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### Investigation of some morphological traits in studied lentil (*Lens culinaris* Medik.) genotypes grown with foliar application of nanosized ferric oxide

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Badania nad niektórymi cechami morfologicznymi wybranych genotypów soczewicy  
(*Lens culinaris* Medik.) hodowanych w obecności nanocząstek tlenku żelaza

#### ABSTRACT

Interest in growing lentil (*Lens culinaris* Medik.) is increasing due to its potential returns relative to other legume crops in semi-arid areas. An experiment was conducted to examine the important traits on lentil under application of nano-fertilizer by using eight genotypes with application of the biplot technique in visualizing research data. Nano-iron oxide (2 g L<sup>-1</sup>) was utilized as foliar spray during vegetative and reproductive stages. The study revealed that genotype by trait (GT) biplot can graphically display the interrelationships among traits and facilitate visual comparison of genotypes. The first two principal components (PC1 and PC2) accounted for 76% of the total variation. The polygon view of GT biplot suggested four sections for the lentil genotypes as well as traits. The vertex genotypes G1 had plant height, number of branches per plant, number of pods per plant, 100-grains weight and grain yield traits. The most prominent relation were: a strong positive association among biological yield, number of branches per plant, number of pods per plant, grains yield and plant height as indicated by the small obtuse angles between their vectors. The traits' relationship in the semi-arid was highly variable, and grain yield improvement can be achieved by selecting for number of pods per plant, 100-grains weight. We suggest that the GT biplot be used jointly to better understand and more fully explore interaction pattern data.

**Keywords:** genotype × trait (GT) biplot, polygon view, regression site

## STRESZCZENIE

Uprawa soczewicy na terenach półpustynnych budzi wzrastające zainteresowanie ze względu na potencjalne korzyści w porównaniu do innych roślin strączkowych. Eksperyment przeprowadzono w celu zbadania wybranych cech soczewicy, po podaniu nanocząstek tlenku żelaza, u ośmiu genotypów z zastosowaniem techniki biplot do wizualizacji danych. Organy wegetatywne i generatywne w różnych fazach rozwoju spryskiwano nanocząstkami tlenku żelaza ( $2\text{gL}^{-1}$ ). Badania wykazały, że zastosowanie techniki biplot, która umożliwia graficzne wykazanie związku między cechami i ułatwia ich wizualizację jest użyteczne do porównania genotypów. Pierwsze dwa główne czynniki (PC1 i PC2) wyjaśniały 76% całkowitej zmienności. Wielostronne obrazowanie GT biplot sugerowało występowanie czterech grup genotypów soczewicy i ich cech. Wierzchołki reprezentujące genotypy G1 przedstawiały wysokość roślin, liczbę pędów rośliny, liczbę strąków na roślinie, wagę 100 ziaren i wydajność. Najbardziej widoczne relacje to: wyraźnie pozytywny związek między wydajnością biologiczną plonu, liczbą pędów rośliny, liczbą strąków na roślinie, plonem i wysokością roślin. Korelacja cech u półpustynnych roślin była bardzo zmienna, a poprawę plonu ziarna można osiągnąć przez selekcję roślin o określonej liczbie strąków i wadze 100 ziaren. Wykazano, że zastosowanie techniki GT biplot pozwala lepiej zrozumieć i pełniej badać wzory interakcji.

**Slowa kluczowe:** genotyp  $\times$  cecha (GT) biplot, wielostronne obrazowanie, obszar regresji

## INTRODUCTION

Genetically improved genotypes must be evaluated on the basis of multiple traits to ensure that the selected genotypes have acceptable performance and to meet the facets of the demand from the farmers. For this reason, some experiments are conducted throughout the world for major crops in which multiple traits are recorded and effective interpretation of the obtained data in making selection decisions remain a major challenge to breeders and agronomists (7). Effective analysis of data becomes an integral part of effective crop evaluation and the major task in data analysis is to select superior genotypes on the basis of multiple traits in addition to yield performance (12). The fulfillment of this task depends on an understanding of the genotype  $\times$  trait (GT) interaction pattern which has been the focus of numerous studies (2; 3; 8) and the interrelations among the measured traits (15). Yan et al. (15) proposed a GGE biplot model that allows visual examination of the genotype  $\times$  environment (GE) interaction pattern of multi environmental trials data. The method emphasizes two concepts: genotype main effect (G) and GE interaction.

