

Genotype Environment Interaction ($G \times E$) of Heat Tolerant Wheat Genotypes over Locations and Years

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Abstract

Twenty heat tolerant wheat genotypes were evaluated in three heat stress environments of Bangladesh such as Wheat Research Center (WRC), Nashipur, Dinajpur, Regional Wheat Research Center, BARI, Gazipur, Regional Wheat Research Center, RARS, Jashore in 2015-2016. The experiments were conducted in RCBD with three replications in 2015-2016. The stability and response to change of location and sowing time of the genotypes were assessed through AMMI model and regression coefficient analysis on seven characters, viz. days to heading, days to maturity, plant height (cm), spikes m^{-2} , grains spike⁻¹, 1000-grain weight (g) and yield ($kg \cdot ha^{-1}$). Among the linear interactions, sowing time alone exerted the maximum sum of square (335,388,000.00**) and among the non-linear interactions, location \times year paid the highest effect (7,676,490.00**), which was followed by year \times location \times sowing time interaction (3,956,500.00**). The highest grain yield (4475.3 $kg \cdot ha^{-1}$) was obtained from the genotype G13, followed by the genotypes G17 (4460.8 $kg \cdot ha^{-1}$) and G19 (4404.7 $kg \cdot ha^{-1}$). The genotype G8 had the smallest interaction effect, while the genotype G14 followed by G10 responded vigorously to amelioration. The genotypes G13, G18 and G19 showed averagely high yield and showed stability over the environmental modifications. The other two genotypes G20 and G12 exhibited great reduction of yield potential under unfavorable environment. The environment 121 proceeded by J121 and G131

appeared as favorable environments. The genotypes G13 and G19 produced high yield ($\text{kg}\cdot\text{ha}^{-1}$) in the favorable environments. The genotypes G16 and G18 high IPCa1 scores, indicated sensitive to mega-environmental changes. The best genotype with respect to environment was genotype G13. Genotypes G17 and G19 were best for site E1; genotypes G14, G16 and G18 were best for site E2.

Keywords

Wheat Genotype, Over Location, Over Years, Heat Stress

1. Introduction

Wheat (*Triticum* spp.) [1] is a grass that is cultivated worldwide. Globally, it is the most important human food grain and ranks second in total production as a cereal crop behind maize; the third being rice. It is a cereal of the Gramineae family which includes other important cereals as foods such as rice, maize, oats, sorghum, etc. It is a source of protein and ranks second after rice as a source of calories in the diets of consumers in developing countries [2].

Yield and Stability are very important in wheat production. In Bangladesh the yield fluctuation and yielding pattern of genotypes were highly varied with small geographic location. Selecting genotypes in diversified testing locations and assessing yield stability of wheat genotypes is a vital issue for the wheat breeders of our country. $G \times E$ interactions are of major importance, because they provide information about the effect of different environments on cultivar performance and have a key role in assessment of performance stability of the breeding materials [3]. Increasing genetic gains in yield is possible in part from narrowing the adaptation of cultivars, thus maximizing yield in particular areas by exploiting genotype \times environment interaction [4]. Several methods have been proposed to analyze GEI or phenotypic stability [5].

Plant breeders invariably encounter genotype \times environment interactions (GEIs) when testing varieties across a number of environments. Depending upon the magnitude of the interactions or the differential genotypic responses to environments, the varietal rankings can differ greatly across environments. A combined analysis of variance can quantify the interactions, and describe the main effects. However, analysis of variance is uninformative for explaining GEI. Other statistical models for describing GEI such as the additive main effects and multiplicative interaction (AMMI) model are useful for understanding GEI.

The additive main effects and multiplicative interaction (AMMI) model is a powerful multivariate method for multi-environmental trials [6]. Various statistical methods (parametric and non-parametric) have been proposed to study Genotype \times environment interactions [5] [7] [8] [9] [10] [11]. Different concepts and definitions of stability have been described over the years.

Sable wheat production is a major concern in rainfed and irrigated areas that

are affected by different environmental factors. Growing adapted cultivars with high yield stability is an effective strategy for reducing environmental effects on wheat production in different areas. To develop suitable cultivars, evaluation of improved genotypes is a critical phase in wheat breeding programs, because great numbers of genotypes need to be evaluated across locations over several years. Studying the response of genotypes under different conditions may significantly increase their productivity potential and performance. Therefore, plant breeders aim to develop new wheat cultivars that consistently have high yield in a variety of environments. The adaptability of a variety is usually tested by the degree of its interaction with different environments. A variety or genotype is considered to be more adaptive or stable if it has a high mean yield with low degree of fluctuation in yielding ability grown over diverse climatic conditions.

