Study of Heritability and Genetic Advance of Agronomic Traits in Barley (*Hordeum vulgare* L.) and Graphic Analysis of Trait Relations by Biplot

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ABSTRACT

Crop yield is a complex trait determined by several components that may positively or negatively affect this trait and its improvement is a major goal of breeding programs. In this study, a population of 148 barley doubled-haploid lines derived from a cross between "Clipper" and an "Sahara3771" were grown in the experimental field of University of Tabriz, in the 2011 and 2012 barley growing seasons to assess the presence of variability for desired traits and amount of variation for different parameters. Variance components, phenotypic and genotypic coefficient of variation, heritability and genetic advance were estimated for all the traits. The lowest differences between genotypic and phenotypic coefficient of variation observed for awn length, stem diameter and hundred grain weight. Highest value of narrow sense heritability was recorded for hundred grain weight (93.52). High genetic gain were observed in number of grain per plant, grain yield and hundred grain weight, suggesting that selection based on phenotypic performance of this trait would be effective to improvement value of these traits. In this study, the interrelationships among yield and other agronomic traits were also investigated using genotype by trait biplot technique. Positive relations were found between grain yield, number of grain per plant and stem diameter. The results showed that line L75 produced maximum grain yield, number of grain per plant, biomass, plant height and peduncle length. The study revealed that genotype by trait (GT) biplot can graphically display the interrelationships among traits and facilitate visual comparison of lines.

Keywords: Barley (*Hordeum vulgare* L.), GT Biplot Analysis, Heritability, Yield Component.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops that have been crucial to the development of humans. It is used for animal feed, brewing malts, human food and production of starch, either for food or for the chemical industry. Its history as a crop began in the Fertile Crescent, a cradle area that today includes parts of Iran, Turkey, Syria, Jordan, Palestine, and Israel (Badr et al., 2000; Cattivelli et al., 1994; Harlan and Zohary, 1966). Badr et al.(2000) reported that remains of barley grains found at archaeological sites in the Fertile Crescent indicated that the crop was domesticated there from its wild relative (*Hordeum spontaneum*) about 10,000 years ago. The study is based on the assumptions that DNA markers can be used to effectively measure genetic distances, that the wild populations are genetically different, and that they

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have not undergone significant change since domestication. The crop is grown in a range of diverse environments but it favors cool production conditions and moderate precipitation (Bothmer et al., 1991). Barley is the second largest field crop in Iran after wheat and is cultivated in all part of the country. In Iran, cultivation of barley has reached about 1.58 million hectare, with the total production of about 3.2 million tons with an average yield of about 2 tons per hectare (FAO 2010). Therefore, there is a need to increase both area and productivity.

The development of high yielding-varieties is the main objective of any breeding program. Grain yield is a complex trait and highly influenced by many genetic factors and environmental variation. Hence, for efficient selection procedure for higher grain yield, it is necessary to have adequate information about the nature and magnitude of genetic variability present in the available breeding materials. Characteristics such as number of grain per plant, hundred grain weight and number of fertile tillers were positively correlated with grain yield (Zecevic et al., 2010). Breeding of yield components to increase grain yield would be most effective if the components involved were highly heritable and genetically independent or positively correlated (Sürek, Korkut, 1998). Therefore, investigation of genetic variability, components of phenotypic variance and heritability for these traits are very important for the crop improvement and variety development. These investigations make it possible to evaluate the efficiency and reliability of yield components as selection criteria in barley breeding for higher grain yield (Bello et al., 2012). Assessment of variability for quantitative characters such as grain yield becomes absolutely essential before planning for an appropriate breeding strategy for genetic improvement. Genetic parameters such as genotypic coefficient of variation (GCV) and

phenotypic coefficient of variation (PCV) are useful in detecting the amount of variability present in the germplasm. Heritability coupled with high genetic advance (GA) would be more useful tool in predicting the resultant effect in selection of the best genotypes for yield and its attributing traits. It helps in determining the influence environment on the expression the genotypic and reliability of characters.

Grain yield is a complex trait determined by several components that may positively or negatively affect this trait. In plant breeding programs, successful plant selection requires knowledge and understanding of relationships between grain yield, yield components and other important agronomic traits (Evans, Fischer, 1999).

