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BIPLOT ANALYSIS OF TRAIT RELATIONS OF SPINACH (*Spinacia oleracea* L.) LANDRACES

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Interest in growing winter spinach (*Spinacia oleracea* L.) in Iran is increasing due to its good nutritional potential returns relative to other vegetable crops. The objectives of this research were to investigate the interrelationships among different traits of spinach and to evaluate different Iranian spinach landraces with application of the genotype × trait (GT) biplot methodology in visualizing research data. 81 spinach landraces were grown during 2-years according to randomized complete block design with four replications. Ranking of the genotypes based on the ideal entry revealed that genotypes G1, G20, G7, G8, G9, G27, G49 G70 and G79 were higher in the measured traits and could be good candidates for improving most of the measured traits. Ranking of traits for the leaf yield showed that petiole diameter, petiole length, leaf numbers at flowering, 1000-seed weight and root dry weight were the most discriminating traits which influence spinach leaf yield at both years. There were 9 winning genotypes and 4 which-won-where patterns at the first year while there were 8 winning genotypes and 4 which-won-where patterns at the second year. As a result, the findings from our study are as follows: (i) traits leaf numbers at flowering, leaf length, leaf width, leaf area, petiole diameter and petiole length could be as selection indices for spinach leaf yield improvement, (ii) genotypes G1, G20, G7, G8, G9, G27, G49 G70, and G79 were the most favorable and is thus recommended for commercial release or incorporating in breeding programs; (iii) the GT biplot method can be used to identify superior genotypes in other crops and in other parts of the world.

Keywords: correlation, genotype × trait biplot, principal component analysis

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INTRODUCTION

Spinach (*Spinacia oleracea* L.) is one of the most desirable leafy vegetables as a result of its high content of beta carotene, folate, vitamin C, calcium, iron and other useful nutrients (MORELOCK and CORRELL, 2008). It is a native of Southwest Asia (probably Iran) and has been grown in China (since at least the 7th century) and was used in Europe (since at least the13th century). It is an economically important vegetable crop in many countries, and about 940,000 hectares are grown annually worldwide (FAOSTAT, 2012). According to FAOSTAT (2012), five major spinach producing countries in decreasing order are China (20000000 tons), United States (354000 tons), Japan (275000 tons), Turkey (222000 tons) and Iran (110000 tons). In 2012, spinach yield was 231000 Hg ha^{-1} which about 80% of its world harvested areas (751000 ha) are in the China and it harvested areas in Iran are about 5000 hectares. Spinach is currently grown on 18000 ha in the United States annually for both fresh and processed markets (National Agricultural Statistics Service, USDA, 2012). Despite substantial progress in the spinach breeding programs, its genetic variability and traits associations have not been investigated sufficiently.

Although, spinach genetically improved cultivars were introduced in the 1950's and they have become the major type of spinach cultivars (MORELOCK and CORRELL, 2008), but Iranian producers currently use native spinach landraces, which have good adaptability to different environmental conditions (SABAGHNIA *et al.*, 2014). The yield performance of these landraces is relatively low (about 2100 kg ha^{-1}) compared with the highest global yields (12000 kg ha^{-1}, produced in Japan; FAOSTAT, 2012). Therefore, there is important need to have had some spinach breeding programs for increasing the genetic potential of yield as well as other important traits for Iranian spinach landraces. Also, since Iran is a centre of genetic diversity of some important cultivated crops (wheat, alfalfa, spinach and etc), it is essential to conserve these important resources as well as possible. Most of the Iranian spinach accessions are landraces which are highly adapted to specific and different environmental conditions and are useful sources of genetic variation (ASADI and HASANDOKHT, 2007). However, utilization of the genetic potential of these landraces requires some important knowledge about these genetic accessions (PROHENS-TOMAS and NUEZ, 2008; AVSAR, 2011), including characterization, evaluation, classification and traits associations.

Yield improvement of spinach has been one of the objectives of breeders which is largely influenced by the environmental conditions and hence has a low heritability. As a result, the response to direct selection for yield may be unpredictable and breeders are seldom interested in a single trait and therefore, there is the need to examine the associations among various traits, especially between yield and other traits. Routine statistical techniques may be insufficient to explain the associations and some new statistical tools have been used in modelling crops yield. YAN *et al.*, (2000) developed a site regression model which contains genotype main effect plus genotype × environment (GGE) using biplot as a graphical tool for analysis of multi-environment trials. A biplot is a plot that simultaneously displays the effects of entries and the testers and it can also be used for all types of two-way data. It is generated by plotting the first two principal components (PC1 and PC2) derived from singular value decomposition of the tester-centered data (YAN *et al.*, 2001). The genotypes can be generalized as rows or entries and the multiple traits as columns or testers. YAN and RAJCAN (2002), DEHGHANI *et al.*, (2008) and SABAGHNIA and JANMOHAMMADI (2014) used a genotype × trait (GT) biplot to demonstrate application of the GGE biplot technique to study the genotype by trait data.

Some investigations have been performed in the past on Iranian spinach accessions, but most of them studies are limited with either using only univariate statistics, studying samples from a limited geographical range or performed on one growing season (ASADI and HASANDOKHT, 2007; EFTEKHARI *et al*., 2010; SABAGHNIA *et al*., 2013). The objective of this study was to evaluate Iranian spinach landraces based on the multiple traits and to study the interrelationships among traits using GT biplot technique.

