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Selection for High-Yielding adapted and Stable Wheat promising Lines in Egypt

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ABSTRACT

Among breeding program, releasing a new genotype with the desired yield stability and high performance under different environments is the main goal for wheat breeders. This study is aimed to identify the high adaptability and stability bread wheat genotypes with high yielding and resistance to yellow rust disease. Thus, twelve field experiments were conducted with 8 genotypes across 2 seasons (2019/2020 and 2020/2021) at 6 locations, viz. Skha, Etay-Elbaroud, Gemiza, Kafer El-Hamam, Matana and Nubaria in a randomized complete block design. The combined analysis of variance for grain yield cleared the significant effects of genotype (G), environment (E) and GE interaction, accounting for about 2.77%, 89.72%and 7.49% of the total variation, respectively. Additive main effect and multiplicative interaction (AMMI) analysis confirmed significant of the first two IPCA's and greatness the environmental variation. Genotype main effect plus GE interaction (GGE) biplot showed main two mega-environments, the first had most environments with winner stable genotype G3 (Line 3) as well as G1 (Line 1).Second mega-environment contained only two environments with winner check varieties. GGE biplot for the genotypes comparison, illustrated that genotypes G3 (Line 3) was found to be most adaptable and ideal stable across all tested environments, and then it should be recommended for releasing with wider environmental adaptability. Three molecular markers were used to evaluate the selected breeding materials for detecting the presence of different rusts resistance genes. Then, it can be used in the bread wheat breeding program to pyramid different resistance gene using Marker Assisted Selection (MAS).

Key words: GGE-biplot, Grain Yield, Resistance, Stability.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered the most strategic cereal crop in Egypt. Egyptian people depend on wheat as a main food. Currently Egypt is the largest wheat-importing country in the world (FAO2020). The Total cultivated area of wheat is about 1.42 million hectare which produced 9.34 million tons with an average 6.85 ton /hec.(Economic Affair sector, 2021). Egypt's imports about 13 million tons of wheat (FAO 2020). These imports increased annually as results to increase growing population by 2.2%. Many efforts have been paid to overcome the gap between consumption and production. Therefore, increasing wheat production is the main challenge facing wheat breeder. This increasing can be achieved by using new technologies system and developed new wheat varieties. Thus, many researches has been done to development new cultivars with high yield potentiality under various environmental which considered as a main target to Wheat National Program. Hence, using multi-environment trials (MET) seems to be the most important tool for predicting new cultivar performance. These trials are conducted in multiple environments (seasons and locations), measuring genotype-by-environment interaction (GEI) by trait three-way data (**Yan and Tinker, 2006**) to provide essential information for selecting wide adapted genotypes.

Recently, MET are widely used to evaluate the relative performance of genotypes over the target environments and to quantify adaptability and stability of genotypes (**Zhang *et al*., 2006; Jha *et al*., 2013**). Presence of a significant GEI qualified data across MET to stability analysis. Many methods could be used to study the stability based on univariate approach as analysis of variance (ANOVA). Also, multivariate approach based on principal component analysis (PCA) as additive main effects and multiplicative interaction (AMMI) and genotype + genotype by environment (GGE) biplot.

AMMI biplot showed linear line defined by the genotype mean yield on the x-axis and its interaction principal components axis (IPCA) score on the y-axis. Meanwhile, GGE biplot is able to illustrate the highest genotype with the highest yield across identical locations (Mega-environments) with best stability, ideal genotype and ideal location to increase yield, and specific location (**Farshadfar and Sadeghi, 2014**).

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Due to impending climate change, theincreasing damage of wheat production by wheat rustsare expected. Leaf rust caused by *Puccinia reccondita*, stem rust caused by *Puccinia graministritici* and Stripe rust of wheat caused by *Puccinia striiformis*, which considered as the most destructive diseases of wheat special in the Delta region. Thus, selection high yielding and resistant cultivars considered to be the most effective, economical and environmentally-friendly strategy for controlling this disease (**Bariana *et al.*, 2007**). Different protocols may be taken for controlling rust diseases and more effort is needed to find resistant genotype adapted to different environments (**Stoddard *et al*., 2010**).

The main objective of this investigate is identified the most adapted, stable, and resistant genotypes across environments by using multivariate statistical analysis of AMMI and GGE biplot methods across different locations over Egypt.

MATERIALAND METHODS

Twelve experiments were performed under recommended conditions using randomized complete block design with three replications during 2019/2020and 2020/2021growing seasons in six different locations (Skha, Etay-Elbaroud, Gemiza, Kafer El-Hamam, Matana and Nubaria) of Egypt. Eight bread wheat genotypes consisting of five advanced lines and three varieties as checks. These checks are included Giza 171 as a wide adaptability check, Shandaweel 1 as a heat tolerance check, and Miser 2 high yielding check (**Table 1**).The features of agricultural locations soil and climate where the research was conducted are shown in(**Table 2**).

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Table 1. Code, pedigree and characteristics of studied eight bread wheat genotypes.

Code	Genotype	Pedigree or selection history
G_1	**Line 1**	KIRITATI/2*WBLL1 CGSS02B00118T-099B-099Y-099M-099Y-099M-18WGY-OB-OGM
G_2	**Line 2**	WBLL1*2/VIVITTSI//AKURI/3/WBLL1*2/BRAMBLING CMSS07Y01066T-099TOPM-099Y-099M-099Y-7M-OWGY-OGM
G_3	**Line 3**	PFAU/SERI.IB//AMAD/3/WAXWING*2/4/TECUE#1 CMSS07B00614T-099TOPY-099M-099Y-099M-49WGY-OB-OGM
G_4	**Line 4**	WHEAR/VIVITIS//WHEAR. CGSS03-B00069T-099Y-099M-34WGY
G_5	**Line 5**	SIDS 1/ATTILA/3/KAUZ//BOW/NKT S.16494-032S-031S-14S-0S
G_6	**Giza 171**	SAKHA 93 / GEMMEIZA 9 Gz 2003-101-1Gz-4Gz-1Gz-2Gz-0Gz
G_7	**Shandaweel 1**	Site/Mo/4/Nac/Th.Ac//3*Pvn/3/Mirlo/Buc CMSS93 B00S 67S-72Y-010M-010Y-010M-3Y-0M-0THY-0SH
G_8	**Misr 2**	SKAUZ/BAV92 CMSS96M03611S-1M-0105Y-010M-010SY-8M-OY-OS