Considering the above facts, the present study was carried out on adaptation and genotype \times environment interaction of wheat genotypes grown at different environments in the country to identify the bread wheat genotypes that have both high mean yield and stable yield performance across different environments of our country

2. Materials and Methods

A total of 73 advanced generation (F6) were derived from a series of hybridization between different pairs of combinations to evolve heat as well as drought tolerant wheat varieties. Three varieties namely, G1 (Shatabdi), G2 (Pradip) and G3 (BARI Gom 26) were kept throughout the investigation. At the end of evaluation based on canopy temperature, SPAD value against chlorophyll content and stress susceptibility index, 20 genetically diverged genotypes including three check varieties were to select apparently heat as well as drought tolerant lines. None of the released varieties showed negative stress susceptibility Index (SSI) but all the 17 genotypes showed negative SSI over the locations. The trials were conducted in three locations (Dinajpur, Joydebpur and Jashore) and two sowing times, ITS (irrigated timely sowing) and ILS (irrigated late sowing) were allotted for each of the genotypes. Finally the 20 genotypes were evaluated in three heat stress environments.

Locations: The experimental fields were located in three different districts of Bangladesh. Wheat Research Center (WRC), Nashipur, Dinajpur, Regional Wheat Research Center, BARI, Gazipur, Regional Wheat Research Center, RARS, Jashore.

Experimental design and layout

The field experiments were laid out in an alpha lattice design with two replications. The plot size was 2.5 m \times 0.8 m with 5 rows. The row length was 2.5 m long and 20 cm and 2 cm distances between rows and plants, respectively. The cultivars were randomly distributed to each of the plots within a block.

Experimental seeds were sown under irrigated timely sown (ITS, 22-24 November 2015) and irrigated late sown (ILS, 24-25 December 2015) conditions in each location. The trials were irrigated 3 times at tillering stage, booting stage

and grain filling stage and kept weed free by hand weeding.

Land preparation

The land was prepared by two ploughings and one cross ploughing with a tractor mounted disc plough. Three days later the land was again ploughed and cross ploughed with the country plough followed by laddering to get a good puddle condition. Weeds and stubbles were removed from the field prior to sowing of seeds. Manures and fertilizers were applied as per the recommended doses and irrigation channels were made around each block. Fertilizers were applied @ 100-27-50-20-1-4.5-5000 kg·ha⁻¹ as N-P-K-S-B-Zn-cow dung, respectively. The total cow dung was applied at the beginning of land preparation and all the fertilizers and two third of urea were applied as basal dose at the time of final land preparation. Rest one third of urea was applied at 21 days after sowing as top dressing as recommended by [12].

Seeds sowing

Experimental seeds were sown under irrigated timely sown (ITS, 22-24 November) and irrigated late sown (ILS, 24-25 December) conditions in each location and in each year.

Intercultural operations

The following necessary intercultural operations were taken during entire cropping period for proper growth and development of the plants and to receive a good harvest

Harvesting

Maturity of crops was determined when 80% of the seeds became physiologically mature. The harvested crop of each plot was separately bundled, properly tagged and then brought to the threshing floor. The yield of grain was recorded after thoroughly drying in the sun.

Data collection

Plant height (cm), days to heading (Days), days to physiological maturity (days), number of spikes per square meter, number of grains per spike, spikelet spike⁻¹, 1000 seed wt. and yield.

Analysis of variance

Analysis of variance for each environment, combined analysis of variance across the tested environments and stability parameters: regression coefficient (*b_i*) and Mean square deviations (*S²_{di}*) from linear regression were performed by using CROPSTAT, version 7.2.

Stability analysis

Stability was determined by regression of the mean grain yield of individual genotypes on environmental index and calculating the deviation from the regression according to [13] as:

$$Y_{ij} = K_i + b_i I_j + s2d_{ij}$$

where Y_{ij} was the mean performance of i th variety in j th environment, K_i was the mean of i th variety over all environments; b_i is the regression coefficient which measured the response of i th variety to varying environment; $s2d_{ij}$ was deviation

from regression of i th variety in the j th environment, and I_j was the environmental index of j th environment.

