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Genetic diversity and heritability of chlorophyll content and photosynthetic indexes among some Iranian wheat genotypes

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Abstract

Genetic diversity and heritability of Chlorophyll (Chl) content and some photosynthetic indexes were studied in thirty wheat genotypes through randomized complete block design with three replications at 2009. The maximum and minimum heritability were obtained for chlorophyll (0.75-0.89) and stomatal conductance (0.2), respectively. The least phenotypic and genotypic coefficients of variation were observed for CO₂ levels in boundary layer. Phenotypic and genotypic correlation coefficients between Chl a, Chl b and total Chl were similar and between SPAD Chl and Chl b were significantly high. Chl b content and transpiration rate, stomatal conductance and H₂O levels in boundary layer had positive and significant genotypic correlation. CO₂ levels in boundary layer and stomatal conductance were significantly and linearly associated with photosynthesis rate. 78.62% of data variation was explained by two factors. Genotypes in first group showed appropriate photosynthetic characters for resistance to drought and heat. Genotypes in third group have high chlorophyll content and photosynthesis rate. Maximum (103.19) and minimum (0.98) genetic distance was found between Moghan 3 and Kavir and between star and Tajan, respectively.

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Introduction

Wheat is one of the most important cereals and main source of food in Iran and many different countries (Gohari *et al.*, 2007). One of the important approaches to wheat breeding is hybridization and subsequent selection. Parent selection is the first step in plant breeding programs through hybridization. In order to use transgressive segregation, genetic distance between parents is necessary (Joshi *et al.*, 2004). If genetic distance between parents is high, chances of higher heterosisty in progeny increase (Joshi and Dhawan, 1966).

A survey of genetic variability with the help of suitable parameters such as genotypic coefficient of variation and heritability are absolutely necessary to start an efficient breeding program (Mishra *et al.*, 1988). Although direct selection for various parameters could be misleading, indirect selection via related parameters with high heritability might be more effective than direct selection (Toker and Cigirgan, 2004).

Towards a clear understanding of the type of plant traits, correlation and path coefficient analysis are logical steps (Kashif and Khaliq, 2004). Traditionally, correlation, regression and path coefficient analysis have been used in determining character interrelationships (Toker and Cagirgan, 2003). However, considering many traits simultaneously requires that the dimensions of the data set be reduced. Factor analysis is designed to do this (Awan *et al.*, 2007). The communality is an indication of the proportion of the variation of a variable that is accounted for by the retained factors. An indicator variable with a low communality indicates that the factor model is not practical for that indicator variable. It furthermore suggests that the specific indicator should possibly be removed from the model. A communality of 0.75, however, may seem high, but is meaningless unless the factor on which the variable is loaded is interpretable. Likewise, a communality of 0.25 may seem low, but may be meaningful if the item contributes to a well defined factor. Thus, it is not the communality coefficient per se which is critical, but

rather the extent to which the item plays a role in the interpretation of the factor (Jordaan and Grové, 2007).

There are different algorithms for cluster analysis. Among these algorithms the methods of Unweighted Paired Group Method using Arithmetic averages (UPGMA) and Ward's are most popular cluster analysis used especially for the study of genetic diversity. Efficiency of the Ward method is similar with the UPGMA method but it does not have the chaining effect problem (Mohammadi and Prasanna, 2003).

Direct and indirect methods can be used to investigate primary organic production. Indirect methods are often used for an approximate estimate of the value of organic production because it is fairly difficult to employ direct methods in plant communities. As indirect methods, it is possible to monitor and measure all phenomena and processes correlated with productivity (Kof *et al.*, 2004). Chlorophyll pigments play an important role in the photosynthetic process as well as biomass production. Genotypes maintaining higher leaf chlorophyll a and chlorophyll b during growth period may be considered potential donors for the ability of producing higher biomass and photosynthetic capacity. Higher photosynthesis rate is supported by leaf chlorophyll content in leaf blades (Miah *et al.*, 1997).