MATERIALS AND METHODS

Trial protocol

Eighty-one Iranian spinach accessions were collected as seed from a wide geographical range of Iran, and then planted in the field in a randomized complete block design (RCBD) replicated four times across two growing seasons. Each spinach accession was collected as seed multiplied by the local farmers and the name of geographical sites of the collected spinach landraces are given in Table 1. Field soil was calcareous, loamy structure, low organic matter, low salt content, low nitrogen, and low phosphorous and had adequate potassium. Fertilization was carried out by spreading 80 kg N ha^{-1} (half of N at sowing stage and half of N at seedling emergence). Each plot contained six rows (3 m long and 0.25 m width) and plot size was 4.5 m^2. Four 2.5 m rows at the center of each plot were harvested with 2.5 m^2 area. Control by hand weeding was carried out twice when the weed density was high, in the pre-flowering and post-flowering stages. Nine traits including leaf numbers at flowering (LN), leaf length (LL), leaf width (LW), leaf area (LA), petiole diameter (PD), petiole length (PL), number of lateral branches (LB), root dry weight (RD) and shoot dry weight (SD) were measured on 10 random (vying plant) points per plot. Days to flowering (DF), percent of female plants (FP), 1000-seed weight (TS), and leaf yield (LY) were recorded at each plot.

Table 1. Geographical coordinates of the collected spinach landraces' locations

Code	Name	Longitude	Latitude	Altitude	Code	Name	Longitude	Latitude	Altitude
G1	Abhar	36°08′N	49°13′E	1540	G42	Quchan	37°06′N	58°30′E	1540
G2	Amol	36°28′N	52°21′E	76	G43	Quchan	37°05′N	58°32′E	1555
G3	Arak	34°05′N	49°41′E	1755	G44	Qum	34°38′N	50°53′E	930
G4	Ardestan-1	33°23′N	52°22′E	1205	G45	Qum	34°38′N	50°53′E	930
G5	Ardestan-2	33°25′N	52°24′E	1200	G46	Qum-Khor	34°35′N	50°53′E	910
G6	Azna	33°27′N	49°27′E	1871	G47	Qum-Saleh	34°41′N	50°51′E	880
G7	Baft	29°13′N	56°36′E	2280	G48	Rahimabad	32°28′N	51°57′E	1550
G8	Bam	29°06′N	58°21′E	1060	G49	Rahnan-1	32°41′N	51°36′E	1545
G9	Birjand	32°75′N	59°22′E	1480	G50	Rahnan-2	32°42′N	51°40′E	1525
G10	Birjand	32°78′N	59°21′E	1491	G51	Ravar	31°15′N	56°55′E	1175
G11	Bojnord	37°28′N	57°19′E	1070	G52	Razan	35°23′N	49°02′E	1803
G12	Brojerd	33°53′N	48°45′E	1580	G53	Salehabad	34°31′N	50°57′E	970
G13	Drood	33°40′N	48°70′E	1326	G54	Salmas	36°19′N	44°76′E	1398
G14	Esfaien	37°04′N	57°30′E	1260	G55	Sanandaj	35°31′N	46°89′E	1518
G15	Fasa	28°56′N	53°38′E	1450	G56	Sarasiab	33°30′N	51°54′E	1660
G16	Hamadan	34°48′N	48°31′E	1850	G57	Sari	36°33′N	53°03′E	132
G17	Hamon	31°04′N	61°31′E	425	G58	Saveh-1	35°10′N	50°05′E	998
G18	Isfahan-1	32°35′N	51°42′E	1543	G59	Saveh-2	35°10′N	50°03′E	985

G19	Isfahan-2	32°38′N	51°39′E	1525	G60	Shiraz-1	29°37′N	52°22′E	1540
G20	Jahrom	28°30′N	53°33′E	1100	G61	Shiraz-2	29°27′N	52°12′E	1320
G21	Jajarm	36°57′N	56°22′E	1000	G62	Shirvan-1	37°40′N	57°92′E	1492
G22	Karaj-1	35°82′N	50°97′E	1300	G63	Shirvan-2	37°38′N	57°91′E	1505
G23	Karaj-2	35°80′N	50°85′E	1350	G64	Sirjan	29°27′N	55°40′E	1735
G24	Karaj-3	56°86′N	50°87′E	1230	G65	Sirjan	29°29′N	55°41′E	1739
G25	Kashan-1	33°59′N	51°27′E	950	G66	Tabriz	38°04′N	46°18′E	1366
G26	Kashan-2	33°62′N	51°25′E	970	G67	Tafresh	34°41′N	50°00′E	1973
G27	Kerman	30°17′N	57°05′E	1775	G68	Taft	31°44′N	54°12′E	2200
G28	Kermanshah	34°31′N	47°65′E	1400	G69	Talesh	48°32′N	49°03′E	1585
G29	Khoramabad	33°29′N	48°21′E	1148	G70	Tarom	36°57′N	48°54′E	1540
G30	Kohban	31°24′N	56°16′E	2200	G71	Tonkabon	36°48′N	50°52′E	-20
G31	Lahijan	37°26′N	50°14′E	-11	G72	Urmia	37°33′N	45°04′E	1340
G32	Langrood	37°19′N	50°14′E	-25	G73	Urmia	37°31′N	45°05′E	1344
G33	Mahan	30°03′N	57°17′E	1817	G74	Varamin-1	35°20′N	51°41′E	911
G34	Maragheh	37°21′N	46°16′E	1477	G75	Varamin-2	35°19′N	51°39′E	915
G35	Mobarake	32°20′N	51°30′E	1690	G76	Varamin-3	35°18′N	51°39′E	922
G36	Norabad	34°04′N	47°58′E	2000	G77	Yazd	31°53′N	54°21′E	1215
G37	Pakdasht	35°28′N	51°40′E	1025	G78	Zabol	31°01′N	61°29′E	475
G38	Pakdasht	35°26′N	51°41′E	1020	G79	Zanjan	36°40′N	48°40′E	1650
G39	Pishva	35°18′N	51°43′E	918	G80	Zarinshar-1	32°23′N	51°22′E	1685
G40	Qazvin	36°16′N	50°00′E	1278	G81	Zarinshar	32°22′N	51°20′E	1712
G41	Qrachak	35°22′N	51°44′E	906					

