E-ISSN: 2618-0618
P-ISSN: 2618-060X

www.agronomyjournals.com
2024; SP-7(9): 113-120
Received: 18-07-2024
Accepted: 26-08-2024

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Delineation of genotype x environment interaction for identification of stable genotypes for different physiological traits in wheat

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DOI: https://doi.org/10.33545/2618060X.2024.v7.i9Sb.1464

Abstract
Heat and drought stress are the major environmental constraints to wheat instability. Climate change enhances Genotype × Environment interactions and urges breeders to step up their efforts to breed cultivars that combine high performance and stability. The current study aimed to assess stability of 100 wheat genotypes grown under eight environments in terms of physiological characters as normalized difference vegetation index (NDVI) and chlorophyll fluorescence. These physiological traits serves as a useful tool for high throughput screening under drought and heat stress for selecting stable wheat genotypes. The pooled analysis of variance due to genotypes, environments as well as genotype × environment interactions were highly significant for these physiological traits according to AMMI biplot analysis indicating the substantial differences in genotypic response across the environments. AMMI biplot study indicated that genotypes WH 1192, PBW 661, PBW 726, HD 2967, PBW 729 and PBW 721 were found stable for NDVI whereas for chlorophyll fluorescence genotypes, WH 1136, WH 1061, WH 1139, WH 1235, PBW 502, WH 1192 were stable across the environments. Therefore, selection for stable genotypes for physiological traits can be used as breeding materials in future wheat programmes as improving physiological responses are being considered to accelerate yield and rate of breeding progress.

Keywords: AMMI biplot, chlorophyll fluorescence, normalized difference vegetation index (NDVI), physiological traits, wheat

Introduction
Globally, wheat (*Triticum aestivum* L.) is a significant staple and caloric food crop (Lamba *et al*., 2023, Karki *et al*., 2021) [21, 16]. It is one of the world's top three cereal crops in the world because of its adaptability, nutritional value and high production potential which is mainly used for bread and biscuits purpose satisfying hunger worldwide (Bishwas *et al*., 2021) [5]. However, this crop is subjected to number of biotic and abiotic stresses leading to considerable losses in annual wheat production, among which drought and heat stress are of major significance. However, often drought and heat stresses coincide, which can be severely detrimental to crop yield than effect of individual stress (He *et al*., 2022) [12]. Therefore, it is an immediate requirement to develop stable genotypes of wheat that have the features needed to endure severe drought stress, high temperature episodes (Above optimal), and increased grain yield under a variety of stress situations. The correlation between physiological characters i.e. NDVI, chlorophyll fluorescence and grain yield under heat and drought stress has been well established (Devate *et al*., 2022; Lamba *et al*., 2023) [6, 21]. In the post anthesis phase 'stay-green' is associated with drought and heat tolerance in several crops (Ali *et al*., 2023; Kamal *et al*., 2019) [1, 14]. Normalized Difference Vegetative Index (NDVI) an excellent indicator of green biomass, has been effectively used for quick assessment of drought and heat tolerance in wheat genotypes (Hassan *et al*. 2019, Devate *et al*., 2022) [11, 6].
Conceptually, the phenotype of any plant is a result of the genotype (G), the environment (E) and the genotype- environment interaction (G x E) (Raffo and Jensen, 2023) [26]. Plant breeders always look for genotypes with minimal G x E interaction, but that seldom occurs especially

under the dynamic weather conditions and the fluctuations in the environmental conditions from year to year and location to location. Therefore, assessing a cultivar's stability is a crucial factor to consider before releasing new cultivars (Karaman *et al*., 2023) [15]. Selecting superior genotypes using stability measurements are more reliable across environments with a minimized G x E interaction (Gupta *et al*., 2022) [9]. Over the environmental conditions the additive main effects and multiplicative interaction (AMMI) analysis has been proved as a useful analytic approach for linear and non-linear response of genotypes among different methods available for stability analysis (Verma and Singh, 2021) [28]. It is a hybrid model which combines both the ANOVA (with additive parameters) for main effects and Principal Component Analysis (with multiplicative parameters) for interaction effects of G x E into a single analysis (Gauch and Zobel, 1988) [8] including a biplot for precisely exemplifying adaptive responses (Annicchiarico, 1997) [2]. AMMI model have recently gained more importance by overcoming the limitations of univariate models as well as it is more informative (Balakrishnan *et al*., 2016) [4] and also a powerful tool for effective analysis and interpretation of multi environment data structure in breeding programs (Heidari *et al*., 2016) [13].