Lentil (*Lens culinaris* Medik.) is the most important field crop in Iran, grown for its protein and the routine genotype evaluation was conducted in arid and semi-arid areas. Cohen (1977; cited in (1) reported domestication of lentil have taken place at a site in Iran dating to 9,500 to 8,000 BP. Lentil is an important food source because the seed is rich in protein (25%) and carbohydrates (28%) and it is usually grown as a winter crop (5). Use of lentil in pastures and for soil improvement dates back to the period many years ago and had an important role in different agricultural and natural environments. Genotype evaluation on the basis of multiple traits is an important issue in plant breeding programs especially with nanotechnology application. Fertilizers have a main role in improving the crop production particularly in fertilizer responsive genotypes. Manufacture of nanofertilizer with high nutrient use efficiency and slow/controlled release of nutrients (9) might be a best alternative to meet the food needs of a growing population. The objective of present research was to describe a GT biplot model, which is an application of the GGE biplot model to

investigate pattern of the GT interaction data, and to examine its usefulness in visualizing lentil trait relationships as well as its application in genotype evaluation and selection.

## MATERIALS AND METHODS

Eight lentil genotypes including FLIP 86-35L (G1), FLIP 87-22L (G2), FLIP 89-63L (G3), FLIP 90-25L (G4), FLIP 92-12L (G5), FLIP 92-36L (G6), FLIP 96-15L (G7) and FLIP 96-46L (G8) were planted at field which was mouldboard-ploughed and disked before planting. The experiment was based on a randomized complete block design with three replicates. Seeds were hand planted on the first week of November in eight rows per plots and the unit plot size was 2.5 m × 2.0 m with plant spacing of 25 cm × 8 cm. Weeds were controlled over the growth period with hand hoeing. Nano- $\text{Fe}_2\text{O}_3$  was procured from Pishgaman Iranian, Iran, and used with concentration of 2 g L<sup>-1</sup> as Fe fertilizer. Plants were foliar sprayed two times at vegetative ( $V_5$ : 1st multifoliate leaf unfolded at the 5th node) and the rest at podding stage. Fig. 1 shows a transmission electron microscopy (TEM) image of the nanoparticle sample.

Some agronomic traits including PH, plant height (cm); NSB, number of branches per plant; PN, number of pods per plant; RD, rooting depth (cm); NEP, number of empty pods; HGW, 100-grains weight (g); GY, grains yield and BY, biological yield were recorded on 15 randomly selected plants in each plot. Plots were harvested at the ground level by hand from late June to early July and grain and biological yield was determined by harvesting the middle three rows of each plot.

The GT biplot model (12) was used to show the lentil genotype by trait two-way data in a biplot. Simple correlation coefficients between all possible combinations of traits were worked out. All biplots presented in this study were generated using the software GGEbiplot package (13).

## RESULTS AND DISCUSSION

The GT biplot explained 76% of the total variation of the standardized data and this relatively high magnitude reflects the complexity of the relationships among the measured traits. The first two principal components (PC1 and PC2) explained 57% and 19%, respectively. The biplot Fig. 2, as polygon view, presents data of eight lentil genotypes in nine different traits and the following can be seen: the vertex genotypes in this investigation are G6, G2, G1, G4 and G8. These genotypes are the best or the poorest genotypes in some or all of the traits since they had the longest distance from the origin of biplot. Therefore, G1 had the highest values for NSB, PN, GY, PH and HGW traits; G2 had the highest values for RD and BY traits; G6 had the highest values for SN trait; and G8 had the highest values for NEP trait (Fig. 2). The vertex genotypes and their related genotypes of them which are located in different seven sections of biplot are good candidates for examination heterosis (2; 14) for hybrid production in lentil using these genotypes or pure lines.