Statistical analysis

The datasets were first tested for normality by Anderson and Darling normality test using MINITAB version 16 (2010) statistical package. The genotype × trait (GT) biplot (YAN and RAJCAN, 2002) was used to show the two-way pattern of spinach landraces' traits in a biplot based on the following formula:

$$\frac{\alpha_{ij}-\beta_j}{\sigma_j}=\sum_{n=1}^{2}\lambda_n\xi_{in}\eta_{jn}+\varepsilon_{ij}=\sum_{n=1}^{2}\xi_{in}^{*}\eta_{jn}^{*}+\varepsilon_{ij}$$

where α_{ij} is the mean value of landrace i for trait j, β_j is the mean value of all landraces in trait j, σ_{ij} is the standard deviation of trait j among the landrace means, λ_n is the singular value for principal component n (PCn), ξ_{in} and η_{jn} are scores for landrace i and trait j on PCn, respectively, and ε_{ij} is the residual associated with landrace i in trait j. To achieve symmetric scaling between the landrace scores and the trait scores the singular value λ_n has to be absorbed by the singular vector for landraces ξ_{in} and that for traits η_{jn}. That is, $\xi_{in}^{*}=\xi_{in}\lambda_n^{0.5}$ and $\eta_{jn}^{*}=\eta_{jn}\lambda_n^{0.5}$. Because of n=2, only PC1 and PC2, are retained in the model and such a model tends to be the best for extracting pattern and rejecting noise from the data. All biplots presented in this study were generated using the software GGEbiplot package (YAN, 2001). Up-to-date information on GGE biplot and new version of GGEbiplot package are available at http://www.ggebiplot.com.

RESULTS AND DISCUSSION

The GT biplot for spinach dataset explained 57 and 58% of the total variation of the standardized data at the first and the second years, respectively. This relatively moderate percentage reflects the complexity of the relationships among the measured traits at both experimental years. The first two PCs (PC1 and PC2) explained 43% and 14 %, respectively while the first two PCs described 45% and 15 %, respectively. The polygon view of the GT biplot helps identify genotypes with the highest values for one or more traits and provides the best way for visualizing the interaction patterns between genotypes and traits and to effectively interpret a biplot. For this purpose, the genotypes that are connected with straight lines so that a polygon is formed with all other traits contained within the polygon.

The biplot Figure 1A, as polygon view, presents data of 81 spinach landraces in thirteen traits at the first year and the following information can be understood: the vertex landraces or genotypes in this investigation are G1, G15, G16, G23, G45, G61, G75, G76 and G79. These landraces are the best or the poorest landraces in some or all of the traits since they had the longest distance from the origin of biplot. Therefore, it seems that G1 had the highest values for all of the measured traits except percent of female plants (FP), shoot dry weight (SD) and number of lateral branches (LB). This genotype (G1) and the other genotypes of this sector had good amounts of days to flowering (DF), leaf numbers at flowering (LN), leaf length (LL), leaf width (LW), leaf area (LA), petiole diameter (PD), petiole length (PL), root dry weight (RD), 1000-seed weight (TS), and leaf yield (LY). The vertex landrace G16 and its related genotypes which fall in its sector were good for percent of female plants (FP) while the vertex landrace G61 and its related genotypes which fall in its sector were good for shoot dry weight (SD). The vertex landrace G79 and its related genotypes which fall in its sector were good for number of lateral branches (LB). The other vertex cultivars (G15, G23, G45, G75 and G79) and related sectors were not suitable performance for the measured traits at first year (Figure 1A). Different vertex landraces and their related landraces which are located in different nine sections of biplot are good candidates for examination heterosis (YAN *et al*., 2007; DEHGHANI *et al*., 2008) for hybrid production in spinach using these genotypes or pure lines.

According to polygon view of the biplot at the second year (Figure 1B); the vertex landraces or genotypes in this investigation are G1, G16, G27, G32, G39, G61, G76 and G79. The G1 and its relegated genotypes which are located in its sector had the highest values for leaf length (LL), leaf width (LW), leaf area (LA) and petiole length (PL) while the G27 had the highest values for Days to flowering (DF), leaf numbers at flowering (LN), petiole diameter (PD), root dry weight (RD) and1000-seed weight (TS). The G79 and its relegated genotypes had the highest values for number of lateral branches (LB) and shoot dry weight (SD) while the G16 had the highest values for percent of female plants (FP) at the second year. However, regarding results of both years, genotype G1 is good candidate for improving LL, LW, LA and PL traits; genotype G16 is good candidate for improving percent of female plants and genotype G79 is good candidate for improving number of lateral branches. Little information is available on spinach yield components; the marketable crop is composed of petiole and leaf from the rosette stage. Leaf dimensions (length and width) have been studied and their genetic and phenotypic correlations on yield performance have been demonstrated (PEARSON, 1983). Therefore genotypes G1 and G20 and their sectors' genotypes could be used for improving leaf yield and its components in breeding programs.

