Mega-environment investigation in durum wheat yield trials in Iran

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Abstract Mega-environment (ME) investigation and genotype evaluation are among the most important objectives of multi-environment trials. This study aims to investigate the possible existence of meaningful mega-environments and identify high-yielding and stable genotypes for rainfed durum wheat-growing regions. The GGE (genotypic main effect plus genotype-by-environment interaction) and GGL (genotypic main effect plus genotype-by-location interaction) biplots analyses were applied to durum wheat grain yield data from multi-year trials conducted at six locations differing in climatic conditions across Iran. Yearly multi-location analysis of variance for grain yield revealed that the location was the main source of variation and captured for 78.5–89.8% of total variation and the GL interaction, depending on year, was greater 2.8–4.4 times than genotype effect alone. The collective analysis of yearly GGL biplots revealed repeatable locations grouping across years and showed that the durum wheat growing region can be divided into four MEs: the western ME represented by the moderate cold location, southern-west ME represented by moderate warm location, western-north ME and eastern-north ME both represented for cold locations. The results suggest that durum wheat yield trials should be conducted and genotype recommendation be made according to each ME. The evaluation of genotypes for mean yield and stability performance per each ME, recommended the breeding line G14 for each both western and western-north MEs, breeding line G10 for southern-west ME and breeding line G3 for eastern-north ME. Based on the factorial regression analysis, climatic variables of monthly rainfall in March (17.5%), May (9.5%), June (7.9%), April (6.8%), cumulative rainfall (12.5%), and average temperature of January (6.6%) significantly ($P < 0.01$) affected genotypes performance and contributed to 60.8% of total GE variation. The results confirmed four different mega-environments in durum

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wheat yield trials, suggest genotype recommendation should be made according to each mega-environment.

Introduction

Durum wheat (*Triticum turgidum* L. subsp. durum), is one of the first domesticated food crops; is being the most cultivated cereal crop in the Mediterranean basin, traditionally grown under rainfed conditions, and is cultivated worldwide over almost 17 million ha, with a global production of 38.1 million tonnes in 2019 (FAO 2019). The main producers of durum wheat are located in the Mediterranean Basin, with cultivation of 1.6 million ha in Algeria, 1.5 million ha in Italy, and 0.5 to 0.8 million ha in Morocco, Tunisia, Turkey, Spain, Portugal, and Greece (Able and Sissons 2014; Bonjean et al. 2016; Karabina and Leonardi 2016). In addition, Egypt, Jordan, and Lebanon recognize the importance of durum wheat and cultivate it in large parts of their arable land (El-Areed et al. 2014). Durum wheat has also maintained its importance in West Asia, covering about 0.7 million ha in Iran, Iraq, and Azerbaijan combined (Bonjean et al. 2016).

The Mediterranean environments are characterized by large and unpredictable fluctuations in rainfall and temperature within and among cropping seasons, resulting in high genotype x environment (GE) interaction and crop production instability (Mohammadi et al. 2021), which implicate the interpretation of performance genotypes in multi-location trials from year to year (Gauch and Zobel 1997). The only way to achieve consistent genotype performance in a region is to divide testing locations into different mega-environments. A mega-environment (ME) can be defined as a group of analogous locations delivering similar genotypic responses when sharing the same set of genotypes across the year (Gauch and Zobel 1997; Yan et al. 2000). The ME can be effectively delineated through the "which-won-where" pattern of GGE biplot analysis (Yan et al. 2000). The aim of ME delineation is to better understanding the complex GE interaction patterns that naturally existence in the target region in order to exploring specific adaptations and maximizing the selection (Yan et al. 2011). The

ME investigation and test location evaluation are two important issues for effective evaluation genotypes through multi-year and multi-location variety trials, which are usually highly unbalanced (Yan 2015).

Yang et al. (2009) questioned the robustness of GGE biplot analysis in revealing crossover GE interaction, which was counter-criticized (Yan et al. 2010). Nevertheless, GGE biplot analysis is being increasingly used by researchers, such as in evaluating test locations for a combination of breeding traits instead of a single trait (Yan et al. 2011; Stojaković et al. 2015; da Silva et al. 2021). Yan et al. (2011) used GGE biplot to analyze the mega-environments and test-locations for oat in Quebec, Canada, and suggested two distinct MEs for the Quebec oat-growing regions (Yan et al. 2011). Luo et al. (2015) using the GGE biplot methodology recommended three major ME for Chinese sugarcane production regions represented by the 14 test environments. Stojaković et al. (2015) applied sites regression (SREG) biplot analysis for maize-growing regions production in Serbia and concluded that the grouping pattern of locations across years showed poor repeatability. They concluded that it is better to develop maize hybrids with high mean yield and stability, rather than to develop hybrids specially adapted to distinct MEs.

The GGE biplot model is among the most efficient methods for exploring genotypic diversity in the case of ME investigation, test-locations and selecting genotypes with specific adaptation to agro-ecological zones and has been adopted by numerous researchers (Yan et al. 2000; Rakshit et al. 2012; Munaro et al. 2014; Luo et al. 2015; Swanckaert et al. 2020; da Silva et al. 2021). However, these studies have followed the strategy of "analyze yearly and summarize across years" (DeLacy et al. 1996; Yan 2015). The GGE biplot model combines SREG analysis and biplot technique in order to graphical presenting both genotype and GE interaction which are the two main sources of variability and are most relevant to evaluation of genotype (Yan et al. 2000). A "which-won-where" view of GGE biplot can be used to delineate distinct MEs to identify the best-yielding genotypes in the corresponding environments (Yan et al. 2000, 2007). Once the "which-won-where" pattern is repeatable across years, then the target region should be divided into MEs. Accordingly, the superior genotypes will also identify through the evaluation of test locations on the most effective way (Yan 2015). The

two main issues of evaluation of test locations are the ability of test location to represent target ME and its ability to discriminate genotypes. However, representativeness and discriminating ability of test locations should be evaluated through multi-location trials and should be repeatable across years (Yan and Tinker 2006; Luo et al. 2015).

Annicchiarico (2002) emphasized that collecting climatic variables and measuring the plant traits at test locations in the target region will be useful in characterizing the mega-environments and extending the results to new locations. The analytical modeling of GE interaction through incorporation of environmental variables for better understanding the environmental factors that significantly affects the GE interaction and performance of genotypes in multi-location and multi-year trials seems to be high attractive strategy in breeding programs (van Eeuwijk et al. 1996; Vargas et al. 1998; Crossa et al. 1999). Among the models underling analytical (biological) interpretation of GE interaction, the factorial regression (FR) approach constitutes a simple but informative methodology for GE interaction interpretation (Denis 1988; van Eeuwijk et al. 1996) that can be used readily, in particular to recommendation purposes in breeding programs.

The rainfed durum wheat breeding program in Iran consisting of three climatic conditions that are differing in long-term rainfall, winter temperature and latitude, and accordingly are characterized as moderate warm (corresponding to Ilam location in southernwest), moderate cold (corresponding to Kermanshah location) and cold (corresponding to Maragheh and Urmia locations in western-north, and Shirvan location in eastern-north). Identifying optimal growing regions for new breeding lines based on the knowledge of genetic materials characteristics and agroecological conditions is the ultimate goal of durum breeding program for the target region. The present study aimed at examines the possible existence of mega-environments in rainfed durum wheat yield trials and the possibility of new genotypes recommendation for the target area.