Chlorophyll content is positively associated with photosynthetic rate which increases biomass production and grain yield (Thomas *et al.*, 2005). Therefore, understanding the genetic mechanism of chlorophyll content would be very important for yield improvement. Significant relationships between chlorophyll content and yield and yield components facilitate selection of high yielding genotypes (Singh, 2001). Rates of net photosynthesis of plants generally decrease as leaf temperatures exceed optimum values. The decline in rate of net photosynthesis at high temperature can result from both external (stomatal) and internal biochemical (mesophyll) factors. The

main external factor limiting net photosynthesis at high temperatures is diffusion of gases from outside the leaf to the sites of carboxylation. Internal factors limiting net photosynthesis at high temperatures include increased rates of photorespiration and dark respiration (Mebrahtu *et al.*, 1991). The aims of this study were assess relationship among leaf chlorophyll and photosynthesis indexes, survey of genetic variation among studied genotypes for leaf chlorophyll content and photosynthesis rat and identification of genotypes with high photosynthesis ability.

Materials and methods

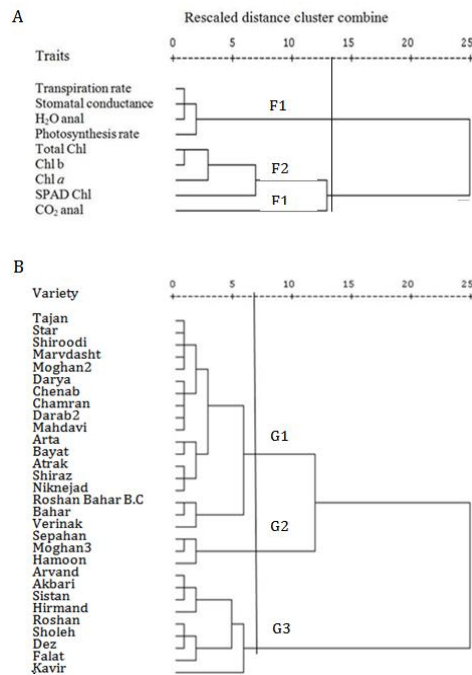
Plant materials and growth condition

Thirty common spring wheat cultivars (Fig 1B) supplied by the Agricultural Research Institutes gene bank in Karaj were prepared and used for this research in the Research Field of Faculty of Agricultural Sciences, Tarbiat Modares University, Tehran, Iran in 2009. A plot length was 1.5 m long with row to row spacing of 20cm on the basis of a randomized complete block design with three replications. Nitrogen and phosphorus fertilizer, were applied at 40 and 60 Kg ha⁻¹, respectively before planting and nitrogen fertilizer at 40 kg ha⁻¹ was also broadcast at tillering and stem elongation.

Fig. 1. Dendrogram showing; (A) The relationships among 9 studied traits, F1 and F2 showing the traits that were significant in factor 1 and factor 2 in factor analysis, respectively, (B) The genetic relationship among 30 spring wheat genotypes, (G1) Members of first cluster, (G2) Members of second cluster, (G3) Members of third cluster; using ward method and squared Euclidean distance

Studied traits and its measuring method

The measured traits include H₂O levels in boundary layer (mbar), CO₂ levels in boundary layer (VPM), leaf boundary layer temperature (C), leaf surface temperature (C), leaf mass (μmol s⁻¹), transpiration rate (mmol m⁻²s⁻¹), stomatal conductance (mol m⁻²s⁻¹) and photosynthesis rate (μmol m⁻²s⁻¹) and were measured by means of photosynthesis meter (model LCi) set. All above traits were measured in approximate CO₂ reference 380 VPM and H₂O reference 0.56 mbar. Relative chlorophyll content was measured by SPAD-502 set. Chlorophyll a, b and total chlorophyll were measured according to following protocol. The material was processed in the fresh state immediately after collection. After fine chopping, portions weighing 0.5 g were measured on an analytical balance. The measured material was then homogenized in a homogenizer with the addition of 10 ml of 80 % acetone. A primary acetone extract containing all chloroplast pigments was obtained in this way. The extract was then centrifuged at 2500 rpm for 5 min. Since the concentration of pigments was in most cases too great for reading on a spectrophotometer, the extract was diluted by adding 9 ml of 80% acetone per ml of extract (Bojovic and Stojanovic, 2005). The extract produced in this way was read on a spectrophotometer. Chlorophyll content was calculated according to Lichtentaler and Wellburn (1985). All of above traits were measured at grain filling stage (Tulio *et al.*, 2007).



