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GGE BIPLOT AN EFFECTIVE TOOL TO STUDY GENOTYPE AND GENOTYPE × ENVIRONMENT INTERACTION; A CASE STUDY IN ONION (*Allium cepa*. L)

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Multi-location trials in breeding program of any crop are important to find the genotype × environment interaction and selection of supreme genotypes across different environments. Present study was conducted to determine the stability and adaptability of 12onion genotypes in six distinct environments. The experiments were laid out in triplicate randomized complete block design at six locations in 2018-19 viz., Faisalabad-1, Bahawalpur-1, Multan, Faisalabad- 2, Bahawalpur-2 and Raiwind. The combined analysis of variance revealed significant differences among the genotypes, environments, and genotype × environment interaction. The scores of principal component 1 (PC1) and principal component 2 (PC2) cumulatively explained 97.13 % of the total variation due to genotype and genotype × environment interaction which were used to draw GGE biplot. Genotypes with score near to zero on both PCs considered as stable one while genotypes with large scores are catagorized as less stable. Phulkara and VRIO-1 were best genotypes in terms of high average yield and stability across multi-location testing. Dark red was the ideal genotype followed by VRIO-1 and Phulkara. The polygon view of GGE biplot exhibits that genotypes: Dark red, Desi red, Early red, Husri and VRIO-2 are highly diverse. Due to more representativeness Bahawalpur-2 was identified as best location among the tested environments for general cultivation of these genotypes. One mega-environment was identified comprised of all tested locations for onion cultivation and all the tested locations are significantly correlated with each other. Thus, GGE biplot technique with graphical display gives an overview of mean performance and stability which is useful for identifying locations with good genotype performance and for efficient use of available resources for the testing program.

Keywords: Onion, GGE Biplot, genotype × environment interaction, multi-location trials.

INTRODUCTION

Onion (*Allium cepa*. L) is an important bulbous crop consumed on daily basis in fresh as well as dried form, throughout the world. It belongs to the family Alliaceae and genus *Allium.* It shows more variation in eastern Mediterranean countries like Tajikistan, Turkmenistan to Pakistan and India. Central Asia is considered its center of origin (Jokanovic *et al.,*2016). Onion has high medicinal values as it prevents heatstroke and heart diseases and helps to fight against cancer. Onion is a rich source of sulfur compounds which are good for human health (Gitin *et al.,* 2014). China is the largest producer of onion with production of 24345 thousand tones and 24.87% share in world production. Pakistan occupies 10th position and 1.87% share in world production (FAO, 2017). In Pakistan, onion is grown on an area of 149 thousand hectares with production 2115 thousand tonnes. The yield per unit area of our country is 14.10 tonnes per hectare (MINFSR, 2017-18). Due to less stability of genotypes over a range of environments our yield is quite low in comparison to leading countries like China (38.85 tonnes/hectare) and Niger (29.33 tonnes/hectare) (FAO, 2017).

Breeding for high yielding genotypes is complex as yield is a quantitative trait which is controlled by genes having small effect and has major influence of environment. Selection for high yielding and stable genotype further becomes difficult due to the significant presence of genotype × environment interaction (GEI). Significant GEI of a genotype for a trait especially yield reduces its usefulness over all environments or locations. Multi-location trials are mostly conducted to check the stability of the genotypes over a range of environments and to identify most suitable location for the cultivation of a genotype (Akter *et al.,* 2015). GEI plays an important role in selection of a genotype for a targeted environment. Some genotypes show significant change in their mean performance over a range of environments due to the presence of GEI (Kaya *et al.,* 2006). Significant GEI is further divided into two types; cross over GEI and non-crossover GEI. In the first type of the GEI, ranking of the genotypes is done on the basis of their mean performance which changes over multi-location testing but in the second one, ranking of the genotypes remains unchanged over changing environments (Liang *et al.,* 2015). So, for selection of high yielding genotypes with stable nature, breeders must consider GEI studies for diverse environments. GGE biplot and additive main effects and multiplicative interaction (AMMI) are commonly used models to study GEI by making biplots.