Materials and methods

Twenty durum wheat genotypes including 18 promising breeding lines, originated from ICARDA and CIMMYT, and two national improved cultivars (Table 1) were evaluated at six test locations representative of rainfed testing sites for the national durum wheat breeding program (Fig. 1) for three cropping seasons (2018–19, 2019–20, 2020–21), which resulted in 17 environments. However, one environment belongs to Shirvan location in 2020–21 due to severe drought was dropped. More details on test environments are presented in Table 2. These experiments serve as elite regional durum wheat yield trials (26th ERDWYT) and represent the final stage of durum wheat breeding program. To identify high-yielding potential genotypes, another set of trials were performed under supplementary irrigation condition, in addition to rainfed trials, in moderate cold location (Kermanshah location) for three cropping seasons. Under irrigation conditions, two irrigations using sprinkler system from heading to grain filling stages to cope with terminal drought stress were applied, each with 30 mm irrigation. In each location, experimental design was a randomized complete blocks design with three replications. The size of each experimental plot was 7.2 m^2 (6 rows, 6 m long, 20 cm row spacing). The experimental management system adopted by the technical recommendations for assessment environments by the Dryland Agricultural Research Institute (DARI) of Iran.

The grain yield data were subjected to combined analysis of variance based on multi-location and multi-year trials to assess the genetic variability among durum wheat genotypes across test environments according to following equation:

$$Y_{ijk} = \mu + b/E_{jk} + G_i + E_j + GE_{ij} + \varepsilon_{ijk}$$

where, Yijk is the observation of ith genotype in the kth replication in jth environment; μ is the grand mean; b/Ejk is the effect of block kth within environment jth; Gi is the effect of the ith genotype considered as fixed; E_j is the effect of the jth environment considered as random; GEij is the random effect of the interaction between genotype ith and environment jth; and ε_{ijk} is the random error associated with observation, assumed to be independent $\varepsilon \sim N(0, \sigma^2)$. Then, to assess the repeatability of GL interaction across years, the grain yield data subjected to ANOVA based on multi-location trials per each year.

When the presence of GE or GL interactions through ANOVA model was observed, the data

Table 1 Code, pedigree/name, origin and type of genotypes tested in the study

Code	Genotypes	Type of genetic materials	Origin
G1	Saji (check)	New cultivar	Iran
G2	Zahab (check)	New cultivar	Iran
G3	ZHONGZUO/2*GREEN_3//SORA/2*PLATA_12/10/PLATA_10/6/MQUE/4/USDA573// QFN/AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/ HUI/POC//BUB/RUFO/4/FNFOOT/11/RISSA/GAN//POHO_1/3/PLATA_3//CREX/ALLA/ 4/JUPARE C 2001/5/ARMENT//SRN_3/NIGRIS_4/3/CA	Breeding line	CIMMYT
G4	LABUD/NIGRIS_3//GAN/3/AJAIA_13/YAZI/10/PLATA_10/6/MQUE/4/USDA573//QFN/ AA_7/3/ALBA-D/5/AVO/HUI/7/PLATA_13/8/THKNEE_11/9/CHEN/ALTAR 84/3/ HUI/POC// BUB/RUFO/4/FNFOOT/11/SOMAT_4/SILVER_1/3/FOCHA_1/ALAS// 4*FOCHA_1/4/SOMAT_3/PHAX_1//TILO_1/LOTUS_4	Breeding line	CIMMYT
G5	BCRIS/BICUM//LLARETA INIA/3/DUKEM_12/2*RASCON_21/5/SRN_2//YAVAUS/HUI/3/ RASCON_19/4/SOMAT_3/PHAX_1//TILO_1/LOTUS_4	Breeding line	CIMMYT
G6	CBC509CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/ AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/8/ SOOTY_9/RASCON_37//WODUCK/CHAM_3/9/ALTAR 84/BINTEPE 85//CAMAYO	Breeding line	CIMMYT
G7	STORLOM/3/RASCON_37/TARRO_2//RASCON_37/4/D00003A/5/1A.1D 5+1–06/3*MOJO/3/AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)//PLATA_13/6/SOOTY_9/ RASCON_37//WODUCK/CHAM_3/3/SOMAT_3/PHAX_1//TILO_1/LOTUS_4/7/ BCRIS/BICUM//LLARETA INIA/3/DUKEM_12/2*RASCON_21	Breeding line	CIMMYT
G8	Icasyr1/3/Bcr/Sbl5//Turartu/4/13376/Bcrch1//Ossl1/Stj5	Breeding line	ICARDA
G9	Ossl1/Stj5/5/Bicrederaa1/4/BEZAIZSHF//SD19539/Waha/3/Stj/Mrb3/6/Stk/Hau//Heca1	Breeding line	ICARDA
G10	Icamilmus5/Miki2//Geromtel1/Icasyr1	Breeding line	ICARDA
G11	Quabrach1/4/IcamorTA0462/3/Maamouri3//Vitron/Bidra1/5/Murlagost2	Breeding line	ICARDA
G12	Azeghar2/Murlagost2//Bicrederaa1/Azeghar2	Breeding line	ICARDA
G13	Plc/Ruff//Gta/Rtte	Breeding line	ICARDA
G14	Korifla/AegSpeltoidesSyr//Mrb5	Breeding line	ICARDA
G15	Mrb5/TdicoAlpCol//Cham1	Breeding line	ICARDA
G16	Sbh/4/D68193A1A//Ruff/Fg/3/Mtl5/5/Marsyr3//Saadi1989/Chan	Breeding line	ICARDA
G17	Amedakul1/TdicoSyrCol//Loukos	Breeding line	ICARDA
G18	Tdicoccum1/Ch1//IcamorTA0469/3/IcamorTA0459//CandocrossH25/Blk204144/4/Mrf1/ Stj2//Bcrch1/5/F413/3/Arthur71/Lahn//Blk2/Lahn/4/Quarmal	Breeding line	ICARDA
G19	Azeghar1/4/IcamorTA0462/3/Maamouri3//Vitron/Bidra1/5/Mgnl3/Ainzen1	Breeding line	ICARDA
G20	Zegrenses1/3/13376/Bcrch1//Ossl1/Stj5	Breeding line	ICARDA

Fig. 1 The research sites as representative of major rainfed durum growing areas of Iran for breeding programs. In Kermanshah location both rainfed and irrigated trials were conducted

were subjected to ME investigation and genotype recommendation using the GGE biplot model (Yan et al. 2000). The yearly and combined GGE biplot analyses were done based on the following model.

$$\overline{Y}_{ij} - \mu_i - \beta_j = \sum_{n=1}^{N} \lambda_n \alpha_{in} \eta_{jn}$$

where Y_{ij} is the grain yield of genotype i in environment j, μ is the grand mean, β_j is the main effect of environment j, n is the number of principal components (PC); λ_n is singular value of the nth PC; and α_{in} and η_{jn} are the scores of genotype i and environment j, respectively, for nth PC; ε_{ij} is the residual associated with genotype i in environment j (Yan et al. 2000).