By keeping in view, the present investigation characterized 100 wheat genotypes for physiological traits across different environments for identification of stable genotypes for general and specific adaptation in different environmental conditions by estimation of genotype × environment interaction and stability parameters.

Materials and Methods

Study site Description

During two consecutive years of Rabi 2019-20 and 2020-21, the experiment was conducted in four environments *viz.* irrigated, rainfed, timely sown and terminal heat stress. The field was sown in the third week of November in timely sown conditions to provide normal temperature to wheat crop in the reproductive and ripening stage. Sowing was delayed by a month (the last week of December) in the field of terminal heat stress, to provide higher temperature to wheat crop in the reproductive and ripening stage. Codes used for different production environments are represented in table 1.

Table 1: Codes used for production environments during 2019-20 and 2020-21

	Timely sown		Late sown	
	Irrigated	**Rainfed**	**Irrigated**	**Rainfed**
2019-20	E1	E2	E3	E4
2020-21	E5	E6	E7	E8

Plant materials

To conduct the experiment, 100 wheat genotypes (Table 2) were used at research area, Wheat and Barley section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar.

Table 2: List of 100 bread wheat genotypes used in the present study

Sr. No.	Genotype	Sr. No.	Genotype	Sr. No.	Genotype	Sr. No.	Genotype
1	WH1182	26	PBW693	51	WH1184	76	WH789
2	PBW725	27	WH1188	52	WH1021	77	PBW750
3	WH1061	28	WH714	53	PBW503	78	DPW621-50
4	PBW729	29	PBW698	54	WH1158	79	WH542
5	PBW560	30	WH1062	55	WH1164	80	PBW486
6	PBW728	31	WH1105	56	WH1129	81	WH147
7	PBW721	32	DBW88	57	UP2902	82	WH1120
8	WH1139	33	PBW527	58	WH1166	83	PBW769
9	UP2565	34	PBW676	59	WH711	84	PB934
10	DBW136	35	WH283	60	WH1181	85	WH1192
11	WH1025	36	WH1138	61	DBW90	86	HD3086
12	WH1152	37	WH1153	62	WH1140	87	PBW163
13	PBW752	38	WH1175	63	WH1132	88	PBW712
14	PBW475	39	WH1235	64	PBW158	89	DBW129
15	PBW621	40	DBW233	65	PBW502	90	WH1124
16	PBW730	41	PBW528	66	UP2338	91	WH1264
17	WH1136	42	PBW88	67	DBW17	92	PBW762
18	WH730	43	PBW706	68	PBW123	93	WH1142
19	PBW343	44	WH1063	69	UP2906	94	WH1186
20	DBW116	45	WH1157	70	PBW681	95	DBW95
21	HD2967	46	PBW550	71	PBW677	96	PBW540
22	WH1151	47	UP2473	72	PBW763	97	PBW542
23	UP2660	48	UP2865	73	WH1123	98	PBW661
24	PBW695	49	PBW726	74	WH1080	99	WH1131
25	PBW709	50	C306	75	DBW16	100	PB533

Experimental design and layout

The genotypes were evaluated using Randomized Block Design (RBD). The genotypes were planted in two replicates with 3-rows of 2-meter length each. For both dates of sowing, row to row distance of 20 cm was maintained.

Observations Recorded

In each replication, from each genotype the observations were recorded on five randomly selected plants.