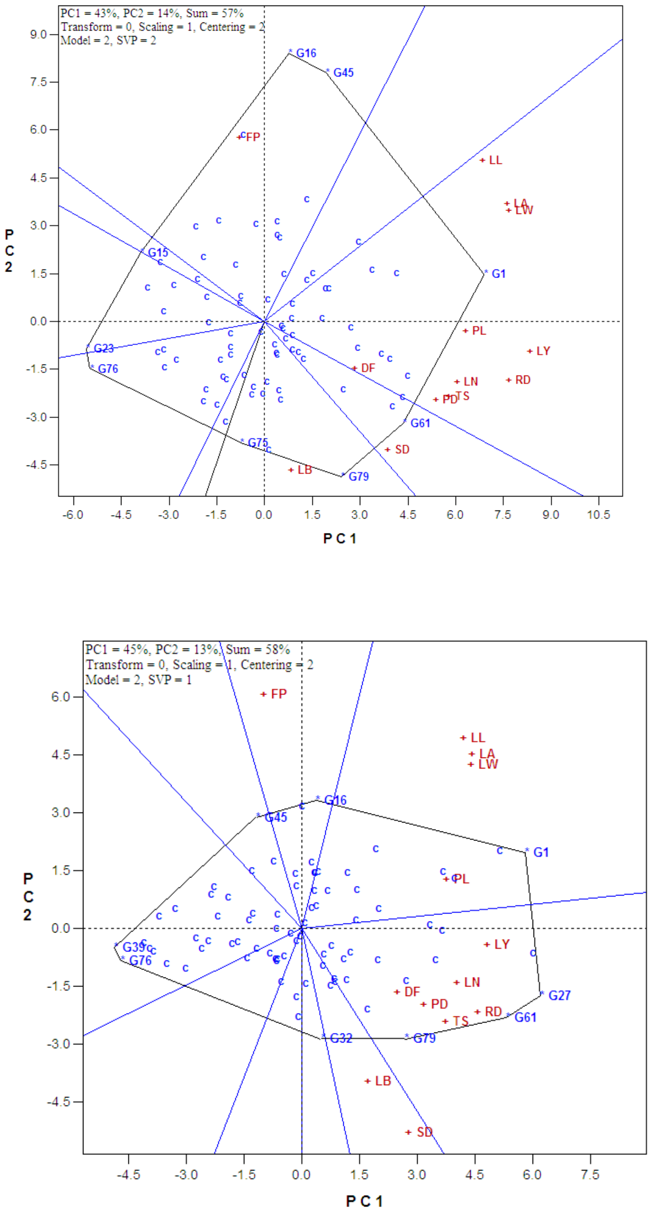

Figure 1. Polygon view of genotype × traits biplot of measured traits of 81 spinach landraces for (up) the first year and (down) the second year, showing which landrace(s) had the highest values for which traits. See Table 1 for codes of the traits.

Provided that the GT biplot model described relatively a sufficient amount of the total variation, the correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors (YAN and KANG, 2003). The most prominent relations by vector-view biplot at the first year (Figure 2A) are: a strong positive association between LA and LW; between PL and LY; between RD and LN; and between TS and PD; as indicated by the small obtuse angles between their vectors (r=cos 0=+1). There was a near zero correlation between FP with PL and LY, between LB with PL and LY, between LL and SD (Figure 2A) as indicated by the near perpendicular vectors (r=cos90=0). There was a negative correlation between FP and SD as indicated by the near an angle of approximately 180 degrees (r=cos180=-1). Some above discrepancies of the biplot predictions and original data were expected because the biplot accounted for <100% of the total variation (Table 2 for the first year and Table 3 for the second year). Bootstrap resampling technique was used to provide estimates of the standard error, bias, and the distribution of correlation coefficients which estimated from a set of 1000 bootstrap samples were in close agreement with observed correlation coefficients among various traits and the low standard error as well as the low bias also indicated the robustness of correlation coefficients. The most prominent relations at the second year (Figure 2B) are: a strong positive association among LL, LA and LW; between LN and RD; among TS, PD and DF; and between LB and SD; as indicated by the small obtuse angles between their vectors (r=cos 0=+1). There was a near zero correlation between FP and PL; between LL, LA and LW with LB and SD (Figure 2B) as indicated by the near perpendicular vectors (r=cos90=0). There was a negative correlation between FP with LB and SD as indicated by the near an angle of approximately 180 degrees (r=cos180=-1). The statistical properties of this technique have been described in detail by YAN *et al*. (2000) and YAN and RAJCAN (2002).

However, regarding results of vector-view biplot at both years, strong positive association between LA and LW; between RD and LN; and between TS and PD; near zero correlation between FP and PL; and between LL and SD; negative association between FP and LB were observed. Also, for improving leaf yield, using PL, LN, RD, DF, PD and TS traits would be useful. As expected, there is high correlation for leaf length and width, but a strong negative correlation with leaf number. Although, the high correlation for leaf length and width with leaf yield, and a negative correlation with leaf number with leaf yield were reported previously (SRIVASTAVA *et al*., 1977), but we found only positive high correlation among leaf numbers at flowering, leaf length, leaf width, leaf area and leaf yield. It seems that two important properties (leaf and petiole) are the most important characteristics would be considered in genetic improvement programs of spinach. Likewise, ASADI and HASANDOKHT (2007) and EFTEKHARI *et al.* (2010) studied some Iranian spinach landraces and found relatively similar results on leaf length, leaf area, leaf numbers in flowering, petiole diameter and petiole length.