GGE biplot methodology is very useful for visually analyzing multi-location trial data and to find out useful information present in it. As compared to AMMI, GGE biplot gives superior mean vs stability view and explain more GGE (Yan *et al.,* 2000). The GGE biplot is based on two concepts, the biplot concept provided by Gabriel (1971) and GGE concept given by Yan *et al.* (2000). It is based on principal component scores and provides graphical display of biplot in many aspects. GGE biplot explains variation present due to genotype and GEI. It is a good tool to assess the adaptability and stability of genotypes in broad environment and performance of different environments and to find most suitable genotype for specific environment (Yan, 2001).

The main objective of the present study was to assess the mean performance and stability of onion genotypes across different locations in Pakistan using GGE biplot analysis. And also, selection of most suitable genotype and environment along with mega-environments for general cultivation of onion in Pakistan.

MATERIALS AND METHODS

***Experimental design and plant material*:** Present study was conducted during 2018-19 at six different locations which were Faisalabad-1 (FSD-1) 31°24'01.0"N & 73°03'56.9"E, Faisalabad-2 (FSD-2) 31°27'28.3"N & 72°58'08.0"E, Bahawalpur-1 (BWP-1) 29°23'11.3"N & 71°39'11.8"E, Bahawalpur-2 (BWP-2) 29°22'34.9"N & 71°38'38.7"E, Multan (MTN) 30°09'03.2"N & 71°26'41.5"E and Raiwind (RWD)31°14'31.0"N 74°13'37.1"E. Twelve onion genotypes were used as experimental material. Names of the genotypes, their origin, codes and mean yield are presented in Table1. The experiment at each location was laid out in randomized complete block design (RCBD) with three replications. Nursery was sown in October,2018 and forty-five days old seedlings were transplanted in the field. Nursery was transplanted on both sides of the ridge with75 cm row to row and 10 cm plant to plant distance keeping plot size 7 x 1.5 m^2. Nitrogen, phosphorus and potash fertilizers were applied at the rate of 125, 90 and 60 kg/ha, respectively. Whole potash and phosphorus fertilizers were applied at the time of land preparation and nitrogen was applied in two splits, 1st at 45 days after transplanting and 2nd at 30 days after first application. All other agronomic practices were applied according to standard recommendations. When most of the bulbs dropped their necks, the bulbs were uprooted and cured in the field for one week. After complete curing, leaves were cut down and bulbs were weighed on electronic balance to record yield (t/ha).

Table 1. Name, number, origin and mean yield of genotypes over six different locations

Code	Genotypes	Origin	Yield (t/ha)
1	Dark Red	VRI	26.51
2	Desi red	VRI	15.01
3	Early red	VRI	15.24
4	Husri	NARC	11.01
5	Local	Local collection	12.06
6	Mirpurkhas	Local collection	14.58
7	Phulkara	VRI	19.54
8	VRIO-1	VRI	21.63
9	VRIO-2	VRI	10.48
10	VRIO-3	VRI	11.06
11	VRIO-4	VRI	12.06
12	VRIO-6	VRI	14.29

VRI= Vegetable Research Institute, Faisalabad, Pakistan; NARC= National Agriculture Research Centre, Islamabad, Pakistan

***Statistical analysis*:** The bulb yield data of 12 genotypes tested across six environments were used to perform a combined analysis of variance to find out the difference among the genotypes regarding genotype (G), environment (E) and G × E interaction. For further analyzing the data visually and to study G ×E interaction, GGE biplot methodology was used which is based on two concepts, the biplot concept (Gabriel, 1971) and the concept of GGE (Yan *et al.*, 2000). R software (6.3.1) was used to perform statistical analysis. The library "Agricolae" was used to perform combined ANOVA and "GGE Biplot GUI" was used to perform GGE biplot analysis. Test centered (G + GE) model keeping SVP (Singular value partitioning) symmetrical was used with scaling based on standard deviation.

RESULTS

***Combined analysis of variance*:** Results of the combined analysis of variance revealed that differences among G, E and GEI were highly significant ($P<0.001$) for bulb yield (Table 2). Highly significant F value of GEI revealed that genotypes should be selected for peculiar environment.

Table 2. Combined analysis of variance of twelve onion genotypes tested across six locations.

