWR									
G1	146.5	e	341.1	d	63.6	a	34.7	ab	46.6	cd	0.38	a	0.85	a	37.1	a	0.540	e
G2	151.0	e	427.7	c	69.8	a	34.3	ab	43.9	de	0.38	a	0.84	a	42.1	a	0.557	e
G3	222.5	c	444.3	c	62.3	a	33.0	b	40.9	e	0.40	a	0.82	a	47.2	a	0.820	c
G4	227.9	c	586.5	a	59.2	a	33.8	b	48.0	bcd	0.38	a	0.88	a	34.8	a	0.840	c
G5	177.2	d	413.2	c	66.6	a	36.8	a	40.5	e	0.40	a	0.87	a	41.9	a	0.653	d
G6	165.5	d	417.5	c	47.1	a	32.8	b	51.5	b	0.43	a	0.85	a	53.8	a	0.610	d
G7	241.5	b	532.3	b	51.1	a	33.3	b	56.7	a	0.40	a	0.88	a	43.0	a	0.890	b
G8	275.8	a	508.4	b	70.2	a	33.6	b	48.8	bc	0.41	a	0.87	a	54.2	a	1.017	a

*: Mean with common letters have no significant difference.

Mean comparison also revealed the presence of different groups of genotypes (Table 2), indicating the presence of genetic variability for the traits under investigation. Comparison of means (Table 2) showed that the genotype G8 had the highest, WUE, RCC, SC, a/b and Ys. Maximum RWC belonged to G7 and

ETE to G4. The high RWC and low excised leaf water loss (RWL) have been suggested as important indicators of water status (El-Tayeb, 2006; Gunaset al., 2008). Rong-Hua *et al.* (2006) concluded that RCC could be considered as a reliable indicator in screening barley genotypes for drought tolerance.

Table 3. Mean and genetic parameters estimated condition

Traits	Mean	σ^2_G	σ^2_p	σ^2_e	h^2_{bs}	PCV	GCV	ECV	GG
Ys	0.741	0.030	0.031	0.001	0.978	23.748	23.484	3.530	47.839
SC	0.397	0.000	0.003	0.003	0.000	13.107	0.000	13.107	0.000
RCC	34.050	0.239	2.924	2.685	0.082	5.022	1.435	4.813	0.845
RWC	47.100	26.541	33.352	6.811	0.796	12.261	10.938	5.541	20.101
ELWR	44.267	7.377	137.450	130.073	0.054	26.485	6.136	25.764	2.928
RWL	61.233	0.000	218.707	218.707	0.000	24.151	0.000	24.151	0.000
ETE	453.988	4289.159	5227.206	938.047	0.821	15.925	14.426	6.746	26.919
WUE	200.988	2226.242	2277.390	51.148	0.978	23.744	23.476	3.558	47.814
a/b	0.854	0.002	0.001	0.000	1.409	4.139	4.914	0.000	12.016

Cluster analysis of genotypes

Using cluster analysis with UPGMA and based on grain yield and physiological criteria (Fig.1), the genotypes classified in three groups. Group 1 (drought tolerance) consisted of genotypes G3, G4 and G8, group 2 (semi-resistance) included genotypes G7 and

group G3 (drought sensitive) discriminated genotypes G1, G2, G5 and G6. As group 1 and 3 showed maximum between group variance, therefore they are recommended for the genetic analysis using diallel or scaling test and QTLs mapping of drought tolerance indices.

Table 4. Phenotypic (Upper off-diagonal matrix) and Genetic (Lower off-diagonal matrix) correlation matrix of the studied traits.

	Ys	SC	RCC	RWC	ELWR	RWL	ETE	WUE	a/b
Ys		-0.129	0.980	-0.049	-0.275	0.086	0.964	-0.538	1.026
SC	1.020		-0.040	-0.091	-0.316	0.103	0.979	-0.577	1.020
RCC	0.135	0.454			0.292	0.306	-0.132	0.571	0.135
RWC	-0.236	-0.002	-0.369			0.379	-0.209	0.536	-0.236
ELWR	0.079	0.757	0.134	0.456			0.178	0.081	0.079
RWL	1.026	-0.028	0.984	-0.116	-0.236			-0.502	1.026
ETE	-0.699	0.037	-0.963	0.337	0.712	0.214			-0.699
WUE	-0.760	-0.443	-0.929	-0.112	0.149	-0.557	-0.971		-0.760
a/b	0.325	-0.091	0.024	0.685	0.514	0.305	0.073	0.664	

Agronomic, morphological and physiological traits are very important with well potential for grouping wheat genetic resources, and also are essential and useful for plant breeders seeking to improve existing

germplasm by introducing novel genetic variation for certain traits into the breeding populations (Pagnotta *et al.*, 2009; Zarkti *et al.*, 2010; Najaphy *et al.*, 2012).