The mean effects of the measured across genotypes were examined by defining an average tester coordinate (ATC) axis and an average or virtual genotype is indicated by a circle and shows the positive end of the ATC axis. Ideal genotype is it that should have large PC1 scores (high traits' means) and small (absolute) PC2 scores (low variability). Genotypes with above-average means was selected, whereas the rest were discarded. At the first year, genotype G1 was the most favorable genotype regarding all of the measured traits due to its low distance from horizontal axis (Figure 3A). Ranking of the best genotypes based on the ideal genotype was G8> G20 > G70 > G61 > G27 and the ranking of the most unfavorable genotypes based on the ideal genotype was G23> G76 > G15 > G5 at the first year (Figure 3A).

Table 2. Pearson's correlation coefficients among measured traits of 81 spinach genotypes at the first year.

		DF	LN	LL	LW	LA	PD	PL	LB	FP	RD	SD	TS
LN	PC	0.20*											
	Bias	0.00											
	SE	0.11											
LL	PC	0.13	0.31										
	Bias	-0.01	-0.01										
	SE	0.11	0.11										
LW	PC	0.13	0.45	0.84									
	Bias	-0.01	-0.01	0.00									
	SE	0.11	0.10	0.03									
LA	PC	0.17	0.47	0.92	0.90								
	Bias	-0.01	-0.01	0.00	0.00								
	SE	0.09	0.10	0.01	0.02								
PD	PC	0.27	0.47	0.21	0.43	0.39							
	Bias	0.00	0.00	0.00	0.00	0.00							
	SE	0.11	0.07	0.13	0.11	0.11							
PL	PC	0.02	0.49	0.50	0.57	0.51	0.36						
	Bias	0.00	0.00	0.00	0.00	0.00	0.00						
	SE	0.17	0.10	0.10	0.09	0.09	0.12						
LB	PC	-0.06	0.21	-0.19	-0.03	-0.02	0.08	0.13					
	Bias	0.00	0.00	0.00	0.00	-0.01	0.00	0.00					
	SE	0.11	0.12	0.12	0.14	0.14	0.10	0.11					
FP	PC	0.01	-0.06	0.17	0.12	0.08	-0.22	-0.16	-0.21				
	Bias	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
	SE	0.11	0.09	0.12	0.10	0.11	0.10	0.11	0.09				
RD	PC	0.32	0.50	0.53	0.59	0.58	0.41	0.53	0.17	-0.16			
	Bias	-0.01	0.00	-0.01	0.00	-0.01	0.00	0.00	0.00	0.00			
	SE	0.10	0.08	0.09	0.08	0.08	0.12	0.07	0.09	0.11			
SD	PC	0.22	0.33	0.12	0.17	0.14	0.21	0.22	0.15	-0.11	0.48		
	Bias	0.00	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.02		
	SE	0.07	0.12	0.09	0.11	0.10	0.09	0.09	0.10	0.11	0.10		
TS	PC	0.22	0.33	0.31	0.38	0.33	0.36	0.39	-0.08	-0.19	0.59	0.39	
	Bias	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	
	SE	0.09	0.09	0.10	0.09	0.09	0.10	0.08	0.13	0.10	0.05	0.09	
LY	PC	0.27	0.58	0.61	0.71	0.73	0.53	0.56	0.12	-0.16	0.87	0.35	0.67
	Bias	0.00	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	SE	0.09	0.07	0.07	0.05	0.06	0.08	0.06	0.12	0.10	0.03	0.11	0.05

PC, Pearson correlation coefficient; Bias and SE (Standard Error) are the Bootstrap parameters

* Critical values of correlation P<0.05 and P<0.01 (D.F. 79) are 0.22 and 0.28, respectively.

Traits are; leaf numbers at flowering (LN), leaf length (LL), leaf width (LW), leaf area (LA), petiole diameter (PD), petiole length (PL), number of lateral branches (LB), root dry weight (RD), shoot dry weight (SD); days to flowering (DF), percent of female plants (FP), 1000-seed weight (TS), and leaf yield (LY).

Table 3. Pearson's correlation coefficients among measured traits of 81 spinach genotypes at the second year.

