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Genetic variability, Genotype × Environment interaction for grain yield of wheat (*Triticum aestivum*) backcross inbred lines population under different moisture regimes

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ABSTRACT

Drought stress is well known phenomenon that affects the productivity of wheat (*Triticum aestivum* L). Knowledge on genetic variation, genotype × environment interaction and association between physiological and yield component traits is crucial for the development of improved varieties having high yield and water use efficiency. The present study consists of 280 backcross inbred lines (BILs) population evaluated for grain yield and morpho-physiological traits for two years at three locations. Combined ANOVA unfolded significant variability among traits in BILs population for yield and morpho-physiological traits.Grain yield showed significant association with normalized difference vegetation index (NDVI), soil plant analysis development (SPAD), thousand grain weight (TGW), and canopy temperature (CT). The genotype, environment and genotype ×environment interaction for yield was highly significant ($p< 0.01$). ASV (AMMI stability value) was calculated and top 29 genotypes were selected and further analyzed with AMMI and GGE biplot analysis for dissecting out genotype × environment interaction. The results classified genotypes G82, G202, G234, G263, G6, G192 and G77 are most stable and high yielding genotypes.

Key words: Drought, GGE biplot, Ideal genotype, Physiological traits, Yield stability

A global Wheat Yield Consortium (WYC) was formed to look into the problem of productivity under major abiotic stresses such as drought and heat (Reynolds *et al.* 2011). The improvement in drought tolerance is limited by complex nature of the trait being controlled by many genes and genotypic × environment interaction (Ahmed Sallam *et al.* 2019). Many physiological phenotyping tools have been developed to allow precise and efficient selection of drought-tolerant genotypes. Thorough understanding of the genetic mechanism of morphological and physiological trait variability for water use efficiency will improve the breeding of wheat (*Triticum aestivum* L) for drought tolerance.

The BILs population has benefit over RILs on selection of genotypes in advance generation. First, major portion of genome will be of recurrent parent type (3:1) with desirable traits transferred from the drought tolerant parent. Second, the undesirable traits from donor parent will be replaced by recurrent parent genome. Therefore, this population outfit the breeder, when he wants his most of the genotype from desired parent with superior traits from donor. NDVI and CT are successfully used for rapid screening of drought tolerance in wheat. Drought susceptible genotypes suffered under greater water stress and warmer canopy temperature at mid-day leads to relatively great yield loss (Blum *et al.* 1989). Relative water content (RWC) is important parameter, which measures degree of stress expressed under drought condition. The genotypes that maintain turgid condition will have physiological advantage under stress. Additionally, integrating whole canopy chlorophyll content (NDVI) with point reading from SPAD chlorophyll meter will have a better impact on evaluation of genotypes physiologically under drought stress.

Additive Main effects and the Multiplicative Interaction (AMMI) model and GGE biplot is highly effective in capturing major section of interaction of sum of squares, meanwhile this separates main as well as interaction

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components and shows that which genotype is suitable for which environment. This highly supports the evaluation of multilocation data in breeding programs and meaningful interpretation of the data and results graphically (Jeberson *et al.* 2017). The current study explains the evaluation of 280 BILs population of the parental crosses HD2733* 1/ C306 was evaluated in three locations for two consecutive years to characterize of drought tolerance using yield and physiological component traits and also to select stable genotypes with high yield over different moisture regimes.

MATERIALS AND METHODS

Plant materials: The BC_1F_5 BIL population comprising 280 lines was developed by crossing C306 (a well-known drought tolerant variety from late sixties), with wheat variety HD2733 (released for irrigated timely sown condition of north eastern plain zone), HD2733 was used as female with C306 as a male parent during crossing.The F_1 from the cross was backcrossed to female HD2733 and the resulting BC_1F_1 progenies were selfed over generations to get BC_1F_5.