The "which-won-where" pattern of the GGE biplot analysis serve as a useful tool for delineating ME, when it is based on multi-location and multi-year trials. It also allows identifying superior genotypes for each ME, and facilitatesd ranking of genotypes for mean yield and stability within each ME (Yan et al. 2007). For this, the axis of average environment coordination (AEC) abscissa denoted by a single arrowed line, represented by higher mean yield and AEC ordinate denoted by a line perpendicular to the AEC abscissa and passing through the biplot origin, indicated genotype stability. Stability is assessed by projections that connect the individual genotypes to the AEC abscissa. The shorter the projections or distance from the AEC is, being the more genotype stability is (Yan and Tinker 2006).

Representativeness and discriminating ability of test locations are two main issues of test location evaluation which must be investigated through the multi-locations data and should be repeatable across years. Test environments with longer vector are more discriminating among genotypes and those with short vector are less discriminating. Thus, in environments with less discrimination ability, genotypes express similar performance, do not present enough information on genotypic differences, and such environments should not be regarded for as test environment for genotype evaluation in breeding programs (Yan 2014). The representativeness of a target environment is shown by the angle between the environment vector and the AEC. The smaller the angle is, being the stronger the representativeness of the location is (Yan 2014).

For further evaluation of test locations, a heat map of pair-wise genetic correlation yield data matrix was applied to study the relationships between environments. The correlation values were highlighted by color according to the selected color scale; and ranged from blue (positive correlations) to red (negative correlations).

Table 2 Description of the experimental locations and their overall agro-climatic conditions

Environments				Coordinates			Position in the map	Soil type	Tem (°C)	Rainfall + IR† (mm)
Code	Cropping season	Location /station name (climatic condition)	Status	Longitude	Latitude	Altitude (m)				
KHR19	2018–19	Kermanshah/Sararood (Moderate cold)	Rainfed	47°17′N	34°19′E	1351	West	Clay-loam	11.9	782.8
KHR20	2018–19		Rainfed						12.0	518.8
KHR21	2019–20		Rainfed						13.0	317.5
KHI19	2019–20	Kermanshah/Sararood (Moderate cold)	Irrigated	47°17′N	34°19′E	1351	West	Clay-loam	11.9	782.8+60
KHI20	2020–21		Irrigated						12.0	518.8+60
KHI21	2020–21		Irrigated						13.0	317.5+60
MAR19	2018–19	Maragheh/Maragheh (Cold)	Rainfed	46°15′ N	37°33′ E	1725	Western-north	Clay-loam	6.0	494
MAR20	2019–20		Rainfed						5.5	323.8
MAR21	2020–21		Rainfed						6.7	245.5
URM19	2018–19	Urmia/Khoramabad (Cold)	Rainfed	46°04′ N	37°15′ E	1350	Western-north	Clay-loam	9.9	578.5
URM20	2019–20		Rainfed						9.2	346.2
URM21	2020–21		Rainfed						10.5	290.9
ILA19	2018–19	Ilam/ Chardavel (Moderate warm)	Rainfed	46°29′ N	33°34′ E	975			12.6	878.4
ILA20	2019–20		Rainfed				Southern-west	Sandy-loam	15.3	603.9
ILA21	2020–21		Rainfed						16.3	302.5
SHI19	2018–19	Shirvan/Shirvan (Cold)	Rainfed	58°07′ N	37°13′ E	1131	Eastern-north	loam-sand	9.2	340.9
SHI20	2019–20		Rainfed						9.4	252.9
SHI21*	2020–21		Rainfed						9.0	152.0

†IR: supplemental irrigation (two times irrigation from flowering to mid-grain filling period, each with 30 mm). *In this environment, due to severe drought condition which resulted in crop failure, no data recorded

Further analyses through the factorial regression, as described by Denis (1988), was applied to identify the most important environmental variables among 19 climatic factors consisting of cumulative rainfall and monthly rainfall and average temperature during cropping season (October to June), that affects GE interaction and location groupings in the region. The all analyses were performed using the packages of META-R (Alvarado et al. 2015), GEA-R (Pacheco et al. 2016) and PAST (Hammer et al. 2001).

Results

Weather condition

The cropping seasons varied in the amount of annual rainfall and in their monthly distribution, providing different growing conditions. Among the environments, rainfall varied among 17 environments from 152 to 878 mm. The rainfall for each location varied from year to year and ranged from 317.5–782.8 mm in Kermanshah, 302.5–878.4 mm in Ilam, 245.5–494 mm in Maragheh, 290.9–578.5 mm in Urmia and from 152–340.9 mm in Shirvan station (Table 2). The average temperature was remarkably different from location to location during three cropping seasons. The average temperature varied from 12.6–16.3 °C in Ilam, 11.9–13 °C in Kermanshah, 9.2–10.5 °C in Urmia, 9–9.4 °C in Shirvan and 5.5–6.7 °C in Maragheh. In all three years, the minimum and maximum temperatures recorded in January and June, and monthly average temperatures ranged from −5.9 (corresponding to January) to 29.2 °C (corresponding to June). The low rainfall and its unfavorable monthly distribution as well as low temperature particularly in cold locations resulted in both drought and cold stresses, which are both limiting factors for durum wheat production in cold and moderate cold locations.

Combined analysis of variance

The combined analysis of variance for grain yield revealed that the effects of genotype, environment and GE interaction were highly significant ($P < 0.01$; Table 3). The environment effect was the main source of variation and contributed to 86.1% of the total sum of squares, while genotype and GE interaction effects contributed to 0.7% and 4.4% of total variation, respectively. The variance explained by GE interaction was greater about six-folds than genotype effect. The large GE interaction effect relative to genotype reveals the possible existence of different MEs in the region. The small portion of variance captured by genotype effect indicates these genotypes are already selected for high-yielding compared to national checks in previous years during durum wheat breeding program.

The mean yield of genotypes ranged from 2227 to 2697 kg ha^{-1} across environments, with an average value of 2483 kg ha^{-1} (data not shown). Genotype G14 had the highest mean yield, while G2 (Zahab check cultivar) exhibited for the lowest mean yield. The average yield per environment ranged from 586 kg ha^{-1} (corresponding to G20 in environment MAR21) to 5635 kg ha^{-1} (corresponding to G16 in KHI19). Most of the genotypes had maximum yield in the moderate environments correspond to Kermanshah and Ilam locations, while the lowest average yield for the genotypes observed in cold environments correspond to Maragheh, Urmia and Shirvan locations.