		DF*	LN	LL	LW	LA	PD	PL	LB	FP	RD	SD	TS
LN	PC	0.47											
	Bias	0.00											
	SE	0.07											
LL	PC	0.30	0.44										
	Bias	0.00	0.00										
	SE	0.10	0.10										
LW	PC	0.20	0.47	0.84									
	Bias	0.00	-0.01	0.00									
	SE	0.10	0.10	0.03									
LA	PC	0.22	0.48	0.88	0.90								
	Bias	0.00	0.00	0.00	0.00								
	SE	0.09	0.10	0.02	0.02								
PD	PC	0.37	0.57	0.20	0.37	0.36							
	Bias	-0.01	0.00	0.00	0.00	0.00							
	SE	0.10	0.07	0.13	0.12	0.11							
PL	PC	0.26	0.53	0.57	0.54	0.52	0.34						
	Bias	0.00	0.00	-0.01	0.00	0.00	0.01						
	SE	0.14	0.09	0.09	0.09	0.09	0.13						
LB	PC	0.17	0.29	0.04	0.18	0.15	0.12	0.19					
	Bias	0.00	-0.01	0.00	0.00	0.00	0.00	0.00					
	SE	0.09	0.11	0.10	0.12	0.11	0.09	0.09					
FP	PC	0.00	-0.07	0.06	0.02	0.01	-0.20	-0.08	-0.22				
	Bias	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
	SE	0.10	0.11	0.11	0.10	0.10	0.09	0.11	0.09				
RD	PC	0.27	0.56	0.60	0.61	0.62	0.40	0.47	0.33	-0.30			
	Bias	0.00	0.00	0.00	0.00	0.00	0.01	0.00	-0.01	0.01			
	SE	0.07	0.08	0.08	0.07	0.07	0.09	0.09	0.11	0.11			
SD	PC	0.24	0.37	0.19	0.19	0.16	0.18	0.24	0.25	-0.24	0.73		
	Bias	0.00	0.00	-0.01	-0.02	-0.01	0.00	-0.01	-0.01	0.01	-0.01		
	SE	0.08	0.10	0.10	0.11	0.11	0.10	0.10	0.12	0.13	0.07		
TS	PC	0.32	0.46	0.40	0.40	0.41	0.41	0.42	0.11	-0.26	0.54	0.45	
	Bias	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	
	SE	0.09	0.08	0.10	0.09	0.09	0.09	0.09	0.12	0.11	0.07	0.08	
LY	PC	0.31	0.60	0.63	0.68	0.72	0.55	0.56	0.22	-0.26	0.78	0.39	0.69
	Bias	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.00
	SE	0.07	0.07	0.08	0.06	0.06	0.07	0.06	0.12	0.11	0.06	0.10	0.06

PC, Pearson correlation coefficient; Bias and SE (Standard Error) are the Bootstrap parameters

* Critical values of correlation $P<0.05$ and $P<0.01$ (D.F. 79) are 0.22 and 0.28, respectively.

Traits are; leaf numbers at flowering (LN), leaf length (LL), leaf width (LW), leaf area (LA), petiole diameter (PD), petiole length (PL), number of lateral branches (LB), root dry weight (RD), shoot dry weight (SD); days to flowering (DF), percent of female plants (FP), 1000-seed weight (TS), and leaf yield (LY).

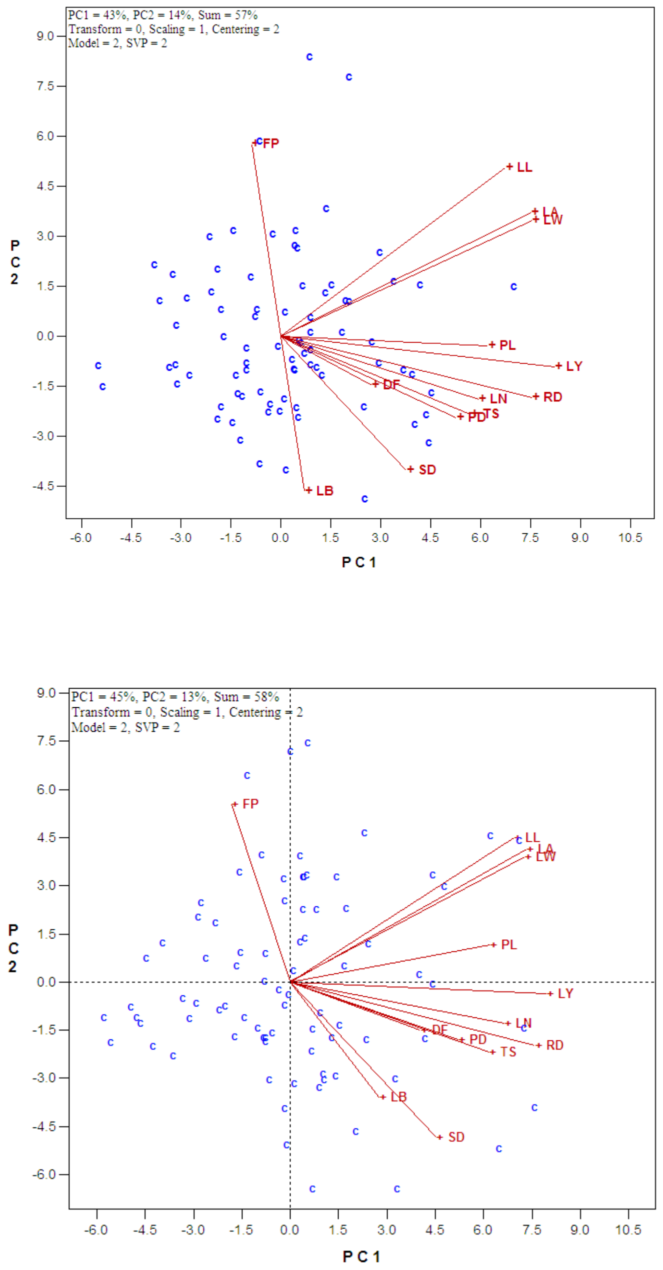

Figure 2. Vector view of genotype × traits biplot of measured traits of 81 spinach landraces for (up) the first year and (down) the second year, showing interrelationships among traits. See Table 1 for codes of the traits.