Evaluation of genetic parameters

High heritability (> 0.5 ; Stanfield, 2005) and genetic gain was observed for Ys, RWC, ETE, WUE and a/b indicating that the major part of phenotypic variation is attributed to genotypic variation (Table 3). High genotypic and phenotypic coefficient of variation (GCV and PCV) were observed for RWC, ELWR, ETE and WUE (Table 3) showing little environment effect on the expression of them. The progress of any breeding program is conditioned by the magnitude and the nature of the genotypic and non-genotypic variation in the various characters. Heritability and genetic advance are also very useful in order to estimate the scope for improvement by selection. Heritability magnitude indicates the reliability with

which the genotype will be recognized by its phenotype expression (Chandrabha and Sharma, 1999). A comparatively low value of heritability was observed for the characters SC, RCC, ELWR and RWL ($< 40\%$) (Table 3). The heritability estimates for different characters depend upon the genetic make up of the breeding materials studied. Therefore, knowledge about these values in the materials in which breeders are interested is of great significance. High broad sense heritability indicates high genetic potentials for these traits, low effect of the environment and existence of predominant role of additive genes. High broad sense heritability seems to be a suitable basis for a reliable selection (Kandasamy *et al.*, 1989; Thiyagarajan, 1990).

Table 5. Co-heritability estimates between agro-physiological characters.

	Ys	SC	RCC	RWC	ELWR	RWL	ETE	WUE
SC	0.548							
RCC	1.198	-7.492						
RWC	1.036	-0.144	1.042					
ELWR	1.318	0.372	0.434	0.361				
RWL	5.042	0.335	0.109	0.926	-0.034			
ETE	1.033	0.085	0.540	0.940	0.581	0.449		
WUE	0.978	0.532	1.204	1.033	1.316	5.317	1.033	
a/b	1.567	-0.738	1.971	1.253	-0.517	-0.341	1.544	1.557

The results of phenotypic and genetic correlation coefficient (Table 4) displayed that RCC, ETE, SC and a/b contributed significantly towards Ys. It can be concluded on the basis of the results obtained in the present investigation that, the range of variability was quite appreciable for most of the characters studied among different genotypes.

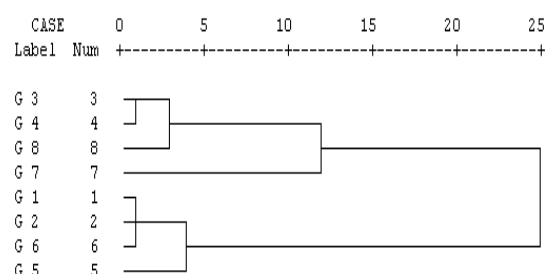


Fig. 1. Dendrogram resulted from cluster analysis based on the traits investigated.

Phenotypic and genotypic matrix

Evolution by natural selection requires heritable

variation. The most common way to represent the pattern and magnitude of the genetic basis of a series of traits is the genetic variance–covariance matrix, also known as the G-matrix. G-matrix is extremely useful for predicting the response to selection over the short term. A population will evolve most rapidly along axes that have the most genetic variation, and more slowly in directions with little genetic variance. Because G accounts for genetic covariance as well, G can also help predict the indirect response to selection on one character from selection on another trait. If the genetic covariance between two traits is different from zero, selection on one trait will affect response to selection on the other (Guillaume and Whitlock, 2007). According to the results (Table 4), the highest genotypic and phenotypic covariance observed between RCC, ETE, a/b and SC indicating significant contribution of these traits to increase grain yield in wheat under raised condition

(correlated response). High values of genetic and/or phenotypic covariance between two traits may represent a high level of variation (genetic, phenotypic or both) between two traits. High values of genetic variation in breeding programs can be very useful (Farshadfar *et al.*, 2013a).

Co-heritability

The lower diagonal of Table 5 has the co-heritability values for pairs of characters. The range of co-heritability was from -7.492 (between SC and RCC) to 19.27 (between WUE and RWL). High co-heritability was observed between RWL and Ys(5.042), RWL and WUE (5.317). Positive co-heritability estimate was observed between Ys and all other physiological traits. This suggests that selection of either of the characters would simultaneously affect the others positively. Romena and Najaphy (2012) suggested that improving grain yield is related to the balance of SC and RWC in wheat under rain-fed condition.

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