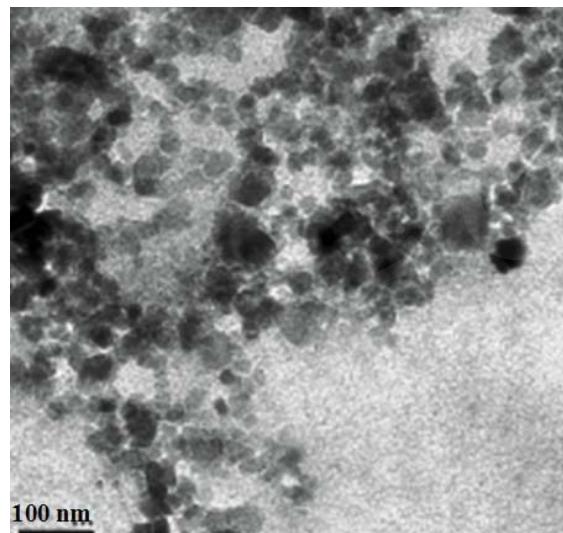
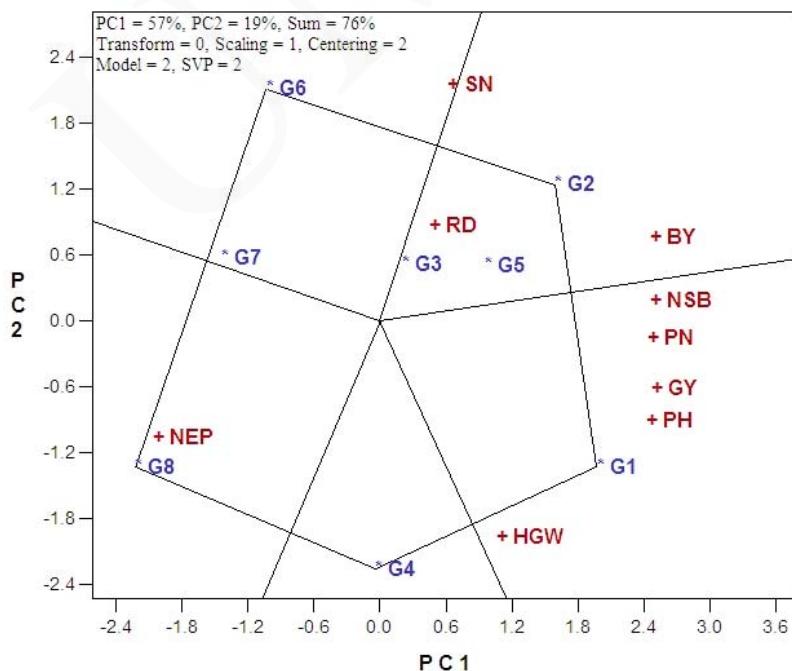
Fig.1. Large area TEM image of  $\text{Fe}_2\text{O}_3$  nanoparticles.

Fig. 2. Polygon view of the genotype by trait (GT) biplot which shows the which-won-where pattern of genotypes and traits in lentil. Traits abbreviations are: PH, plant height; NSB, number of branches per plant; PN, number of pods per plant; RD, rooting depth (cm); NEP, number of empty pods; HGW, 100-grains weight; GY, grains yield and BY, biological yield.

In Fig. 3, the most prominent relations are: a strong positive association among BY, NSB, PN, GY and PH as indicated by the small obtuse angles between their vectors ( $r=\cos 0=+1$ ). There was a near zero correlation between NEP and HGW; between NEP and SN; BY and HGW; and SN with GY and PH (Fig. 3) as indicated by the near perpendicular vectors ( $r=\cos 90=0$ ). A strong negative association between SN and HGW and between NEP and BY is indicated by the large obtuse angles between their vectors ( $r=\cos 180=-1$ ). Most of the mentioned results can be verified using correlation coefficients of Table 1 while some discrepancies between the biplot predictions and original data were expected because the biplot accounted for <100% of the total variation (about 76%). Similar to vector view of testers, the relations among studied genotypes are investigated via vector view of entries (Fig. 4) and show that the lentil genotypes are very variable regarding measured traits.