Table 3 Combined analysis of variance for grain yield of 20 durum wheat genotypes across 17 environments

**Significant at 1% level of probability. df: degree of freedom; %TSS: percentage relative to total sum of squares

Source	df	Sum of squares	Mean squares	F-value	Prob	%TSS
Environment (E)	16	1,504,671,449	94,041,966	101.8**	0.0000	86.1
Rep/E	34	31,422,124	924,180			1.8
Genotype (G)	19	12,529,154	659,429	3.5**	0.0000	0.7
GE interaction	304	76,765,241	252,517	1.3**	0.0012	4.4
Error	646	121,845,656	188,616			7.0
Total	1019	1,747,233,624				
CV(%)		17.5				

"Which-won-where" patterns of GE interaction

Mega-environment identification

According to Yan et al. (2000), if the patterns of GE interactions are repeatable across years, MEs can be delineated; otherwise the environments are belonging to a single ME. For this, the yearly multi-location trials data were subjected to analysis of variance and the results presented in Table 4, which presents an overall picture of the relative magnitude effects of genotype, location and GL interaction. Location was always main source of grain yield variation and captured for 78.5–89.8% of total variation across years. The GL interaction in all three years was greater than genotype effect and its magnitude was between 2.8 and 4.4 times than G effect alone. The large variation captured by location effect, which is irrelevant to genotype evaluation and ME identification, justified selecting GGE biplot analysis as an appropriate method for analyzing multi-environment trial data (Gauch and Zobel 1997; Yan et al. 2000). The greater magnitude GL relative to genotype effect, confirms the possible existence of different MEs.

The yearly GGL biplots (Fig. 2) captured for 67.4–75.4% of the total variation. Figure 2 shows the genotype and GL interaction (GGL) biplots for each year of yield trials. In each year, the locations were clustered into groups with different winning genotypes (Fig. 2a-c, Table 5). Based on the 2018–19 data, GGL biplot suggest four MEs (Fig. 2a): The first mega-environment (ME-1) represented by Kermanshah location consisting of two rainfed (KHR19) and irrigated (KHI19) environments; the next ME (ME-2) represented by Ilam (ILA19); while the third ME represented by Maragheh (MAR19) and Urmia (URM19) locations and the last one (ME-4) represented by Shirvan location. According to Fig. 2a, the G14, G10, G16 and G12 were best performing genotypes in ME-1, ME-2, ME-3 and ME-4, respectively.

In 2019–20 (Fig. 2b), Maragheh (MAR20) and Urmia (URM20), as first year, made up a single ME (ME-1), while each of rainfed (KHR20) and irrigated (KHI20) environments corresponding to Kermanshah location made up different MEs (ME-2 and ME-3); and Ilam (ILA20) and Shirvan (SHI20), as first year, made up the two single MEs (ME-4 and ME-5). The genotypes G7, G14, G1, G16 and G9 were being the winners for ME-1, ME-2, ME-3, ME-4 and ME-5, respectively.

In 2020–21, GGL biplot analysis suggested three MEs. The first (ME-1) consisted of two rainfed (KHR21) and irrigated (KHI21) environments corresponding to Kermanshah location, while the second

Table 4 Yearly ANOVA for grain yield of 20 durum wheat genotypes across locations

Year	Source	df	Sum of squares	Mean squares	F-value	%TSS
2018–19	Location (L)	5	410,200,059	82,040,012	58.6**	78.5
	Rep/L	12	16,799,389	1,399,949		3.2
	Genotype (G)	19	11,993,291	631,226	2.9**	2.3
	GL interaction	95	34,399,653	256,838	1.7**	6.6
	Error	228	49,472,232	260,843		9.5
	Total	359	522,864,625			100
2019–20	Location (L)	5	739,825,992	147,965,198	212**	89.8
	Rep/L	12	8,376,569	698,047		1.0
	Genotype (G)	19	7,198,003	378,842	2.1**	0.9
	GL interaction	95	26,873,563	282,880	1.6**	3.3
	Error	228	41,261,600	180,972		5
	Total	359	823,535,727			100
2020–21	Location (L)	4	170,450,170	42,612,542	68.2**	78.7
	Rep/L	10	6,246,166	624,617		2.9
	Genotype (G)	19	3,542,282	186,436	1.7*	1.6
	GL interaction	76	15,287,602	201,153	1.8**	7.1
	Error	190	21,111,824	111,115		9.7
	Total	299	216,638,044			100

*, **Significant at 5% and 1% level of probability, respectively. df: degree of freedom; %TSS: percentage relative to total sum of squares
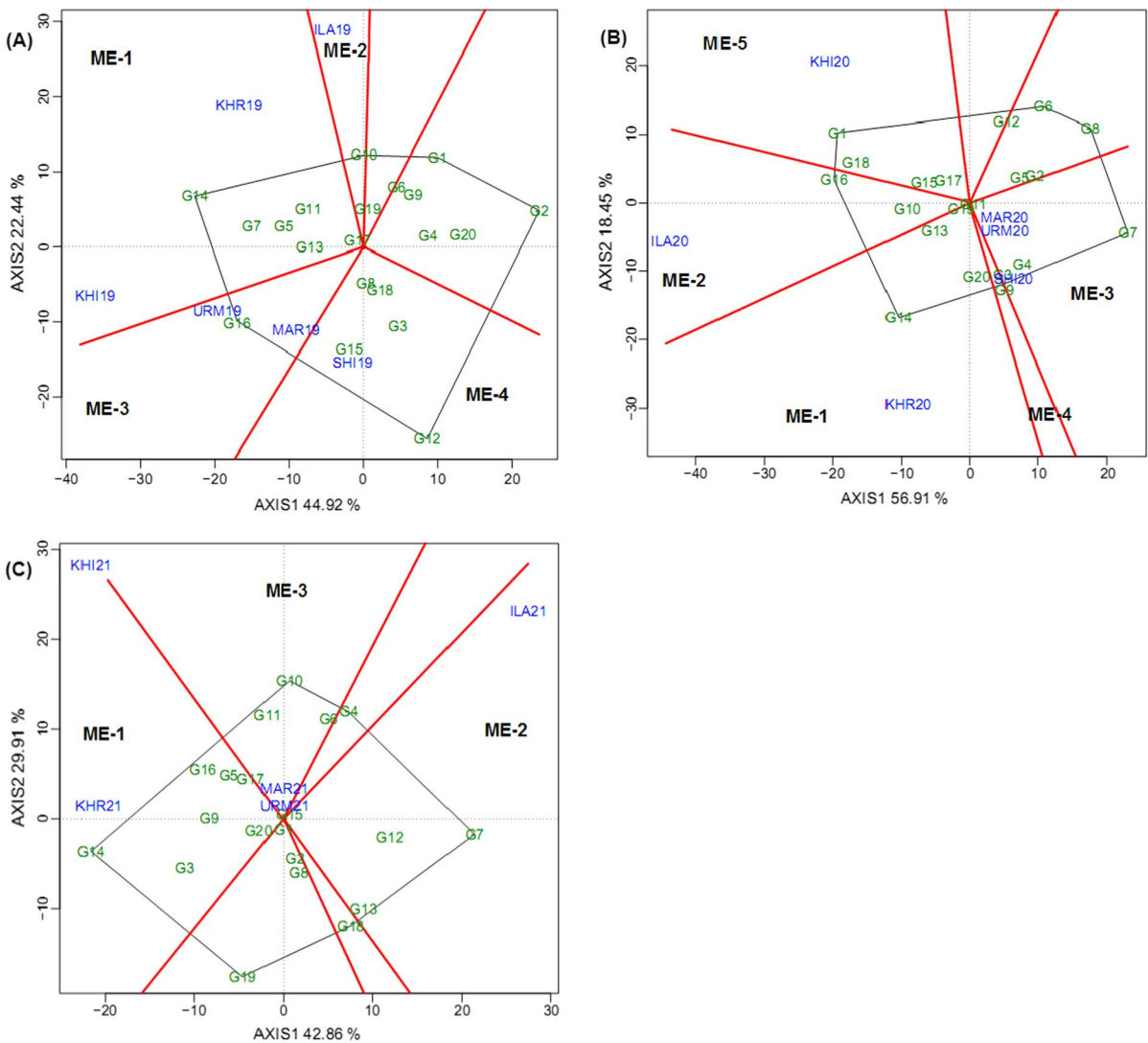
Fig. 2 The GGL biplots showing the "which-won-where" patterns based on the gain yield data of 20 durum wheat genotypes in each cropping season. **a**: 2018–19; **b**: 2019–20; **c**: 2020–21. G1-G20 are genotypes and letters in blue are locations. For genotype and location names see Tables 1 and 2

(ME-2) represented by Ilam location (ILA21), and the third ME (ME-3) represented by Urmia (URM21) and Maragheh (MAR21) locations, as two previous years. However, in this year, due to severe drought condition the data for Shirvan location was not included in analysis.