Source	Df	MS	Explained SS (%)
Replication	12	2.48	
Environment (E)	5	70.43***	6.30
Genotype (G)	11	437.43***	86.13
G x E interaction	55	7.68***	7.50
Error	132	1.65	
Total	215		

***Indicate significance at $P<0.001$; Df= degree of freedom; SS= Sum of Squares; MS= Mean Square

The combined ANOVA also depicted that bulb yield was significantly affected by genotypes that contributes 86.13 % of the total variation (G + E + GEI). Environment affects 6.3 % and GEI 7.5 % to the total variation (Table. 2). Significant GEI indicated that breeder must select the genotypes with better stability across wide range of environments.

***GGE biplot based on tested locations and genotypes*:** The GGE biplot based on principal component 1and principal component 2 scores is shown in Fig.1. Biplot explains the type of variations that is due to genotype, and GEI present in multi-location trials (MLT) data. Biplot explains 97.13% of the total variation that is due to GGE (Fig. 1). The distance of the genotypes from the biplot origin indicates yield performance across tested locations. Genotypes present on the left side of biplot origin are above average yielders i.e. 1,7 and 8 are high yielding genotypes, 2 and 3 are average yielders and remaining are poor yielders (Fig. 1).

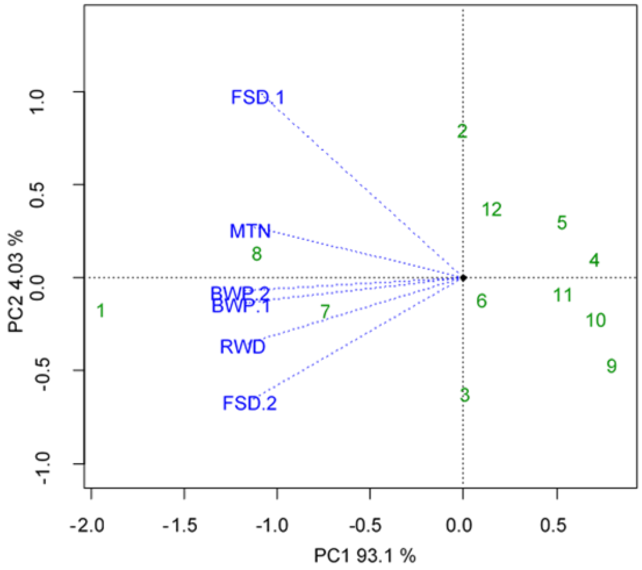

Figure 1. The GGE biplot of twelve onion genotypes tested across six locations. Genotypes are represented by numbers and environments by their abbreviations. PC1 and PC2 are first and second principal components respectively. The genotypes are represented by numbers and locations are represented by their abbreviations, given in material and method.

***Selection of best cultivar for every location*:** A convex hull was drawn by connecting the vertex (genotype present at the vertex of polygon) genotypes with straight line. In this biplot, genotypes 1, 2, 3, 4 and 9are the vertex (more responsive) genotypes possessing more distance from the biplot origin (Fig. 2). The biplot is divided into seven sections with perpendicular lines which are intersecting the convex hull. These sections are grouping the environments into one group and the vertex genotypes for these sections are winning genotypes for targeted environments present in the section. In section 1 the genotype 1 is the vertex genotype and all environment fall in this section and this is the high yielding genotype for all the tested locations. In section 2 the genotype 2 is the winning genotype for this section but no location falls in this sector. In section 4, 5 and 6 the genotypes 4, 9 and 3 are the vertex genotypes but no environment falls in these sections. This biplot also suggests that onion growing locations consist of only one mega-environment which is more suitable for its production. No location fell into the section of genotypes 2, 4, 9 and 3 suggesting that these genotypes did not perform well in any of the environment and none of the studied location is suitable to them (Fig. 2).