The field experiment was ploughed to a depth of 50 cm, three times, organic manure was incorporated into the ploughed layer at the rate of 40m^3/feddan. Super phosphate (15.5%) at the rate of 15.5 kg P_2O_5/feddan was added and mixed into the upper-15 cm layer of soil during the second ploughing. Each environment was sown by dividing the field into plot with size by 3m x 3.5m (10.5m^2). Each plot including 15 rows, row was 3.5 m long and the spaces apart rows were 20 cm. All cultural practices for growing wheat were applied as recommended. Plots were surrounded by spreader area planted with a mixture of highly susceptible wheat genotypes to rusts, *i.e.*, Morocco and Max to spread rust inoculums. Data of rust reaction were transformed to average coefficient of infection (ACI) according to the Cobb's scale adopted by **Pathan and Park (2006).**The final rust severity (FRS) was recorded as outlined by **Das *et al.* (1993)** as the disease severity (%) when highly susceptible check cultivar was severely rusted and disease rate reached the highest and final level of stripe rust severity.

At harvest, the two external rows from each plot were eliminated to avoid the border effect. Thus, 8 rows were harvested, threshed and their grain yields were weighed and adjusted to ardab per fedan (ard fed^{-1}).

The main chemical and physical properties of the soil with the climatic characteristics, relative humidity (RH %), air temperature (TC°),

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wind speed (Ws, m / sec at 2 m height) and rainfall (mm month^{-1}) rainfall during the two seasons are shown in (**Table 2**).

Table 2. Data of the experiment soil properties and climatic environments where the experiments were conducted.

No.	Location	Season	Environment	Code	Soil properties	temperature mean (℃)	Rain-fall (mm)
1	Skha	2019/20	Skha-1	Sk1	pH= 7.5, clay-loam	19.5	72.65
2		2020/21	Skha-2	Sk2	pH= 7.5, clay-loam	21.65	170.36
3	Etay El-baroud	2019/20	Etay-1	Ety1	pH= 7.5, clay	16.18	58
4		2020/21	Etay-2	Ety2	pH= 7.5, clay	18.61	100.6
5	Gemiza	2019/20	Gemiza-1	Gem1	pH= 8.0, clay	16.07	55.01
6		2020/21	Gemiza-2	Gem2	pH= 8.1, clay	16.52	233.41
7	KaferHamam	2019/20	Hamam-1	Ham1	pH= 7.5, clay	17.56	3.58
8		2020/21	Hamam-2	Ham2	pH= 7.5, clay	17.0	108
9	Matana	2019/20	Matana-1	Mat1	pH= 7.5, clay-loam	18.5	0
10		2020/21	Matana-2	Mat2	pH= 7.5, clay-loam	19.96	0
11	Nubaria	2019/20	Nubaria-1	Nub1	pH= 7.5, sandy clay	14.18	57
12		2020/21	Nubaria-2	Nub2	pH= 7.5, sandy clay	16.46	99.12

DNA Extraction

DNA was extracted from fresh young leaves 15-20 days old seedlings and 20 - 50 mg of powdered tissue was used for isolation of total genomic DNA using the following CTAB (Cetyltrimethyl ammonium bromide) method as modified by (Allen, et al., 2006). The DNA was diluted to a final concentration of 20 mg/µl and quantified in 0.8% agarose gel

PCR amplification and marker analysis

Three SSR and STS markers linked to stripe, stem and leaf rust resistance genes were used for identifying the stripe and stem rust resistance genes in selected eight advanced breeding materials. The PCR reaction was carried out in a 20 ml reaction volume containing 3.0 µl of template DNA (20mg/µl stock), 0.2µl (1 unit) of GRS Taq DNA polymerase (grisp, Portugal), 1.5 µl of 25 mM MgCl2 (total 1.5 to 2.5 mM MgCl2 per reaction), 3.0 µl of each dNTP (Promega, USA), 1.5 µl of each SSR marker (5mM) stock and 6.3 µl distilled H2O.

Amplification was carried out in a Veriti™ 96-well Thermal Cycler PCR (Applied Bio systems) at 1 cycle of 4 min at 94°C, 94°C for 1 min, 50-61°C (depending on marker) for 1 min. and 72°C for 1:30 min (35 cycles) and a final extension step of 72°C for 10 min (1 cycle). PCR products were resolved on 2 to 3% agarose gel at 120v for1.5 to 2h.

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Gels were stained in safe red and photographed on a digital gel documentation system (UVP-Multi Doc-It System, UVP-UK). 100 bp DNA ladder (GeneDirex) was used (3µl) for determining the molecular size of the DNA bands

Biometrical analysis

Obtained data from RCBD were subjected individually for each environment to analysis of variance according to **Gomez and Gomez (1984)**. **Levene test (1960)** was performed prior to the combined analysis to test the homogeneity of individual errors. Accordingly, the combined analysis of variance over twelve environments (two years and six locations) was done. Duncan's test was used to detect multiple range test at 5% probability level (**Duncan, 1955**). Data from all locations were pooled and tested for the presence of significant G×E by using analysis of variance. To evaluate the interaction effects, the data were subjected to multivariate analysis using additive main effect and multiplicative interaction (AMMI) model as previously described by **Gauch *et al* (2008).** To evaluate the stability and adaptability, the genotype and genotype by environment (GGE) biplot analysis was performed, considering the simplified model for two main components. These analyses were carried out with the help of the **GenStat (version 18)** package program (**Payne *et al*., 2015**) according to **Yan and Tinker (2006)**.