Statistical analysis

Analysis of variance, phenotypic correlation coefficients, factor analysis based on principal component method and varimax with kaiser

normalization rotation method, cluster analysis, discriminant analysis and the significance difference between cluster means was tested by Duncan's new multiple range test (DMRT) at 5% level of probability using the statistical software SPSS version 16.0 (SPSS, Chicago, USA) program. Group means were compared by Duncan's multiple range test ($P < 0.05$) and the averages of five data were used for statistical analysis. Genotypic correlation coefficients were computed according to the formula used by Steel and Torrie (1984). Path analysis was done by means of Path 0.1 software and based on Dewey and Lu (1956). The mean squares were used to estimate genotypic and phenotypic variance according to Johnson *et al.* (1955). The coefficient of variation was calculated according to the formula suggested by Burton (1952). The genotypic and phenotypic coefficient of variation and broadcast heritability were calculated according to the formula used by Singh and Choudhury (1985).

Results and discussion

Analysis of variance and heritability

Analysis of variance showed significant difference among genotypes for all traits with except of leaf mass, leaf surface temperature, and stomatal conductance. The present results of transpiration rates are in accordance with those previously reported by Solomon and Labuschagne (2003). Photosynthesis rates are in accordance with obtained results by Carver and Nevo (1990). Therefore, the traits that did not showed variation among genotypes eliminated. Chl a (0.82), b (0.89), total Chl (0.92), and SPAD Chl (0.84) showed high broadcast heritability. It is indicated that these traits were under genetic control and fewer genes control these traits (Mohammadi *et al.*, 2010). In addition, the previous study on chlorophyll heritability showed that chlorophyll content controlled by a few genes (Ghain *et al.*, 1969). Therefore, to improve these traits the breeding methods without progeny test can be applied.

Phenotypic and genotypic coefficients of variation for Chl b and total Chl were high and close together. The least phenotypic and genotypic coefficients of

variation were observed for CO₂ levels. It is indicated that this trait does not have enough variation among genotypes. Stomatal conductance showed the least heritability and the highest coefficient of variation that are approximately in accordance with Rebetzke *et al.* (1996). Therefore, the portion of environmental conditions used for definition of this trait is high.

Traits relations

Phenotypic and genotypic correlation coefficients between Chl a, Chl b and total Chl together with and between SPAD Chl and Chl b were significantly high. In addition, SPAD Chl was significantly associated with total Chl and photosynthesis rate by genotypic and phenotypic correlation coefficients, respectively (Table 1). Similar to this study, results of previous study on *Amaranthus vlitus* L. SPAD readings showed linearly and positively relation to total Chl concentration in leaves (Kapotis *et al.*, 2002). The present results about relation between transpiration rate and chlorophyll content are in accordance with those previously reported by Thomas *et al.* (2005) and Zhang *et al.* (2009). Some studies have demonstrated that chlorophyll content correlated with photosynthetic rate (Thomas *et al.*, 2005). SPAD had positive and significant phenotypic correlation with photosynthesis rate and this result is in accordance with Zhang *et al.* (2009). Chl b content and transpiration rate, stomatal conductance and H₂O levels had a positive and significant genotypic correlation, but this relation between Chl b and CO₂ level was negative and significant. Photosynthesis characters including transpiration rate, photosynthesis rate, stomatal conductance, and H₂O showed significant and positive genotypic and phenotypic correlation coefficients while CO₂ levels were had negative and significant genotypic and phenotypic correlation coefficients with other photosynthesis characters. It is obviously clear that increases of photosynthesis rate and transpiration rate will increase stomatal conductance and this action leads to a decrease in levels of CO₂ in leaf boundary layer in agreement with Del Blanco *et al.* (2000) who observed positive and significant

correlation between photosynthesis rate and stomatal conductance. This is not what they found about relation between photosynthesis rate and total Chl. Because of estimating genotypic correlation

coefficients from mean squares, some genotypic correlation coefficients were higher than 1. Similar results were obtained by Saleem *et al.* (2006).

Table 1. Phenotypic (rp) and Genotypic (rg) correlation coefficients among studied traits in spring wheat.