Phenotyping and analysis of phenotypic data: The BIL population was evaluated for, NDVI, SPAD, CT, RWC, Days to heading (DH), Plant height (PH), average grain weight per spike (GWS), TGW, biomass, and yield at three locations for two years, which makes six environments. Phenotypic trails were conducted at Delhi under rainfed condition 2016-17 (DRF17), 2017-18 (DRF18), and, irrigated condition during 2016-17 (DELIR17), 2017-18 (DELIR18). The Research farm at Division of Genetics, IARI was situated at the altitude of 228 m above mean sea level with 28° 40' N latitude and 77° 13' E longitude representing northern India. A trial under restricted irrigated condition (two irrigations) was conducted during 2017-18 (INDORE17), 2018-19 (INDORE18) at Indore. This location was located at an altitude of 553 m above mean sea level, 22° for N° 75 E' placed at central India.All trials were conducted using Alpha Lattice design with two replications. Each entry was planted in triple-row plot of 1m long and 0.8m wide in a bed planting system.The parental genotypes were used as checks in the design. All the agronomic practices were taken up to raise a healthy and uniform crop trials. Plant materials were harvested after they attained full physiological maturity when grains were totally dry in the field. NDVI was measured as NDVI-1 (Heading stage), NDVI-2 (Anthesis stage) and NDVI-3 (Grain milk stage) measured with the help of hand held Trimble Green Seeker. SPAD readings were taken using Minolta SPAD-502 chlorophyll meter. Randomly 10 spikes harvested from each plot consisting of three rows of each genotype were threshed and grains weighed using electronic balance constituted average grain weight per spike. RWC was measured using formula Leaf RWC (%) = ((Fresh Weight-Dry Weight) / (Turgid Weight-Dry Weight)) × 100. CT recorded as CT-1 (Heading stage) and CT-2 (Grain filling stage) using hand held Infrared thermometer. CT and NDVI were recorded by referring standard trait dictionary of CIMMYT (Pask *et al.* 2012). TGW was measured manually by counting randomly selected thousand seeds from each plot and weighed using electronic balance.

Statistical analysis: Data for physiological, yield and its component traits of six environments were used to estimate analysis of variance (ANOVA), and heritability using META-R (Multi Environment Trail Analysis with R for Windows) Version 6.04, Software (CIMMYT). Pearson's correlation coefficient among different traits was estimated by OPSTATsoftware package. GenStat Software, 17th edition was used to calculate the AMMI test and to derive AMMI1 plots and GGE Biplots. ASV was estimated for each genotype according to the relative contributions of the principal component axis scores (IPCA1 and IPCA2) to the interaction sum of squares. The AMMI stability value (ASV) as described by Purchase *et al.* (2000), was calculated as follows:

$$ASV = \sqrt{\left[\frac{IPCA1_{Sum\ of\ squares}}{IPCA2_{Sum\ of\ squares}}\left(IPCA1_{score}\right)\right]^2 + \left(IPCA2_{score}\right)^2} 1$$

RESULTS AND DISCUSSION

Genotypic variance and G × E interaction

The analysis of variance from present experimentation on BILs manifest highly significant differences among genotypes and genotype× environment interaction for all thirteen traits across the environments (Table 1). Among the physiological traits NDVI, CT, SPAD, GWS and TGW, have low genotypic variance compared PH, Biomass, DTF, RWC and Yield. The physiological traits NDVI-2, NDVI-3 and RWC were found to have higher heritability (>0.60) compared to NDVI-1, SPAD, CT-1,and CT-2. Whereas, PH, DTF, and yield were found to be more heritable among agronomic traits in comparison to biomass, GWS, HI and TGW. The pooled broad sense heritability across environment ranged from 0.31 (CT-2) to 0.91(PH). In overall, across six environments broad sense heritability for all traits is ranged between0.30-0.60. The data of RWC taken in single location (DRF 17) was found promising with high heritability (0.90) with significant genotypic variance.