Both AMMI and Eberhart and Russel models were computed using Agrobases software [14].

Additive main effect and multiplicative interaction (AMMI) model

The additive main effect and multiplicative interaction (AMMI) analysis was performed using the model suggested by [8] as:

$$Y_{ij} = \mu + g_i + e_j + \sum_{n=1}^h \lambda_n \alpha_{ni} \gamma_{nj} + R_{ij}$$

GGE biplot/PCA using R program [15]

The concept of GGE

The concept of GGE originates from analysis of METs of crop cultivars. The yield of a cultivar (or any other measure of cultivar performance) in an environment is a mixed effect of genotype main effect (G), environment main effect (E), and genotype \times environment interaction (GE). In normal METs, E accounts for 80% of the total yield variation, and G and GE each account for about 10% [16] [17]. For the purpose of cultivar evaluation, however, only G and GE are relevant [16]. Furthermore, both G and GE must be considered in cultivar evaluation, thus the term GGE [17].

3. Result and Discussion

AMMI Analysis

The analysis of variance (ANOVA) and AMMI model of twenty wheat genotypes for eight characters including yield ($\text{kg}\cdot\text{ha}^{-1}$) across three environments are presented in **Table 1**. Based on the combined analysis of variance, a significant effect of year (Y) was observed for all characters except plant height but location (L) was significant for all the characters. The grain yield of wheat genotypes in the tests was highly significant $Y \times L$ interaction effect, possibly due to changes in environmental conditions, which vary from one environment (year \times location combination) to another. Genotypes demonstrated significant effect, which could be due to changes in genotype characteristics, varying from one genotype to another. The interaction effects ($G \times L$) and ($G \times Y$) and the three way interaction of factors ($G \times L \times Y$) were significant against all the characters excluding grains spike^{-1} . [18] reported that $G \times E$ interaction with location is more important than $G \times E$ interaction with year. However, the partitioning of variance components for environment revealed that both predictable (locations) and unpredictable (year) components were important sources of variation. When $G \times E$ (ST) interaction was due to variation in predictable factors, a plant breeder has the choice of either developing specific genotypes for selected environments or broadly adapted genotypes that can perform well under variable conditions [19].

However, the results of combined ANOVA for the yearly datasets were shown in **Table 2** which provided a general picture of the relative magnitudes of effects of genotype, location and the interaction ($G \times L$) due to $G + L + G \times L$ variations.

Table 1. Combined analysis of variance for different characters of 20 wheat genotypes across location.

Source of variation	Degree of freedom (df)	Mean sum of squares (MS)							
		HD	MD	PH. (cm)	Spike m ⁻²	Spikelet spike ⁻¹	Grains spike ⁻¹	TGW (gm)	Yield (kg·ha ⁻¹)
REP	1	8.02*	8.67*	106.57**	22,972.9**	0.04ns	16.17ns	0.03ns	2429.47ns
YEAR	1	299.02**	143.11**	10.51ns	543,729.00**	3.76*	76.51	33.72**	8,137,380.00**
LOCATION (L)	2	3655.90**	5063.08**	5841.10**	691,924.00**	168.19**	463.38	361.77**	2,160,390.00**
SEEDING TIME (ST)	2	2738.51**	19,807.20**	3651.91**	15,886.60**	84.71**	5535.07	9810.35**	335,388,000.00**
GENOTYPE (G)	19	206.34**	74.018**	355.14**	34,403.30**	13.18**	391.47	586.57**	1,620,170.00**
YEAR × L	2	119.67**	1347.20**	21.16ns	133,294.00**	15.29**	1173.12	48.76**	7,676,490.00**
YEAR × ST	2	349.44**	30.22**	184.09**	25,839.70**	12.26**	49.87	0.19ns	58,626.80*
L × ST	4	99.36**	116.86**	131.23**	6822.44**	13.28**	85.75	220.11**	839,563.00**
YEAR × G	19	6.6479**	6.4137**	37.95**	1326.57ns	1.42*	34.08	2.27**	202,348.00**
L × G	38	11.58**	8.21**	29.17**	2414.93ns	1.93**	65.15	32.59**	355,551**
ST × G	38	8.15**	8.25**	13.29ns	2073.92ns	1.70**	16.24	15.69**	339,741.00**
YEAR × L × ST	4	39.51**	365.56**	183.12**	19,228.10**	31.35**	238.41	7.29**	3,956,500.00**
YEAR × L × G	38	5.10**	4.49**	17.52*	3719.22**	1.74**	23.59	3.56**	108,427.00**
YEAR × ST × G	38	4.84**	4.78**	23.92**	1941.59ns	1.14ns	18.34	3.31**	74,789.30**
L × ST × G	76	2.18ns	4.47**	14.02*	2253.29ns	1.07ns	16.47	12.09**	122,911.00**
YEAR × L × ST × G	76	3.47**	3.67*	16.67**	2040.17ns	0.95ns	15.64	3.68**	84,129.40**
RESIDUAL	359	1.86	2.65	10.88	1836.24	0.89	3439.18	1.02	18,130.40