Traits	r	Total Chl	Chl b	SPAD Chl	Transpiration Rate (TR)	Photosynthesis Rate (PR)	Stomatal Conductance (SC)	H ₂ O level	CO ₂ level
Chl a	r _p	0.81***	0.48**	0.18	-0.04	-0.03	-0.07	-0.09	-0.05
	r _g	0.84***	0.54**	0.32	-0.10	-0.08	-0.19	-0.19	-0.05
Total Chl	r _p		0.84***	0.33	0.25	0.16	0.12	0.21	-0.24
	r _g		0.87***	0.43*	0.41*	0.23	0.23	0.26	0.34
Chl b	r _p			0.43*	0.35	0.19	0.22	0.31	-0.28
	r _g			0.55**	0.56**	0.29	0.48**	0.52**	-0.53**
SPAD Chl	r _p				0.15	0.36*	-0.02	0.16	-0.12
	r _g				0.18	0.17	-0.07	0.28	-0.20
TR	r _p					0.86***	0.90***	0.90***	-0.85***
	r _g					0.94***	1.00***	1.06***	-1.08***
PR	r _p						0.80***	0.77***	-0.92***
	r _g						1.08***	0.90**	-1.00***
SC	r _p							0.87***	-0.81***
	r _g							0.97***	-1.07**
H ₂ O level	r _p								-0.77***
	r _g								-1.08***

*, ** and *** significant at 5%, 1% and 0.1% level of probability, respectively

The results of regression analysis indicated that CO₂ level in the boundary layer (b₁= -0.35) and stomatal conductance (b₂= 1.44) was significantly and linearly associated with photosynthesis rate and explained 87% of the total variation of photosynthesis rate. Also, the study of Peri *et al.* (2003) on modeling net photosynthetic rate of field-grown *Dactylis glomerata* leaves during regrowth duration revealed that there was a positive linear relationship between stomatal conductance and photosynthesis rate. Should be noted that the relationship between photosynthesis and consumed CO₂ is positive (Bhatt *et al.*, 2010) but

in this study CO₂ levels in the boundary layer were measured, and as the amount of CO₂ in boundary layer increased, subsequently photosynthesis rate decreased.

Path coefficient analysis (Table 2) revealed that Chl a showed negative and positive direct effect on photosynthesis rate based on phenotypic and genotypic correlation coefficients, respectively and had a total negative effect based on both correlation coefficients. The indirect effects via other traits were positive and negative. Total Chl had the positive

direct and total effect on photosynthesis rate based on both correlation coefficients. The indirect effects of total Chl were negative and positive via Chl b and transpiration rate, respectively. Chl b had a direct negative effect on photosynthesis rate while it had a positive total effect based on both correlation coefficients. Thus, the indirect effects of Chl b via other traits are positive and greater than direct effect of Chl b. SPAD Chl had a positive and negative direct effect on photosynthesis based on phenotypic and genotypic correlation coefficients, respectively. The effect of SPAD Chl on photosynthesis rate was indirect and via H₂O. However SPAD Chl had a positive total effect on photosynthesis rate based on two types of correlation coefficients. Transpiration rate had a positive direct and high total effect on photosynthesis rate based on both phenotypic and genotypic correlation coefficients. Stomatal conductance had a positive and high negative direct effect on photosynthesis based on phenotypic and

genotypic correlation coefficients, respectively. Also it had a high positive total effect on photosynthesis rate. H₂O levels had a negative and high positive direct effect on photosynthesis based on phenotypic and genotypic correlation coefficients, respectively. Stomatal conductance and H₂O levels had a positive indirect effect on photosynthesis rate via total Chl, transpiration rate and CO₂ levels and negative indirect effect via Chl b based on two types of correlation coefficients. CO₂ levels had a negative direct effect on photosynthesis and positive and negative indirect effects via Chl b and transpiration rates based on both phenotypic and genotypic correlation coefficients. There is limited information about leaf chlorophyll content and photosynthesis as contributing characters of wheat in the literature. Therefore, further investigation is required on the leaf chlorophyll content and photosynthesis contributing characters.

Table 2. Direct (Bold) and indirect effects of various traits on photosynthesis rate in wheat.