Association between yield with its component and physiological traits across environment

The best linear unbiased prediction (BLUPs) values were calculated for each genotype across the environment. The BLUP values were used for calculation of Pearson's correlation for phenotypic correlation among all traits (Table 2). The correlation between morpho-physiological traits with grain yield for individual environment is represented in Table 3. The physiological traits NDVI-1, NDVI-2, NDVI-3, SPAD, RWC positively correlated with yield, whereas CT-1 and CT-2 of reproductive stages were negatively correlated.These results are in accordance with (El-Hendawy *et al.* (2015), who reported significant negative correlation between CT grain yield, and positive correlation with leaf water content.The CT1 found non significant association with grain yield under irrigated condition. The major agronomic traits GWS, TGW, biomass,

Table 1 Analysis of variance for morpho-physiological and yield traits wheat BILs in two years (2016-17 to 2017-18) of tested in three locations under different moisture regimes, viz RF, RI, IR

Statistic	NDVI-1	NDVI-2	NDVI-3	CT-1	CT-2	PH	BIOMASS	GWS	DH	SPAD	TGW	RWC	YIELD
Heritability	0.53090	0.75990	0.88550	0.36744	0.31401	0.91700	0.53100	0.44190	0.88350	0.42490	0.51600	0.90538	0.60320
Genotype variance	0.00030	0.00090	0.00180	0.06791	0.01871	64.94630	7689.39510	1.13480	37.33200	1.17540	4.49980	51.73716	1934.80900
Gen×Loc variance	0.00080	0.00070	0.00040	0.07015	0.23932	16.01300	33714.82570	4.83530	13.85440	3.28280	16.30640		6138.95150
Residual variance	0.00160	0.00220	0.00190	1.02874	1.26650	38.48200	14073.69630	7.52620	11.65520	12.52260	18.04130	10.81387	2997.37070
Grand mean	0.69140	0.57550	0.52060	24.67875	24.44110	108.33910	1223.92430	17.94480	95.84680	49.27320	47.97680	77.46556	412.43100
LSD	0.03270	0.04240	0.04050	0.58088	0.35804	6.50970	166.95530	2.21880	5.83500	2.29140	4.10950	6.52300	77.05100
CV	5.71140	8.18300	8.29410	4.10988	4.60449	5.72590	9.69280	15.28800	3.56190	7.18180	8.85320	4.24504	13.27450
n replicates	2	2	2	2	2	2	2	2	2	2	2	2	2
n environments	6	6	6	6	6	6	6	6	4	6	6	1	6
Genotype significance	5.00E-18	3.00E-67	2.00E-167	9.59E-07	0.098351	4.00E-229	1.00E-18	5.00E-11	4.00E-127	4.00E-10	5.00E-17	1.66E-65	1.00E-27
Gen×Env signifxicance	7.00E-38	2.00E-18	5.00E-13	0.031345	1.96E-09	2.00E-31	2.00E-232	2.00E-54	1.00E-72	1.00E-15	2.00E-88		3.00E-202

are positively correlated with yield. Apart from this CT was also found to be negatively correlated with RWC, NDVI and TGW. Biomass was found positively correlated with NDVI and PH. SPAD also found positively correlated with NDVI, GWS, and biomass. Ramya *et al.* (2016) conducted recurrent selection programme for physiological traits like NDVI, CT and Chlorophyll content for developing superior drought tolerant lines with high yield. Therefore, best way to improve genetic gain of wheat under drought stress by selecting and combining economic physiological traits, *viz* NDVI, CT and SPAD.

Genotype and environment analysis

The BILs population exhibited significant variation for yield among the experimental conditions.The AMMI analysis of variance showed significant effects of genotype, environment, and their interaction (GEI) for yield. The significance of GEI has influence on magnitude of difference between genotypes in different environment and itsanalysis of GEI gives estimate of stability (Hill *et al.* 1998). Genotype, Environment and genotype × environment interaction accounted for 20.39%, 39.05%, and 40.55% of the total variation, which is comparable to the results reported by Mehari *et al.* (2015) and Verma *et al.* (2015). Interaction variation was again differentiated into interaction principle component axis (IPCA). The IPCA1 and IPCA-2 explained 37.95% and 20.51% of interaction sum of square, respectively over yield components (Table 4). Both the IPCAs were significant. The sum of square of GEI was 1.99 times higher than that of sum of square of genotypes and has more proportion of total variance, suggesting that the experiment carried out in divergent environmental conditions resulted in significant differences in genotypes response across the environments (Jeberson *et al.* 2017, Verma *et al.* 2015, Mohammadi *et al.* 2009). The best 10 genotypes in terms of yield for each environment is represented in Table 5. Many genotypes out performed transversely under irrigated, restricted irrigation and rainfed conditions. Genotype 4, and 86 consistently picked over E2, E4, E5, and E6 environment and ranked within 10 for their higher mean yield. This was followed by G28, G70, G109, G265 and G116 over three environments and genotypes G7, G26, G264, G243 and G171 between two environment conditions.AMMI analysis and mean performance (grain yield per plot) bestowed G86 as most desirable, stable and high yielding genotype over all the three different moisture stress environments followed by G4, G109, G265, G116, G28 and G70. An outstanding genotype needs to combine more grain yield and stable performance across a range of crop production environments. The AMMI Biplot helps us to visualize the GEI and provides details on association between environment. The INDORE17, INDORE18 and DELIR18 environments clustered together and influence the genotypes in identical way. Indore 17, Indore 18 and DRF18, DELIR18 had positive correlation and were present in the same sector in biplot (Fig 1), whereas DELIR18, DRF18 and DRF17, DRF18 have negative correlation as they were