*P < 0.05, **P < 0.01, ns = non-significant. HD = Heading Days, MD = Maturity Days, PH = Plant Height.

Table 2. Genotypes means at three locations (L), three sowing date (D) for grain yield (kg) over two years.

Genotype	2015									2016									Mean
	Dinajpur			Gazipur			Jashore			Dinajpur			Gazipur			Jashore			
	ITS	ILS	IVLS	ITS	ILS	IVLS	ITS	ILS	IVLS	ITS	ILS	IVLS	ITS	ILS	IVLS	ITS	ILS	IVLS	
D121	D122	D123	G121	G122	G123	J121	J122	J123	D131	D132	D133	G131	G132	G133	J131	J132	J133		
1	5680.0	3598.3	3325.0	5282.0	4416.0	3004.0	5320.0	3755.0	2935.0	4140.0	3590.0	2025.0	5070.0	3730.0	3115.0	5290.7	4048.2	2842.6	3953.7
2	5766.7	3503.3	2370.0	5096.3	4131.0	3040.0	5435.0	3905.0	3280.0	4750.0	3395.0	2315.0	4800.0	4465.0	2915.0	5607.4	4281.5	3262.4	4017.7
3	6493.3	3670.0	2986.7	4424.4	3960.5	2875.0	4990.0	4160.0	2995.0	4620.0	3695.0	2740.0	4895.0	4270.5	3540.0	5509.3	3755.6	2905.6	4027.0
4	6513.3	3730.0	3306.7	4844.0	3924.5	3285.0	4995.0	3760.0	2945.0	4425.0	3420.0	2310.0	5180.0	3635.0	3175.0	5253.7	3587.0	2957.2	3958.1
5	6430.0	3766.7	3103.3	5285.0	3845.5	2950.0	5745.0	4200.0	3290.0	4640.0	3495.0	2960.0	5220.0	3645.0	3235.0	5944.4	3464.8	3185.2	4133.6
6	6256.7	4253.3	3473.3	5245.0	4205.0	3100.0	5300.0	4130.0	3436.0	4750.0	3810.0	2705.0	5575.0	4415.0	3520.0	5487.0	3706.5	2846.3	4234.1
7	6610.0	4213.3	3173.3	5415.0	4116.0	2620.0	5585.0	3955.0	3307.5	4875.0	3610.0	2485.0	5410.0	4180.0	2445.0	5972.2	3165.0	2895.0	4112.9
8	5735.3	3786.7	3096.7	5212.0	4095.0	3481.0	5135.0	3525.0	3150.0	4530.0	3390.0	2585.0	5440.5	4067.0	3490.0	5381.5	3074.1	2855.6	4001.7
9	5700.0	4303.3	3333.3	5415.0	4180.0	3405.0	5075.0	3941.5	3071.5	4745.0	3825.0	2835.0	5380.0	4470.0	3335.0	5633.9	3653.7	3010.7	4184.1