Based on the yearly biplots results, Kermanshah, Ilam and Shirvan represent three different single mega-environments; and Maragheh and Urmia also clustered in a same ME. These observations suggest four mega-environments. The rainfed and irrigated environments correspond to Kermanshah location tend to grouped in same mega-environment in two out of three years, suggests that these two environmental conditions are not independent and are related to the same ME.

Based on the results, grouping of locations across years revealed a repeatable pattern, showing that GL interaction patterns were consistent across years and can be explained through geographical positions. However, the both strategies of the "by-year and multi-year analyses", to evaluate the regional

Table 5 The results of yearly GGE biplot based on "which-won-where" pattern in the study

Cropping season	Locations group	Winner genotype
2018–19	KHR19, KHI19	G14
	URM19, MAR19	G16
	ILA19	G10
	SHI19	G12
2019–20	KHR20	G14
	MAR20, URM20	G7
	ILA20	G16
	KHI20	G1
	SHI20	G9
2020–21	KHR21, KHI21	G14
	MAR21, URM21	G10
	ILA21	G7

yield trials data indicated the existence of different mega-environments and confirmed that it is needed to divide the durum-growing regions into sub-regions to make genotype recommendations. Although the yearly GGL biplots allowed us to select superior genotypes for each ME, the winning genotypes for each ME was not consisted across years, except for breeding line G14 that was superior for western ME (corresponding to Kermanshah location) in all three years (Fig. 2).

Identification of promising genotypes based on mega-environments

When MEs are defined for a target region, then genotypes should be evaluated through each single ME (Yan 2014). The AEC view of the GGE biplot for each mega-environment is presented in Fig. 3. The breeding line G14 recorded the highest mean yield in Western ME (Fig. 3a). The breeding lines G14, G11, G5, G10, G15 and G19 were the most stable genotypes with above-average yields in this ME. Thus, the breeding line G14 followed by G11 was the most ideal genotype as exhibited the highest mean yield and stability performance. The breeding lines G10 and G17 were the most stable genotype with above-average yield in Southern-west ME (Fig. 3b). Genotype G1 (Saji cultivar) recorded the highest mean yield but its performance was found relatively variable across environments in this ME, while breeding line G10 was the ideal genotype, as it showed high mean yield and stability performance. Other stable genotypes with above-average yields in this ME were G17, G13 and G20. In the case of western-north ME, breeding line G14 recorded the highest mean yield and stability, while G16 and G17 exhibited most stability with above-average yields (Fig. 3c). According to eastern-north ME breeding lines G3, G4 and G12 were the most stable genotypes with above-average yield (Fig. 3d), while breeding lines G16, G14 and G18 were above-average yielder genotypes but were relatively unstable. When taking all environments into consideration (Fig. 3e), breeding line G14 was the most stable genotype with highest mean yield. Genotype G13 was the most stable genotype with near average yield. Breeding lines G16, G11 and G5 were also above-average yielder genotypes but were relatively unstable.

Discriminating and representativeness view of test locations

Figure 4 presents discriminating ability and representativeness view of test locations. In 2018–19, among the six locations, KHI19, ILA19 and KHR19 with longest vectors were the most discriminating (most informative) (Fig. 4a), while in 2019–20 (Fig. 4b), ILA20, KHR20 and KHI20 were the most discriminating environments; and in 2020–21, KHI19, ILA19 and KHR19 were the most discriminating environment (Fig. 4c). When taking all three years data into consideration (Fig. 4d), ILA20 and KHI19 were the most discriminating environments; and URM19, KHR19, KHI20, KHR20 and KHR21 showed relatively high values of discrimination power, and URM21, MAR20, MAR21 showed low values of discrimination ability (Fig. 4d).