Table 2 Pearson correlation coefficient among the morpho-physiological and yield traits of BILs population

	GY	DH	NDVI-1	NDVI-2	NDVI-3	RWC	CT-1	CT-2	GWPS	PH	Biomass	SPAD	TGW
GY	1												
DH	0.052	1											
NDVI-1	0.316*	0.014	1										
NDVI-2	0.251*	0.112	0.264*	1									
NDVI-3	0.285*	0.298*	0.125*	0.234*	1								
RWC	0.158*	-0.05	0.012	0.115	0.056	1							
CT-1	-0.152	-0.03	0.05	-0.234*	-0.125	-0.112	1						
CT-2	-0.367**	0.01	0.157*	-0.122*	-0.223	-0.203*	0.148	1					
GWS	0.371**	0.215*	0.07	0.148*	0.233*	0.115*	0.04	-0.396*	1				
PH	0.071	0.236*	0.012	0.025	0.04	0.06	0.05	0.115	0.221*	1			
Biomass	0.373**	0.200*	0.234*	0.104	0.365*	0.121	-0.124	0.405**	0.089	0.433**	1		
SPAD	0.231**	0.153	0.557**	0.342*	0.452*	0.234*	0.05	0.045	0.315**	0.103*	0.373**	1	
TGW	0.285**	0.04	0.331**	0.212*	0.101*	0.145*	-0.235*	-0.562*	0.224*	0.127**	0.296**	0.541**	1

*Significant at 0.05, **Significant at 0.01.

Table 3 Pearson correlation coefficient among the morpho-physiological traits with grain yield across different locations in BILs population

	DRF17 (GY)	DRF18 (GY)	DIR17 (GY)	DIR18 (GY)	IND17 (GY)	IND18 (GY)
GY	1	1	1	1	1	1
NDVI-1	0.116	0.213*	0.312*	0.102*	0.214*	0.127*
NDVI-2	0.256*	0.325*	0.115	0.126*	0.163*	0.226*
NDVI-3	0.314*	0.204*	0.263*	0.241**	0.227*	0.204*
CT-1	-0.224*	-0.105*	-0.057	-0.046	-0.254*	-0.231*
CT-2	-0.367*	-0.312*	-0.141*	-0.135*	-0.325*	-0.269*
GWS	0.331*	0.423*	0.332*	0.422**	0.241*	0.324**
PH	0.279*	0.147	-0.138	0.034	0.025	0.054
Biomass	0.673*	0.210*	0.602*	0.301*	0.131*	0.268*
SPAD	0.431*	0.248*	0.321*	0.287*	0.354*	0.249*
TGW	0.351*	0.415**	0.283*	0.317*	0.407**	0.337*

*Significant at 0.05, **Significant at 0.01.

present in opposite sectors of the biplot (Yan *et al.* 2006). The collective contribution of IPCA1 and IPCA2 is 58.46% to the total GEI. However, as testimony for the variation revealed by G×E, the first two IPCA axes were sufficient as has been previously reported (Gauch 2006; Fufa 2013; Verma *et al.* 2015). Since most of G×E left out from first two multiplicative component axes, insufficient accounting of total interaction variance in judging genotypes stability over all environment may happen. Therefore, further on dissection of 280 genotypes, the top stable genotypes were selected using ASV for further AMMI stability analysis and GGE biplot analysis. This stability measure has a significant correlation with other noted stability measures like Shukla, Wricke (Wi) and Eberhart and Russel (S^2d) (Purchase *et al.* 2000).