Traits		Chl a	Total Chl	Chl b	SPAD Chl	Transpiration Rate (TR)	Stomatal Conductance (SC)	H ₂ O level	CO ₂ level	Total effect
Chl a	P ¹	-0.24	0.35	-0.24	0.07	-0.01	-0.03	0.02	0.03	-0.03
	G ²	0.52	0.03	-0.29	-0.17	-0.07	0.27	-0.38	0.004	-0.08
Total Chl	P	-0.20	0.44	-0.41	0.13	0.05	0.04	-0.05	0.15	0.16
	G	0.44	0.03	-0.46	-0.22	0.28	-0.33	0.52	-0.03	0.23
Chl b	P	-0.12	0.37	-0.49	0.17	0.08	0.08	-0.08	0.18	0.19
	G	0.28	0.03	-0.53	-0.29	0.39	-0.68	1.04	0.05	0.29
SPAD Chl	P	-0.04	0.14	-0.21	0.41	0.03	-0.01	-0.04	0.08	0.36
	G	0.17	0.01	-0.29	-0.52	0.12	0.10	0.56	0.02	0.17
TR	P	0.01	0.11	-0.17	0.06	0.22	0.31	-0.22	0.54	0.86
	G	-0.05	0.01	-0.30	-0.09	0.69	-1.41	1.99	0.09	0.94
SC	P	0.02	0.05	-0.11	-0.01	0.20	0.35	-0.22	0.52	0.80
	G	-1.00	0.01	-0.25	0.04	0.69	-1.41	1.93	0.09	1.00
H ₂ O level	P	0.02	0.09	-0.15	0.06	0.20	0.30	-0.25	0.49	0.77
	G	-1.00	0.01	-0.28	-0.15	0.69	-1.37	1.99	0.09	0.90
CO ₂ level	P	0.01	-0.11	0.14	-0.05	-0.19	-0.28	0.19	-0.64	-0.92
	G	-0.03	0.01	0.28	0.10	-0.70	1.41	-1.99	-0.10	-1.00

1 and 2 are path analysis results based on phenotypic and genotypic correlation coefficients, respectively. Residual effects for genotypic and phenotypic correlation were 0.38 and 0.08 respectively.

The data presented in Table 3 show that two main factors were significant (Eigen value>1) (Jordaan and

Grové, 2007) and accounted for 78.62% of the total variability in the dependent structure. This implies

that the factor analysis model used in this study was effective in illuminating the unique variance of each variable. In this study KMO value was estimated 0.67 therefore traits were suitable for inclusion in the factor analysis (Berghaus *et al.*, 2005). Communalities ranged from 0.27 to 0.94. The observed high communalities indicate that the role of the studied traits are important (Garson, 2004) and the communalities of all the traits except SPAD Chl are more than 0.71 which indicates that the factors

explain more than 71% of the variation in the traits. These traits also contribute significantly to the interpretation of the respective factors. Generally, factor loadings of 0.7 - 0.9 would be considered high loadings and those from 0 to 0.2 as low loadings (Denis and Adams, 1978). In accordance with Ariyo (1995) in this study for the purpose of interpretation only characters exhibiting factor loadings of 0.5 or larger were considered important.

Table 3. Results of factor analysis in spring wheat.

Traits	C ₁ ⁺	C ₂ ⁺	communalities	Rotation Sums of Squared Loadings				KMO [§]
				C ⁺	Eigen values	Variance %	Cumulative Variance %	
Chl a	-0.14	0.83*	0.71	C ⁺				0.67
Total Chl	0.12	0.96*	0.94	1	4.44	49.28	49.28	
Chl b	0.23	0.86*	0.79	2	2.64	29.34	78.62	
SPAD Chl	0.09	0.51*	0.27					
Transpiration Rate	0.96*	0.13	0.94					
Photosynthesis Rate	0.92*	0.06	0.85					
Stomatal Conductance	0.94*	0.002	0.88					
H ₂ O level	0.92*	0.10	0.86					
CO ₂ level	-0.92*	-0.14	0.87					

+: Components by principal component extraction method and varimax with Kaiser Normalization rotation method; §: Kaiser-Meyer-Olkin Measure of Sampling Adequacy; *: significant at 5% level of probability.

The first factor included transpiration rate, photosynthesis rate, stomatal conductance, H₂O levels and CO₂ levels, which explained 49.28% of total variance. The suggested name for this factor is photosynthesis characters. The second factor included Chl a, Chl b, total Chl and SPAD Chl that explained 29.34% of total variance was the chlorophyll content.