In 2018–19, the vectors KHI19, URM19 and KHR19 are very close to AEC axis, showing their strong representativeness, while the test environments SHI19, MAR19 and ILA19 are in near-right angle with AEC axis, indicating their poor representativeness (Fig. 4a). For the second cropping season (2019–20), ILA20 and KHR20 showed high representativeness and MAR20, URM20 and SHIR20 showed relatively weak representativeness (Fig. 4b). In 2020–21, KHI21 showed strong representativeness and remaining locations showed relatively weak
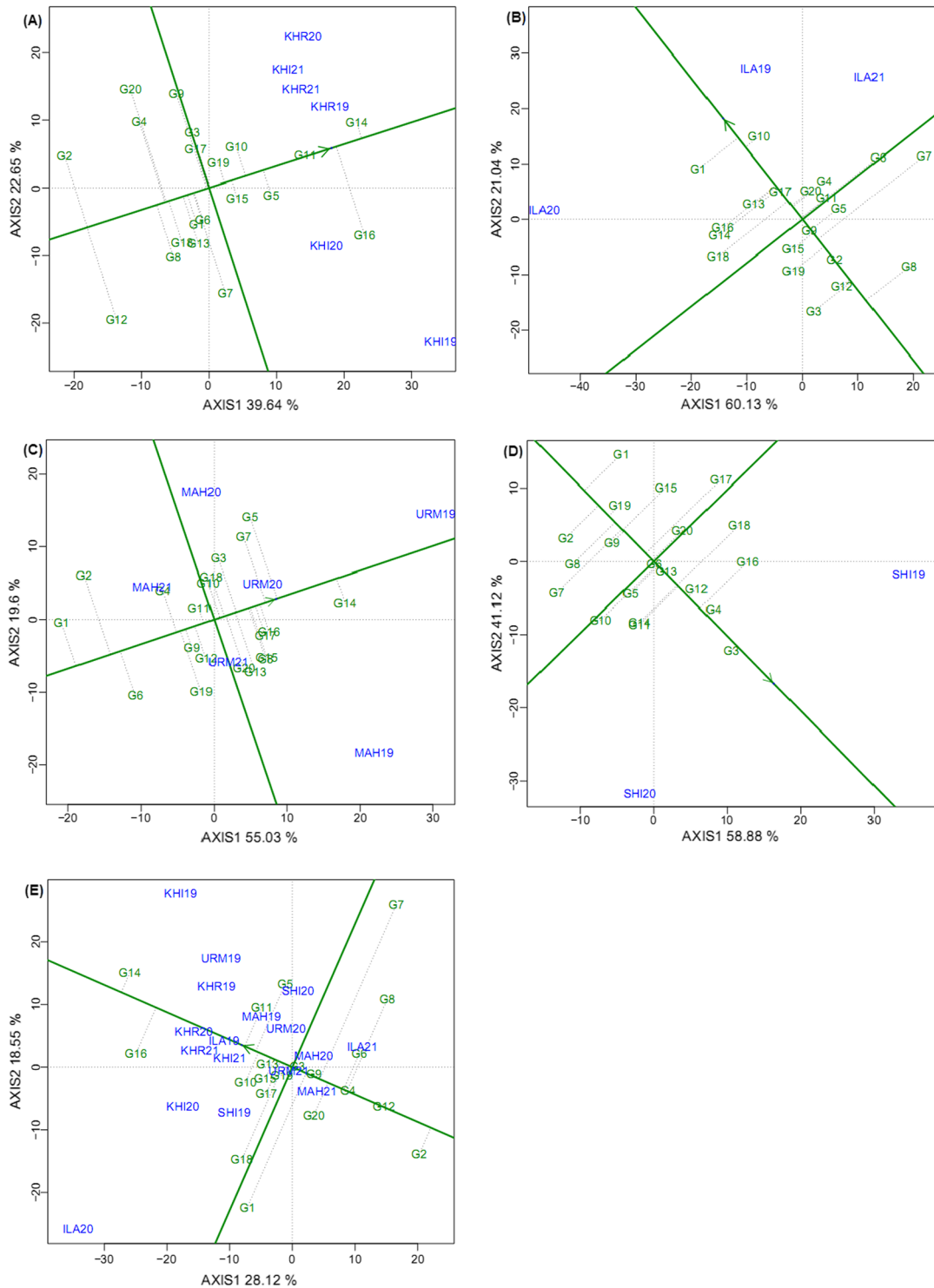
Fig. 3 Mega-environment based GGE biplots to show the mean yield and stability performance of 20 durum wheat genotypes in: **a** Western mega-environment, **b** southern-west mega-environment, **c** western-north mega-environment, **d** eastern-north mega-environment, and **e** all environments
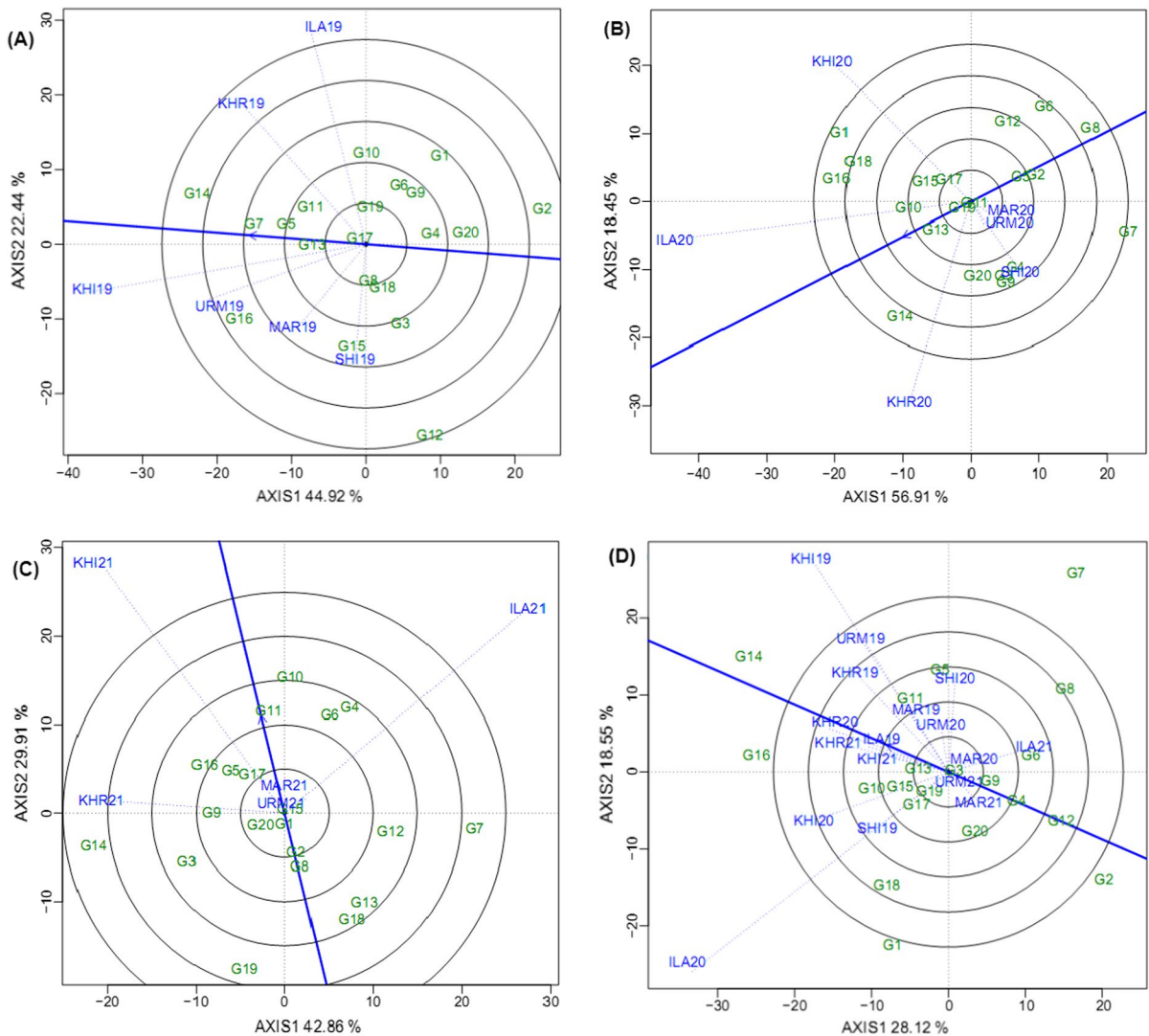
Fig. 4 GGE biplot showing "discrimination ability vs. representativeness" view of test locations for grain yield data of durum wheat genotypes evaluated during three cropping seasons: **a**: 2018–19; **b**: 2019–20; **c**: 2020–21; **d**: across three years (2018–21). G1-G20 are genotypes and letters in blue are environments. For genotype and environment names see Tables 1 and 2

to no representativeness (Fig. 4c). Across three years (Fig. 4d), all environments correspond to Kermanshah location showed a relatively strong representativeness, followed by ILA19 (Ilam location), URM19 (Urmia location), whereas environments belong to Maragheh, Shirvan and some environments belong to Ilam and Urmia locations showed a relatively weak representativeness. In general the locations Maragheh, Urmia, Shirvan and Ilam are in near-right angle with AEC axis, indicating their poor representativeness,

showing these locations come out as undesirable test locations if the whole region is considered as a single mega-environment. These locations clearly deviate from the AEC axis. The obtuse angle between test locations, suggesting that the locations may belong to different mega-environments.