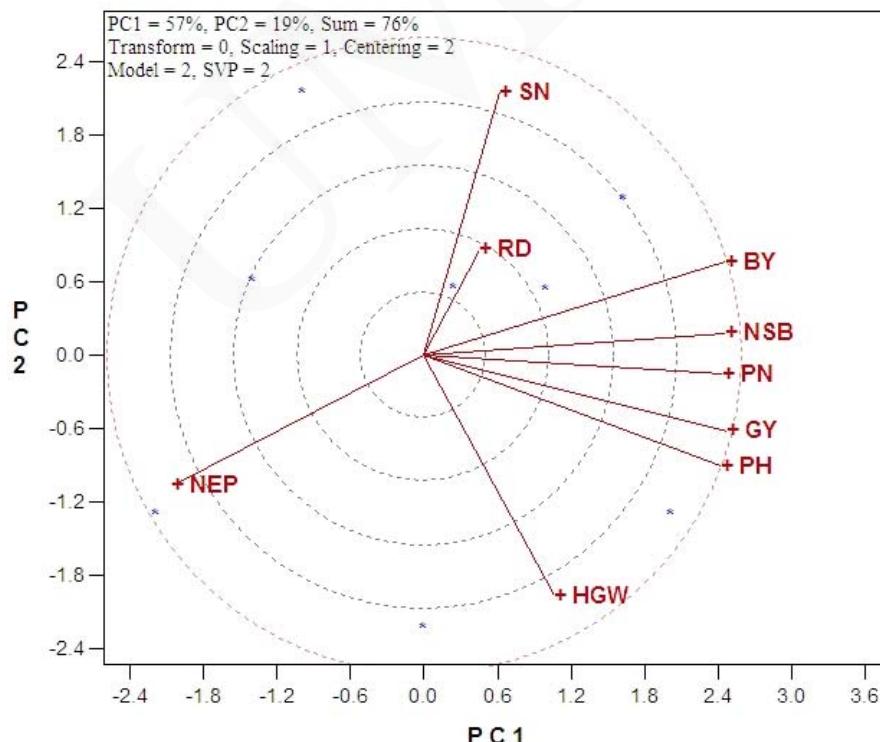


Fig. 3. Vector view of the genotype by trait (GT) biplot which shows the correlation among measured traits. Traits abbreviations are: PH, plant height; NSB, number of branches per plant; PN, number of pods per plant; RD, rooting depth (cm); NEP, number of empty pods; HGW, 100-grains weight; GY, grains yield and BY, biological yield.

Table 1. Phenotypic coefficients of correlation between measured traits

	PH	NSB	PN	SN	RD	NEP	HGW	GY
NSB	0.79†							
PN	0.86	0.84						
SN	-0.04	0.13	0.10					
RD	0.14	0.25	-0.10	0.20				
NEP	-0.52	-0.78	-0.68	-0.47	-0.12			
HGW	0.65	0.27	0.23	-0.33	0.06	-0.05		
GY	0.94	0.80	0.88	0.15	-0.03	-0.60	0.58	
BY	0.78	0.89	0.86	0.41	0.33	-0.75	0.12	0.80

† Critical values of correlation  $P < 0.05$  and  $P < 0.01$  (df 6) are 0.71 and 0.83, respectively.