Numerous methods have been used to understand of the data patterns, and study of interrelationship among traits. Yan et al. (2000) developed a GGE biplot methodology (genotype main effect plus genotype × environment effect) for graphical analysis of multi-environment trial data. Although the GGE biplot methodology was originally proposed for analyzing multi-environment trials data for a given trait, it is equally applicable to all types of two-way data that assume an entry-by-tester structure, such as a genotype-by-trait two-way dataset (Yan, Kang, 2003). Biplots are useful for summarizing and approximating patterns of response that exist in the original data. The biplot technique (Gabriel, 1971) is a multivariate analytical technique that graphically displays the two-way data and allows visualization of the interrelationship among traits, and the interrelationship between genotypes.

In the current study, an analysis of variability was conducted among the traits and the phenotypic and genetic coefficients of variation and narrow sense heritability were accounted for of several agronomic traits in doubled haploid barley population developed from a cross between Clipper and Sahara3771 cultivars.

The interrelationships among yield and its components were also investigated by Genotype by Trait (GT) biplot technique.

MATERIALS AND METHODS
Experimental Design and Genetic Materials

The 148 doubled-haploid lines derived from a cross between Australian cultivar "Clipper" and an Algerian landrace "Sahara3771" and parental lines were conducted in 2011 and 2012 at the Research Farm of the University of Tabriz, Iran (latitude 38°05_N, longitude 46°17_E, altitude 1360 m above sea level). The climate of research area is characterized by mean annual precipitation of 285 mm, mean annual temperature of 10°C, mean annual maximum temperature of 16.6°C and mean annual minimum temperature of 4.2°C. The Clipper/Sahara population was produced by the *Hordeum bulbosum* method using embryo culture followed by chromosome doubling through colchicines treatment (Islam, Shepherd, 1981). The population kindly was provided by the University of Western Australia. The DH lines and parental lines were sown in RCBD with two replications. The experimental plots consisted of four rows of 3 m length. Row to row and plant to plant distances was kept at 20 and 3 cm, respectively. Ten competitive plants from the two middle rows were used for phenotyping. Plant height (PH), spike length (SL), awn length (AL), peduncle length (PL), stem diameter (SD), number of fertile tillers (NFT), number of grain per plant (NGP), hundred grain weight (HGW), biomass (BM) and grain yield (GY) were recorded in both years.

Statistical Analysis.

Analysis of variance was conducted by SAS 9.0 (2002), to determine the effect of year (Y), line (L) and LY interaction among these factors, on measured traits.

The variance components, genotypic and phonotypic coefficient of variation were determined as suggested by Burton and De Vane (1953) and Johnson et al. (1955). Heritability in narrow sense (h^2) was estimated according to Falconer (1989). Due to the fact that lines were DH, narrow sense heritability was estimated by dividing genetic variance by phenotype variance. Genetic advance was calculated with the method suggested by Allard (1960) and was estimated from the following formula:

$$GA = k.\sigma_{ph}.h^2$$

Where k is the selection differential in standard units in the present study and it was 2.06 at 5% level of selection, σ_{ph} is standard deviation of the phenotypic variance of lines and h^2 is narrow sense heritability.

Genetic advance expressed as percentage of mean (GAM) was measured by the following formula:

$$GAM = (GA/mean) \times 100$$

A GGE biplot model based on singular value decomposition (SVD) of first two principal components was employed to display the line × trait two-way data in a biplot based on the formula

$$\frac{T_{ij} - \overline{T}_j}{s_j} = \lambda_l \zeta_{i1} \tau_{j1} + \lambda_l \zeta_{i2} \tau_{j2} + \varepsilon_{ij} \qquad \text{(Yan,}$$

Rajcan, 2002). Where T_{ij} is the average value of line *i* for trait *j*, $\overline{T}_j$ is the average value of trait *j* overall lines, s_j is the standard deviation of trait *j* among the line averages, λ_1 and λ_2 are the singular values (SV) for the first and second principal component (PC1 and PC2), respectively, ζ_{i1} and ζ_{i2} are eigenvectors of line *i* for PC1 and PC2, respectively; τ_{j1} and τ_{j2} are eigenvectors of trait *j* for PC1 and PC2, respectively and ε_{ij} is the residual associated with the line *i* in trait *j*. Biplots analysis was

carried out using the GGEbiplot software (Yan 2001).

RESULTS AND DISCUSSION

Analysis of Variance

Combined analysis of variance over two years revealed significant differences among DH lines for all the studied traits (Table 1) indicating the presence of

sufficient genetic variability among the DH lines. The year effect was significant for PL and GY and BM. Line × Year interaction was significant for SL, PL, NGP, HGW, GY, PH and BM and showed the influence of changes in year on these traits of the lines evaluated. Lines 75 and 143 have the highest grain yield, at two years, respectively.