Fig. 5 Heat map for correlation coefficients between mean yields observed in different environments. See Table 2 for full environment names. The threshold correlation for $P < 0.05$ is 0.46 and that for $P < 0.01$ is 0.60
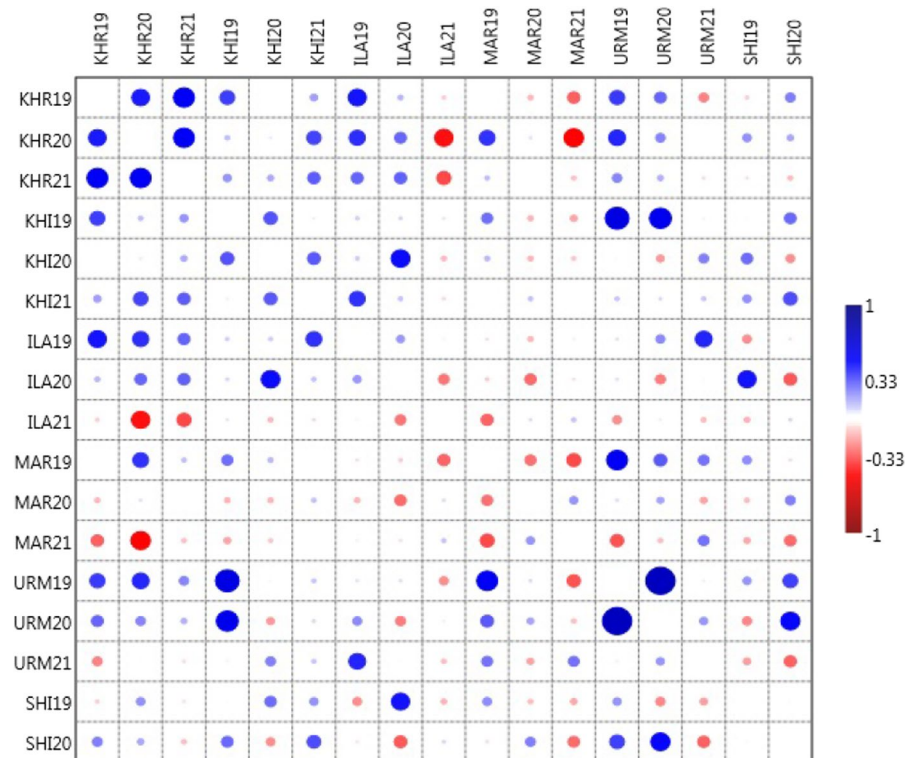
Genetic correlation analyses between test locations

The genetic correlation analyses between locations in each year were estimated and expressed graphically using the heat map yield correlations (Fig. 5). It can be observed that some locations were significantly correlated in some years, e.g., Kermanshah (KHR19) *vs.* ILam (ILA19), suggesting little GE between them; some locations were not correlated in any of the years, e.g., Ilam vs. Maragheh and Urmia, suggesting repeatable GE interaction between them. The relationships among test locations can be more easily investigated through GGE biplot (Fig. 4). It can be seen that the test locations show relatively low correlations as can be also verified by the right-obtuse angles between their vectors. The Maragheh and Urmia across years showed shorter vectors. This indicates that their correlations with other locations were weak and not fully displayed in the biplot. We refer to this group of locations as Western-north ME. In this ME, there was a both negative and positive correlation between environments across years. Shirvan location, which was generally same in latitude but different geographical position with Maragheh and

Urmia, did not showed consistent correlation with these two locations and tended to separate in a single ME (Eastern-north ME). In this ME a weak positive correlation was observed between environments across years. Similar trends were also observed for Ilam and Kermanshah locations that fell into different groups with different winning genotypes. In mega-environment represented by Kermanshah location, that we refer to it as Western ME, there was a positive and moderate magnitude correlation between environments across years. For mega-environment represented by Ilam location, that we refer to it as Southern-west ME, there was a both positive and negative correlation between environments across years. In general, there was both positive and negative correlations between environments across years in the study and correlations were classified as moderate to low.

Environmental factors affecting GE interaction and locations grouping

The results of factorial regression underling the identification of environmental factors that significantly

Table 6 The results of factorial regression analysis based on grain yield data of 20 durum wheat genotypes across 17 environments that enriched with 19 climatic factors

Source	df	Sum of squares	Mean squares	F-value	AIC	(%GE)
GE interaction	304	76,765,241	252,517			
G x Rain5	19	13,462,307	708,542	4.48**	15,404.4	17.5
G x RainT	19	9,622,051	506,424	3.20**	15,404.9	12.5
G x Rain8	19	7,262,213	382,222	2.41**	15,405.9	9.5
G x Rain9	19	6,075,726	319,775	2.02**	15,409.5	7.9
G x Rain6	19	5,205,433	273,970	1.73**	15,410.5	6.8
G x Tem3	19	5,064,357	266,545	1.68**	15,414.1	6.6
Residual	190	30,073,155	158,280			39.2

AIC selected model 15,414.159

Rain: rainfall; numbers 3, 5, 6, 8 and 9 stand for January, March, April, May and June; and T stand for total

affected the GE interaction and locations grouping are presented in Table 6. Using the F-test and Akaike's (1974) information criterion (AIC) environmental factors that significantly contributed to GE interactions were identified. Based on the results, the monthly rainfall in March (17.5%), cumulative rainfall (12.5%), monthly rainfalls in May (9.5%), June (7.9%) and April (6.8%) and average temperature in January (6.6%) significantly ($P < 0.01$) contributed to GE interaction and captured for 60.8% of the total variations in GE interaction (Table 6). Monthly rainfall in March is important for durum wheat as it coincided with the change of growing phase from tillering to stem elongation in moderate location (Kermanshah), and development of tillering in cold locations (Maragheh, Urmia and Shirvan) and stem elongation in moderate warm location (Ilam). Monthly rainfalls through May, June, and April are also critical, respectively, in moderate cold (coinciding with grain filling), cold (coinciding with early heading) and moderate warm location (coinciding with early grain filling). Temperature in January also significantly contributed to GE interaction mainly through limit crop growth in cold locations.

Discussion

The analysis of variance for grain yield revealed that the genotype, environment and GE interaction were highly significant, showing that the genotypes performance significantly varied across environments. The environment had the highest impact on genotypic performance as it was the main source of variation in yield which captured 86% of total variation. This magnitude effect of environment, often is observing in durum wheat yield trials and it is near to the values 76%-85% observed from our earlier studies on durum wheat regional yield trials (Mohammadi et al. 2010, 2021). The mean performance of genotypes significantly varied among environments. This shows high difference in genotypes responses to environmental conditions that can be supported by the six-fold larger effect of GE interaction compared to genotype effect. This also can be observed by the climatic features of each location (Table 2 and Fig. 1) which were differed in climate conditions including unpredictable factors such as rainfall, temperature, altitude and latitude, soil type, etc.