Chlorophyll fluorescence

The Chlorophyll fluorescence measurements, original fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v) and maximum quantum yield (F_v/F_m) were taken about 4 cm from the base of abaxial surface of flag leaves using a portable handy chlorophyll fluorometer, model OS-30p

(Opti-Sciences) under timely and late sown environments, it was recorded at anthesis by adopting 2 min dark period on fully expanded flag leaves. Data was recorded on clear sunny days between 12:00 pm to 14:00 pm. The fully expanded leaves were first acclimatized to the dark for 2 minutes by fixing clips. The fluorescence signals were detected as F_o, F_m and F_v/F_m.

Normalized difference vegetation index (NDVI)

Active remote sensing based NDVI measurements were acquired using a Greenseeker® Model 505 hand held optical sensor about 90 cm above the crop canopy and walking in the centre of each plot. Each plot was sensed for approximately two to five seconds. The strength of the detected light is a direct indicator of the health of the crop; the higher the reading, the healthier the plant. The reflectance measurements were acquired at anthesis between 11:00 AM and 2:00 PM on cloud-free days.

Statistical Analysis

The combined analysis of variance of physiological parameters was undertaken across all test environments, using Genotype-Environment interaction data for stability analysis using AMMI model (Table 3) with the help of INDOSTAT and PB tools developed at IRRI.

The model equation of AMMI is:

$$Y_{ij} = \mu + g_i + e_j + \Sigma \lambda_n \alpha_{in} \gamma_{jn} + \theta_{ij}$$

Where,

Y_{ij} = mean value of ith genotype in the jth environment

M = general mean

g_i = ith genotypic effect

e_j = jth location effect

λ_n = eigen value of the Principal Component Axis n

$\alpha_{in}\gamma_{jn}$ = ith genotype, jth environment Principle component analysis (PCA) scores for the PCA axis

θ_{ij} = residual

n = number of PCA axes retained in the model

Table 3: Data collected was analyzed as combined over the environments using the following ANOVA outline

Source	Df	MSS	F
Total	(ger-1)		
Treatment	(ge-1)		
Genotypes	(g-1)	MS1	MS1/MS3
Environment	(e-1)	MS2	MS2/MS3
Genotype x Environment	(g-1)(e-1)	MS3	MS3/Mse
IPCA1	(G+E-1-2n)	MS4	MS4/Mse
IPCA2	(G+E-1-2n)		
Residual			
Blocks	(r-1)		
Error	(r-1)(ge-1)	Mse	

Results

AMMI biplot analysis

The AMMI model's analysis of variance for 100 bread wheat genotypes evaluated in eight environments found that genotypes (G), environments (E) and genotype environment interaction (GEI) were highly significant ($P < 0.001$) for normalized difference vegetation index (NDVI) and chlorophyll fluorescence. The environment explained over 50% of the total variation in NDVI. The proportion of the total variance explained by genotype, environment and G×E interaction for chlorophyll fluorescence were 22.48%, 61.76% and 15.76%, respectively.

Additionally, the analysis of variance of the AMMI model indicated that the first two AMMI principal components (IPCA1 to IPCA2) were very highly significant which demonstrated significant variation in normalized difference vegetation index (NDVI) and chlorophyll fluorescence among the wheat genotypes across the tested environments. Normalized difference vegetation index (NDVI) cumulatively capturing 94.76% of total GEI as the first principal component of AMMI, explaining 85.77% of the genotype–environment interaction, whereas the second principal component explained 8.99% of the genotype–environment interaction. In case of chlorophyll fluorescence the first two AMMI principal components captured 88.45% of total variation (Table 4).

Table 4: Pooled analysis of variance for normalized difference vegetative index (NDVI) of 100 wheat genotypes across different environments using AMMI model.