RESULTS AND DISCUSSION

The combined analysis of variance for grain yield (ard/fed) was performed after **Levene test (1960)** for homogeneity of errors across different locations and seasons **(Table 3**). The combined data revealed that grain yield was highly significantly influenced by locations and seasons accounted for 39.10 and 10.86 %, respectively (about 50%) of the total variation. This showed that, locations were more influence on the wheat grain yield larger than seasons. Regarding to genotypes, it had highly significant differences with account2.46%, indicating to presence of genetic variability in grain yield performance. However, location*season*genotype explained highly significant contribute (3.71%) of the total variation, pointing to the big influence of environment on yield performance of bread wheat genotypes. Therefore, genotype recorded higher interaction with location and more important than interaction with season **(Darwish, *et al* 2022**). Based on the significance of interaction, stability of studied genotypes can be preceded and estimated (**Farshadfar and Sutka, 2006**).

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Table 3. Combined analysis of variance for grain yield of bread wheat across different location and seasons.

Source of variation	d.f.	s.s.	m.s.	ExplainedSS (%)
Location	5	1900.65	380.13**	39.10
Location*Replication(Error1)	12	28.70	2.39	
Season	1	528.18	528.18**	10.87
Location* Season	5	1430.06	286.01**	29.42
Location*Season*Replication (Error2)	12	53.30	4.44	
Genotype	7	119.35	17.05**	2.46
Location*Genotype	35	131.75	3.76	2.71
Season*Genotype	7	10.38	1.48	0.21
Location*Season*Genotype	35	180.24	5.15**	3.71
Residual	168	478.29	2.85	9.839
Total	287	4860.90		

* and ** significant at 0.05 and 0.01 probability levels, respectively.

AMMI and principal component analysis (PCA)

Additive main effect and multiplicative interaction (AMMI) model considered as an effective way to investigate and interpretation the most part of significant GE interaction by principle component analysis (PCA). Results of analysis of variance for grain yield of eight bread wheat genotypes across twelve environments were presented in **Table (4)**. The results of pooled analysis of variance cleared sources of variation for each (Treatments, Genotypes and environment and genotypes x environments interaction). Environment as the main sources of variation sum of square with 89.72% followed by interactions with 7.49% and genotypes with 2.77% of the whole effect of grain yield variation **(Mahgoub *et al* 2022)**.

Table 4. AMMI analysis of variance for grain yield of eight bread wheat genotypes across twelve environments.

Source	d.f.	s.s.	m.s.	Explained %
Block	24	82	3.42	1.69
Treatments	95	4301	45.27**	88.48
Genotypes	7	119	17.05**	2.77
Environments	11	3859	350.81**	89.72
Interactions	77	322	4.19*	7.49
IPCA 1	17	152	8.92**	47.20
IPCA 2	15	77	5.16*	23.91
Residuals	45	93	2.07	28.88
Error	168	478	2.85	9.83
Total	287	4861	16.94	100

*** and ** significant at 0.05 and 0.01 probability levels, respectively.**

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Therefore, environments had the largest effect in grain yield, which is in harmony with the findings of Mahgoub ***et al*** **(2022), Darwish, *et al* (2022), Dejene (2016) and Sabaghnia *et al*. (2013).** Small portion ratio of genotypes in total wheat grain yield variation may be due to the complex nature of the yield trait, that controlled by a large number of components or the divergence of selected genotypes. Based on highly significant of genotype-environment interaction (GEI), AMMI model suggested the extension analysis by partitioning interaction among the significant first two interactions principal component axis (IPCA). Both of $IPCA_1$ and $IPCA_2$ accounted for 47.20% and 23.91%, respectively of the variation caused by interaction. These results were in agreement with the **Darwish, *et al* 2022, Mohamed and Ahmed (2013), Ilker *et al*. (2011) and Gauch and Zobel (1996)** recommendation with achievement the most accurate AMMI model by using the first two IPCAs.

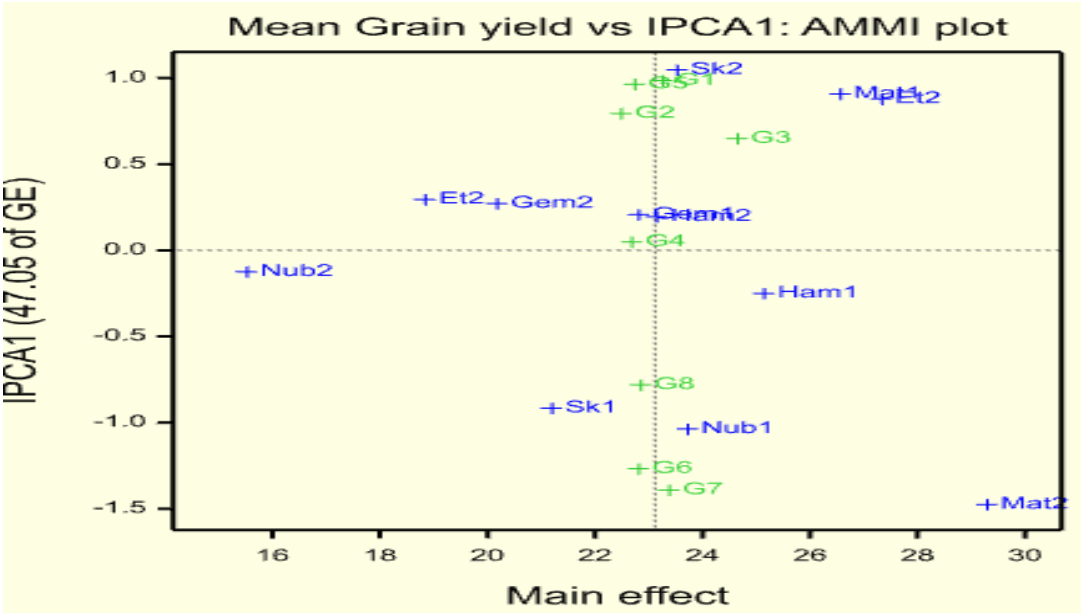

Figure 1. AMMI biplot showing the main and interaction (PC1) effects of both genotypes and location by seasons on wheat grain yield.