Continued

10	6453.3	3993.3	2800.0	5790.0	4104.0	3220.5	5135.0	3403.0	2975.5	4915.0	3880.0	3095.0	5880.0	3825.0	3080.0	5927.0	3508.5	2407.4	44132.9
11	5980.0	3466.7	2940.0	5596.5	3685.0	2920.0	5015.0	2903.5	2065.0	4925.0	3255.0	2460.0	5605.0	3405.0	2765.0	5401.9	2877.8	1772.2	3724.4
12	5753.3	4206.7	3257.3	4620.0	3500.0	2700.0	4860.0	3955.0	3055.0	4540.0	3525.0	2165.0	4390.0	3405.0	2525.0	5101.9	3316.7	2235.2	3728.4
13	5886.7	3981.7	3020.0	5780.0	4281.0	3420.0	5230.0	4905.0	4010.0	5505.0	4085.0	2856.0	6000.0	4530.0	3690.0	5972.2	4361.1	3042.6	4475.3
14	5400.0	3139.2	2932.5	5615.0	3985.5	3131.0	5040.0	4030.0	3235.0	4675.0	3315.0	2895.0	5800.0	4110.0	3130.0	5838.9	3646.3	2829.6	4041.6
15	5651.7	4213.3	3235.0	4815.0	4280.0	3225.0	4991.5	4100.0	3421.0	4700.0	3550.0	3045.0	4755.0	4210.0	3160.0	5181.5	3502.6	2958.0	4055.3
16	5730.8	4480.0	3275.0	5866.5	4485.0	3415.0	5795.0	3810.0	3220.0	5005.0	4085.0	3000.0	6085.0	4435.0	3515.0	5642.0	3955.6	2966.7	4375.9
17	5857.5	4266.5	3315.0	5500.0	4326.5	3500.0	5380.0	4300.0	3443.0	5428.5	4045.0	3166.5	5925.0	4555.0	3520.0	6079.6	4584.3	3101.4	4460.8
18	5646.0	4082.0	3050.0	5525.0	4200.0	3120.0	5315.0	4300.0	3275.0	4645.0	3645.5	3014.5	5920.0	4110.0	3160.0	5896.3	4006.0	2677.8	4199.3
19	6004.5	4455.0	3460.0	5609.0	4590.0	3420.0	5357.0	4125.0	3498.5	5125.0	3920.0	3035.0	5736.5	4525.0	3400.0	5689.1	4112.5	3223.3	4404.7
20	5313.3	3406.7	3060.0	4905.0	4091.0	3170.0	4745.0	3890.0	3207.5	4520.0	3325.0	2595.0	4895.0	3870.0	3275.0	5681.5	3955.6	2730.2	3924.2
Mean	5943.1	3925.8	3125.7	5292.0	4120.1	3150.1	5222.2	3952.7	3190.8	4772.9	3643.0	2714.4	5398.1	4092.9	3199.5	5624.6	3728.2	2835.2	4107.3
F-test										**									
5% LSD										264.767									
CV										3.3									

The residual mean sum of squares ranged from 0.89, 1092 (spikelets spike⁻¹) to 3439.18 (grains spike⁻¹). The highest residual mean sum of square was exaggerated due to non-significant effects of all kinds of interactions in combined analysis for grains spike⁻¹.

The presence of main effect variation was observed. However, according to IPCA1 genotype array, significant G × E interaction occurred. The genotype G8 had the smallest interaction effect, while the genotype G14 followed by G10 responded vigorously to amelioration. The genotypes G13, G18 and G19 gave averagely high yield and showed stable over the environmental modifications. The other two genotypes, G20 and G12 exhibited great reduction of yield potential under unfavorable environment (Figure 1). A remarkable grain yield variation explained by environments indicated that environments tested in the study were diverse, with large differences among environmental effects causing the most variation in grain yields of wheat genotypes [20].

AMMI biplot analysis

By modeling the above shown AMMI1 results, the effects of the experiment locations were removed, and the yields of the genotypes were calculated according to the scores of the first principal component (Figure 2). The stations were presented on the y-axis according to their scores on the principal component of AMMI1, and the nominal yield is represented on the y-axis. The grouping of the experiment sites is shown.

Genotypes or Location placed in the right side of the midpoint of the perpendicular line have higher yields than genotypes or location placed to the left side of the perpendicular line (grand mean). The genotypes, G13, G17 and G19 were higher yielder located to the right of the perpendicular line (grand mean).