Source	Degree of Freedom	NDVI	% Explained	Chlorophyll fluorescence	% Explained
Trials	799	0.016^{***}		0.009^{***}	
Genotypes	99	0.026^{***}	20.28	0.017^{***}	22.48
Environments	7	1.104^{***}	59.88	0.661^{***}	61.76
G x E interaction	693	0.004^{***}	19.84	0.002^{***}	15.76
PCA I	105	0.021^{***}	85.77	0.008^{***}	72.57
PCA II	103	0.002^{***}	8.99	0.002^{***}	15.88
PCA III	101	0.001^{***}	4.69	0.001	6.90
Pooled error	800	0.0004		0.001	

*** = significance at 0.001 level.

NDVI

The AMMI 1 Model

The AMMI biplot has the main effect (genotype and environment means), as normalized difference vegetation index in the abscissa (X coordinate) and the Y coordinate denotes the interaction effects (IPCA1); where the genotypes or environments that lie on the same vertical line have the same NDVI, and those that lie on the same horizontal line have the same interaction pattern. In the AMMI 1 biplot, the elite genotypes namely, WH 1192, PBW 661, PBW 726, HD 2967, PBW 729 and PBW 721 are relatively stable showing even performance across environments that are broadly adapted lines (Figure 1). The wheat genotypes WH 1123, WH 711, WH 1021, PBW 695, PBW 762, PBW 681, PBW 712, PBW 621, PBW 88, WH 1063, WH 1152 are relatively unstable because these lines are far from the origin and can be specifically adapted to particular environment. Especially, genotypes WH 711, WH 1021 and WH 1123 had NDVI on right hand side of the main effect axis with large positive score on IPCA1 and therefore, likely to perform better in E1, E5 and E6 environment but the performance varied from one environment to another environment whereas the genotypes WH 1062 and PBW 763 were identified as specially adapted to environments E3 and E7. The environments E2, E4 and E8 were the most responsive for genotypes PBW 343 and PBW 486 having lower NDVI with negative IPCA1 score (Figure 1).

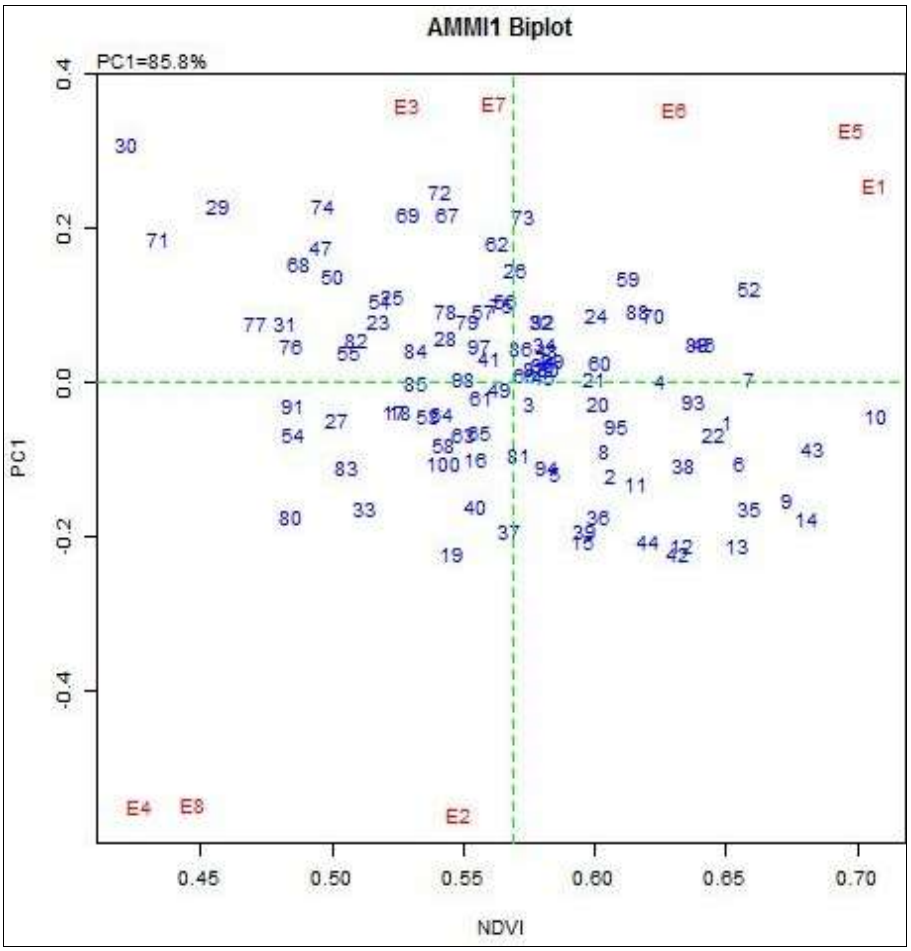

Fig 1: AMMI 1 biplot for NDVI of 100 wheat genotypes and 8 Environments using genotypic and Environmental scores.