AMMI-biplot showed the main and interaction (PC1) effects of both genotypes and environments on wheat grain yield in **Figure (1).** Genotypes of G3 (Line 3), G1 (Line 1) and G7 (Shandweel 1) were placed on the positive right side of the graph. Wherever, G3 (Line 3) genotype was the highest yielding with most stable one. On the other hand, G4 (Line 4) was near the origin but on the left side of the vertical line of the genotype and environment means, so it considered as the stable genotype with the poorest yielding. Meanwhile, G2 (Line 2), G5 (Line 5), G6 (Misr 2)

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and G8 (Giza 171) located far away from the origin on the left side and also were unstable and poor yielding genotypes.

The best performing environments that placed on the right side were Sk2, Mat1, Mat2, Et1, Gem1, Ham1, Ham2 and Nub1. Meanwhile, the poor performing environments that placed on the left side were Nub2, Et2, Gem2 and Sk1, recording performance less than grand mean.

Means yield of wheat genotypes and environments

Mean performance of grain yield of eight bread wheat genotypes tested in twelve environments was shown in **Table (5).** Results revealed that the significant differences were found among the studied genotypes under different environments, indicating a wide range of genotypes and environmental effects as shown in **Table (5) and Fig (2).** Grain yield trait varied from 14.05 to 31.46 ard/fed across genotypes x environments interaction with yield grand mean 23.11 ard/fed.

Table 5. Average grain yield (ard/fed) of eight bread wheat genotypes tested in twelve environments.

Env. / Geno.	Line 1	Line 2	Line 3	Line 4	Line 5	G171	Sh.1	Misr 2	Geno-Mean
Sk1	21.02ab	18.42b	23.70a	20.70ab	19.62ab	21.90ab	23.50a	20.75ab	**21.20^{fg}**
Sk2	25.07ab	22.37c	25.62a	23.06bc	25.16ab	22.29c	21.89c	22.81bc	**23.54^{def}**
Et1	28.20ab	28.20ab	29.61a	26.32b	27.39ab	25.04c	26.92ab	27.05ab	**27.34^{ab}**
Et2	19.35a	18.57a	20.18a	18.58a	18.70a	18.33a	18.10a	18.99a	**18.85^{h}**
Gem1	23.50a	21.35b	24.37a	23.36a	23.11a	23.25a	22.65ab	20.99b	**22.82^{ef}**
Gem2	20.94a	20.82a	21.91a	21.21a	18.56ab	20.70a	20.45a	16.91b	**20.19^{gh}**
Ham1	25.17a	23.89b	26.68a	24.16b	24.93ab	25.09a	25.74a	25.56a	**25.15^{cd}**
Ham2	22.80b	24.07a	24.40a	22.26b	22.76b	22.31b	22.67b	24.00a	**23.16^{def}**
Mat1	27.03ab	26.93ab	28.68a	25.80ab	27.50ab	24.39b	25.80ab	26.41ab	**26.57^{bc}**
Mat2	27.15b	27.11b	29.64ab	28.63ab	28.81ab	**31.46a**	30.60a	31.02a	**29.30^{a}**
Nub1	23.29bc	24.18b	23.20bc	23.20b	21.33c	23.55b	26.22a	24.89ab	**23.73^{de}**
Nub2	15.63bc	14.05d	17.87a	15.05c	15.05c	15.52bc	16.15b	14.93c	**15.53^{i}**
Env-mean	**23.26^{ab}**	**22.50^{b}**	**24.65^{a}**	**22.70^{b}**	**22.74^{b}**	**22.82^{b}**	**23.39^{ab}**	**22.86^{b}**	***23.12***

Means of the same column (environments) or row (genotypes) followed by the same letter (s) are not significantly different (**Duncan, 1955**).

Regarding to genotypes mean across environments, genotype main effects were illustrated in **Fig (2).** Graph showed that the highest grain yield mean was determined by Line 3 with 24.65 ard/fed followed by Shandweel 1 and Line 1 (23.39 and 23.26 ard/fed, respectively) and the lowest value is 22.50 ard/fed in Line 2. These results confirmed data in**Table** (**5**).

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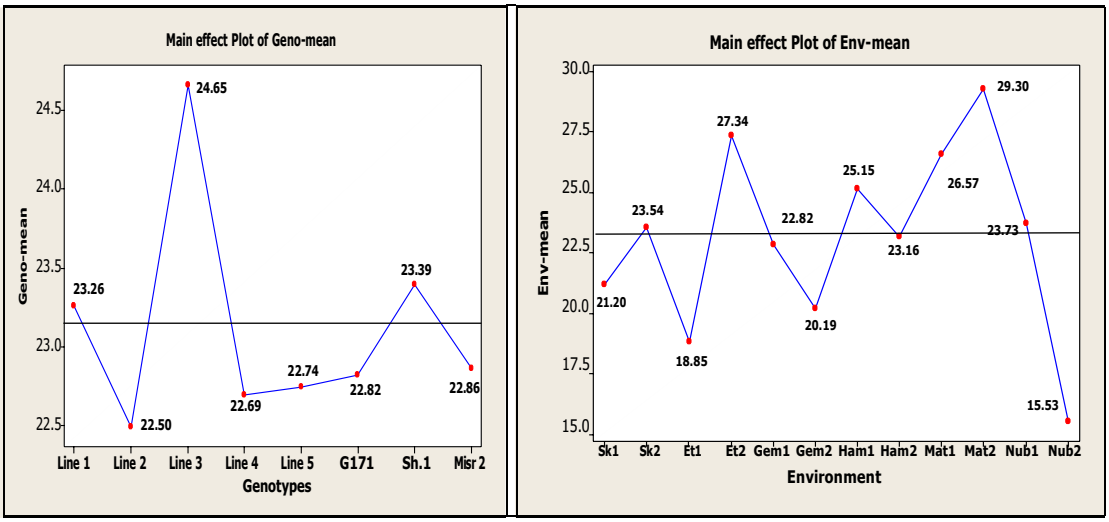

Fig 2. Main effects Plot for grain yield among eight Egyptians bread wheat genotypes and twelve environments**.**

Concerning on a wide range of environments effects, Matana environment had the highest mean values among all environments for grain yield/feddan in both seasons, recording 29.30 and 26.57 ard/fed, respectively. Also, Etay El-Baroud mean value (27.34 ard/fed) followed by Matana environment for grain yield. Meanwhile, Nubaria environment in the 2nd season with15.53ard/fed had the lowest one.

