Summarizing the genotype × environment interaction and mega-environments delineation using LG biplot analysis of unrepeatable multi-environment bread wheat yield trials data of southern warm and dry agro-climatic zone in Iran

S. Tahmasebi [1]*, M. Esmaeilzadeh Moghaddam[2], M. Tabib Ghaffari [3], M. Sayyahfar [4], Kh. Miri[5], Gh. A. Lotfali Ayeneh[6], H. Akbari Moghaddam[7] and S. Osroosh[8]

[1]Seed and Plant Improvement Department, Fars Agriculture and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization, Shiraz, Iran.
[2]Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization, Karaj, Iran.
[3]Seed and Plant Improvement Department, Safiabad Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization, Dezful, Iran.
[4]Seed and Plant Improvement Department, Lorestan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization, Khorramabad, Iran.
[5]Seed and Plant Improvement Department, Baloochestan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization, Iranshahr, Iran.
[6]Seed and Plant Improvement Department, Khuzestan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization, Ahvaz, Iran.
[7]Seed and Plant Improvement Department, Sistan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization, Zabol, Iran.
[8]Seed and Plant Certification and Registration Institue, Agricultural Research, Education and Extension Organization, Karaj, Iran.
*Corresponding author's Email address: stahmasebi2000@yahoo.com

ABSTRACT

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In this study LG (location-grouping) biplot analysis, as a new method, was used to identify repeatable and unrepeatable GEI patterns and to delineate mega-environments using grain yield data of five multi-environment bread wheat trials from six southern warm and dry agro-climatic zone of Iran including Khorramabad (KHR), Darab (DAR), Dezful (DEZ), Iranshahr (ISH), Ahvaz (AHV) and Zabol (ZAB). The trials included 18, 32, 28, 28 and 28 elite bread wheat genotypes. Each of genotype sets was evaluated in two successive cropping seasons of 2012-14, 2013-15, 2014-16, 2015-17 and 2016-18, respectively. The highest (7.99 ton ha^{-1}) and lowest (4.33 ton ha^{-1}) grand mean of testing locations across ten trials were observed in KHR and AHV, respectively. Results of the yearly GGE biplots based on the grain yield data from the 2012-13 to 2016-18 cropping seasons of 10 bread wheat yield trials across six locations varied from cropping cycle to cropping cycle, thus it was difficult to extract the common patterns across cropping seasons and grouping the test locations using two-year grain yield data. When these datasets were incorporated in a LG biplot analysis, six locations were divided into four MEs. The LG biplot explained 49.86% of the total variation of the two-way correlation table. KHR ZAB locations formed ME1 and ME2, respectively. AHV and Iranshahr ISH formed ME3, while DAR and DEZ grouped in ME4. Unlike ME1 and ME2, which had negative correlation with each other and with other MEs, ME3 and ME4 were weakly correlated, therefore, a genotype with the highest grain yield in ME3 may perform well in ME2, and vice versa. Result of this study can help bread wheat breeders to understand the bread wheat growing MEs in the southern warm and dry agro-climatic zone of Iran, and lead to better decision-making for the analysis of multi-environments yield trial data and to identify and release high-yielding bread wheat cultivars adapted to each ME.

Keywords: bread wheat, Cropping season, LG biplot, high yielding cultivar, total variation

INTRODUCTION

Increasing grain yield potential and improving yield stability for sustainable crop production is one of the most important objectives of bread wheat breeding programs. Due to the high genotype × environment interactions (GEI) in diverse regions, no crop cultivar performs the best in all environments, therefore, achieving yield stability is a major challenge of breeders in crop breeding programs (Cattivelli *et al*., 2008). Multi-environment trials are used for evaluating GEI and selecting the desirable genotypes (Sabaghnia *et al*., 2013).

In multi-environment trials (METs), evaluating genotypes in multi-location and multi-year yield trials is necessary to be able to verify GEI. Identifying repeatable GEI and accommodating non-repeatable GEI are two approaches to dealing with GEI (Yan, 2016). Identifying repeatable GEI is used to group the heterogeneous target areas into meaningful mega-environments (ME) with more homogeneous conditions, which could be used for breeding ME-specific cultivars (Yan *et al*., 2019). Non-repeatable GEI is used to select high mean yield with high yield stability cultivars within a ME (Yan, 2016).

Genotype plus GEI (GGE) biplot analysis (Yan *et al*., 2000) is one of the most known and appealing multiplication approaches for modelling GEI in multi-environment trials (METs) and can help breeders to determine whether the target cropping region is homogeneous or should be grouped into different mega-environments (Dardanelli *et al*., 2006). GGE biplot analysis has been used by numerous researchers in ME analysis for different regions and crops (Voltas *et al*., 2005; Dardanelli *et al*., 2006; Dehghani *et al*., 2006; Mohammadi *et al*., 2010; Rakshit *et al*., 2012; Munaro *et al*., 2014; Xu *et al*., 2014; Luo et al., 2015; Swanckaert *et al*., 2020; Tahmasebi *et al*., 2021).

In this method, delineation of a target region or regions into mega-environments must be based on repeatable GEI patterns, which can be obtained only from multi-year, multi-location crop yield trials (Yan, 2015).

However, data from multi-location yield trials, in all major crops, conducted every year in every region have rarely been utilized to extract repeatable GEI patterns, because different sets of genotypes are tested in different years (Yan, 2019). In most multi-location yield trials one set of genotypes is only tested in two years. In these cases, summarization across years is difficult and subjective (Yan *et al*., 2021). Some attempts have been made to utilize such datasets to summarize the GEI patterns across years. LG (location-grouping) biplot is a new method recently developed (Yan, 2019) and can be used to summarize the results of GEI patterns with different genotypes across years. This new method allows utilization of existing yield trial data to identify repeatable GEI patterns, to delineate mega-environments, and to understand the scope of unrepeatable GEI at a location and within a mega-environment (Yan, 2019).

Bread wheat is the most widely grown and the most important staple food crop in Iran. Wheat production in the country is frequently influenced by unpredictable environmental conditions particularly in southern warm and dry agro-climatic zone (Zone II) (Jalal Kamali and Duveiller, 2006). Breeding the high yielding and widely adapted wheat cultivars resistant to biotic and abiotic stresses is the main objective of breeding programs in these environments. Under these conditions, bread wheat breeders are always challenged by significant GEI that complicates the identification of superior genotypes. Mega-environment analysis of bread wheat yield trials in these environments is difficult, because of the geographical extent and different climatic and agro-ecological conditions of this agro-climatic zone. Analysis of bread wheat yield trials data using the strategy of yearly analysis and summarizing across two years in many cases does not reveal the repeatable GEI patterns for the ME differentiation.

The main objective of this study was to identify MEs covering southern warm and dry agro-climatic zone of wheat production areas of Iran using different and long term unrepeatable multi-year and multi-location bread wheat trial data, which can be useful for developing the national bread wheat breeding strategies for these areas.

MATERIAL AND METHODS
Grain yield data

Grain yield data of the five multi-

environment bread wheat adaptation trials from 2012-13 to 2017-18 cropping seasons from Darab (DAR), Dezful (DEZ), Ahvaz (AHV), Khorramabad (KHR), Zabol (ZAB) and Iranshahr (ISH) were compiled and used in this study. The sites are representative of the major irrigated wheat production in the southern warm and dry agro-climatic zone of Iran. Description of testing locations and their average meteorological data from 2012-13 to 2017-18 cropping seasons is given in Table 1.