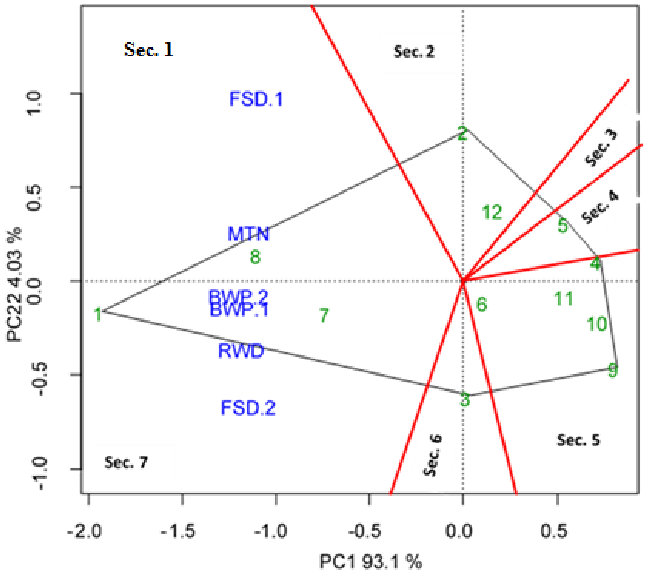

Figure 2. The polygon view of the GGE biplot. Genotypes are represented by numbers and environments by their abbreviations. PC1 and PC2 are first and second principal components respectively

***Stability and average yield performance of genotypes*:** The average yield and stability of the genotypes were estimated through average environment coordinate (AEC) method in GGE biplot methodology (Yan and Tinker, 2006). Average of PC 1 and PC 2 scores across all environments are used to define x-axis of AEC which passes through the biplot origin and the circle present on it shows the average environment. Absolute length of the genotype vectors from AEC in direction of arrow shows their yield performance, the more the distance the more will be yield and vice versa. The cultivars 1, 8 and 7 are high yielding respectively while the cultivars 9 and 10 are poor yielder (Fig. 3). Another line (y-axis) perpendicular to AEC passes through biplot origin and tells about the stability of the genotypes. Estimation of stability has been done by projection of genotypes from its origin, cultivars near to origin will be considered as more stable and those away from origin in both directions will be considered least stable. In this biplot the genotypes 4, 8and 6 are more stable while the genotypes 2 and 3 are least stable and showed more GEI. Selection of genotypes will be done based on high yield performance and more stability. In our study the genotypes 1, 7 and 8 are high yielder and more stable (Fig. 3).

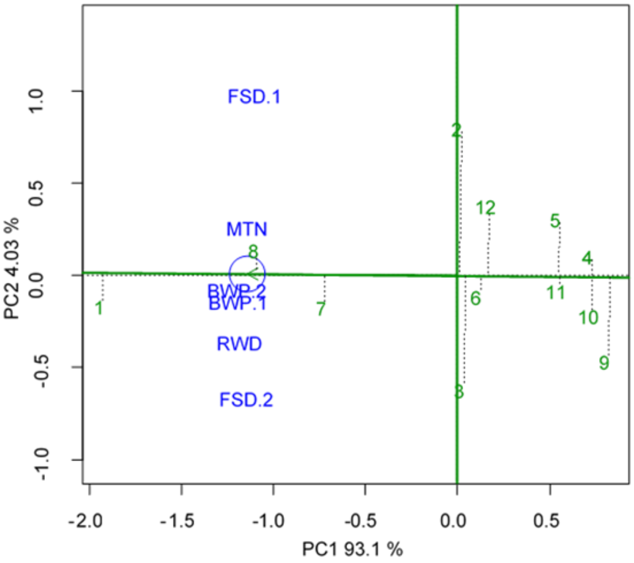

Figure 3. Average tester(environment) coordinate view of the GGE biplot. Genotypes are represented by numbers and environments by their abbreviations. PC1 and PC2 are first and second principal components respectively.

Comparison of genotypes with ideal genotype**:** A genotype which shows stable performance and high mean yield across all tested locations is considered as ideal genotype. Such an ideal genotype rarely exists in nature, but its position could be used as reference for selection of desirable genotypes. In GGE biplot, concentric circles are made which show the distance from the ideal genotype and between each genotype. First circle of GGE biplot graph contains ideal genotype and the genotypes close to it are considered as desirable genotypes and could be selected for breeding purpose. Genotype 1 could be considered as ideal genotype as it is present in first circle and the genotypes 8 and 7 are close to it which are considered as most desirable genotypes (Fig. 4) The genotypes 4, 9, 10 and 11 are predicted as poor yielding genotypes as they showed maximum distance from the position of the ideal genotype (Fig. 4).