Therefore, some researchers emphasized that environments had great effects on bread wheat genotypes (**Darwish, *et al* 2022 andKadir *et al* 2018)**.

GGE biplot analysis

GGE biplot analysis of MET data can help researchers to better understand their target environment to detect the genotype by environment interaction pattern and classify mega environments that are widely or specifically. Also, it was used to identify superior genotypes based on stability and mean yield, and to establish more effective breeding.

Which-Won-Where view

Connecting the vertex genotypes of the furthest away from the bi-plot origin formed the GGE polygon biplot (**Figure 3**). This polygon-view of GGE biplot showing which genotypes had the highest values for which environments. Environments with the same winning genotype were considered as a mega-environment. In the polygon biplot view, the vectors from the biplot origin divided the graph into main six sectors, showing the main two different wheat growing mega-environments.

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The first mega-environment included the most stable ten environments (E1, E2, E3, E4, E5, E6, E7, E8, E9 and E12), which were in the locations of (Sk1, Sk2, Et1, Et2, Gem1, Gem2, Ham1, Ham2, Mat1 and Nub2). Genotype G3 (Line 3) was the most positively responsive at the vertex with the highest yielding in first mega-environment, followed by G1 (Line 1). However, G1 (Line 1) had specific adaptability for Sk2, Et1 and Mat1. Meanwhile, the second mega-environment, containing environments (E10 and E11) which were the lowest stable under (Mat2 and Nub1) locations with the vertex checks varieties genotypes G6 (Giza 171) and G7 (Shandweel 1). Our findings were similar to those of **Darwish, *et al* 2022 and Kadir *et al* 2018**. Generally, G3 (Line 3) was wider adaptable genotype followed by Line 1, meanwhile all checks varieties genotypes fall in the same mega-environment.

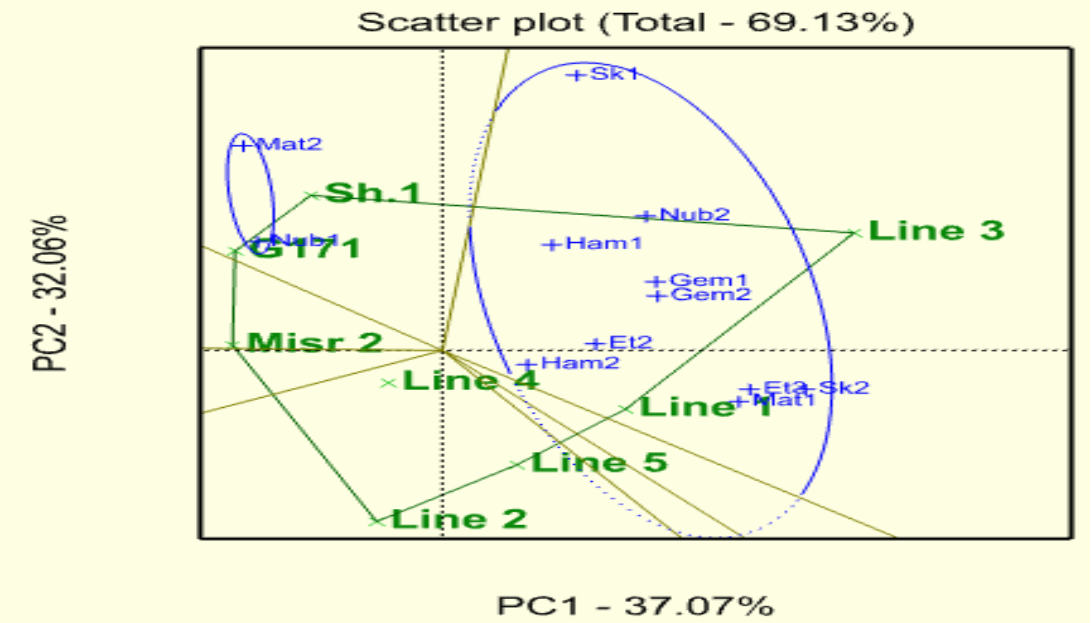

Figure 3. Polygon-view of GGE biplot graph showing which genotypes had the highest values for which environments.

Ideal genotype

GGE-biplot for comparison of the studied genotypes with the ideal one was shown in **Figure (4)**. The ideal genotype that had both stable and high mean yield performance across environments was plotted in the concentric circle in the biplot graph. The middle concentric circle pointed with drawn arrow help in recognizing the distance between genotypes and the ideal genotype (**Yan and Tinker, 2006**).

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From the obtained graph illustrated that G3 (Line 3) was the ideal genotype with the highest mean grain yield and most stable across variable environments. Nearest genotypes to the ideal genotype were the stable ones, while others far from the ideal were the unstable. Then, G1 (Line 1) that located above the yield average and close to the ideal genotype can considered as desirable genotype. It was observed that genotype Line 3 was adapted for all environments, especially locations in north Egypt mostly. Meanwhile, G2 (Line 2) that was far from the ideal genotype and below the yield average can considered as the unstable low yield one. Similar result was reported by **Darwish, *et al* 2022, Kadir *et al* 2018 and Asnake *et al*., (2013)** who suggested that ideal bread wheat genotypes were determined for different locations.

As seen from results, the ideal genotype G3 (Line 3) had high mean performance coupled by high stability to give wide adaptability in the different regions.