Each dataset included grain yield of 18, 32, 28, 28 and 28 elite bread wheat genotypes tested in six test locations in two successive cropping seasons of the 2012-14, 2013-15, 2014-16, 2015-17 and 2016-18, respectively. In each year, the trials at DEZ and KHR were grown under optimum irrigation conditions while trials at DAR, AHV, ZAB and ISH were grown under terminal drought stress conditions.

In optimum irrigation conditions, genotypes were well irrigated throughout the cropping cycle, while in terminal drought experiments, irrigation was ceased from late booting stage. In each trial, genotypes were planted in an alpha lattice design (Patterson & Williams, 1976) with four replications. Plots consisted of six rows that were six m long with row spacing of 0.20 m. The seeding rate was 400 seeds m^{-2} at each trial. Agronomic practices including fertilizer application and weed management carried out as recommended at each location. Following harvest, grain yield (ton ha^{-1}) was determined for each genotype at each testing environment.

Table 1. Geographical coordinates of test locations and their average meteorological data from 2012-13 to 2017-18 growing seasons

Location	Code	Moisture management	Altitude (m)	Latitude	Longitude	Temperature (°C)			Rainfall (mm)
						Min.	Max.	Ave.	
Ahvaz	AHV	Terminal drought	18	31°24´ N	48°52´ E	14.1	27.3	20.7	157.2
Darab	DAR	Terminal drought	1080	28°47´ N	54°17´ E	8.6	23.3	15.9	340.1
Dezful	DEZ	Optimum irrigation	83	32°15´ N	48°25´ E	17.0	25.7	21.4	343.6
Iranshahr	ISH	Terminal drought	591	27°15' N	60°40' E	15.3	29.5	22.4	69.9
Khorramabad	KHR	Optimum irrigation	1148	33°30' N	48°25' E	4.4	18.6	11.5	438.5
Zabol	ZAB	Terminal drought	490	31° 18´ N	61° 15´ E	9.7	25.7	17.7	37.4

GGE biplot analysis

GGE biplot analysis was conducted for the yearly yield data from the 2012-13 to 2017-18 trials using GenStat 15 software (Payne et al., 2012). The data of genotype × environment interaction effect on mean values of grain yield in each of test locations and year were standardized before subjecting to GGE biplot analysis. The standardization was conducted using the following equation (Yan, 2019):

Equation (1) $$Y_{ij} = \frac{T_{ij} - \bar{T}_j}{S_j}$$

Where, Y_{ij} is the standardized grain yield of genotype i in environment j, T_{ij} is the original grain yield of genotype i in environment j, $\bar{T}_j$ is the mean grain yield of genotypes in environment j, and S_i is the standard deviation in environment j.

Totally ten yearly standardized data of the six test locations were subjected to GGE biplot analysis. Before that, two-way genotype-to-environment tables of six locations were constructed for each trial (original data not shown). GGE biplots were performed based on the first two principal components (PC)

resulting from subjecting the standardized genotype-by-environment table (Pij) to SVD for each data series. This process decomposes the table into genotype eigenvalues, environment eigenvalues, and singular values (Yan, 2013):

$P_{ij} = (d\lambda_1{}^\alpha \zeta_{i1})(\lambda_1{}^{1-\alpha} \tau_{1j}/d) + (d\lambda_2{}^\alpha \zeta_{i2})(\lambda_2{}^{1-\alpha} \tau_{2j}/d) + \varepsilon_{ij}$,

where ζ_{i1} and ζ_{i2} are the eigenvalues for PC1 and PC2, respectively, for genotype i; τ_{1j} and τ_{2j} are the eigenvalues for PC1 and PC2, respectively, for environment j, and ε_{ij} is the residual from fitting PC1 and PC2 for genotype i in environment j; λ_1 and λ_2 are the singular values for PC1 and PC2, respectively. α is the singular value partitioning (SVP) factor. When $\alpha = 1$ (i.e., SVP = 1), the biplot is genotype-focused, and use for comparing genotypes. When $\alpha = 0$ (i.e., SVP = 2), the biplot is environment-focused, and is suitable for visualizing correlations among environments (Yan, 2013). The scalar d is chosen such that the length of the longest vector among genotypes equals to that among environments; this is important for generating

a functional biplot. The GGE biplot was constructed by plotting ($d\lambda_1^\alpha \tau_{i1}$) against ($d\lambda_2^\alpha \tau_{i2}$) for genotypes and plotting ($\lambda_1^{1-\alpha}\tau_{1j}/d$) against ($\lambda_2^{1-\alpha} \tau_{2j}/d$) for environments in the same plot (Yan, 2013).

LG biplot analysis

LG biplot analysis was employed based on the method developed by Yan (2019). This method includes two steps. First, the yearly Pearson correlations among six test locations across tested genotypes were calculated to form a location by trial table of correlations. This table was subjected to SVD (Singular Value Decomposition) and displayed in a LG biplot in the second step.

The process of generating a LG biplot is the same as generating a GGE biplot (Equation 2) except to define Y_{ij} as the Pearson correlation coefficient between location i and location-year combination j and replace "genotypes" with "locations" and "environments" with "trials" or "location-year combinations" (Yan, 2019). No scaling or centering was performed before subjecting the correlation table to SVD (denoted as "Scaling = 0" and "Centering = 0"). The LG biplot, therefore, approximates the correlation values of test locations. In this method, information of tested genotypes as random samples in the population is not required in LG biplot analysis (Yan, 2019).

RESULTS AND DISCUSSION
Grain yield means of the wheat trials

Mean grain yield of genotype sets at each location in each cropping season were presented in Table 2. Genotype means of trials ranged from 3.27 ton ha^{-1} (in ERWYT2015-Y2 at ZAB) to 8.86 (in ERWYT2016-Y2 at KHR). The highest (7.99 ton ha^{-1}) and lowest (4.33 ton ha^{-1}) grand mean of testing locations across ten trials were observed in KHR and AHV, respectively (Table 2). In general, at the most trials, KHR had the highest mean of grain yield. AHV and ZAB had the lowest means of grain yield in most trials, respectively (Table 2).

Table 2. Mean grain yield (±SE) of bread wheat genotypes at each location in each elite regional wheat yield trials from 2012-13 to 2017-18 cropping seasons

| | | | Locations | | | | | | | | | | |
| | | | KHR | | DAR | | DEZ | | ISH | | AHV | | ZAB | |
Trial	Cropping season	Number of genotypes	Genotypes mean (ton ha^{-1})	SE	Genotypes mean (ton ha^{-1})	SE	Genotypes mean (ton ha^{-1})	SE	Genotypes mean (ton ha^{-1})	SE	Genotypes mean (ton ha^{-1})	SE	Genotypes mean (ton ha^{-1})	SE
ERWYT2012-Y1	2012-13	18	6.93	0.44	7.47	0.50	4.31	0.73	5.81	0.42	5.25	0.38	6.75	0.67
ERWYT2012-Y2	2013-14	18	7.32	0.38	6.97	0.40	5.34	0.44	5.24	0.66	4.16	0.49	6.60	0.92
ERWYT2013-Y1	2013-14	32	7.42	0.68	6.10	0.59	5.62	0.91	6.14	1.10	4.37	0.47	6.63	0.69
ERWYT2013-Y2	2014-15	32	7.72	0.54	5.73	0.86	5.82	0.76	6.37	0.67	3.93	0.42	3.36	0.49
ERWYT2014-Y1	2014-15	28	7.76	0.89	6.34	0.48	6.02	0.29	7.37	0.71	4.57	0.32	4.16	0.54
ERWYT2014-Y2	2015-16	28	8.85	1.01	5.54	0.45	5.31	0.45	6.93	0.63	4.31	0.41	4.14	0.38
ERWYT2015-Y1	2015-16	28	8.54	0.76	4.74	0.35	5.21	0.61	5.98	0.67	4.28	0.28	4.86	0.60
ERWYT2015-Y2	2016-17	28	8.66	0.58	4.93	0.62	6.23	0.46	4.84	0.40	4.21	0.30	3.27	0.71
ERWYT2016-Y1	2016-17	28	7.81	0.52	5.20	0.49	5.88	0.53	4.89	0.62	4.17	0.47	3.54	0.74
ERWYT2016-Y2	2018-19	28	8.86	1.12	6.90	0.59	5.26	0.88	3.90	0.51	4.03	0.44	3.62	0.54
Grain yield grand mean			7.99		5.99		5.50		5.75		4.33		4.69	

SE: standard error of mean grain yield of genotypes in each trial.