Table 4 AMMI analysis of variance of main effects and interactions for wheat BILs population for grain yield

Source	df	SS	MS	F	F_prob
Total	3359	58008823	17270		
Treatments	1679	52918101	31518	10.27	<0.001
Genotypes	279	10790613	38676	12.6	<0.001
Environments	5	20666323	4133265	3940.72	<0.001
Interactions	1391	21461165	15429	5.03	<0.001
IPCA	283	8146004	28784	9.38	<0.001
IPCA	281	4403219	15670	5.1	<0.001
Residuals	827	8911942	10776	3.51	<0.001
Error	1656	5084429	3070		

Based on this, top 29 highly stable genotypes with relatively high average yield were chosen. AMMI ANOVA

Table 5 Mean yield performance in an environment (Em) and first 10 AMMI selections per environment

ENV	Em	1	2	3	4	5	6	7	8	9	10
E1(DELIR17)	525.04	G243	G171	G71	G265	G80	G244	G261	G119	G227	G123
E2(DELIR18)	493.5	G86	G265	G28	G264	G109	G171	G4	G7	G26	G243
E3(DRF17)	517.48	G216	G108	G93	G54	G50	G64	G156	G116	G219	G102
E4(DRF18)	521.1	G86	G70	G53	G5	G4	G247	G176	G15	G126	G26
E5(INDORE17)	317.5	G86	G28	G109	G265	G4	G264	G205	G7	G116	G70
E6(INDORE18)	379.8	G4	G205	G70	G86	G108	G28	G35	G109	G116	G68

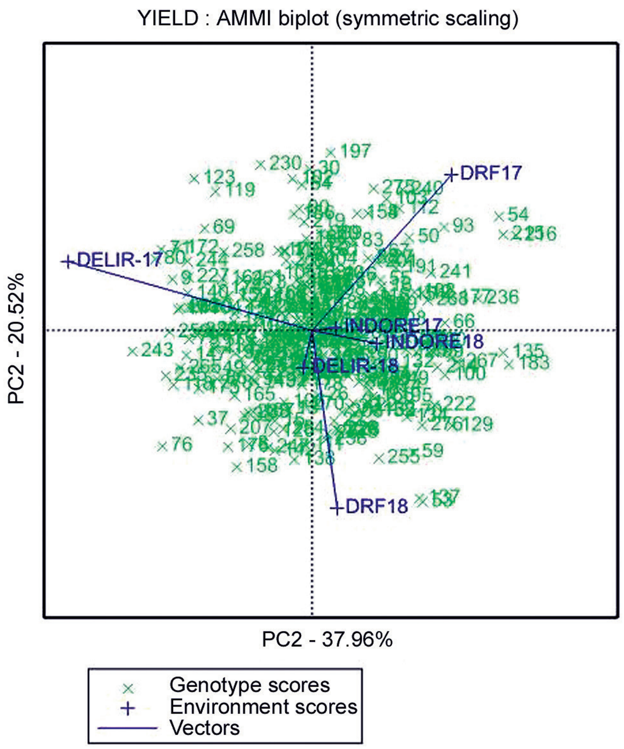

Fig 1 AMMI Biplot for grain yield showing the interaction of IPCA2 against IPCA1 scores of 280 wheat genotypes (G) in six environments.

of selected stable genotypes accounted 13%, 64.50% and 22.49% of significant genotypic, environmental, GEI variance (Table 6). The environment has more influence in genotypes stability. The partition of interaction into IPCA explained IPCA1 (45.37%), and IPCA2 (21.88%) interaction of sum of square. The total variance of first two multiplicative component axes is 67.25%. According to Yang *et al.* (2009), if biplot can represent at least 60% of the total data variance, it can be used to identify positions of mega environments. This excise narrows down our investigation from 280 genotypes to bunch of 29 highly stable genotypes. Analyzing selected genotypes using GGE biplot helps to filtrate and interpret performance of genotypes in terms of stability and high mean yield.