Table (1): Combined analysis of variance for yield and related traits in barley DH population derived from a cross between Clipper and Sahara3771

Source of variance	df	Mean of square (MS)									
		GY	PH	SL	AL	PL	SD	NFT	BM	NGP	HGW
Year (Y)	1	9582*	3649.5 ns	8.7 ns	54.8 ns	746.1**	0.46 ns	3.2 ns	270.1*	4013108ns	24.2 ns
Rep./ Y	2	291	296.2	0.5	7. 6	2.3	0.04	3. 6	2.9	444488	1.9
Lines (L)	149	139**	54.2**	2.7**	2.9**	21.8**	0.16**	1.2**	3.9*	243100**	1.3**
L × Y	149	37*	24.3*	0.6**	0. 6 ns	8.5**	0.05 ns	0.7 ns	2.9*	35128**	0.2**
Pooled error	298	29	17.9	0.4	0.5	5.3	0.04	0.6	2.2	25183	0.1

ns: not significant difference; **, * Significance at 0.01 and 0.05 level respectively.
AL: awn length; BM: biomass; GY: grain yield; HGW: hundred grain weight; NFT: number of fertile tillers; NGP: number of grain per plant; PH: plant height; PL: peduncle length; SD: stem diameter; SL: spike length.

Coefficient of variation, heritability and genetic advance

The estimates of variance components, GCV, and PCV, heritability and genetic advance are presented in Table 2. The considerable amount of variation was observed for all the traits indicating enough scope for bringing about improvement in the desire direction. Due to polygenic genetic control of grain yield and its components and influence environmental conditions on expression of these traits, PCV and GCV could not be sufficient for comparison of relative amount of phenotypic and genotypic variations among different traits but these parameters could provide information for improvement by selection (Bello et al., 2012). The estimates of PCV and GCV were high for number of grain per plant and grain yield. Similar results have also been reported in the previous studies (Kole, 2006; Jalata et al., 2011; Singh,

2011). The minimum PCV and GCV were estimated for plant height (5.57 and 4.77, respectively). The difference between PCV and GCV was small for awn length, stem diameter and hundred grain weight indicate that the effect of environmental condition on these traits was less compared with others. Bello et al. (2012) mentioned that high ratio of GCV/ PCV is desirable for breeding of the trait. For all the traits except biomass, genotype × year interaction variance (σ^2_{gy}) was low (Table 2). These show that for most traits, genotype × year interaction have negligible role in phenotypic value. Genotype × environment (GE) interaction often results in the lack of correlation between genotypic performance and phonotypic performance. Significant GE interaction result from changes in the magnitude of the differences among genotype in different location or years (environments). A significant GE interaction for a quantitative trait such as

grain yield can reduces the correlation between phenotype and genotype, and decreases selection progress.

Estimate of narrow sense heritability (h^2) was high for most of the traits. The higher values of heritability estimations of trait are indicators of a greater proportion of genetic components in relation to the environmental and genotype × environment interaction components in the total phenotypic variance of a trait (Falconer 1989). According to Dabholkar (1992) classification of

heritability estimates, all of the measured traits showed high heritability. Highest value of h^2 was recorded for hundred grain weight (93.52%) followed by number of grain per plant (92.76%) and awn length (91.37). High observed heritability for these traits were in agreement with the findings of earlier workers (Singh et al., 1986; Vimal, Vishwakarma, 1998; Mohammadi et al., 2006; Jalata et al., 2011; Singh, 2012).

Table(2): Estimation of phenotypic, genetic and environmental variances; phenotypic and genetic coefficient of variability, heritability for the measured traits in 148 barley DH and two parents in two years.