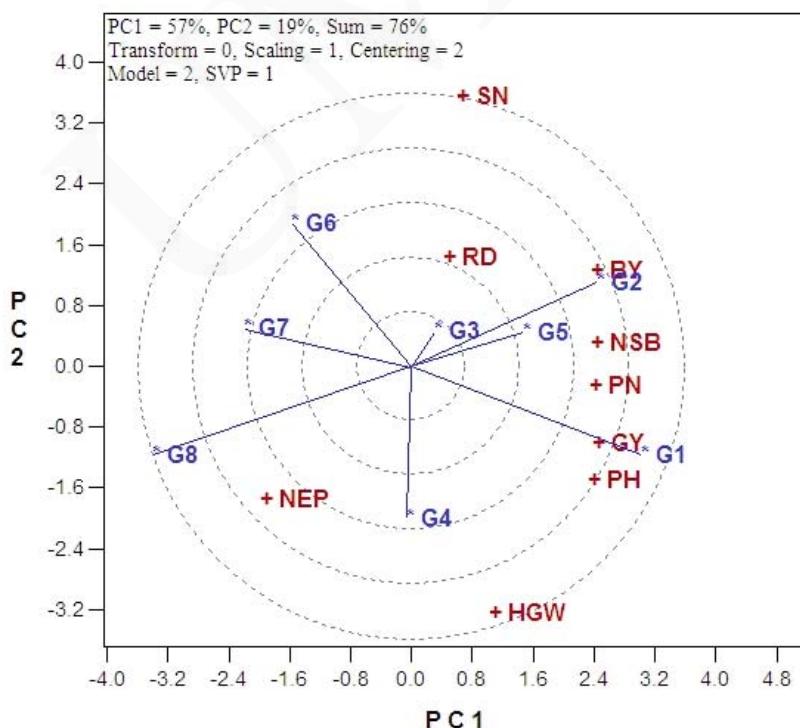


Fig. 4. Vector view of the genotype by trait (GT) biplot which shows the correlation among studied genotypes. Traits abbreviations are: PH, plant height; NSB, number of branches per plant; PN, number of pods per plant; RD, rooting depth (cm); NEP, number of empty pods; HGW, 100-grains weight; GY, grains yield and BY, biological yield.

In lentil, improvement for achieving high grain yield as an important desirable trait is the purpose of many breeding programs. In the Fig. 5, lentil grain yield (GY) was compared with other measured traits. Ranking of other traits based on the GY was PH > PN > NSB > BY > HGW > RD > NEP. In other words, the most important traits for producing high yielding lentil varieties are PH, PN, and NSB and BY while the least important trait on lentil grain yield was NEP. 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 (i.e. soybean (12), white lupin (3), rapeseed (2), canola (4) and wheat (6)).

Genotype G1 (FLIP 86-35L) which is improved by International Center for Agricultural Research in the Dry Area (ICARDA) is one of the important candidates for commercial cultivar release for arid and semi-arid areas. Therefore, other genotypes were compared with this genotype via biplot (Fig. 5). Ranking of other