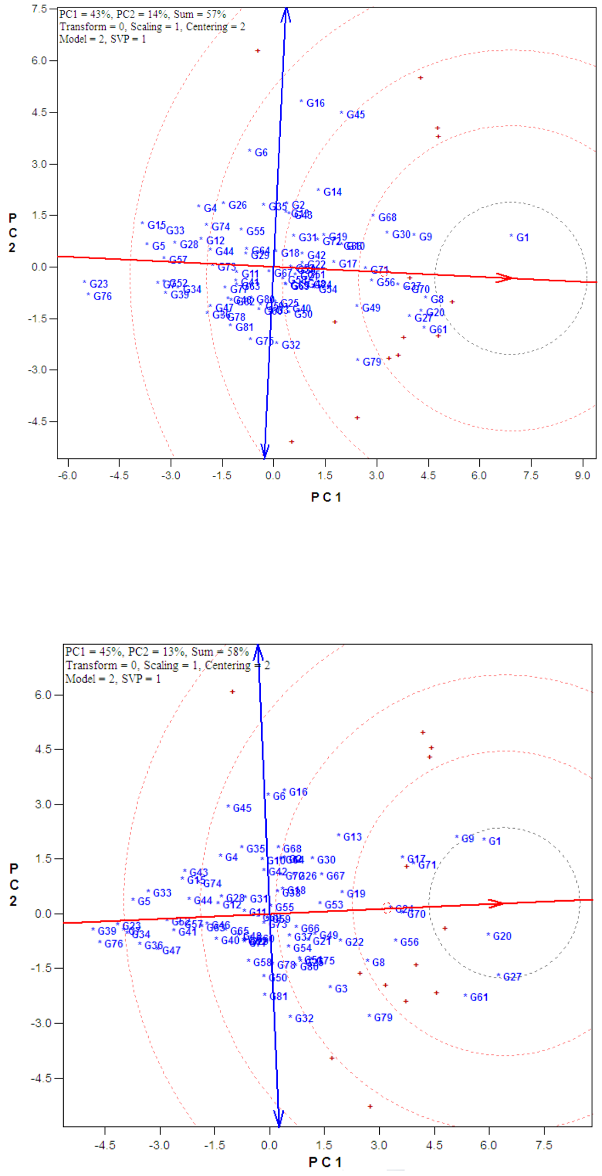

Figure 3. Ideal-entry view of genotype × traits biplot of measured traits of 81 spinach landraces for (up) the first year and (down) the second year, showing ranking of landraces according to the best supposed genotypes. See Table 1 for codes of the traits.

The requirement for the use of site regression based GT biplots in the identification of most superior genotypes is to facilitate the identification of such genotypes (CROSSA *et al.*, 2002). At the second year, genotype G20 was the most favorable genotype regarding all of the measured traits due to its low distance from horizontal axis (Figure 3B). Ranking of the best genotypes based on the ideal genotype was G1> G27 > G9 > G71 > G70 > G17 > G24 > G61 > G56 and the ranking of the most unfavorable genotypes based on the ideal genotype was G39 > G76 > G23> G7 > G34 > G5 > G33 > G36> G47 at the second year (Figure 3B). The present research has clearly shown that the site regression model can analyze patterns and relationships of genotypes and traits successfully as well as provide a valuable prediction. However, regarding results of ideal-entry view biplot at both years, almost half of studied genotypes including G1, G20, G7, G8, G9, G27, G49 G70, and G79 are good candidates for improving most of the measured traits and therefore there are good genetic variability in our plant materials. SABAGHNIA *et al.* (2010), however, mentioned that multivariate methods and their graphical tools are too sophisticated to provide a simple measure of genotypes favorability.

In spinach, improvement for achieving high leaf yield as an important desirable character is the purpose of many breeding programs. In the Figure 4A, spinach leaf yield (LY) was compared with other measured traits and the ranking of measured traits based on the LY was RD > PL > LN > TS > PD following to LW > LA > SD > DF > LL > LB > FP at the first year. In the second year (Figure 4B), comparing spinach leaf yield with other measured traits showed this ranking: LN > RD > PL > TS > PD > DF following to LW > LA > LL > SD > LB > FP. In other words, the most important traits for producing high yielding spinach landraces are RD > PL > LN > TS > PD while the least important trait on spinach dry yield was FP. it is notices that the FP is important for improving seed yield and so it seems that defining breeding strategies for genetic improvement of seed yield and leaf yield must be performed separately. BHAGCHANDANI *et al.* (1979) reported no average heterosis for leaf size properties (leaf length, leaf width and leaf area) while showed practically 29% heterosis for leaf yield and concluded that leaf size was controlled by some of minor genes with additive genetic effects, and that there was a partial dominance genetic effects for increased leaf yield. However, we found the most important traits on spinach leaf yield were leaf numbers at flowering, petiole diameter, petiole length, 1000-seed weight and root dry weight, although leaf size properties (leaf length, leaf width and leaf area) also had important effects on spinach leaf yield. Similarly, BAYRAKTAR *et al.* (1978) found that leaf area and single plant weight were correlated with leaf yield performance and that visual selection would be valid. The relative contributions of different traits of economical crop yield to the identification of desirable genotype found in this study by the traits comparing biplot procedure of the GT biplot are similar to those found in other crop studies; soybean (YAN and RAJCAN, 2002), white lupin (RUBIO *et al*., 2004), and rapeseed (SABAGHNIA *et al*., 2010).

Potential sources of genetic variation are important to allow plant breeders to deal with changing environmental condition. Large collections of genetic stocks do not exist for spinach and this point is best illustrated by the fact that in the past decade a few downy mildew cultivars have developed (IRISH, 2004). While large collections of genetic stocks do not exist, there are some local landraces available to breeders but variation exists for most traits have not been investigated. Although, leaf yield and seed yield were the most important target traits for breeders but in future breeding for horticultural type disease resistance such as downy mildew is the most common focus of breeding efforts with spinach (MORELOCK *et al*., 2007). Also, the recent increased interest in human nutrition has put spinach in a very enviable position due to its excellent profile of nutrients, while spinach has long been recognized as a good source of nutrients the more recent increased interest is in lutein and phenolic compounds (MURPHY and MORELOCK, 2000). Preliminary investigations have shown considerable variation in spinach for lutein content which indicate that breeding for higher lutein content should be possible (MURPHY, 2001). Spinach has some very unique phenolic compounds such as patuletins and spinacetins and suitable variation of them indicated that it should be possible to breed for these traits in future breeding efforts (HOWARD *et al*., 2002).