Cluster analysis and cluster properties

In order to grouping studied traits and identify traits that have a close relation, cluster analysis was done and two separate clusters diagnosed (Fig 1A). This analysis was in accordance with the results of correlation and factor analysis so that the traits with

positive and significant relation were grouped. As shown in Fig 1A the traits that significantly contributed to the first factor belonged in the first cluster with the exception of CO₂. It had a negative but significant relation with other photosynthesis characters and was closer to chlorophyll content characters than photosynthesis characters therefore CO₂ belonged in to the second cluster.

Based on cluster analysis, thirty genotypes were grouped into three completely separate clusters (Fig 1B) suggesting considerable amount of genetic diversity in the material. The accuracy of grouping was confirmed by both discriminant analysis and multivariate analysis of variance based on complete

randomized design so that the genotypes correctly belonged in their groups and the difference among group means were significant ($P < 0.001$) based on Pillai's trace, Wilks' Lambda and Roy's Largest Root tests. Thus, the grouping was correct. Results of mean comparison (Table 4) showed the significant ($P < 0.05$) difference between the first cluster and other two clusters for traits that had the meaningful contribution in the first factor. Means of the third cluster for H₂O levels and transpiration rate had a significant ($P < 0.05$) difference with means of other two cluster means. The difference among means of overall clusters were significant ($P < 0.05$) for CO₂ levels and photosynthesis rate. In this study, moreover clustering by cluster analysis with ward method and squared Euclidean distance measure, genotypes were grouped by biplot graph using discriminant function and factor scores (Fig. 2). By comparison the results of three grouping methods indicated that results of mentioned methods were the same. Nevertheless, in discriminant function biplot method (Fig 2A) genotypes could be separated. Therefore, for the subjects grouped in the three clusters we can use discriminant scores for grouping and in other subjects in which we have more than three clusters we can use discriminant scores for

cluster analysis with squared Euclidean. According to Fig 1A and Fig. 2 the first cluster was the largest having 18 genotypes indicating overall genetic similarity among them. Trait means in the first cluster were less than total mean except for CO₂ levels that were higher than total mean (Table 4). Havaux and Trady (1999) reported that the correlation between low level of chlorophyll and stomatal conductance was positive therefore the genotypes with low chlorophyll are adaptive to drought conditions. Mohammadi *et al.* (2009) explained the reason of this observation. They said that low level of chlorophyll leads to decline light absorption and its destructive effects. They observed that correlation between chlorophyll content and seed yield in various wheat genotypes in heat and drought stress conditions was negative and significant ($P < 0.01$). In addition, mean of transpiration rate in the first cluster was less than total mean photosynthesis rate is dependent to chlorophyll content (Kura Hotta *et al.* 1987) therefore mean of photosynthesis rate in this cluster was less than total mean. According to the above explanation, the genotypes of this cluster are suitable for using in drought and heat resistance breeding programs.

Table 4. Mean, standard deviation, Deviation from total mean and mean comparison results.

Group	Statistics	Chl a	Chl b	Total Chl	SPAD Chl	H ₂ O level	CO ₂ level	Transpiration Rate	Photosynthesis Rate
1	mean	4.82 ^b	0.50 ^b	5.33 ^b	47.58 ^b	4.21 ^b	342.01 ^b	3.83 ^b	18.01 ^b
	DfTM	-0.43	-0.39	-0.94	-1.74	-0.58	3.14	-0.30	-1.04
	Std. Deviation	0.82	0.25	0.88	3.44	1.39	4.69	0.57	2.50
2	mean	6.10 ^a	1.82 ^a	8.29 ^a	51.98 ^a	2.56 ^b	350.42 ^a	3.16 ^b	11.92 ^c
	DfTM	0.84	0.93	2.02	2.66	-2.23	11.55	-0.98	-7.14
	Std. Deviation	1.03	0.66	1.27	0.44	0.93	6.07	0.20	3.41
3	mean	5.85 ^a	1.37 ^a	7.48 ^a	51.91 ^a	6.70 ^a	328.72 ^c	5.06 ^a	23.52 ^a
	DfTM	0.59	0.48	1.21	2.59	1.90	-10.15	0.93	4.46
	Std. Deviation	0.63	0.61	1.16	4.12	1.52	8.52	0.93	4.68