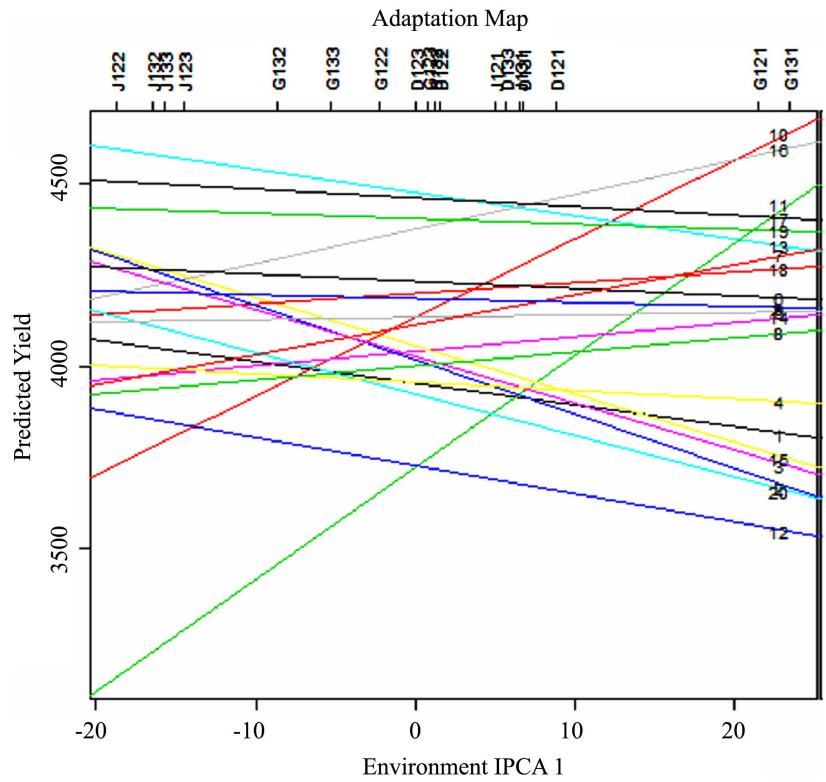


Figure 1. Sensitiveness of the genotypes across the environments for grain yield ($\text{kg}\cdot\text{ha}^{-1}$).

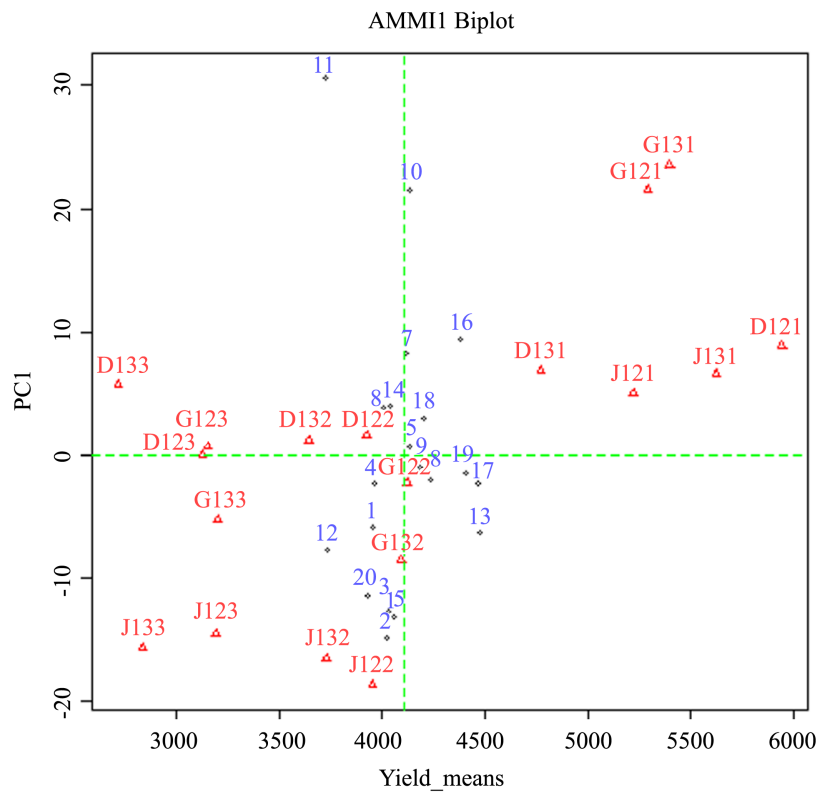


Figure 2. AMMI 1 model for grain yield ($\text{kg}\cdot\text{ha}^{-1}$) showing the means of genotypes and locations against their respective IPCA 1 scores.