Components of variance	GY	PH	SL	AL	PL	SD	NFT	BM	NGP	HGW
σ^2_{ph}	29.42	10.17	0.60	0.63	4.45	0.03	0.19	0.56	56053.19	0.31
σ^2_{g}	25.51	7.48	0.53	0.58	3.31	0.03	0.13	0.24	51992.95	0.29
σ^2_{gy}	4.17	3.14	0.09	0.05	1.62	0.00	0.03	0.37	4972.56	0.03
σ^2_{e}	7.29	4.49	0.09	0.11	1.32	0.01	0.15	0.55	6295.85	0.02
PCV (%)	21.68	5.57	13.12	7.60	10.82	6.52	11.08	10.05	33.91	14.85
GCV (%)	20.19	4.77	12.36	7.26	9.33	6.05	9.37	6.55	32.66	14.36
h^2 (%)	86.72	73.53	88.65	91.37	74.38	86.14	71.54	42.49	92.76	93.52
GA	9.69	4.83	1.42	1.50	3.23	0.32	0.64	0.66	452.39	1.07
GAM	38.74	8.43	23.97	14.30	16.58	11.56	16.33	8.80	64.80	28.62

σ^2_{ph}: phenotypic variance; σ^2_{g}: genetic variance; σ^2_{gy}: genotype by environment interaction variance; σ^2_{e}: environmental variance; PCV: phenotypic coefficient of variation; GCV: coefficient of variation; h^2: heritability in narrow sense; GA: genetic advance and GAM: genetic advance expressed as percentage of mean.

AL: awn length; BM: biomass; GY: grain yield; HGW: hundred grain weight; NFT: number of fertile tillers; NGP: number of grain per plant; PH: plant height; PL: peduncle length; SD: stem diameter; SL: spike length.

The expected genetic advance (GA) values for the measured traits in the population are presented in Table 2. Due to different scale of traits, genetic advance was calculated as percentages of the genotypes mean (GAM). High heritability along with high genetic advance is an important factor for predicting the resultant effect for selecting the best individuals (Al-Tabbal, Al-Fraihat, 2012). In other words, genetic advance gives an idea of possible improvement of new population through

selections, compared with the original population. The genetic gain depends on the amount of genetic variability and magnitude of the masking effect of the environment. Values of genetic advance as percentage of mean ranged from 8.43 to 64.80. High values of genetic advance as percentage of mean estimates coupled with high estimates of heritability expected in number of grain per plant, grain yield and hundred grain weight indicate the preponderance of additive gene action for

the expression of these traits (Falconer 1989). Biomass exhibited low heritability and genetic advance indicating that this trait is controlled by non-additive genetic effects suggesting thereby that its improvement could be achieved through heterosis breeding and selection based on phenotypic performance of this trait would not be effective to improvement value of this trait.

Biplot Analysis

The biplot for the 148 DH lines and parental lines data explained 55% (31% and 24% by PC1 and PC2, respectively) of the total variation (Fig. 1A). The vector view of a GGE biplot provides a succinct summary of the interrelationships among the traits (Yan, Kang, 2003). Figure 1 is the vector view of the GGE biplot, in which the traits are connected with the biplot origin via lines. This view of the biplot aids understanding of the interrelationships among the traits. The cosine of the angle between the vectors of two traits approximates the correlation coefficient between them. Two traits are positively correlated if the angle between their vectors is <90°, negatively correlated if the angle is >90°, independent if the angle is 90° (Yan, Rajcan, 2002). In our study, the most prominent positive relations were between HGW and AL, between GY, NGP and SD, and between PH, SL, NFT and PL. Whereas HGW and AL were negatively associated with GY, NGP and SD. If the higher percentage of variation is explained by PC1 and PC2, the conclusions would be accurate. In the present study, 55% of the total variation was explained by two first PC. This percentage indicates the complexity of the relationships among the measured traits. Nevertheless, these results are approximately consistent with Table 3.

Table (3): Phenotypic Pearson's correlation coefficients among measured traits in 148 barley DH and two parents in two years.

Traits	GY	PH	SL	AL	PL	SD	NFT	BM	NGP
PH	0.15								
SL	0.10	0.56							
AL	-0.24	0.21	0.25						
PL	-0.03	0.46	0.03	-0.04					
SD	0.24	0.05	0.00	-0.35	0.16				
NFT	0.18	0.15	0.09	0.34	-0.02	-0.41			
BM	0.59	0.45	0.30	0.02	0.04	0.22	0.46		
NGP	0.92	0.00	-0.05	-0.43	-0.04	0.27	0.02	0.43	
HGW	-0.54	0.23	0.27	0.59	0.05	-0.19	0.21	-0.07	-0.80

AL: awn length; BM: biomass; GY: grain yield; HGW: hundred grain weight; NFT: number of fertile tillers; NGP: number of grain per plant; PH: plant height; PL: peduncle length; SD: stem diameter; SL: spike length.

Visual Identification of the Best Lines Based on the Measured Traits.