Yearly correlations and GGE biplot analysis

The yearly correlations among test locations in each of the 60 trials (5 genotype data set × 6 location × 2 repeated cropping seasons for each genotype data sets) from the evaluation of promising bread wheat lines in elite regional uniform yield trials (ERWYT) in southern warm and dry agro-climatic zone of Iran is presented in Table 3. Some locations (e.g., DAR and DEZ) showed more positive and significant correlation with the other locations in different trials (Table 3). On the contrary, the correlation of KHR and ZAB with other locations were negative or not significant (Table 3) in most trials. Also, when correlations in two consecutive years with the common genotypes were examined, in most cases the same pattern of correlation could not be extracted. Varied correlation between each pair of locations in different years, indicating considerable non-repeatable GE interaction.

Table 3. Pearson correlations coefficients between mean grain yield of the elite regional wheat yield trials of test locations in 2012-13 to 2017-18 cropping seasons

Location	Trial name*	Trial code	Cropping season	Pearson correlation					
				KHR	DAR	DEZ	ISH	AHV	ZAB
AHV	ERWYT2012-Y1	AHV-1	2012-13	-0.44*	0.00	-0.22	0.00	1.00	-0.03
AHV	ERWYT2012-Y2	AHV-2	2013-14	0.09	-0.27	0.60**	0.29	1.00	0.43*
AHV	ERWYT2013-Y1	AHV-3	2013-14	-0.07	0.33*	0.52	0.60**	1.00	0.08
AHV	ERWYT2013-Y2	AHV-4	2014-15	-0.38*	0.71**	0.65**	0.60**	1.00	0.45**
AHV	ERWYT2014-Y1	AHV-5	2014-15	0.22	0.51**	0.11	0.08	1.00	0.28
AHV	ERWYT2014-Y2	AHV-6	2015-16	0.38*	0.22	0.12	0.30	1.00	-0.12
AHV	ERWYT2015-Y1	AHV-7	2015-16	-0.19	-0.13	0.20	-0.18	1.00	-0.03
AHV	ERWYT2015-Y2	AHV-8	2016-17	0.25	-0.11	0.02	-0.11	1.00	0.07
AHV	ERWYT2016-Y1	AHV-9	2016-17	0.28	0.48**	0.11	0.21	1.00	-0.03
AHV	ERWYT2016-Y2	AHV-10	2017-18	0.14	0.03	-0.03	0.20	1.00	-0.11
DAR	ERWYT2012-Y1	DAR-1	2012-13	-0.06	1.00	0.30	0.35	0.00	-0.24
DAR	ERWYT2012-Y2	DAR-2	2013-14	0.01	1.00	-0.34	-0.08	-0.27	-0.13
DAR	ERWYT2013-Y1	DAR-3	2013-14	0.33*	1.00	0.72**	0.54**	0.33*	0.31
DAR	ERWYT2013-Y2	DAR-4	2014-15	-0.33	1.00	0.79**	0.54**	0.71**	0.25
DAR	ERWYT2014-Y1	DAR-5	2014-15	0.32	1.00	0.01	0.44**	0.51**	0.67**
DAR	ERWYT2014-Y2	DAR-6	2015-16	0.18	1.00	0.25	0.38*	0.22	0.02
DAR	ERWYT2015-Y1	DAR-7	2015-16	-0.07	1.00	-0.03	0.09	-0.13	-0.16
DAR	ERWYT2015-Y2	DAR-8	2016-17	0.06	1.00	0.39*	0.31	-0.11	-0.14
DAR	ERWYT2016-Y1	DAR-9	2016-17	0.56**	1.00	0.24	0.34	0.48**	0.28
DAR	ERWYT2016-Y2	DAR-10	2017-18	0.15	1.00	0.13	0.23	0.03	0.21
DEZ	ERWYT2012-Y1	DEZ-1	2012-13	0.49*	0.30	1.00	0.03	-0.22	-0.43*
DEZ	ERWYT2012-Y2	DEZ-2	2013-14	0.43*	-0.34	1.00	0.42	0.60**	0.65**
DEZ	ERWYT2013-Y1	DEZ-3	2013-14	0.29	0.72**	1.00	0.74**	0.52**	0.37*
DEZ	ERWYT2013-Y2	DEZ-4	2014-15	-0.34*	0.79**	1.00	0.51**	0.65**	0.14
DEZ	ERWYT2014-Y1	DEZ-5	2014-15	-0.08	0.01	1.00	0.11	0.11	0.34
DEZ	ERWYT2014-Y2	DEZ-6	2015-16	0.34	0.25	1.00	-0.13	0.12	0.20
DEZ	ERWYT2015-Y1	DEZ-7	2015-16	-0.17	-0.03	1.00	-0.23	0.20	0.01
DEZ	ERWYT2015-Y2	DEZ-8	2016-17	0.05	0.39*	1.00	0.17	0.02	-0.38*
DEZ	ERWYT2016-Y1	DEZ-9	2016-17	0.37*	0.24	1.00	0.03	0.11	-0.07
DEZ	ERWYT2016-Y2	DEZ-10	2017-18	0.17	0.13	1.00	0.25	-0.03	-0.20
ISH	ERWYT2012-Y1	ISH-1	2012-13	-0.02	0.35	0.03	1.00	0.00	0.34
ISH	ERWYT2012-Y2	ISH-2	2013-14	-0.19	-0.08	0.42	1.00	0.29	0.32
ISH	ERWYT2013-Y1	ISH-3	2013-14	0.22	0.54**	0.74**	1.00	0.60**	0.17
ISH	ERWYT2013-Y2	ISH-4	2014-15	-0.41**	0.54**	0.51**	1.00	0.60**	0.37*

Table 3. Continued.