The AMMI2 Model

In AMMI2 biplot, the representation of the stability of the lines across the environment were reported by the Interaction Principal Component Axes 1 (IPCA1) and Interaction Principal Component Axes 2 (IPCA2) scores that is, the lines with the least PC scores have high stability and vice versa, i.e., the more IPCA scores that approximate to zero, the more stable the genotypes are across all the environments. Inspection of the Fig. 2 voted that the environments E3, E5, E6 and E7 had comparatively short spokes and they did not exert strong interactive force while environment E1, E2, E4 and E8 having long spokes exert strong interactions. In this case, the best adapted genotypes UP 2902, WH 1061 and WH 711 were tightly grouped in environments E3, E5, E6 and E7 (Figure 2).

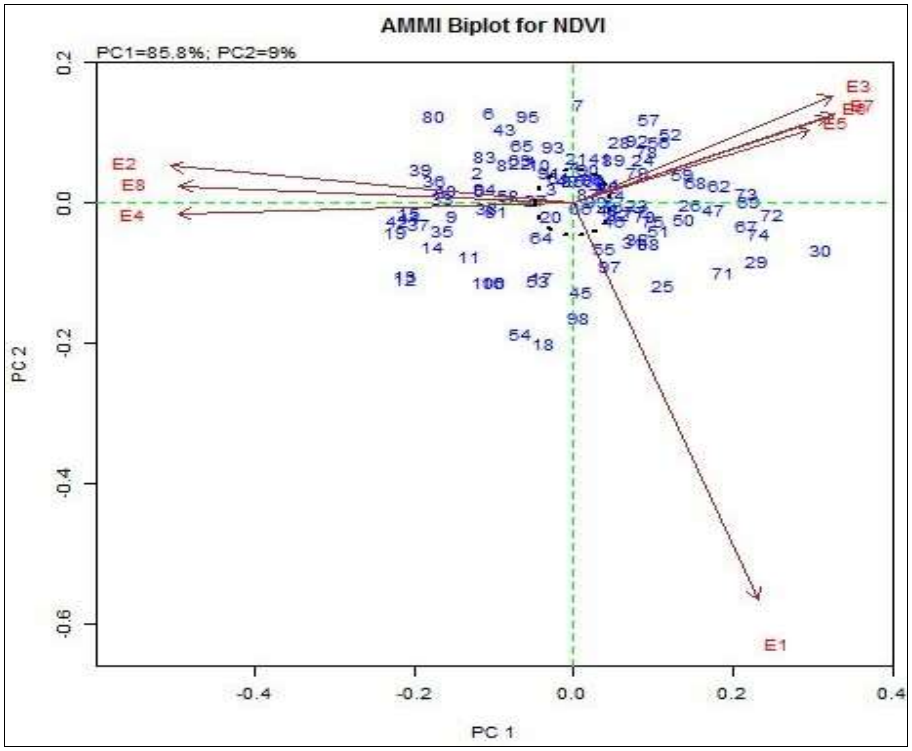

Fig 2: AMMI 2 biplot for NDVI showing interaction of IPCA 2 against IPCA 1 scores of 100 wheat genotypes in 8 environments.

Chlorophyll fluorescence

The AMMI 1 Model

The genotypes, WH 1136, WH 1061, WH 1139, WH 1235, PBW 502 and WH 1192 were nearly placed to the horizontal line implying that the genotypes were stable. The genotypes, WH 1124, DPW 621-50, DBW 88, PBW 550, DBW 136, HD 3086, PBW 729, WH 789, WH 1131, WH 147, DBW 233, WH 1063, UP 2473 and WH 1235 were located distant from the horizontal line contributing much to the increasing magnitude of genotype by environment interaction and they were unstable.