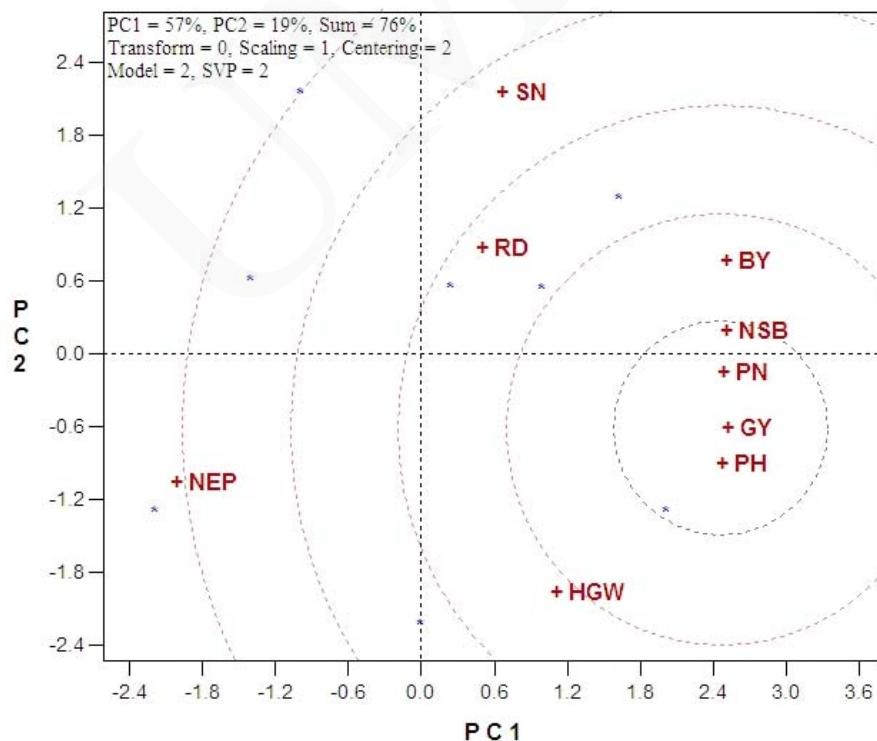


Fig. 5. Genotype by trait (GT) biplot for comparison of different traits according to grain yield (GY). Traits abbreviations are: PH, plant height; NSB, number of branches per plant; PN, number of pods per plant; RD, rooting depth (cm); NEP, number of empty pods; HGW, 100-grains weight; GY, grains yield and BY, biological yield.

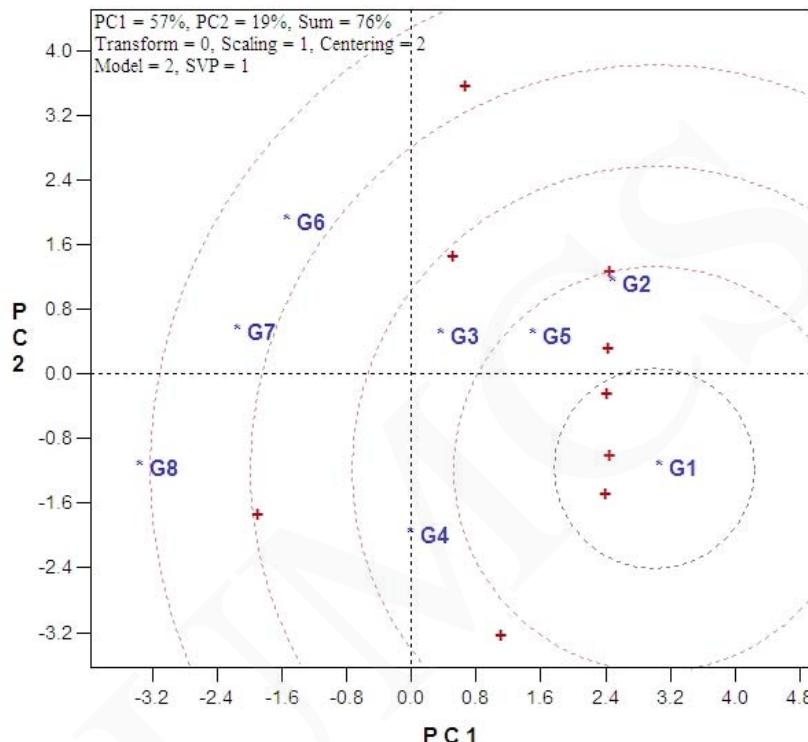


Fig. 6. Genotype by trait (GT) biplot for ranking of genotypes based on G1.

remained genotypes based on the genotype G1 was  $G5 > G2 > G4 > G3 > G7 > G6 > G8$ . Therefore, the most favorable genotypes regarding all of the lentil measured traits are G5 and G2 while the least favorable genotypes were G7, G6 and G8. In this research, interpretation of the genotype by trait interaction was based on the GT biplot technique (12) in lentil genotypes. Considering all of the generated bi-plots, at least plant height (PH), number of branches per plant (NSB), 100-grains weight (HGW) and number of pods per plant (PN) traits which related to grain yield were identified as most influencing traits on lentil yield performance. Therefore, for future lentil breeding programs it is essential to define selection indices based on above traits. Polygon biplot suggested that there exist four possible sections for lentil traits for which this section patterns needs verification through other spinach trials for this target region (14).