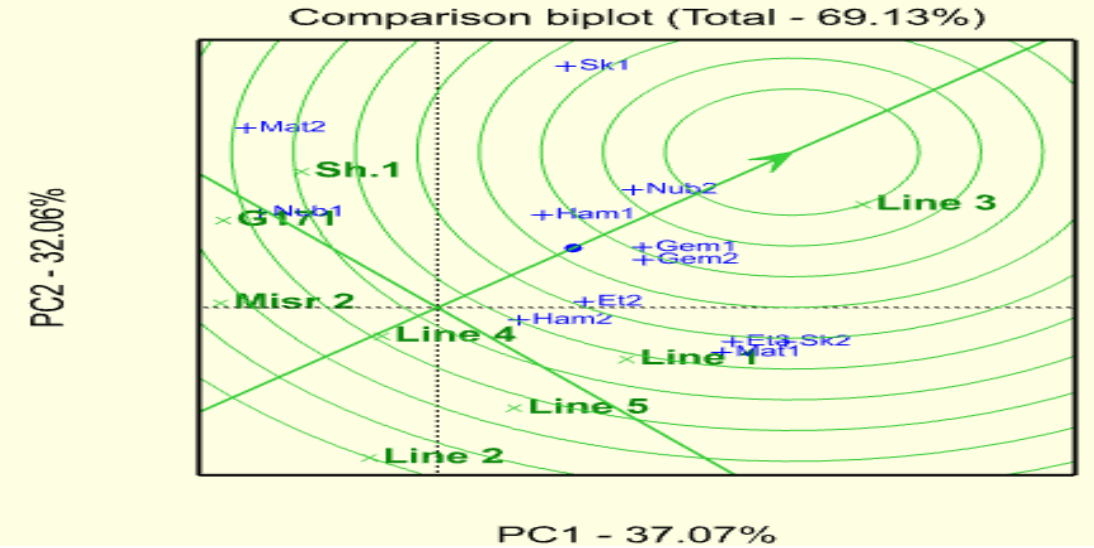

Figure 4. GGE-biplot for the genotypes comparison with the ideal genotype.

Ideal environment

GGE-biplot for comparison of the tested environments with the ideal one was shown in **Figure (5)**. As similar to the ideal genotype, Nub2 environment was the ideal environment (stable effect on the genotypes regardless its low mean) which located in the middle concentric circle with an arrow passing through it in the biplot graph. The closest environments to the ideal one and had desirable performance were (Ham1, Gem1, Gem2, Et1, Ham2 and Sk1), suggesting the widely adapted bread wheat genotypes **(Asnake *et al*., 2013 and Muez *et al*. 2015**). Meanwhile, the environments (Mat2 and Nub1) which had high performance can be considered as

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discriminating power to distinguish between the examined genotypes. These results were similar to those obtained by **Bhartiya *et al*. (2017) andAsnake *et al*., (2013)**.

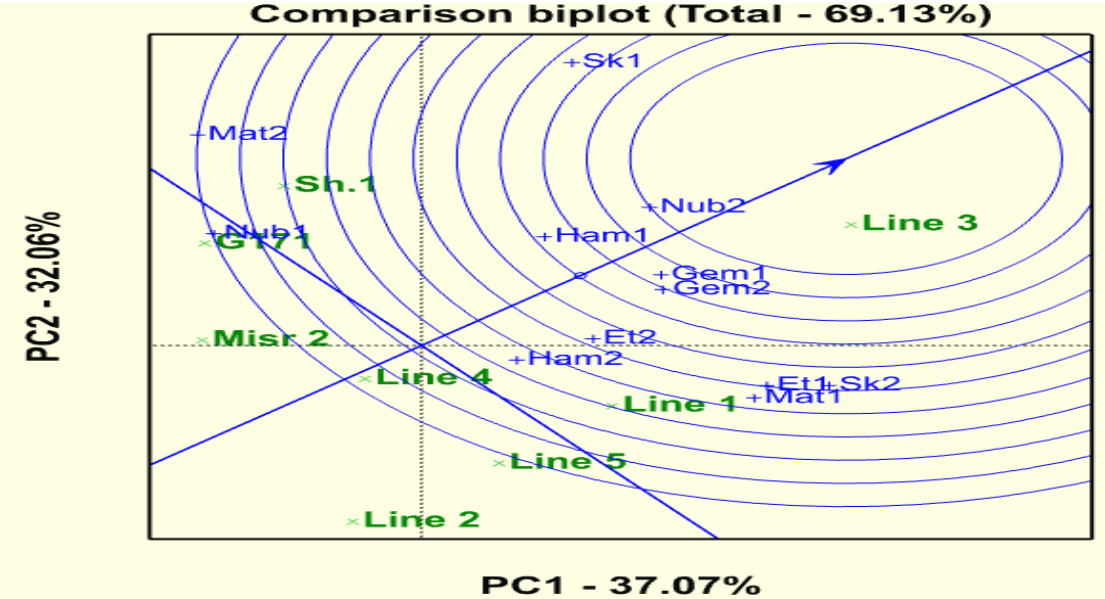

Figure 5. GGE-biplot for environments comparison with the ideal environment.

In the present study, Nub2 was the most discriminating environment followed by Gem1, Gem2, Ham1 and Sk1 (**Fig. 5**). Although the environments of Mat 2 and Nub1 that fall on the outer circle in graph had high yielding, however they were non-discriminating and less representative. It was observed that the differences of genotypes response across same location among seasons. These results referred that the genotype stability could be challenged not only due to the change in the test environment but also due to change in growing season per environment. Similarity results were obtained by **Odewale *et al*. (2013)** who pointed to only one environment which was stable, representative and discriminating among many environments for the tested genotypes.

Ranking genotypes based on environments Vector view

GGE biplot graph showing the ranking of examined genotypes on the average environment coordination (AEC) based on mean and stability for grain yield of bread wheat was illustrated in **Figure** (**6**). The AEC ordinate was the line that passes through the origin and separated genotypes according to average means and direction (**Bhartiya *et al*., 2017**).