Comparison of tested locations with ideal environment**:** In Fig. 5 arrow present on x-axis represents the ideal environment and the locations near to it will be considered as desirable locations. The concentric circles present in GGE biplot graph shows the distance present between each environment and the ideal environment. In Fig. 5 the locations BWP-2, BWP-1 and MTN were close to ideal environment so considered as best test locations followed by RWD but FSD-2 and FSD-1 were the poor locations.

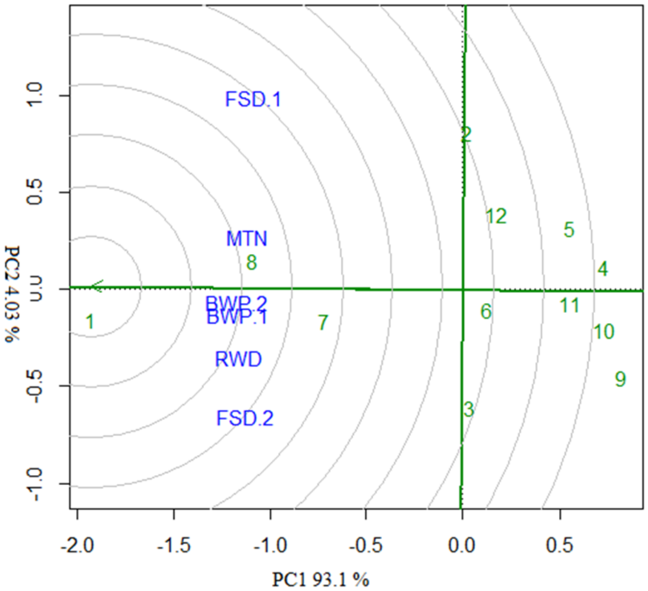

Figure 4. Ranking of genotypes with respect to ideal genotype. Genotypes are represented by numbers and environments by their abbreviations. PC1 and PC2 are first and second principal components respectively

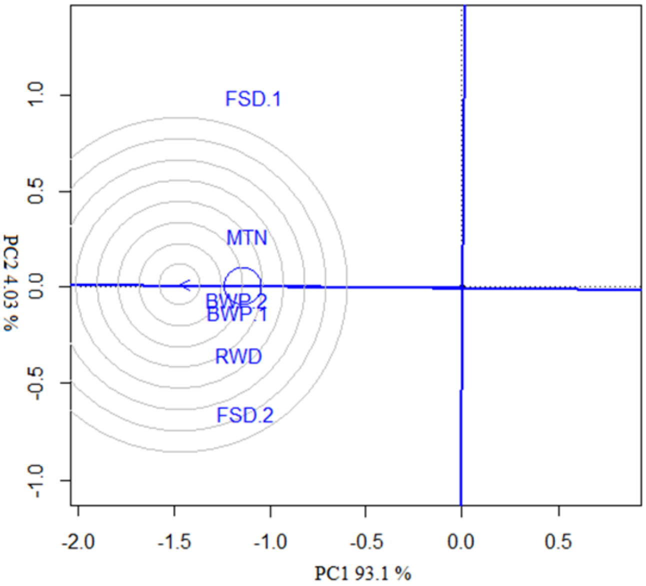

Figure 5. Ranking of the environments based on discriminating ability and representativeness of the average environment. Environments are represented by their abbreviations. PC1 and PC2 are first and second principal components respectively.

Relationship among tested locations**:** The GGE biplot also explains the differentiating ability and representativeness of the studied environments. The lines joining the environment markers and the biplot origin are environment vectors and length of these vectors explain the distinguishing ability of the environment. The more the vector length the more discriminate is that environment (Fig. 6).