Genotypes namely WH 1124, DPW 621-50 and DBW 88 had chlorophyll fluorescence present on right hand side of the main effect axis with large positive score on IPCA1 and therefore, likely to perform better in E2 environment which seems to be most favorable environment whereas the genotypes PBW 752 and PBW 475 were identified as specially adapted to environment E1 and the genotypes WH 1164, WH 714 and WH 711 were adapted to E6 having above average chlorophyll fluorescence with negative IPCA1 score. E7 is the most responsive environment for genotypes UP 2660 and PBW 709 having low chlorophyll fluorescence with negative IPCA1 score (Figure 3).

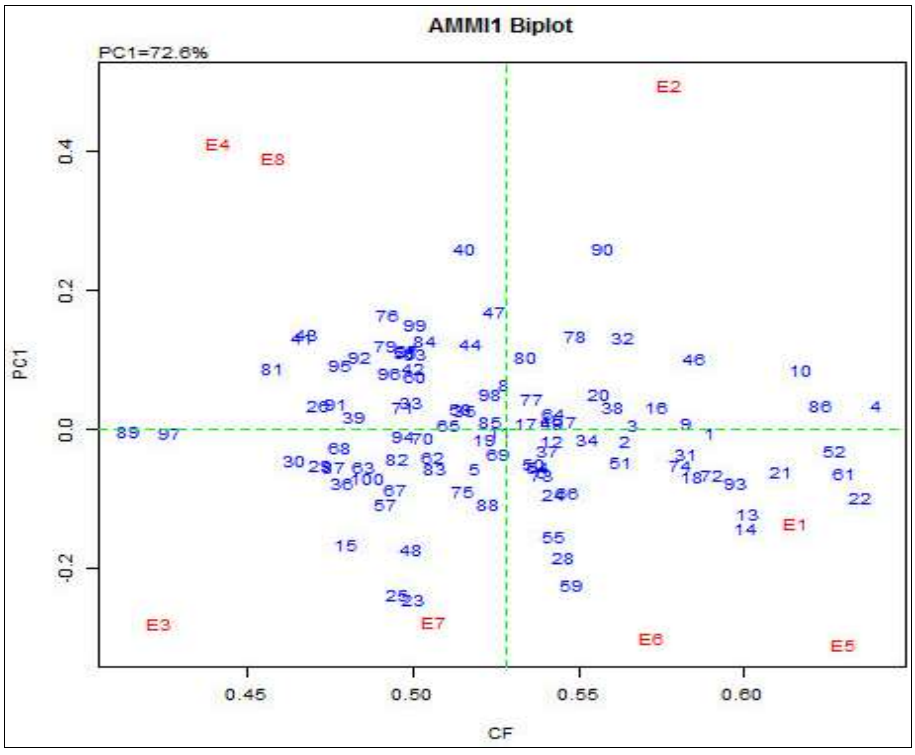

Fig 3: AMMI 1 biplot for chlorophyll fluorescence of 100 wheat genotypes and 8 Environments using genotypic and Environmental scores.

The AMMI 2 Model: In AMMI2 biplot, the environments E3, E5, E6, E7and E8 did not exert strong interactive force while environment E1, E2 and E4 exert strong interaction. Genotypes WH 1153, PBW 721, WH 1175, DBW 129 and WH 1182 were non-sensitive to environmental interactive forces while genotypes WH 1152, PBW 752, WH 1025, WH 1124, DBW 116, WH 1151 and PBW 695 were most responsive. In this case, the best adapted genotypes with respect to environment E4 was WH 1124 whereas the genotype DBW 233 was adapted to the environments E8. The genotypes WH 714, WH 711 and PBW 695 were adapted to the environment E3, E5, E6 and E7 (Figure 4).