Figure 1B represents GT biplot with a polygon view of 148 DH lines and parental lines based on GY, PH, SL, AL, PL, SD, NFT, BM, NGP and HGW data. The polygon view of a biplot is the best way to visualize the interaction patterns between lines and traits and to effectively interpret a set data (Yan, Kang, 2003). The

polygon is drawn joining the lines that are located farthest from the biplot origin, so that all other lines are contained in the polygon. The vertex lines in this investigation were L75, L143, L12, L20, L21 and L57 (Fig. 1B). A line perpendicular to each side of the polygon is drawn and polygon divides to the different sectors. The vertex line for each sector is the one that gave the highest value for the traits that fall within that sector. There are six vertex lines in Fig. 1B and three of them (L143, L75 and L12) were favorable for some traits but the rest of them (L21, L57 and L20) were unfavorable for any traits.

The scores of five traits GY, NGP, BM, PH and PL fell in the L75 sector suggested that line L75 had highest or near-highest values for these traits. Based on the measured data, line L75 had the high value for GY, NGP, BM, PH and PL. Line L75 scored the first rank in the GY, second in NGP, third in BM and sixth in PH. Similarly, line L143 showed the highest means of NGP and SD. Line L143 scored the first rank in the NGP, second in GY and third in SD. Line L12 was the third

vertex line and it showed the first rank in the SL and AL, second in PH and fourth in HGW. It is observed that the GT biplot technique could be useful for selecting lines based on several traits.

By reversing the sign of the traits and redrawing the polygon, the poorest lines for each of the traits was indicated (Fig. 2A). Figure 2A shows that the scores of five traits BM, NFT, PH, SL and PL fell in the L57 sector, suggesting this line had lowest or near-lowest values for these traits.

After selecting a line, it is better to estimate its relative levels with respect to the measured traits. For this purpose, a line is drawn that passes through the biplot origin and the marker of line. The traits are ranked along the line axis. Figure 2B indicates that line L75 had higher means compared with average for all the traits expected the AL and HGW. Both AL and HGW were negatively correlated with grain yield and low value for these traits is most suited for the selected line. Choosing lines having desirable attributes of both parents were important purpose of this study.