DfTM: Deviation from total mean

The second cluster included 3 genotypes (Fig 1B). Trait means in the second cluster were more than the

total mean except for H₂O levels, transpiration rate and photosynthesis rate which were less than total

mean (Table 4). In this cluster, chlorophyll content was more than total mean therefore leaf surface became murky and light absorption increased and subsequently led to increase leaf temperature. By leaf temperature, increasing the stomata closed and led to decreasing transpiration rate therefore H₂O levels in the boundary layer became less than total mean. Due to stomata closing consumption of CO₂ levels in the boundary layer were decreased subsequently the amount of CO₂ was increased in the leaf boundary layer. Hence, transpiration rate and photosynthesis

rate declined in genotypes of this cluster. Previously Del Blanco *et al.* (2000) obtained similar results. They observed that maximum photosynthesis positively correlated with stomatal conductance and low leaf temperature. According to the above explanation, the genotypes of this cluster are suitable for using in breeding programs with purpose of breeding for culturing in the regions with high light intensity and temperate climates.

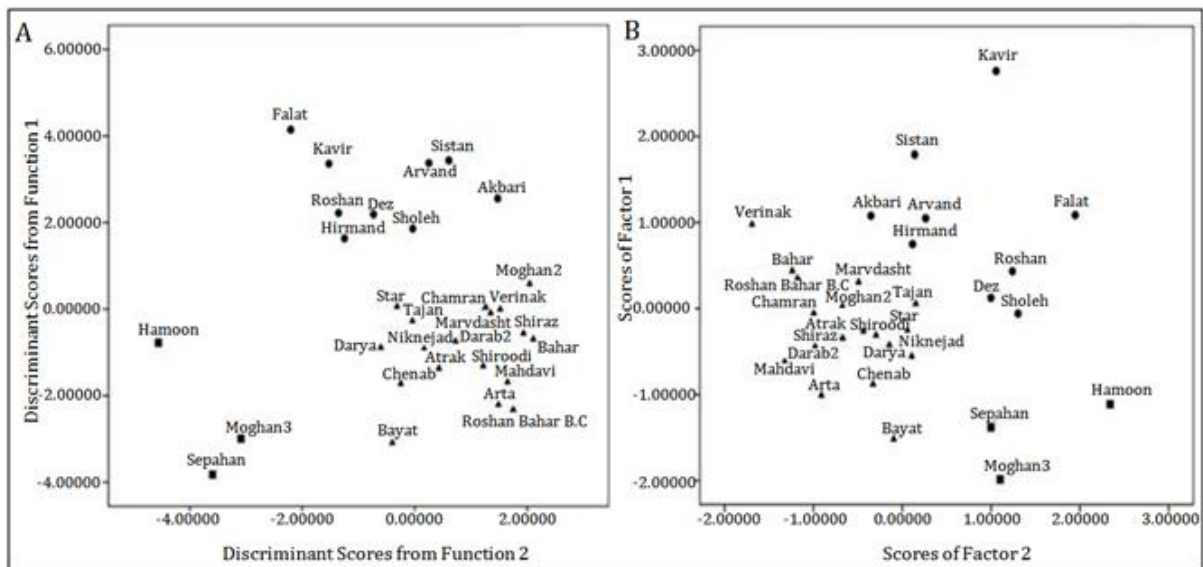


Fig. 2. Grouping of 30 spring wheat genotypes using biplot based on (A) discriminant function scores, (B) factor scores estimated by regression method; (▲) genotypes in the first cluster, (●) genotypes in the Second cluster, (■) genotypes in the third cluster.

The third cluster included nine genotypes (Fig 1B). Trait means in the third cluster were more than the total mean except for CO₂ levels which were less than total mean (Table 4). The photosynthesis characters in genotypes of the third cluster were better than genotypes of other clusters. Although a chlorophyll content in this cluster members were higher than other clusters the stomatal conductance rates were not affected by increasing leaf temperature due to rising chlorophyll content which subsequently leads to increase in photosynthesis rate by increases in H₂O and CO₂ exchange in this cluster members. Hence, these cluster members are adapted to high

temperature and they can used in breeding programs with purpose of breeding for tolerance to high temperatures. The greatest genetic distance (103.19) was observed between Moghan 3 and Kavir genotypes. These genotypes can be used in breeding programs with aim of reaching maximum heterosis and transgressive segregation for photosynthesis rate. The least genetic distance (0.98) was observed between Star and Tajan genotypes. These genotypes can be used in back cross program for gene transfer.

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