Location	Trial name*	Trial code	Cropping season	Pearson correlation					
				KHR	DAR	DEZ	ISH	AHV	ZAB
ISH	ERWYT2014-Y1	ISH-5	2014-15	-0.12	0.44**	0.11	1.00	0.08	0.39*
ISH	ERWYT2014-Y2	ISH-6	2015-16	-0.02	0.38*	-0.13	1.00	0.30	0.27
ISH	ERWYT2015-Y1	ISH-7	2015-16	0.12	0.09	-0.23	1.00	-0.18	0.31
ISH	ERWYT2015-Y2	ISH-8	2016-17	-0.07	0.31	0.17	1.00	-0.11	-0.15
ISH	ERWYT2016-Y1	ISH-9	2016-17	0.23	0.34	0.03	1.00	0.21	0.02
ISH	ERWYT2016-Y2	ISH-10	2017-18	0.56**	0.23	0.25	1.00	0.20	-0.18
KHR	ERWYT2012-Y1	KHR-1	2012-13	1.00	-0.06	0.49*	-0.02	-0.44*	-0.03
KHR	ERWYT2012-Y2	KHR-2	2013-14	1.00	0.01	0.43*	-0.19	0.09	0.28
KHR	ERWYT2013-Y1	KHR-3	2013-14	1.00	0.33*	0.29	0.22	-0.07	0.08
KHR	ERWYT2013-Y2	KHR-4	2014-15	1.00	-0.33	-0.34*	-0.41*	-0.38*	-0.09
KHR	ERWYT2014-Y1	KHR-5	2014-15	1.00	0.32	-0.08	-0.12	0.22	0.09
KHR	ERWYT2014-Y2	KHR-6	2015-16	1.00	0.18	0.34	-0.02	0.38*	0.07
KHR	ERWYT2015-Y1	KHR-7	2015-16	1.00	-0.07	-0.17	0.12	-0.19	-0.14
KHR	ERWYT2015-Y2	KHR-8	2016-17	1.00	0.06	0.05	-0.07	0.25	-0.16
KHR	ERWYT2016-Y1	KHR-9	2016-17	1.00	0.56**	0.37*	0.23	0.28	0.08
KHR	ERWYT2016-Y2	KHR-10	2017-18	1.00	0.15	0.17	0.56**	0.14	0.11
ZAB	ERWYT2012-Y1	ZAB-1	2012-13	-0.03	-0.24	-0.43*	0.34	-0.03	1.00
ZAB	ERWYT2012-Y2	ZAB-2	2013-14	0.28	-0.13	0.65**	0.32	0.43	1.00
ZAB	ERWYT2013-Y1	ZAB-3	2013-14	0.08	0.31	0.37*	0.17	0.08	1.00
ZAB	ERWYT2013-Y2	ZAB-4	2014-15	-0.09	0.25	0.14	0.37*	0.45**	1.00
ZAB	ERWYT2014-Y1	ZAB-5	2014-15	0.09	0.67**	0.34	0.39*	0.28	1.00
ZAB	ERWYT2014-Y2	ZAB-6	2015-16	0.07	0.02	0.20	0.27	-0.12	1.00
ZAB	ERWYT2015-Y1	ZAB-7	2015-16	-0.14	-0.16	0.01	0.31	-0.03	1.00
ZAB	ERWYT2015-Y2	ZAB-8	2016-17	-0.16	-0.14	-0.38*	-0.15	0.07	1.00
ZAB	ERWYT2016-Y1	ZAB-9	2016-17	0.08	0.28	-0.07	0.02	-0.03	1.00
ZAB	ERWYT2016-Y2	ZAB-10	2017-18	0.11	0.21	-0.20	-0.18	-0.11	1.00

* and **: significant at the 5% and 1% probability levels, respectively.
Y1 and Y2 in the trial name column are the first and second year of the elite regional wheat yield trials (ERWYT) with the same genotypes. AHV: Ahvaz, DAR: Darab, DEZ: Dezful, KHR: Khorramabad, ISH: Iranshahr, ZAB: Zabol

The yearly GGE biplots summarizing the yield data from the 2012-13 to 2016-18 growing season of 10 wheat trials across six locations are presented in Figs. 1-5. In each cropping season (except 2012-13 and 2017-18), there were two trials in each test location with different genotypes; one trial with common genotypes with previous year (repeated last year trial) and other with new genotypes which are repeated in the next cropping season. The aim of yearly GGE biplot in this study was to present the degree of similarity/dissimilarity among test locations in ranking genotypes.

The studied genotypes in each year were assumed as random samples of the genotype population, so all genotypes were designated numerically (G1, G2, G3…) in the GGE biplots. The GGE biplot analyses were based on location-standardized data (Scaling =1, Centering = 2) and the singular values were partitioned entirely to the location vectors ("SVP = 2"). The cosine of the angle between two locations in the biplot approximates the Pearson correlation between them for all tested genotypes. Acute and obtuse angles indicated positive and negative correlation,

respectively. Right angles between two location vectors means lack of correlation between two locations for all tested genotypes (Yan and Kang, 2002). Moreover, the locations with longer vectors are more responsive to the genotypes.

GGE biplots based on the 2012–14 yield data are presented in Fig. 1a (2012-13 cropping season) and Fig. 1b (2013-14 cropping season). The biplots explained 55.19 and 63.54 percent of the total variation of the GEI two-way table in the first and second year, respectively. In the first year, AHV and ZAB showed the highest positive correlation. Based on the GGE pattern, there were three distinct groups in this year including; AHV/ZAB, DAR/ISH and KHR/DEZ. Each group shared common genotypes. In the second year, ZAB and DEZ showed a high positive correlation. The test location of ZAB, DEZ and AHV were placed in the same group, while other locations did not form any distinct group. There was a large GEI (indicated by the obtuse angles in Fig. 1a and Fig. 1b) between some trials in both years. In these two trials (2012-14), with common genotypes, the patterns of GEI were not same (Fig. 1).
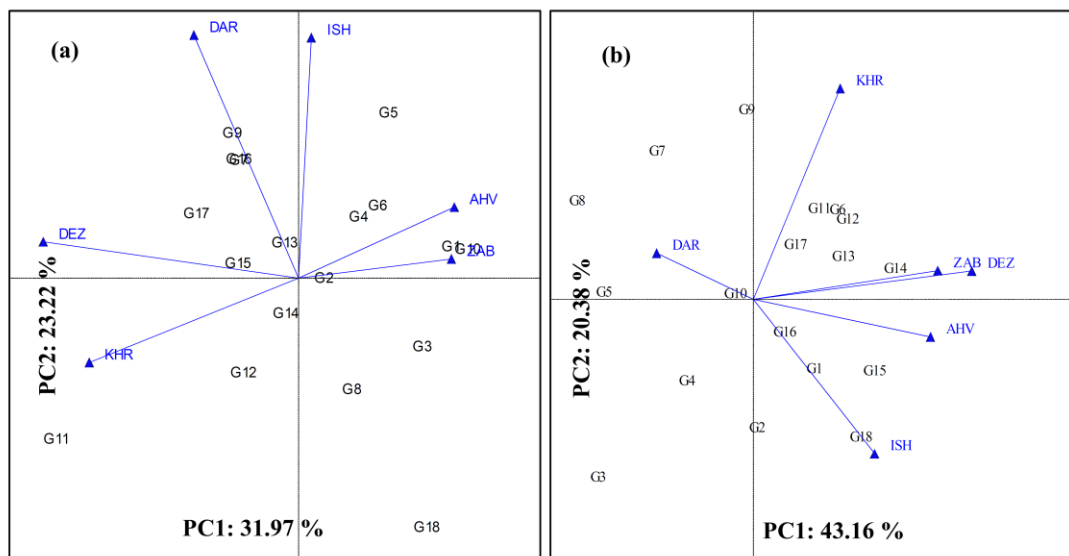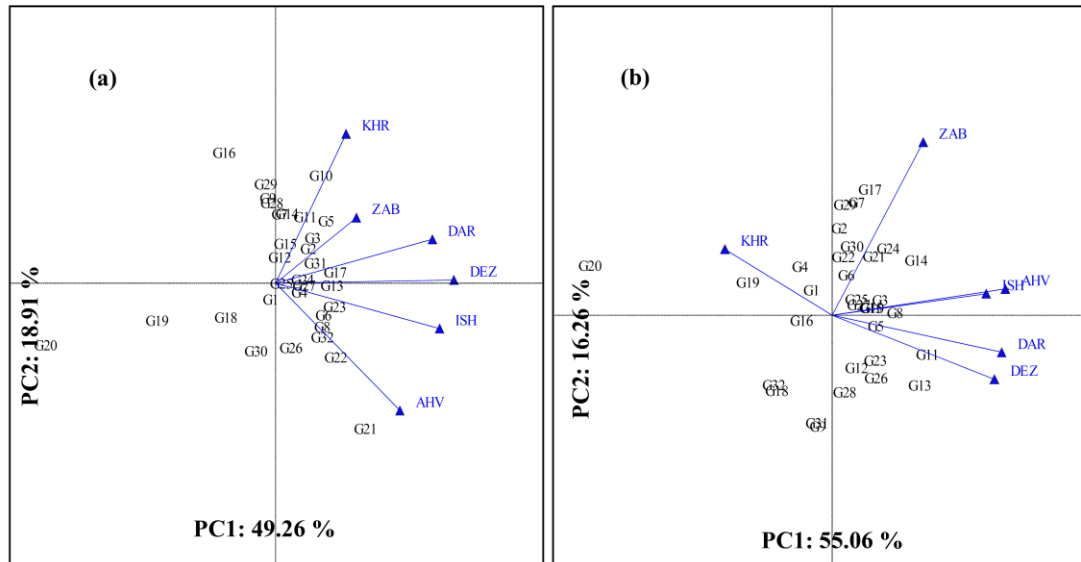