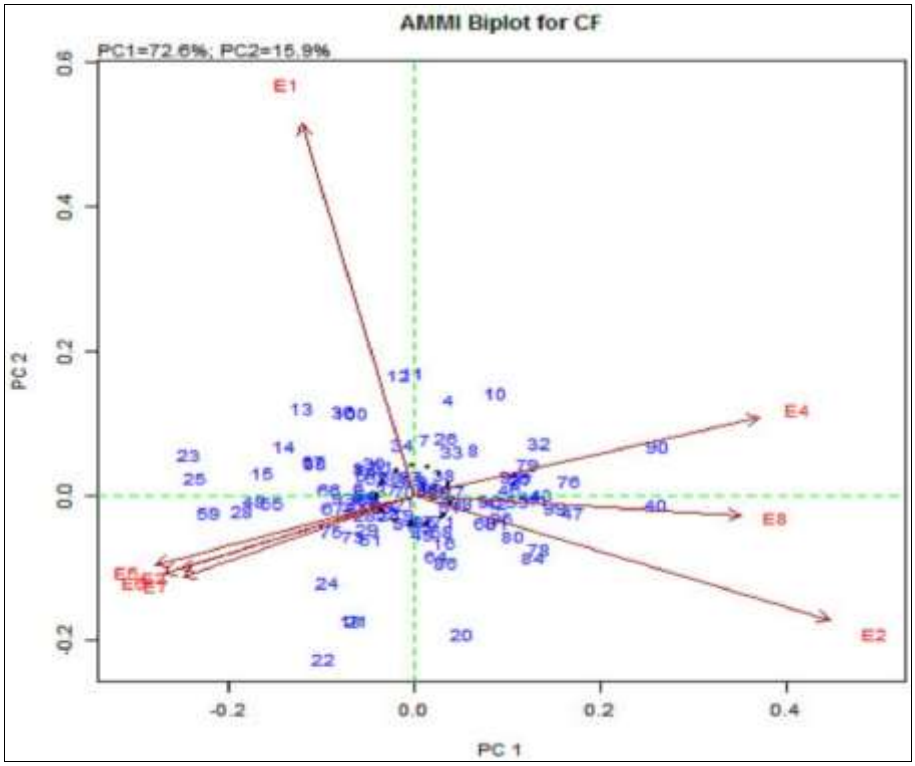

Fig 4: AMMI 2 biplot for chlorophyll fluorescence showing interaction of IPCA 2 against IPCA 1 scores of 100 wheat genotypes in 8 environments.

Discussion

Due to various agro-ecologies in our country, crop varieties don't show uniform performance across different environments and often confounded by differences in plant physiology under different environments (Pankaj *et al*., 2022) [24]. Under such circumstances, the G × E study and stability analysis has always been considered as an important topic in plant breeding. The results of pooled analysis of variance for stability as devised by AMMI model showed that the variance due to genotypes and environments were significant for normalized difference vegetation index (NDVI) and chlorophyll fluorescence indicating that genotypes were rich in variation for these physiological traits and micro environments created through production systems were different from each other. The mean sum of squares due to genotype x environment interaction when tested against pooled error was also significant for these traits indicating genotype x environment interaction components showed wide differential behaviour of genotypes under changing environments. Similar results were reported by Gupta *et al*., 2023 [10].

The literature provides many examples of AMMI model application in the studies of genotype–environment interaction for important crops as wheat, maize, barley etc. which provides a base of better use for other models (Latifi *et al*., 2020, Katsenios *et al*., 2021, Omrani *et al*., 2022) [22, 17, 23]. In this study AMMI model was used to examine bread wheat stability for normalized difference vegetation index (NDVI) and chlorophyll fluorescence for the purposes of breeding program. AMMI revealed that a major part of the variation in NDVI and chlorophyll fluorescence was explained by environment, indicated a high environmental diversity showed that the environments were diverse. Similarly, AMMI for yield indicated that more than 50% of variation contributed by environment; E, G and G × E contributed to the tune of 71.66, 14.79, and 13.56%, respectively (Yashavanthakumar *et al*., 2021) [29]. The results are in line with the findings of Kumar *et al*., 2022 [19], Gajghate *et al*., 2021 [7].