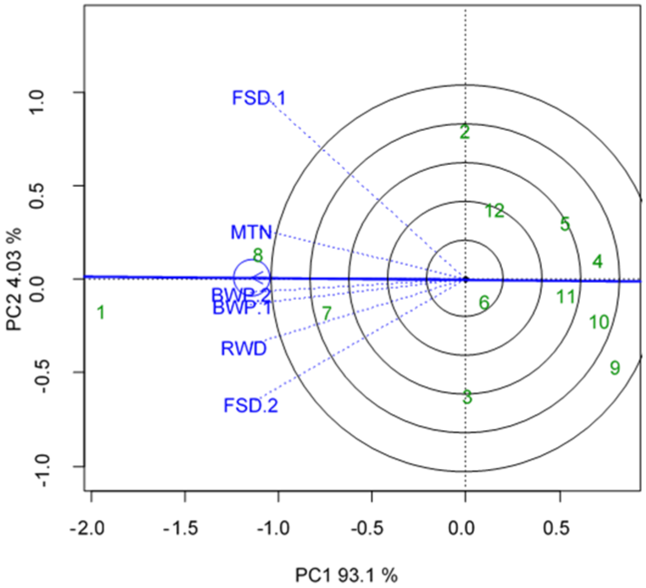

Figure 6. Representativeness and differentiating ability of the environments. Genotypes are represented by numbers and environments by their abbreviations. PC1 and PC2 are first and second principal components respectively.

In Fig. 6 the locations FSD-1 and FSD-2 are more discriminant. The projection of the environment markers from y-axis in both directions explains about their representativeness. Environments with less projection are more representative. In the current study the locations BWP-1, BWP-2 and MTN are more representative. Angle present among these vectors show correlation among environments, locations with acute angle values have significant correlation while the locations with obtuse angle values have non-significant correlation. In this study all the tested locations are significantly correlated with each other.

Comparison between two contrasting genotypes**:** The GGE biplot has another feature of comparing any two genotypes visually. In Fig.7 two circles show compared genotypes and a straight line is joining these two genotypes. A perpendicular line passes from biplot origin called the equality line telling that the compared genotypes must be equal in all environments present on this line. Fig. 7 shows that all the locations are on the side of genotype 1suggesting that this genotype perform well in these locations. There is no location on the side of genotype 4 indicating that this genotype did not perform well in any of the environment.

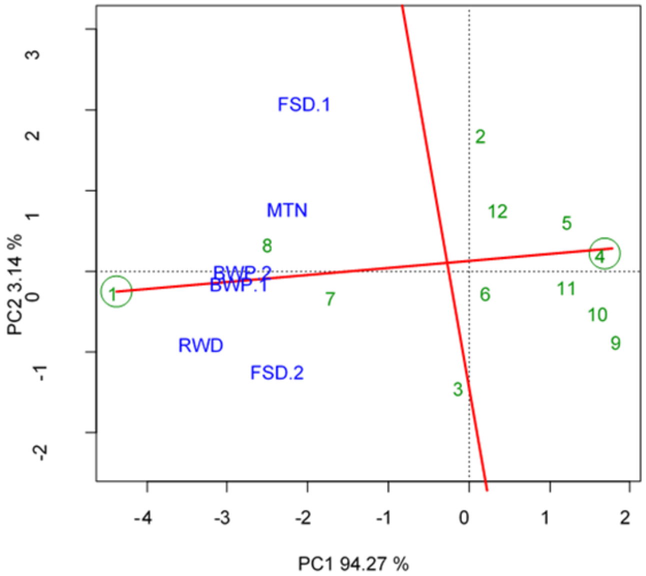

Figure 7. Comparison of two cultivars in different environments. Genotypes are represented by numbers and environments by their abbreviations. PC1 and PC2 are first and second principal components respectively.

DISCUSSION

Adaptability and stability of a genotype are pre-requisite to release a cultivar for general cultivation in a specific area. Presence of GEI creates hurdles in the selection of variety which remain stable over changing environments (Khar *et al.*, 2007). Multi-locational trials are a crucial part of breeding programs as they generate data for depicting GEI. As GEI changes the performance of genotypes in multiple environments so it is necessary to study GEI effects on yield (Kaya *et al.*, 2006). Combined analysis of variance showed highly significant (at $P<0.001$) GEI indicating the presence of crossover and non-crossover interaction and suggesting breeders must study behavior of genotypes in multiple environments either to develop or select a variety for the targeted environment or choose a genotype which exhibited stable performance across a range of environments. More than 86% of the total variation was exhibited by genotypes representing the diverse nature of germplasm. Other studies of multi-environment trials also found significant genotype × environment interaction in different crops i.e. wheat, rice, garlic, chickpea, lentil, barley and maize (Dehghani *et al.*, 2006; Koutis *et al.*, 2012; Batth *et al.*, 2013; Karimizadeh *et al.*, 2013; Munawar *et al.*, 2013; Akter *et al.*, 2015; Erdemci, 2018; Bocianowski *et al.*, 2019).