Fig. 1. The GGE biplot for grain yield based on location standardized data (Scaling = 1, Centering = 2) in elite regional wheat yield trial (ERWYT12) in (a): the first cropping seasons (2012-13) and (b): the second cropping seasons (2013-14). Environmental codes; DAR, ZAB, DEZ, AHV, KHR and ISH represent the field stations of Darab, Zabol, Dezful, Ahvaz, Khorramabad and Iranshahr, respectively.

In 2013-15 cropping seasons, with 32 genotypes in common, the biplots explained 68.17 and 71.32 percent of the total variation of the GEI two-way table in the first and second year, respectively. In two consecutive years, the test location of DAR

and DEZ, as well as AHV and ISH placed in the same groups in both growing seasons of 2014-15 and 2015-16. However, KHR and ZAB, which had a positive correlation in the first year, had no correlation in the second year (Fig. 2a and Fig. 2b)

Fig. 2. The GGE biplot for grain yield based on location standardized data (Scaling = 1, Centering = 2) in elite regional wheat yield trial (ERWYT13) in (a): the first cropping seasons (2013-14) and (b): the second cropping seasons (2014-15). Environmental codes; DAR, ZAB, DEZ, AHV, KHR and ISH represent the field stations of Darab, Zabol, Dezful, Ahvaz, Khorramabad and Iranshahr, respectively**.**

For the 2014-16 cropping season, the GGE biplots of yield data are presented in Fig. 3a (2014-15 cropping season) and Fig. 1b (2015-16 cropping season). The biplots explained 60.59 and 52.79% of the total variation of the GE two-way table in the first and second year, respectively. In these two trials with 28 common genotypes, no common GEI pattern was identified. KHR and DEZ, which had the highest negative correlation in the first year, showed the highest positive correlation in the second year. However, some locations had positive correlation in both years (e.g. DAR and DEZ as well as ISH and ZAB).

As shown in Fig. 5a and Fig 5b, in 2015-16 and 2016-18 cropping seasons each with 28 genotypes in common, the biplots explained 55.92 and 52.16% of the total variation of the GEI two-way table in the first and second year, respectively. Despite some similarities in GEI patterns (e.g. DEZ and ZAB negative correlation in both years), there was no common GEI pattern in two

consecutive years.