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A

Model 2 PC 1 = 31% PC 2 = 24% Sum = 55%

B

PC1
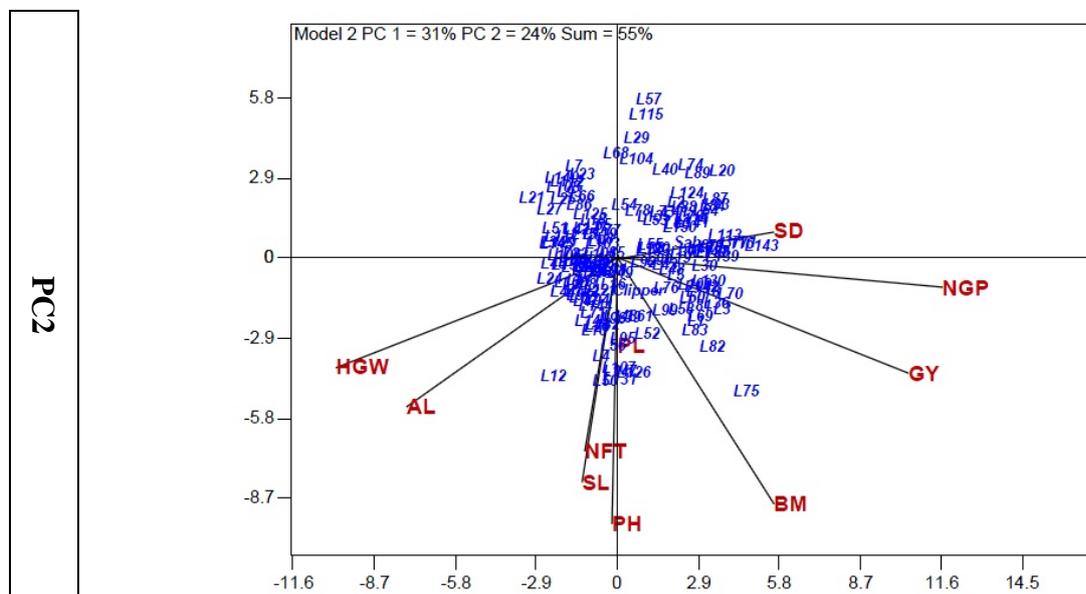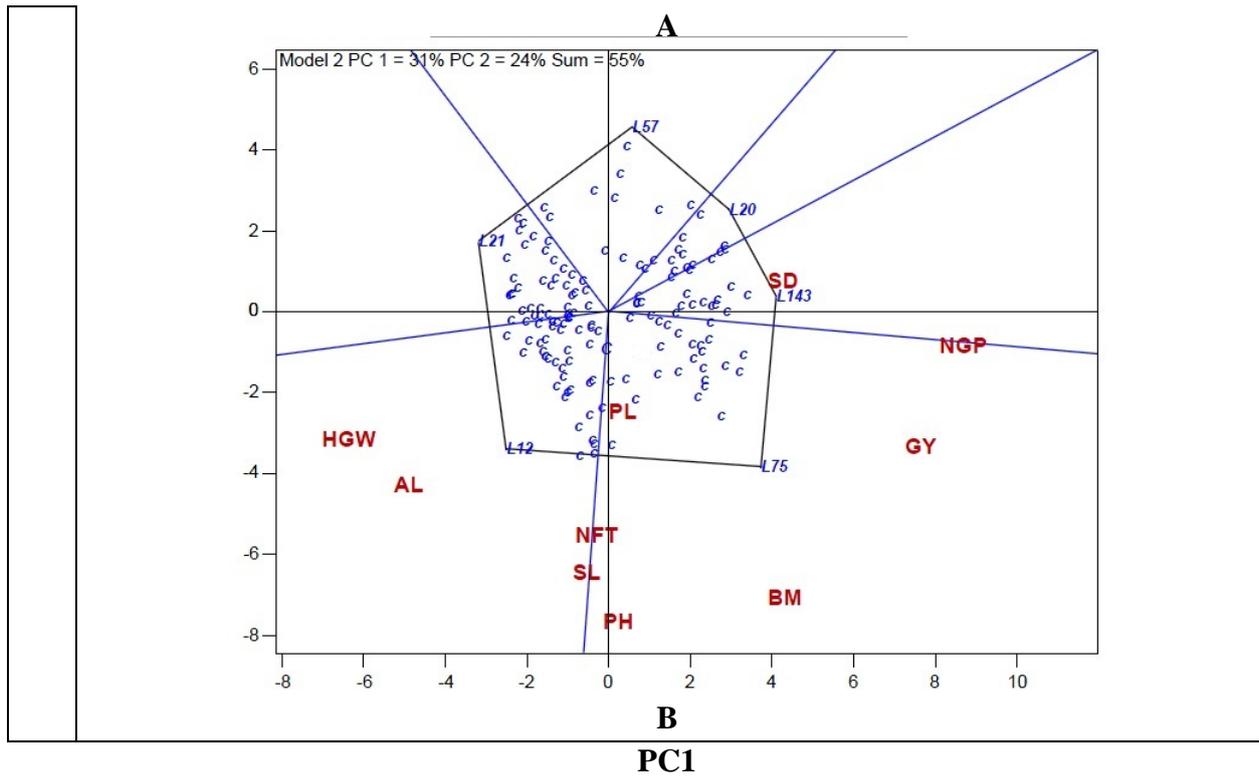
Figure (1): (A) Vector view of line by trait biplot for 148 DH lines and two parent data, showing the interrelationship among all measured trait, (B) Polygon view of the biplot, showing which line had the highest values for which traits.
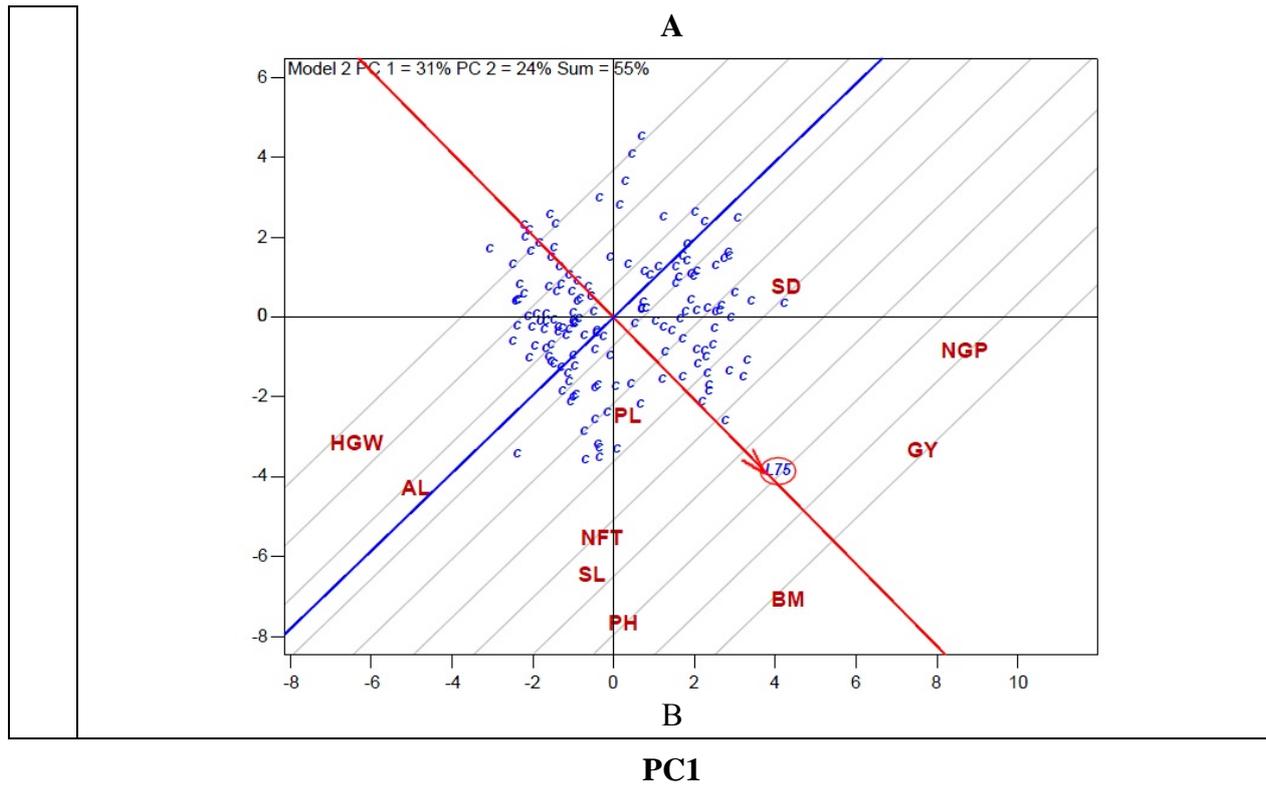