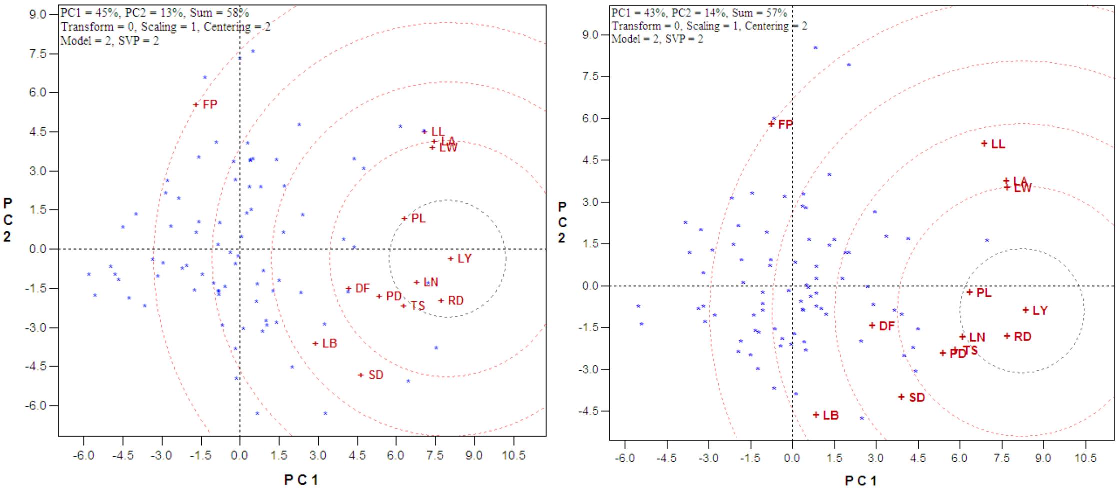

Figure 4. Ranking of measured traits of 81 spinach landraces based on leaf yield (LY) in the genotype × traits biplot for (up) the first year and (down) the second year. See Table 1 for codes of the traits.

It is clear that the GT biplot method is an excellent tool for visual genotype by trait data analysis. It is a powerful data visualization tool and can be used to graphically address many research questions but it has not been used properly in many studies because it is still a new technique and its utilities are still being developed (YAN, 2014). Compared with conventional methods of the genotype by trait data analysis, the GT biplot approach has some advantages (YAN and KANG, 2003; YAN *et al*., 2007). The first advantage of the biplot is its graphical presentation of the data, which greatly enhances our ability to understand the patterns of the data. The second is that it is more interpretative and facilitates pair-wise genotype or trait comparisons and effectively reveals the interrelationships among the spinach traits. Based on different trait relationships, selection for leaf yield or any target trait is not only the simplest, but also the most effective strategy in the early stages of Iranian spinach landraces breeding. The third advantage of the GT biplot method is that it facilitates identification of possible which-won-where patterns or which-lost-where patterns. Fourth, the GT biplot method can be used in independent culling based on multiple traits and in comparing selection strategies.

In conclusion, based on the GT biplot, leaf numbers at flowering, leaf length, leaf width, leaf area, petiole diameter and petiole length were identified as traits suitable for selection for leaf yield improvement in spinach. Thus, selecting for these traits is expected to lead to improved leaf yield and this suggests that selection index that incorporates these traits will not only result in the development of high leaf yielding cultivars but with other desirable agronomic traits. From my observations, it appears possible to improve Iranian spinach landraces by selecting for genotypes with higher values of the above mentioned traits. Also, the advanced lines produced by intercrossing the pure landrace lines could be further crossed with exotic germplasm (for example from IPK, Germany) to broaden the genetic basis of high potential spinach breeding programs. In addition, this research demonstrated that the GT biplot is an excellent tool for visualizing genotype by trait data. Also, almost half of studied genotypes including G1, G20, G7, G8, G9, G27, G49 G70, and G79 are good candidates for improving most of the measured traits due to existence of good genetic variability in our plant materials.

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BIPOLOT ANALIZA ODNOSA OSOBINA DOMAĆIH VRSTA SPANAĆA (*Spinacia oleracea* L.)

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Izvod

Vršeno je gajenje osamdeset jedne (81) domaće sorte spanaća u toku dve godine u potpunom slučajnom bloku u četiri ponavljana. Rang genotipova prema idealnim dobijenim podacima je potvrdilo da genotipovi G1, G20, G7, G8, G9, G27, G49 G70 i G79 su bili višeg ranga u merenim osobinama i da mogu da budu dobri kandidati u poboljšaanju većine merenih osobina. Rezulati istraživanja kao što su osobine: broj listova i cvetova, dužina lista, širina lista površina lista, dijametar drške lista i dužina drške lista mogu da budu indikatori u selekciji spanaća u oplemenjivanju. Genotipovi G1, G20, G7, G8, G9, G27, G49 G70 i G79 su preporučeni za komercijalno testiranje i uključivanje u programe oplemenjivanja. GT biplot metod može da se koristi u identifikaciji superiornih genotipova i kod drugih biljnih vrsta u drugim delovima sveta.

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