The main purpose of mega-environment analysis is to optimize future variety evaluation and recommendation for the target region (Yan, 2019). However, high GEI complicates the prediction of response of genotypes to environments, and the variety selection process (Subira *et al*., 2015). The problem of both correlation (Table 3) and GGE biplot (Fig. 1-5), however, is that most of GEI patterns and correlations for test locations varied in successive cropping seasons. Thus, it is difficult to extract the common patterns across years. Esmailzadeh *et al*. (2018) and Tahmasabi *et al*. (2021) reported different results for grouping of test locations in warm and dry agro-climatic zone of Iran using two-year data analysis of regional wheat variety trials. Results of the present study indicated that it is difficult to group the test locations and separation of mega-environments using two-year yield data,
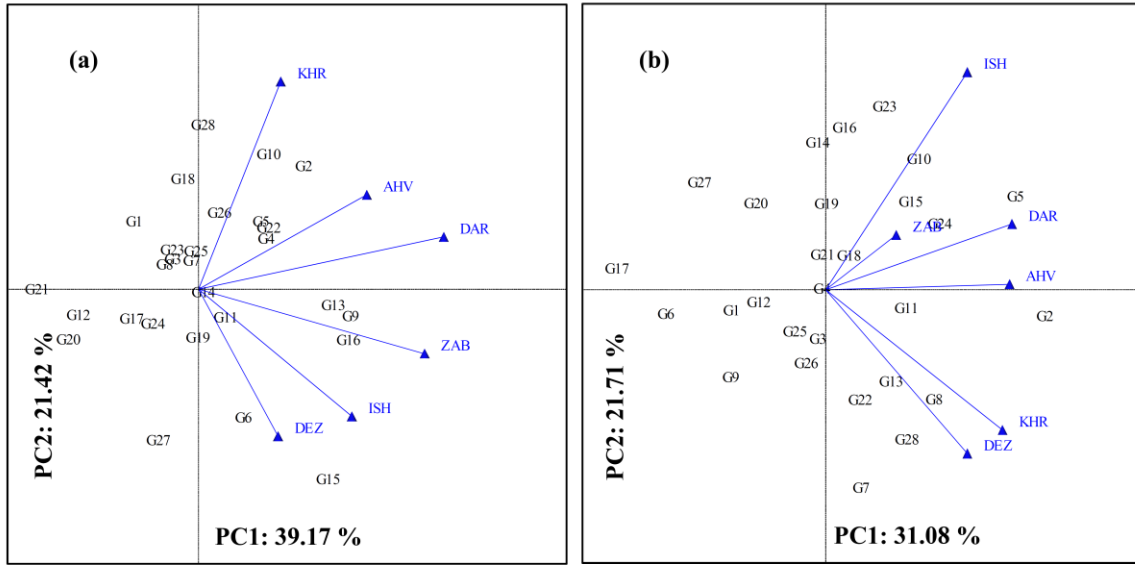
Fig. 3. The GGE biplot for grain yield based on location standardized data (Scaling = 1, Centering = 2) in elite regional wheat yield trial (ERWYT14) in (a): the first cropping seasons (2014-15) and (b): the second cropping seasons (2015-16). Environmental codes; DAR, ZAB, DEZ, AHV, KHR and ISH represent the field stations of Darab, Zabol, Dezful, Ahvaz, Khorramabad and Iranshahr, respectively.
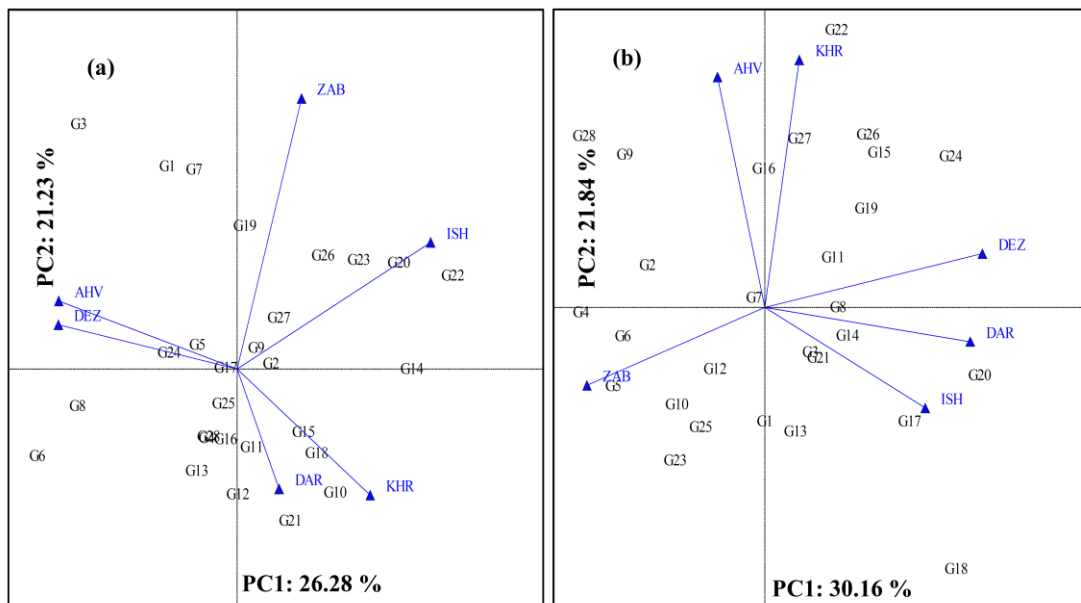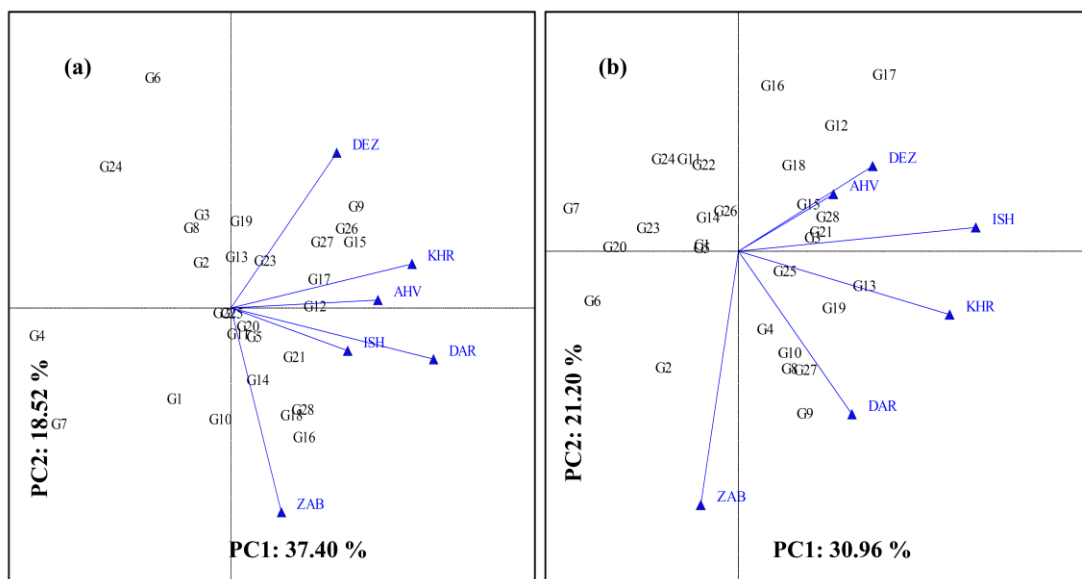

Fig. 4. The GGE biplot for grain yield based on location standardized data (Scaling = 1, Centering = 2) in elite regional wheat yield trial (ERWYT15) in (a): the first cropping seasons (2015-16) and (b): the second cropping seasons (2016-17). Environmental codes; DAR, ZAB, DEZ, AHV, KHR and ISH represent the field stations of Darab, Zabol, Dezful, Ahvaz, Khorramabad and Iranshahr, respectively.

Fig. 5. The GGE biplot for grain yield based on location standardized data (Scaling = 1, Centering = 2) in elite regional wheat yield trial (ERWYT16) in (a): the first cropping seasons (2016-17) and (b): the second cropping seasons (2017-18). Environmental codes; DAR, ZAB, DEZ, AHV, KHR and ISH represent the field stations of Darab, Zabol, Dezful, Ahvaz, Khorramabad and Iranshahr, respectively.

and the results vary from year to year. Because of high yearly variations at individual test locations and overlaps among test locations in different years, it is difficult to extract the common patterns of GEI across years and difficult to separate repeatable GEI from unrepeatable GEI (Yan, 2019; Yan *et al*., 2021). Therefore, long-term monitoring of the GEI in varied environments is essential for meaningful mega-environment delineation and grouping of environments to the sub-zones.

The LG biplot analysis to delineate mega-environments

Correlations between locations (Table 3) in each of the 10 trials from 2012-14 to 2016-18 in six locations (5 dataset ×2 year × 6 locations = 60 trials) was used to the LG (location-grouping) biplot analysis (Fig. 6). This LG biplot approximates the correlation values of Table 3. The rows and columns of the Table 3 are presented in LG biplot as location-year combinations and location points, respectively. The LG biplot explained 49.86% of the total variation of the two-way correlation table. This LG biplot allows visualization of the similarity (repeatable GEI) and variability (unrepeatable GEI) of a location in correlation with other locations

across years (Yan, 2019).

Fig. 6 can be used to form a more functional LG biplot and delineate the mega-environments as Fig. 7. For this purpose, the mean coordinates of all trials conducted at each location were used to determine the placement of the same location (Yan, 2019). For example, the placement of "AHV" was determined by the placements of the ten trials conducted at Ahvaz i.e., AHV-1 to AHV-10 trial numbers. Each cluster represents mean placement and variation of associated location across years. The overlapping or non-overlapping of clusters determines the formation of MEs (Yan *et al*., 2021). If the clusters of two locations do overlap, the two locations are considered as the same ME, and if the clusters do not overlap, the locations belong to separate MEs.