Table 6 AMMI analysis of variance of 29 selected stable genotypes based on ASV of wheat BILs population for grain yield

Source	df	SS	MS	F	F_prob
Total	347	3725737	10737		
Treatments	173	3158650	18258	5.6	<0.001
Genotypes	28	410768	14670	4.5	<0.001
Environments	5	2037475	407495	110.41	<0.001
Interactions	139	710406	5111	1.57	0.0028
IPCA1	32	288809	9025	2.77	<0.001
IPCA2	30	211079	7036	2.16	0.0012
Residuals	77	210518	2734	0.84	0.8084
Error	167	544944	3263		

'Which won where' feature of GGE biplot helps us to find out genotypes that perform well in each environment and in each mega environment. The biplot divided into 7 sectors and three-mega environment. The one mega environment comprised INDORE 18 while the other includes INDORE17 and DELIR18. The remaining environments combined to form third mega environment. Harikrishna *et al.* (2016) identified three mega environments by evaluating RILs population of wheat under different moisture stress based on the GGE biplot analysis.The hexagon has six genotypes, viz. G98, G149, G124, G45, G36, and G273 at the vertices (Fig 2). These are most responsive to environmental change and specifically adapted genotypes, because they have longest distance from the origin (Yan and Tinker 2006). As now environment markers placed into different sectors, this shows that different cultivars won in different sectors (Yan *et al.* 2007).The G273 winning genotype in first mega environment (INDORE18). G36 represented in second mega environment (INDORE17 and DELIR18). Third mega environment (DRF18, DRF17 and DELIR17) comprised G45 as winning genotype. Therefore, these genotypes should be selected in these mega environments and deployed for each. Third mega environment includes DRF17 and DRF18Delhi rainfed repeated across years, hence G45 genotype can be exploited by selecting in it.Further genotypes namely G115, G202, G234 and G20 are centered at polygon having average performance, high stability, less responsive across all environments. The genotype having high mean yield and absolute stable performance is considered ideal genotype (Yan and Kang 2003, Farshadfar *et al.* 2012). A genotype which is closer to ideal genotype is most desirable for selection (Mitrovic *et al.* 2012). Hence ideal genotype will be indicated by representing point on average environment axis towards positive direction and having highest vector length of high yielding genotypes with zero G×E, as indicated by an arrow pointing towards ideal genotype. In order to visualize the distance between ideal genotype to each genotype, keeping ideal genotype in center concentric circles have been drawn placing other genotypes in it (Yan and Tinker 2006). Hence forth, G82 was closure to ideal genotype followed by G6, G192, G77, G202, G234 and G263 ranked for closest to ideal genotype and most desirable genotypes (Fig 3). Through the biplot it's possible to assess the mean yield and stability performance. The projection onto ATC vertical axis represent stability of genotype similarly projection of their markers on to the ATC horizontal axis indicate average yield of genotypes (Yan and Tinkler 2006). Thus, genotype G82, G45, G36 and G6 had the highest average yield, and G98, G29 and G34 had the lowest. G120 had a mean yield similar to grand mean. Whereas genotypes G149, G77, G273 and G124 were the least stable and G82, G202, G234, G120, G263and G239 were the most stable. However, by considering both mean yield and stability performance, genotypes, G82, G263, G202, G234, and