The genotypes G11 and G12 were lower mean grain yield located to the left of the perpendicular line (grand mean). The genotypes, which nearly placed to the origin (G6 and G8) with lower contribution to the magnitude of genotype by environment interaction implying that the genotypes were stable. The genotypes, G2, G3, G15, G11 and G12 were located distant from the origin which was interactive genotypes contributing much to the increasing magnitude of genotype by environment interaction and they were the most unstable. The environment 121 proceeded by J121 and G131 appeared as favorable environments. The genotypes G13 and G19 produced high yield (kg·ha⁻¹) in the favorable environments. The genotypes G16 and G18 high IPCA1 scores, indicated sensitivity to mega-environmental changes.

The results in a graph (Figure 3) showed the environmental effect on each genotype [16] [21] [22] [23]. This bidimensional figure (Figure 3) was divided into four sections in which cultivars and environments were distributed according to the sign of their respective vectors obtained in the analysis [24] [25]. In general, cultivars exhibit a high degree of yield variability reflected by high dispersion in the biplot. Thus, interesting cultivars were those with IPCA1 values higher than zero and classified as high yield, while those with IPCA1 values lower than zero are classified as low yield and low adaptability [25]. Low yielding genotypes and environments were located in quadrants III and IV while, high

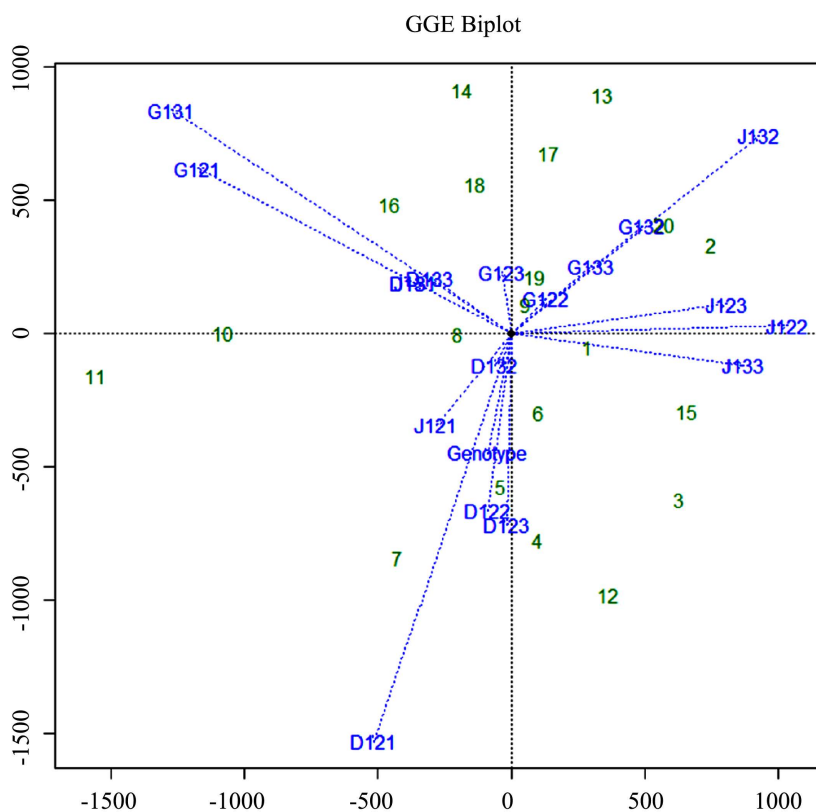


Figure 3. AMMI 2 model for grain yield (kg·ha⁻¹) showing IPCA1 vs IPCA2 scores of wheat genotypes sown across the locations.

yielding genotypes are located in quadrants I and II. The genotypes G1, G6 and G8 had low IPCA scores, indicated low adaptability and the lowest yield. On the other hand, genotypes that were stable or less sensitive to environmental influences were found near the biplot point of origin [25]-[30]. Since, the environments with positive IPCA1 score near zero had small interaction effects indicating that all the genotypes performed well in that locations. [31] and [32], reported similar pattern of interactions.