The six locations involved in 10 trials formed four distinct groups. Each of KHR (located in the west of Iran) and ZAB (located in the south-east of Iran) was placed in a distinct group and can be named ME1 and ME2, respectively. These two locations were weakly correlated and had no overlap with any other locations. AHV and ISH locations were highly correlated and felled into the same group referred to as ME3. AHV and ISH are
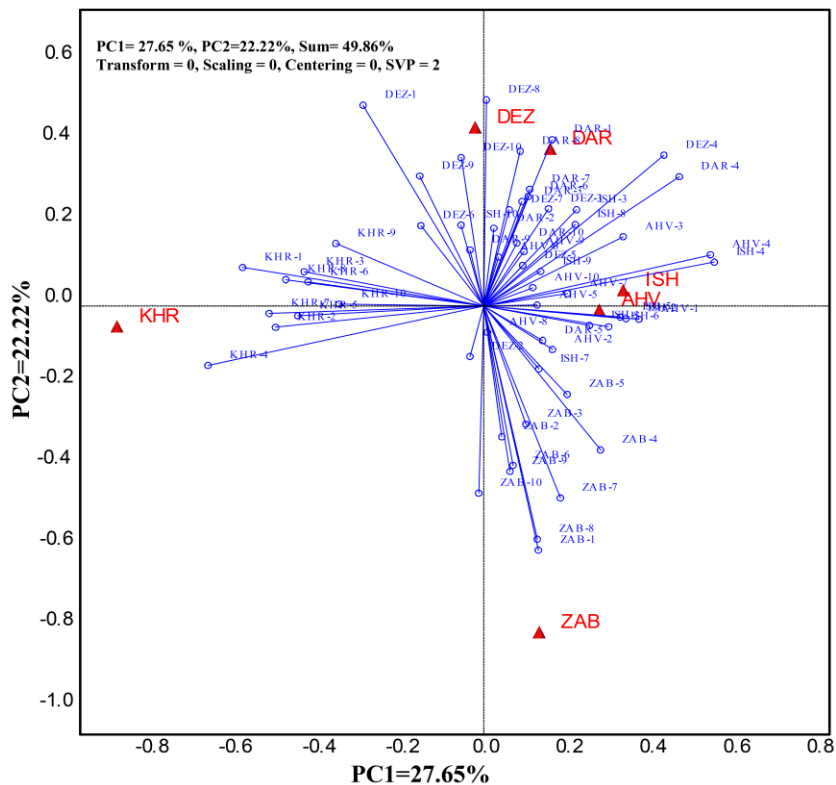
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Fig. 6. The LG (location-grouping) biplot displaying the correlations between locations in five two-year datasets from 2012-14 to 2016-18 in 6 locations (5 data set ×2 year × 6 locations = 60 trials). Each trial is presented in blue point with a location name and a number which represents the dataset and year of trial. For example, DAR-5 represents the trial of ERWYT2014-Y1 at Darab (see Table 3 for full trial description). The locations are presented in red.

located in the south-west and the south-east of the country, respectively. Both locations have low average annual rainfall and high mean temperature (for more details see Table 1).

The other group including DAR and DEZ, which had a high positive correlation, could be referred to as ME4. The GEI between MEs and GEI within a ME represents repeatable and unrepeatable GEI, respectively. Repeatable GEI can be utilized by breeders for each ME while unrepeatable GEI must be verified by testing adequately within the ME (Yan, 2016). The sum of the yearly variations represents the unrepeatable GEI for each location within a mega-environment. Magnitude of this variation determines how many years and locations are required to identify superior cultivars with yield stability for the target mega-environments.

In general, for a successful breeding program, it is not only important to evaluate the genotype, but also to identify and classify

the testing locations with optimal discriminating ability to select the desirable genotypes (Mirosavljevic *et al*., 2018). Mega-environments identification helps breeders to assess the discriminating ability and representativeness of the environments while retaining important information of the genotypes (Akinwale *et al*., 2014).

The classification of wheat growing regions of the southern warm and dry agro-climatic zone of Iran into four MEs can be consistent with the differences in many factors such as climatic factors (rainfall, air temperature, humidity, radiation,…), crop management, geographical factors (altitude, latitude, longitude), soil physico-chemical properties, biotic and abiotic stresses of the locations (Mohammadi and Amri, 2016; Mohammadi, *et al*., 2020; Tahmasebi *et al*., 2020). A mega-environment can be small in acreage and consist of only a single location if the genotypic differences in that location are not

correlated with those in any other locations within the target region (Yan, 2015).

In our study, KHR (ME1) and ZAB (ME2) were not correlated with any other location. Thus, wheat cultivars popular in each of ME1 and ME2 most likely have not a high performance in ME3 or ME4, and vice versa. These two MEs were negatively correlated with the other mega-environments, thus for each ME1 and ME2, different cultivars with specific adaptation must be selected and recommended (Yan, 2019). Unlike ME1 and ME2, which have negative correlation with each other and with other MEs, ME3

(includes AHV and ISH) and ME4 (includes DAR and DEZ) are weakly correlated (Fig. 7). Accordingly, the widely adapted cultivars with highest yield in ME3 may yield well in ME2, and vice versa. This provides an opportunity for sharing cultivars between these two MEs (Yan, 2019). For the two ME3 and ME4, two locations in the same mega-environment were not closely correlated every year and had relatively high unrepeatable GEI. Thus, multi-year multi-location tests are needed to identify superior and cultivars with yield stability for each target mega-environment.
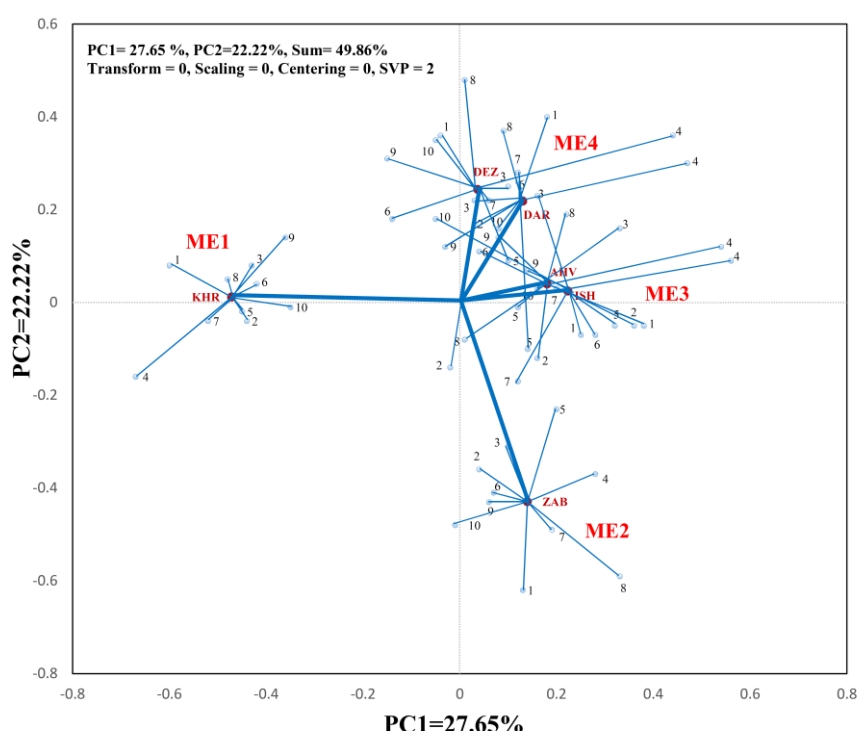

Fig. 7. The mega-environment view of the LG biplot based on subsets of the 2012 – 2016 Elite Regional Wheat Trials at six test locations. The position of each location presented in the biplot is determined using the mean coordinates of all trials conducted at that location. The trial numbers for each location are numbered from 1 to 10. For example, for DAR location the number 1 to 10 are the DAR-1 to DAR-10 trials, respectively.

CONCLUSION

In the present study, the long-term grain yield data of the Elite Regional Wheat Yield Trials (ERWYT) of six locations of the southern warm and dry agro-climatic zone of Iran including 60 environments from 2012-13 to 2017-18 cropping seasons was used for mega-environment delineation. Results showed that because of high yearly variations

at individual test locations and overlaps among test locations in different years, it is difficult to extract the common patterns of GEI across years and difficult to separate repeatable GEI from unrepeatable GEI from the yearly GGE biplot analysis.

When all trials data were incorporated in a LG biplot analysis, the six test locations were formed four distinct groups or MEs. KHR and

ZAB formed ME1 and ME2, respectively. AHV and ISH locations formed ME3, while DAR and DEZ locations made ME4. Result of this study can help breeders to better understanding of the bread wheat growing MEs in the southern warm and dry agro-climatic zone of Iran, and lead to better decision-making for the variety trial data analysis and release of high-yielding wheat cultivars with yield stability adapted to each ME.