Model 2 PC 1 = 31% PC 2 = 24% Sum = 55%

PC2
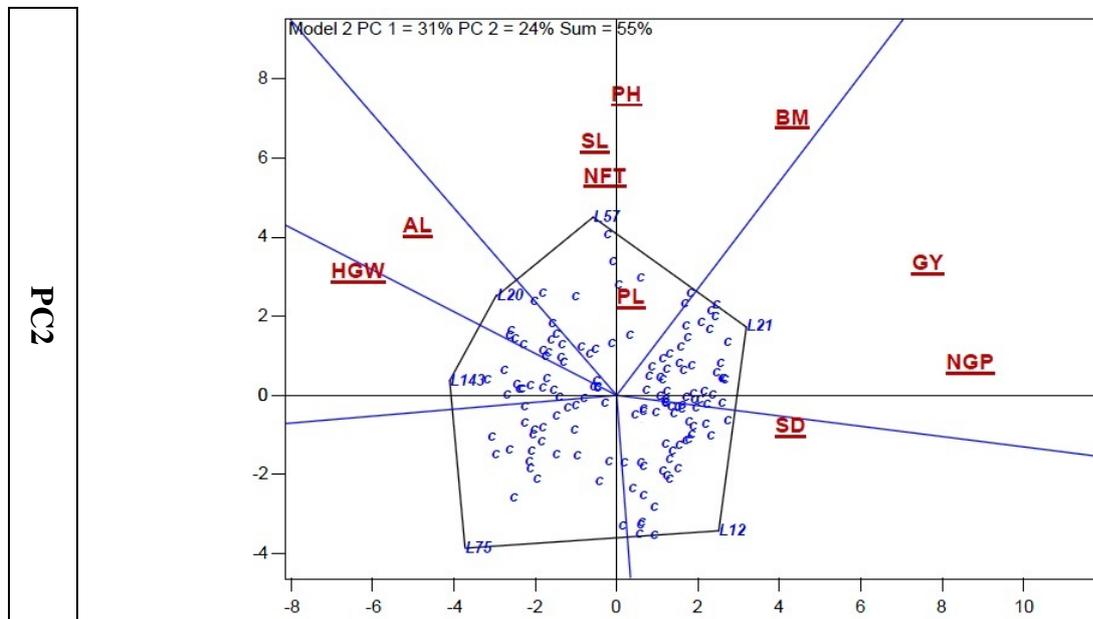
Figure 2. (A) Polygon view of the biplot after the signs are reversed, showing which line had the smallest values for which traits (B) Visualizing the relative magnitudes of line L75for various traits.