Based on the GGE biplot analysis, when different genotypes are adapted to different groups of environments, and the variation between groups is higher than within groups, then a ME can be defined (Gauch and Zobel 1997; Yan et al. 2000; Luo et al. 2015). Using this methodology, Yan et al. (2000) investigated the ME analysis and test location for winter wheat in Canada and recommended two winter wheat MEs in Ontario, and Luo et al. (2015) identified three major MEs for sugarcane in China with the same methodology. Based on the present study, using the GGE biplot analysis the six locations representing for durum wheat regional yield trials were clustered into four MEs. This result is in agreement with our previous study which four out of six locations in this study involved, but with different genetic materials (Mohammadi et al. 2010). Based on the results,

Maragheh and Urmia locations with generally same in climate, geographical position and altitude tended to make the Northern-west ME. The two irrigated and rainfed environments belonging to Kermanshah location also formed the Western ME. This suggests no remarkable difference in adaptation of genotypes to rainfed and irrigated environments in this ME across years. The Suthern-west ME represented by Ilam location possessing some main different features from other MEs for its agro-climate conditions such as moderate warm climate, lower altitude, more rainfall, and soil type. The Eastern-north ME represented by Shirvan location characterized as cold location with annually low rainfall and geographically positioned far from other locations tend to separate in a single ME.

Based on the results a genotype x ME interaction was also observed in the study, as the winning genotypes for each ME, except for western ME, was varied from year to year. Breeding line G14 was consistently a winner genotype for the Western ME, while for southern-west ME breeding lines G10, G16 and G7, depending on year, were being the winners; G9 and G12 were performers for the Eastern-north ME; and breeding lines G7, G10 and G16 were best yielders for the western-north ME. The definition of ME is that there should be a sufficient number of sites such that the response of genotypes remains the same across a subset of sites within an ME, but it was difficult to prove this in this study due to limited number of sites under investigation. Therefore, it is suggested that this investigation be carried out in some future studies with a larger number of sites, in order to have more concrete evidence of the existence of MEs for durum wheat in the country. However, the year-to-year variation in yield performance for regional cultivar trials inevitably causes yearly fluctuations in genotypic and environmental scores, thereby trivializing and obscuring any "which-won-where" pattern that is identified in a single year (Yang et al. 2009).

The correlations coefficients between environments from different mega-environments varied from highly negative to positive; as a result, the four MEs were slightly negatively correlated. This indicates that different genotypes should be selected and recommended specifically to each ME (Yan 2019). In other wise, a superior genotype with high mean yield and stability performance across all environments should be developed. However, the main goal of ME

investigation is to the explore the GE interaction patterns (repeatable and unrepeatable) in breeding programs (Munaro et al. 2014; da Silva et al. 2021). In Fig. 3, the GGE biplots which approximates the grain yield data of 20 genotypes across 17 environments during 2018 to 2021, focuses on this point. When the yield data were analyzed by ME, genotypes G14 followed by G11 expressed highest mean yield and stability across environments in western ME, that was clearly better than other tested genotypes (Fig. 3a). Thus, the breeding line G14 (originated from ICARDA) should be recommended to this mega-environment.

In the southern-west ME, while G14 was still the high yielder genotype on average, it was not best performer in most of the environments. Instead, G1, G10 and G17 well performers, but G10 and G17 also exhibited best stability performance (Fig. 3b). Thus, G1, G10 and G17 showed better responses to the environments within southern-west ME, in comparison with G14 and G11 which well performed in western-ME (Fig. 3a). Thus, in order to stabilize the durum productivity within and across years in southern-west ME, breeding lines G10 and G17 should be recommended to buffer the high and unpredictable GE effects. However, these two genotypes (G10 and G17) were also yielded about average across all environments (Fig. 3e) and performed among the average in the other MEs (Figs. 3a, c and d). Considering that the southern-west ME is a very key durum wheat production region in the country, understanding this issue has important implications on durum wheat productivity in this ME.

In the western-north ME, G14 was the best yielder on average with high stability performance across environments (Fig. 3c), while in the eastern-north ME, G3 followed by G4 and G12 were the highest performers on average with best stability performance across environments. Note that G3 performed only about average across all environments (Fig. 3e) and poorly yielded in the southern-west ME (Fig. 3b). Based on the overall results (Fig. 3e) when evaluating genotypes simultaneously for highest mean yield and stability performance, the breeding lines G14 and G16 expressed highest yielding and stability across all trials.

Considering both discriminating and representativeness view of test locations, Kermanshah location was an "ideal" location for testing advance breeding

materials for mean yield and stability performance during early breeding stage. The existence of non-crossover GE interaction (consist performance of a genotype i.e., G14 for western ME, Fig. 2a, b and c) suggested the existence of positive relationships among test environments (Fig. 4d). Thus GGE biplot recommended as most precise method for the test location evaluation with the purpose of identifying ideal test location for the target region (Yan et al. 2000; Yan and Tinker 2006). It also provided a meaningful and useful summary of GE interaction data, and helped in assessing the relationships of test locations and variations in genotypes performance across environments. The GGE biplot has successfully detected superior genotypes for high mean yield and stability performance in various crops (Yan et al. 2000, 2021; Rakshit et al. 2012; Munaro et al. 2014; Xu et al. 2014; Luo et al. 2015; Swanckaert et al. 2020).

The use of genotypic and climatic factors as external covariates for analytical analysis of GE interaction and understanding factors most affecting genotype performance are important (Vargas et al. 2001; Voltas et al. 2005). According to Voltas et al. (2005), environmental modeling of GE interaction using factorial regression can be considered as a predictive strategy for the recommendation purposes and should be integrated into crop breeding programs. The results of factorial regression revealed that about 61% of the total GE interaction in the present study was explained by monthly rainfalls in March, May, June and April, and cumulative rainfall, as well as average temperature in January as it corresponding to coldest month during cropping season. Vargas et al. (2001) using multiple factorial regression model identified the minimum temperatures in January, February, and March and the maximum temperature in April as the most important environmental variables that explained year x nitrogen interaction in wheat. Branković et al. (2014) found the climatic factors of maximum temperature, precipitation, and sunshine hours as the most influential variables in explaining GE interaction during grain filling and grain ripening stages in durum wheat.

Conclusion

The collective analysis of the yearly biplots suggested four mega-environments for rainfed durum wheat-growing regions in Iran. These results provided some implications for future durum wheat breeding. The new breeding lines from this study should be recommended for each mega-environment to maximize productivity and stability. The four mega-environments identified in this study namely western, southern-west, western-north and eastern-north mega-environments suggests that specific adaptation could be positively exploited. High-yielding and stable genotypes in each of the mega-environments were generally different which implies specific adaptation of superior genetic materials to each specific mega-environment that leads to positive exploitation of the GE interaction. Based on the findings, the superior genotype G14 originated from ICARDA was specifically adapted to both western and western-north mega-environments, while G10 originated form ICARDA best adapted to southern-west mega-environment, and G3 originated from CIMMYT mostly adapted to eastern-north mega-environment. Environmental modeling of GE interaction by climatic variables showed that the most influential environmental factors in explaining GE interaction and locations grouping were monthly rainfalls in March, May, June, April, cumulative rainfall and average temperature in January. In summary, the GGE biplot method provided an adequate graphical tool for visual analysis of GE interaction in durum wheat multi-location and multi-year trials, and allowed separation of repeatable from unrepeatable GE interaction patterns, and mega-environments delineation with targeted genotypes recommendation. Such information is crucial and useful for improving plant breeding efficiency, crop cultivar deployment, and crop productivity.

Declarations

Conflict of interest   There is no conflict of interest.

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