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The average of grain yield of the tested genotype with increase direction arrow was located on the AEC and approximated stability was determined around axis **(Yan and Rajcan, 2002)**. Genotypes were ranked according to mean performance from right side to left around AEC axe (Line 3, Shandweel1, Line 1, Giza 171, Line 5, Line 4, Misr2 and Line 2). Genotypes grain yield performances significantly changed according to the environments. Meanwhile, the stability ranking was formed based on the genotype farness from AEC axe (vector length), whereas the shortest vector was the most stable. Graph view showed that only G3 (Line 3) was above average of the all environments and was the nearest (shortest vector) to the AEC followed by G8 (Misr2) and G1 (Line 1). Then, Line 3which was the best genotype for general adaptability. It was the ideal one across all environments (locations/seasons) as it was high yielding and stable. In many of previous studies conducted in multi-environments, stable and unstable bread wheat genotypes were identified (**Kadir *et al* 2018, Akcura *et al*., 2017; Farshadfar, *et al*., 2012 and Yan and Hunt, 2001**).

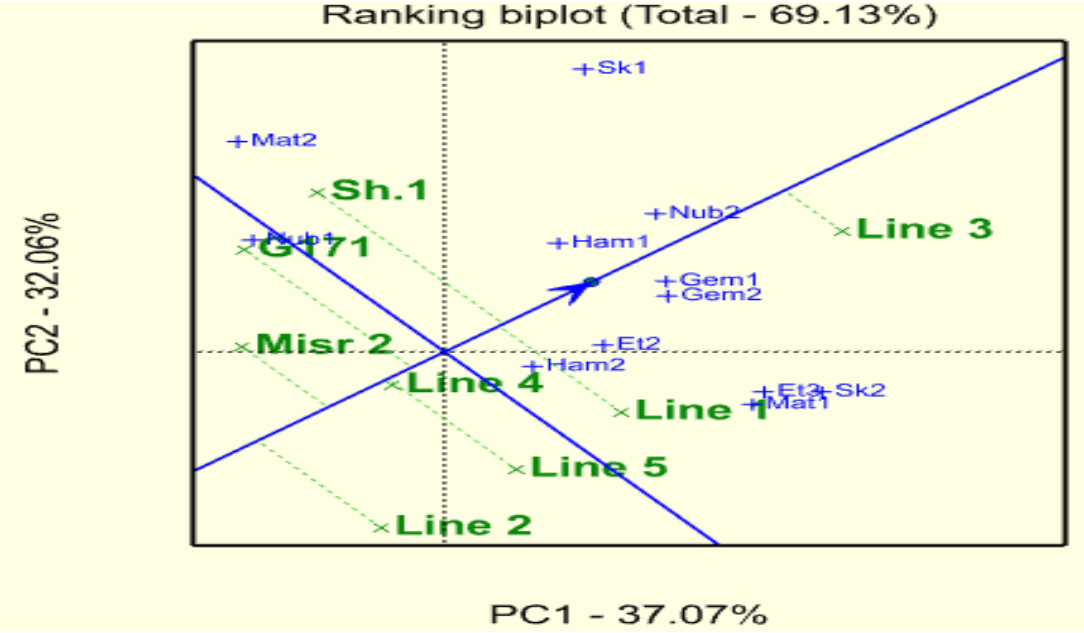

Figure 6. GGE biplot graph showing the ranking of eight genotypes on the average environment coordination (AEC) based on mean and stability of bread wheat grain yield.

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Reaction to rust diseases

Phenotype under field conditions

Under natural filed condition, the tested wheat lines showed different reaction to stripe rust Line 2, line 3, line 5 and local cultivars Giza171 were mostly resistant to stripe rust (**Table 6**). However, Line 4 and two local cultivars Shandaweel 1 and Misr 2 were susceptible to stripe rust disease under natural field conditions. Meanwhile, all genotypes revealed resistance to the leaf rust except line 1 and line 5 over all locations. On the other side, no stem rust infection was detected over studied materials and locations.

Table 6. Different reaction average to stripe and leaf rust of eight bread wheat genotypes tested in six locations.

Genotypes	Rust type	Gemmeiza	Sakha	Etai-Elbaroad	Nubaria	Kafr - Elhamam	Mataana	Mean
Line 1	**Stripe**	10.00	10.00	5.00	5.00	5.00	0.01	5.84
	Leaf	4.00	8.00	4.00	8.00	4.00	0.01	4.67
Line 2	**Stripe**	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Leaf	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Line 3	**Stripe**	0.40	0.01	0.01	2.00	0.40	0.01	0.47
	Leaf	0.01	0.2	0.01	1	0.01	0.01	0.21
Line 4	**Stripe**	20.00	30.00	10.00	20.00	10.00	0.01	15.00
	Leaf	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Line 5	**Stripe**	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Leaf	4.00	4.00	4.00	8.00	4.00	0.01	4.00
Giza 171	**Stripe**	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Leaf	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Shandaweel 1	**Stripe**	80.00	70.00	60.00	70.00	50.00	0.01	55.00
	Leaf	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Misr 2	**Stripe**	40.00	50.00	40.00	30.00	30.00	0.01	31.67
	Leaf	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Amplification results of SCoT marker analysis

Eight advanced breeding wheat line were screened using diagnostic markers based on protocols for Marker-Assisted selection (MAS) (http://maswheat .ucdavis .edu/) to identify the stripe and stem rust resistance genes presence in the selected breeding materials.