The GT biplot method as the multivariate statistical tool has been introduced to explore the multi-directionality aspect of data and to extract more information from interaction component. The GT biplot also identifies superior genotypes with favorable traits effects which would be useful for the breeding of new lines

for each target entry because it will help researchers for exploiting the interactions among entries and subsets of testers (2).

## CONCLUSIONS

For several reasons, we prefer to the use of GT biplot to evaluate the genotypes performance grown with application of nano-Fe fertilizer: (i) by using this method the main effect of the genotype is not omitted; (ii), biplot graph is easy to use and to interpret; and (iii) other information such as the best mating partner for each genotype, groups of similar genotypes and the best crosses that are superior to their genotypes can be graphically visualized to rapidly identify suitable genotypes and crosses for further investigation.

## ACKNOWLEDGMENTS

We wish to thank Dr. W. Yan for making available a time-limited version of GGE biplot software as "Test Biplotxlsx."

## REFERENCES

1. Bryan J.A. 2000. Nitrogen-fixing trees and shrubs: a basic resource of agroforestry, [in:] The Silvicultural Basis for Agroforestry Systems. Eds M.S. Ashton, F. Montagnini (CRC Press, Baton Rouge, LA), pp. 41–60.
2. Dehghani D., Omidi H., Sabaghnia N. 2008. Graphic analysis of trait relations of canola (*Brassica napus* L.) using biplot method. *Agronomy Journal* 100: 760–764.
3. Rubio J., Cubero J.I., Martin L.M., Suso M.J., Flores F. 2004. Biplot analysis of trait relations of white lupin in Spain. *Euphytica* 135 (2): 217–224.
4. Sabaghnia N., Dehghani H., Alizadeh B., Mohghaddam M. 2010. Genetic analysis of oil yield, seed yield, and yield components in rapeseed using additive main effects and multiplicative interaction biplots. *Agronomy Journal* 102: 1361–1368.
5. Sabaghnia N., Karimizadeh R., Mohammadi M. 2014. Graphic analysis of yield stability in new improved lentil (*Lens culinaris* Medik.) genotypes using nonparametric statistics. *Acta Agriculturae Slovenica* 103: 113 – 127.
6. Sabaghnia N., Janmohammadi M. 2014. Interrelationships among some morphological traits of wheat (*Triticum aestivum* L.) cultivars using biplot. *Botanica Lithuanica* 20 (1): 19–26.
7. Sabaghnia N., Karimizadeh R., Mohammadi M., 2013. GGL biplot analysis of durum wheat (*Triticum turgidum* spp. *durum*) yield in multi-environment trials. *Bulgarian Journal of Agricultural Science* 19 (4): 756–765.
8. Sabaghnia N., Dehghani H., Alizadeh B., Moghaddam M. 2011. Yield analysis of rapeseed (*Brassica napus* L.) under water-stress conditions using GGE biplot methodology. *Journal of Crop Improvement* 25: 26–45.
9. Sekhon B.S. 2014. Nanotechnology in agri-food production: an overview. *Nanotechnology, Science and Applications* 7: 31–53.
10. Yan W., Cornelius P.L., Crossa J., Hunt L.A. 2001. Comparison of two types of GGE biplots for studying genotype by environment interaction. *Crop Science* 41: 656–663.

11. Yan W., Kang M.S. 2000. GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC Press, Boca Raton, FL.
12. Yan W., Rajcan I. 2002. Biplot evaluation of test sites and trait relations of soybean in Ontario. *Crop Science* 42: 11–20.
13. Yan W. 2001. GGEbiplot – A Windows application for graphical analysis of multienvironment trial data and other types of twoway data. *Agronomy Journal* 93: 1111–1118.
14. Yan W., Kang M.S., Ma B., Woods S., Cornelius P.L. 2007. GGE biplot vs. AMI analysis of genotype-by-environment data. *Crop Science* 47: 643–655.
15. Yan W., Hunt L.A., Sheng Q., Szlavnics Z. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science* 40: 597–605.