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REFERENCES

Akinwale, R. O., Fakorede, M. A. B., Badu-Apraku, B. and Oluwaranti, A. 2014. Assessing the usefulness of GGE biplot as a statistical tool for plant breeders and agronomists. Cereal Res. Commun. 42 (3): 534-546.

Cattivelli, L., Rizza, F., Badeck, F. W., Mazzucotelli, E. A. M., Francia, E., Marè, C., Tondelli, A. and Stanca, A. M. 2008. Drought tolerance improvement in crop plants: an integrated view from breeding to genomic. Field Crops Res. 15: 1-14.

Dardanellia, J. L., Balzarinic, M., Martíneza, M. J. and Cuniberti, M. 2006. Soybean maturity groups, environments, and their interaction define mega-environments for seed composition in Argentina. Crop Sci. 46: 1939-1947.

Dehghani, H., Ebadi, A. and Yousefi, A. 2006. Biplot analysis of genotype by environment interaction for barley yield in Iran. Agron. J. 98: 388-393.

Esmaeilzadeh Moghaddam, M., Tahmasebi, S., Lotf Ali Ayeneh, G. A., Akbari Moghadam, H., Mahmoudi, K., Sayyahfar, M., Tabib Ghaffari, S. M. and Zali, H. 2018. Evaluation of grain yield stability of bread wheat (*Triticum aestivum* L.) promising lines in warm and dry regions of Iran. Iran. J. Crop Sci. 20 (1): 270-283.

Jalal Kamali, M. R. and Duveiller, E. 2006. Wheat production and research in Iran: A success story. pp. 54. In: Reynolds, M. P. and Godinez, D. (eds.) Extended abstracts of the International symposium on wheat yield potential "challenges to International wheat breeding" March 20-24th, 2006 Cd. Obregon, Mexico. CIMMYT, Mexico, DF.

Luo, J., Pan, Y. B., Que, Y., Zhang, H., Grisham, M. P. and Xu, L. 2015. Biplot evaluation of test environments and identification of mega environment for sugarcane cultivars in China. Sci. Rep. 5: e15505.

Mirosavljevic, M., Canak, P., Momcilovic, V., Jockovic, B., Zoric, M., Acin, V., Dencic, S. and Przulj, N. 2018. Identification of favorable testing location for barley breeding in south Pannonian Plain. J. Agri. Sci. 24: 303-311.

Mohammadi, R. and Amri, A. 2016. Genotype × Environment Interaction Implication: A Case Study of Durum Wheat Breeding in Iran. pp. 515-558. In: Al-Khayri, J. M., Jain, S. M. and Johnson, D. V. (eds.) Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits. Springer International Publishing.

Mohammadi, R., Sadeghzadeh, B., Poursiahbidi, M. M., Ahmadi, M. M. 2020. Integrating univariate and multivariate statistical models to investigate genotype × environment interaction in durum wheat. Ann. Appl. Biol. 178(3): 450-465.

Mohammadi, R., Haghparast, R., Amri, A. and Ceccarelli, S. 2010. Yield stability of rainfed durum wheat and GGE biplot analysis of multi-environment trials. Crop Pasture Sci. 61: 92-101.

Munaro, L. B., Benin, G., Marchioro, V. S., de Assis Franco, F., Silva, R. R., da Silva, C. L. and Beche, E. 2014. Brazilian spring wheat homogeneous adaptation regions can be dissected in major mega environments. Crop Sci. 54: 1374-1383.

Patterson, H. D. and Williams, E. R. 1976. A new class of resolvable incomplete block designs. Biometrika 63 (1): 83-92.

Payne, R. W., Murray, D. A., Harding, S. A., Baird, D. B. and Soutar, D. M. 2012. Genstat® for Windows™, 15th Edition, Introduction. VSN International, Oxford.

Rakshit, S., Ganapathy, K. N., Gomashe, S. S., Rathore, A., Ghorade, R. B., Nagesh Kumar, M. V., Ganesmurthy, K., Jain, S. K., Kamtar, M. Y., Sachan, J. S., Ambekar, S. S., Ranwa, B. R., Kanawade, D. G., Balusamy, M., Kadam, D., Sarkar, A., Tonapi, V. A. and Patil, J. V. 2012. GGE biplot analysis to evaluate genotype, environment, and their interactions in sorghum multi-location data. Euphytica 185: 465-479.

Sabaghnia, N., Mohammadi, M. and Karimizadeh, R. 2013. Yield stability of performance in multi-environment trials of barley (*Hordeum vulgare L.*) genotypes. Acta Univ. Agric. Silvic. Mendel. Brun. 61 (3): 787-793.

Subira, J., Alvaro, F. Garcia Del Moral L. F. and Royo, C. 2015. Breeding effects on the cultivar × environment interaction of durum wheat yield. Eur. J. Agron. 68: 78-88.

Swanckaert, J., Akansake, D., Adofo, K., Acheremu, K., De Boeck, B., Eyzaguirre, R., Grüneberg, W. J., Low, J. W. and Campos, H. 2020. Variance component estimations and mega-environments for sweet potato breeding in West Africa. Crop Sci. 60: 50-61.

Tahmasebi, S., Esmaeilzadeh Moghaddam, M., Tabib Ghaffari, S. M., Sayyahfar, M., Lotfali Ayeneh, G. and Akbari Moghaddam, H. 2021. Dissection of genotype × environment interaction and assessment of adaptability and grain yield stability of spring bread wheat genotypes. Crop Breed. J. 11 (1 & 2): 11-24.

Voltas, J., Lopez-Corcoles, H. and Borras, G. 2005. Use of biplot analysis and factorial regression for the investigation of superior genotypes in multi-environment trials. Eur. J. Agron. 22: 309-324.

Xu, N. Y., Fok, M., Zhang, G. W., Li, J. and Zhou, Z. G. 2014. The application of GGE biplot analysis for evaluating test locations and mega-environment investigation of cotton regional trials. J. Integr. Agric. 13: 1921-933.

Yan, W. 2013. Biplot analysis of incomplete two-way data. Crop Sci. 53(1): 48-57.

Yan, W. 2015. Mega-environment analysis and test location evaluation based on unbalanced multiyear data. Crop Sci. 55: 113-122.

Yan, W. 2016. Analysis and handling of G×E in a practical breeding program. Crop Sci. 56: 2106-2118.

Yan, W. 2019. LG biplot: A graphical method for mega-environment investigation using existing crop variety trial data. Sci. Rep. 9 (1): e7130. https://doi.org/10.1038/s41598-019-43683-9

Yan, W. and Kang, M. S. 2002. GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC Press. 286 pp.

Yan, W., Hunt, L. A., Sheng, Q. and Szlavnics, Z. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Sci. 40: 597-605.

Yan, W., Mitchell-Fetch, J., Beattie, A., Nilson, K. T., Pageau, D., De Haan, B., Hayes, M., Mountain, N., Cummiskey, A. and Mac Eachern, D. 2021. Oat mega-environments in Canada. Crop Sci. 61: 1141-1153.

Yan, W., Tinker, N. A., Bekele, W. A., Mitchell-Fetch, J. and Frégeau-Reid, J. 2019. Theoretical unification and practical integration of conventional methods and genomic selection in plant breeding. Crop Breed. Genet. Genom. 1 (2): e190003. https://doi.org/10.20900/cbgg20190003