Which-Won-Where Pattern of genotypes

One of the smartest facial appearance of a GGE biplot is its facility to show the which-won-where model of a genotype by environment dataset (Figure 4). Many researchers find this use of a biplot intriguing, as it graphically addresses important concepts such as crossover $G \times E$, mega environment differentiation, particular adaptation, etc. [33]. In Figure 4, the sites fell into four sectors. The polygon is created by involving the markers of the genotypes that are further away from the biplot source such that all other genotypes are restricted in the polygon. Genotypes located on the vertices of the polygon performed either the best or the poorest in one or more environments since they had the long distance from the origin of biplot. The perpendicular lines are equality lines between adjacent genotypes on the polygon, which facilitate visual comparison of them.

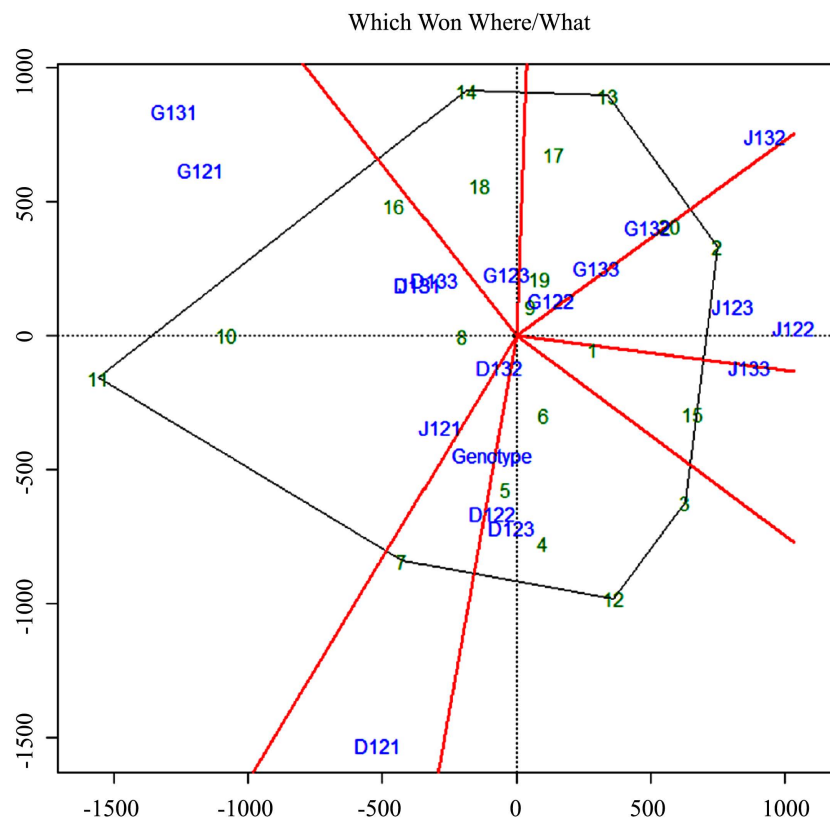


Figure 4. Polygon views of the GGE biplot based on symmetrical sealing for the which-won-where pattern of genotypes and environments.

The best genotype with respect to environment was genotype G13. Genotypes G17 and G19 were best for site E1; genotypes G14, G16 and G18 were best for site E2. Genotypes located near the plot origin were less responsive than the vertex genotypes. Genotypes G13 and G15 gave the highest average yield (largest IPCA 1 scores), but G13 was not stable over the sites, due to the fact that it did not give small absolute IPCA 2 score. In contrast, the non-adapted genotypes G1 and G8 yielded poorly at all sites, as indicated by their small IPCA1 scores (low yielding) and relatively small IPCA 2 scores (relatively stable). The average yield of genotypes G5 was below average (PCA1 score < 0) and highly unstable (large absolute PCA 2 score). The biplot showed not only the average yield of a genotype (IPCA 1 and IPCA2 effects), but also how it was achieved. That is, the biplot also showed the yield of a genotype at individual sites.

4. Conclusion

The AMMI analysis for the additive main effect and multiplicative interaction effect revealed significant difference for genotype, testing location and genotype by testing location and sowing time interaction. The first interaction principal component (IPCA1) captured higher of the interaction as compared to second interaction principal component explained. The AMMI model provided fitness and the wheat genotype by environment interaction were well predicted by this model. In multi-location adaption trial considering both the stability and mean grain yield is important. According to the AMMI biplot the wheat genotypes G13, G17 and G19 were stable but responsive to changing environments coupled with higher mean grain yield greater than the grand mean.

Conflicts of Interest

There were no conflicts of interest.

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