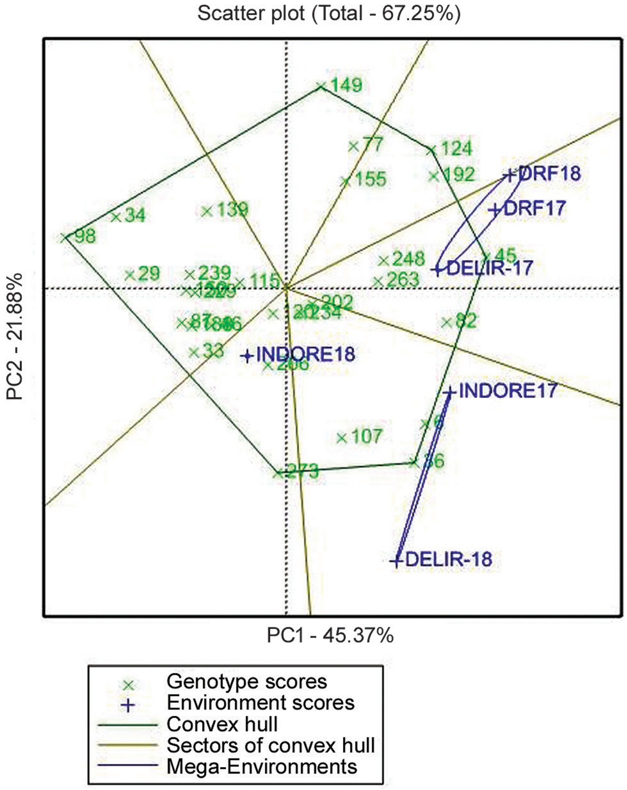

Fig 2 Polygon view of GGE biplot showing "which won where" pattern for genotypes and environments.

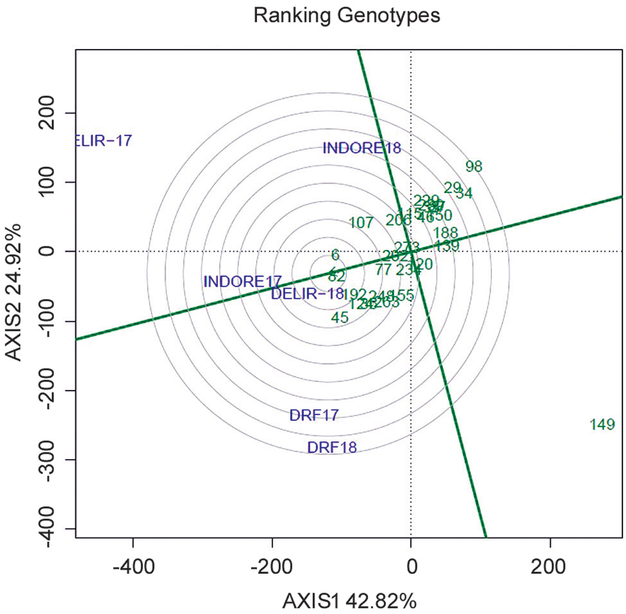

Fig 3 GGE biplot based on genotype-focused scaling for rank genotypes relative to ideal genotype (the center of the concentric circles).

G120 can be considered as the most favorable. Amare *et al.* 2019, based on AMMI and GGE biplot analysis of 24 pearl millet genotypes they selected three stable and high yielding genotypes for advancement of varietal trail. The ranking of genotypes by following procedure has been reported by Yan and Kang (2002); Baxevanos *et al.* (2008); Hamayoon *et al.* (2011); Roostaei *et al.* (2014). These genotypes can be explored for the selection as they have high mean yield and stability across the genotypes. The present study of AMMI and GGE biplot analysis on yield revealed that genotypes G82, G202, G234, G263, G6, G192 and G77 were recognized as superior, most adapted, highly stable and superior yielding genotypes across the locations and over the years. These genotypes can be used as contributor for breeding drought tolerant varieties, and release of these genotypes will manifest stable performance under moisture stress conditions.

Crop yield under drought stress is highly targeted trait for breeding, but this is influenced by so many component traits along with environment. Development of high yielding and stable genotypes for diverse environment, will serve as best option for wheat growers. The present study on wheat BILs population showed segregation and large variability for morph-physiological traits and, also presence of G × E interaction, which can be productively utilized for breeding drought tolerance lines. The positive significant correlation of grain yield with NDVI, SPAD, TGW, as well as negative association with CT specify that these traits can be genetically manipulated to enhance wheat yield under moisture stress. AMMI stability analysis model could be prominent tool for selecting most fit and stable high yielding genotypes for specific and diverse environment. AMMI model has shown that the major proportion of the total variation in grain yield was contributed by environments and GE interactions. Overall on the basis of stability of BILs G82, G202, G234, G263, G6, G192 and G77 were hardly affected by the G×E interaction and thus would perform wellacross a wide range of environments, viz. RF, RI, and Irrigated. These genotypes can be exploited in wheat varietal breeding scheme to develop drought tolerant genotypes.

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