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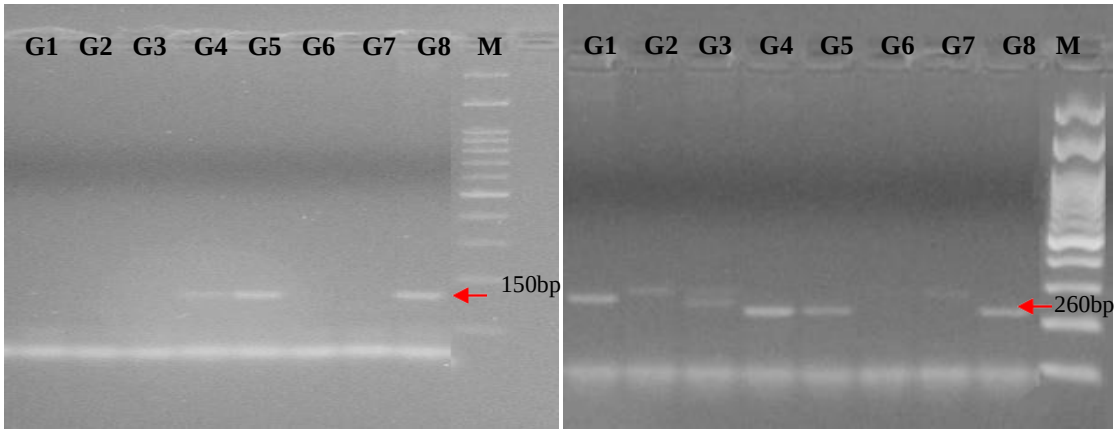
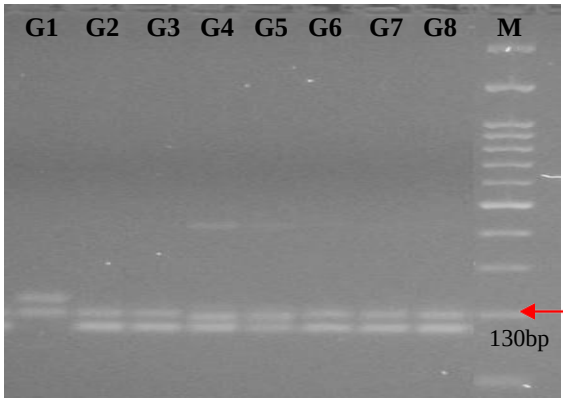


In vitro amplification profile of CSLV marker linked to Yr18-Lr34 genes

In vitro amplification profile of Xpsp3000 linked to Yr10 genes



In vitro amplification profile of Gb marker linked to Sr25/Lr19 genes

Figure (7). In vitro PCR amplification profile of different markers linked to some resistance genes in set of wheat breeding lines and cultivars.

Marker: 100bp DNA ladder, G1: line1, G2: Line2, G3: line3, G4: line4, G5: line5, G6: Giza171, G7: Shandawel 1 and G8: Misr2.

Genotype-specific bands that could be related to foliar disease are mentioned in **Table (6).** SSR (simple sequence repeat) marker analysis revealed amplification profile of the three different markers (Yr18/Lr34, Yr10 and Sr25/Lr19) linked to some resistance genes in the studied eight bread wheat lines and cultivars (**Fig 7 and Table 7**).The positive bands were patterned by used markers as resistance genes in the estimated genotypes as follow:

Yr18/Lr34

To identify the stripe and leaf rust resistance gene Yr18/Lr34 in the selected eight wheat lines the STS (sequence tagged site) marker csLV34 mapped 0.4cM from Lr34 and Yr18was used to genotype the selected breeding materials. Out of the tested breeding materials, two wheat lines and a cultivar were positive with the marker linked to Yr18/Lr34 namely line 4,

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5 and Misr 2. The resistance breeding materials can be used in wheat breeding program.

Yr10

To detect the presences of stripe rust resistance gene Yr10 in the selected wheat lines, the microsatellite marker Xpsp3000was used to the eight wheat genotype. Out of eight tested genotypes three genotypes namely line 4, 5 and Misr2 were positive with the linked marker and produced DNA fragment of 260bp showed the presence of Yr10 in the three bread wheat lines and this line showed resistant to stripe rust under field conditions.

Sr25/Lr19

For stem rust resistance gene Sr25/Lr19, genotyping with marker Gb linked to stem rust resistance gene Sr25/Lr19 yielded positive PCR product in all tested lines (**Table 6**). The dominant marker Gb amplified a 130 bp fragment only in the Sr25/Lr19-positive wheat lines and no PCR product was obtained in wheat lines that lack Sr25/Lr19.

Table 7: The detected markers of the stripe, leaf and stem rust resistance genes presence among the screened wheat material using Marker-Assisted selection (MAS).

Marker / Genotype	Yr18/Lr34	Yr10	Sr25/Lr19
Line 1	-	-	+
Line 2	-	-	+
Line 3	-	-	+
Line 4	+	+	+
Line 5	+	+	+
Giza 171	-	-	+
Shandaweel 1	-	-	+
Misr 2	+	+	+
Resistance bands	3	3	8
Name of linked marker	csLV34	Xpsp3000	Gb
Marker type	STS	microsatellite	EST
Molecular weight	150bp	260bp	130bp
Resistance type	stripe and leaf rust	stripe rust	stem rust

STS: sequence tagged site, microsatellite: type of repetitive sequence, EST: expressed sequence tag

Moreover, these results indicate that the resistance genes present in bread wheat genotypes may be different from each other. However, further genotyping is still required to validate the suitability of these markers for marker-assisted wheat breeding and these loci could be effectively used in breeding programs. **Elkot *et al* (2018 a and b**) demonstrated that molecular

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markers exhibited interest specific loci relating to foliar diseases resistance in wheat genotypes.

Using the molecular marker linked to identify rusts resistance genes is useful tools to identify line resistance to different rust disease. Our results were reported by **Bosily (2018)** detected specific markers for barley leaf rust disease using five Scot primers.

CONCLUSION

There were interactions between locations and seasons in the performance of genotypes. Then, stability of examined genotypes could be estimated. Bread wheat genotype G3 (Line 3) had the highest observed stability, where the average yield was more stable than the average of the all tested genotypes. Then, it was the selected genotype that had the chance to be released as a new superior variety. Tested environment (E12) Nub2 was the most suitable environment for testing the stability of grain yield to discriminate the tested bread wheat genotypes in Egypt, followed by (E7) Ham1, (E5) Gem1 and (E6) Gem2. Meanwhile, Mat2 (E10) could not able to distinguish the testing wheat genotype, especially concerning yield stability. Based on the genetic molecular profiles, using three SSR markers which used to detect the presence of different rusts resistance genes in the selected materials, the molecular markers revealed the presence of some important resistance genes for different rusts. The evaluated materials which carry rust resistance gene showed good level of resistance to stripe and stem rust disease under natural field conditions.

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