CONCLUSIONS

The present study indicated that heritability was high for the most of measured traits showing a greater proportion of genetic components in relation to the environmental factors for these traits. High genetic advance is important parameter to be considered for selection and improvement of the crop. Number of grain per plant and grain yield exhibited high genetic advance indicating that this trait is controlled by additive genetic effects and selection based on phenotypic value of these traits would be effective to improvement value of these traits, similar finding reported by Yadav et al. (2014) and Al-Tabbal and Al-Fraihat (2012).

We used the GT biplot to evaluate 148 DH line derived from a cross between Sahara3771 and Clipper barley varieties on the basis of multiple traits and to identify lines that are superior for the traits of interest. The polygon view of GT biplot grouped the lines into the six groups (Fig. 1B) on the basis of 10 agronomic traits. The average values of lines in each group were higher than the total mean of all lines for all of traits falling in the particular group. Superior lines may be selected from those groups to obtain genetic recombination and transgressive segregation in the subsequent generations. For example, Figure 2B have shown that line L75 have high value for the most interest traits. Because of the high heritability of traits related to yield, selection based on phenotypic performance of

these traits would be effective to selection of lines that have suitable traits of both Clipper and Sahara3771. This study also demonstrated that the GT biplot is a useful method for visualizing genotype by trait data and provides much more information than the other conventional methods.

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دراسة التوريث والتقدم الوراثي للصفات الزراعية في الشعير (.Hordeum vulgare L)
والتحليل البياني لعلاقات الصفات على الطريقة ثنائية الامر Biplot

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ملخص

المحصول الناتج هو سمة المعقدة تحددها العديد من المكونات التي قد تؤثر سلبا أو إيجابا على هذه الصفة وتحسينها هو الهدف الرئيسي لبرامج التربية. في هذه الدراسة تم اختيار عدد 148 من صنف محصول الشعير من الخطوط التي تحتوي على صفة مفردة مضاعفة (doubled-haploid) المستمدة من تزاوج بين صنف كليبر "Clipper" وصنف صحارى 3771"Sahara3771" والمزروعة في الحقل التجريبي في جامعة تبريز، في الأعوام 2011 و2012 حيث تم زراعة الشعير في هذه المواسم لتقييم وجود التباين للصفات المطلوبة ومقدار الاختلاف للمعاملات المختلفة. وقدرت مكونات التباين، المظهري والوراثي لمعاملات الاختلاف، للتوريث والتقدم الوراثي لجميع الصفات. أدنى الاختلافات بين معاملات التباين الوراثي والمظهري تم ملاحظتها لطول السنبلة، قطر الساق ووزن مئة حبة. وسجلت أعلى قيمة لأقل تباين وراثي لوزن مئة حبة (93.52). وقد لوحظ ارتفاع الاكتساب الجيني الوراثي في عدد كل حبه لكل نبات، ناتج محصول الحبوب، ووزن مئة حبة. مما يشير إلى أن هذا الاختيار على أساس الأداء المظهري من هذه الصفة تكون فاعلة لحسين هذه الصفات. في هذه الدراسة، تمت دراسة العلاقات المتبادلة بين المحصول والصفات الزراعية الأخرى أيضا باستخدام النمط الجيني بوساطة تقنية ثنائية الأمر (biplot) للصفة. تم العثور على العلاقات الإيجابية بين محصول الحبوب وعدد الحبوب لكل نبات وقطر الساق. وأظهرت النتائج أن خط L75 انتج أقصى محصول الحبوب وعدد الحبوب للنبات، والكتلة الحيوية، ارتفاع النبات وطول السويقة. وكشفت الدراسة أن النمط الجيني عن طريق سمة (GT) للتقنية ثنائية الأمر (biplot) أنه يمكن عرض العلاقات المتبادلة بوضوح بين الصفات وتسهيل المقارنة البصرية بين الخطوط.

الكلمات الدالة: الشعير (.Hordeum vulgare L)، تحليل سمة GT عن طريق تقنية ثنائية الامر biplot ، التوريث، مكونات المحصول الناتج.