GGE biplot analysis is an effective tool to study and analyze multivariate data for making inferences. It makes possible to visually analyze two-way tables by making different graphs indicating similarities and differences present in the studied genotypes (Frutos *et al.,* 2014). The biplot revealed that variation is present in onion germplasm which could be valuable for breeding of new high yielding most stable onion cultivar. Results of the current study indicated the presence of one mega-environment for onion genotypes. Munawar *et al.* (2013) also studied maize hybrids in multi-location trials through GGE biplot and found the presence of two mega-environments for tested genotypes. In other studies, mega-environments for different genotypes of chickpea, lentil, barley, garlic, wheat and rice were also identified throughout the world (Dehghani *et al.,* 2006; Koutis*et al.,* 2012; Karimizadeh *et al.,* 2013; Akter *et al.,* 2015; Singh *et al.,* 2016; Erdemci, 2018). Fifteen genotypes of chickpea were evaluated in multi-location trials and the tested locations were grouped into two mega-environments by GGE biplot (Erdemci, 2018). Another biplot identified one mega-environment for six rice genotypes in Bangladesh (Akter *et al.,* 2015).

Selection of proper location for a genotype is vital prior to its cultivation, for this purpose which-won-where tool of GGE biplot was used which identified five vertex genotypes and dark red as the winning genotype in all the locations depicting that it performed above average in all the tested locations. According to previous studies, the vertex genotypes are corner genotypes possessing maximum distance from biplot origin and these are the most responsive genotypes either high yielding or poor yielders (Yan and Tinker, 2006). In our study Desi red and Early red are found as average yielders and VRIO-9 as poor genotype among all tested genotypes. Dehghani *et al.*(2006) studied nineteen genotypes of barley and found seven genotypes as vertex genotypes and three out of them as high yielder. Batth *et al.*(2013) evaluated 25 garlic genotypes and seven were identified as vertex genotypes. Stability represents the maintenance of yield performance of a genotype over diverse environments and assessment of mean performance and stability of a genotype is needed prior to its selection (Brdar-Jokanović *et al.,* 2016). VRIO-1 and Phulkara were found as desirable genotypes based on mean performance and stability suggesting that these genotypes could be selected for the studied location. In the tested onion genotypes, dark red found closer to the ideal genotype means that it is the genotype with high bulb yield and greater yield stability in studied locations and performance of other genotypes could be assessed relative to its performance. Nineteen genotypes of barley were compared with reference to ideal genotype in Iran using GGE biplot to find out best performing genotypes (Dehghani *et al.,* 2006). Genotypes of chickpea, garlic and maize were also evaluated with ideal genotype through GGE biplot (Batth *et al.,* 2013; Munawar *et al.,* 2013; Erdemci, 2018).

An environment with large PC1 scores and small value of PC2 scores is considered as an ideal environment meaning that it is more representative of all the tested locations and has greater ability to differentiate the tested genotypes. This environment usually does not exist in nature, but its value could be used as a reference in the selection of most suitable environment. Bahawalpur was found to be the desired location for the tested genotypes as it is more informative and differentiated bulb yield of genotypes more accurately.Bahawalpur-1, Bahawalpur-2 and Multan are closely related to each other and are near to the ideal environment suggesting that these locations are the most appropriate for onion cultivation with Dark red as the most suitable genotype producing high bulb yield. So GGE biplot could be efficiently used to study GEI, relationship among different environments, selection of desirable genotypes, identification of ideal genotype/environment, study diverse environments and to assess adaptability and stability based on mean performance of cultivars.

Conclusion: Twelve genotypes of onion were evaluated based on mean performance of yield over six locations using GGE biplot analysis. Combined ANOVA revealed highly significant G, E and GEI effects on bulb yield. Highly significant GEI intends to study interaction in detail, so by using it is easy to select genotype for a specific environment. Presence of highly significant interaction showed that bulb yield response of genotypes varied with variation in environment. GGE biplot analysis allowed evaluation of interaction effect of a genotype in every location and it assists to identify genotypes which are most appropriate for the targeted environment. The genotypes Dark red, VRIO-1 and Phulkara could be used as parents in further breeding programs as they showed high mean bulb yield and more stability. It is concluded from the current study that different cultivars should be developed for diverse environments.

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