Genotypes and environments close to the origin had a smaller share of the GEI and were generally adaptable to all environments and those far from the origin have a greater role in GEI (Bakare *et al*, 2022) [3]. Thus, for NDVI, the wheat genotypes WH 1123, WH 711, WH 1021, PBW 695, PBW 762, PBW 681, PBW 712, PBW 621, PBW 88, WH 1063, WH 1152 were far from the origin, had the most fluctuations in environmental changes, while genotypes WH 1192, PBW 661, PBW 726, HD 2967, PBW 729 and PBW 721 were within the orient and close to the biplot origin had a smaller share of the GEI and were generally adaptable with all environments. Shashikumara *et al*. (2020) [25] also exploited the AMMI biplot to differentiate between stable and unstable genotypes. E1, E5 and E6 were observed as suitable environments for this trait since in these environments NDVI would have larger values with positive interaction. Similar concepts were used in the report of Gajghate *et al*. (2021) [7].

The AMMI2 biplot has better fit and accuracy for studying the complex GEI pattern. Also, the AMMI2 with two main components justifies the highest rate of GEI changes (Khan *et al*. 2021) [18]. According to the results of the experimental years for NDVI, genotypes WH 1061, DBW 116, UP 2338, PBW 550, PBW 163 were identified as stable genotypes and genotypes

WH 1158, WH 730, PBW 698, WH 1062, PBW 486 and PBW 677 as most responsive genotypes. Similar to this research, Omrani *et al*., 2022 [23] identified G12 and G21 genotypes as superior genotypes while G13 genotype as undesirable genotype. The environments E1, E2, E4 and E8 having long spokes exert strong interactions whereas E3, E5, E6 and E7 environments having short spokes did not exert strong interactive force. AMMI2 biplot indicated environments E2, E4 and E6 were discriminatory and located far away from the biplot origin exerting strong interactive forces (Singh *et al*, 2019) [27]. Yashavanthakumar *et al*., 2021 [29] found that the selections MACS 6729, HD 2932, and MACS 6733 consistently performed under each tested environment based on AMMI biplot. These findings were also in conformity to those of Shashikumara *et al*. (2020) [25].

Based on the performance of genotypes across different environments, AMMI1 classified the genotypes as most stable and having more chlorophyll fluorescence (WH 1136, PBW 721, WH 1061 and UP 2565), less stable and having more chlorophyll fluorescence (WH 1124, DPW 621-50, DBW 88, PBW 550, DBW 136, HD 3086 and PBW 729) and more stable and having low chlorophyll fluorescence genotypes *viz*., WH 1139, WH 1235, PBW 502 and WH 1192. From AMMI2 biplot the highly interactive environment (E1, E2 and E4) and genotypes (WH 1152, PBW 752, WH 1025, WH 1124, DBW 116, WH 1151 and PBW 695) were identified. The precise adaptation of genotype WH 1124 to the appropriate environment E4 and the genotype DBW 233 adapted to the site E8 has also been visualized with the help of biplot. Similarly Kumar *et al*. (2018) [20] identified the wheat genotypes ET127253 and ET127270 were more adaptable to high yielding environment S3 while performing AMMI analysis.

Conclusion

The wheat genotypes used in this study revealed considerable variability for normalized difference vegetation index (NDVI) and chlorophyll fluorescence which can lead to effective and potential utilization in the breeding for drought and heat stress tolerance. On the basis of stability devised by AMMI biplot, genotypes WH 1192, PBW 661, PBW 726, HD 2967, PBW 729, PBW 721 were found to be stable having high NDVI value across all the environments whereas for chlorophyll fluorescence genotypes, WH 1136, WH 1061, WH 1139, WH 1235, PBW 502, WH 1192 These genotypes need to be further tested in heat- and drought-stressed environments to ensure their performance over the years and can be used in wheat breeding programme to develop heat and drought tolerant varieties.

Acknowledgments

We are grateful to Department of Genetics and Plant Breeding, College of Agriculture, CCS HAU, Hisar for their research support, materials and facilities.

Author Contributions

VG: Conducting study and data collection; MK: Planning and guidance; VS: Field Resources; KL: Data analysis; LC & APS**:** Editing the manuscript. All authors reviewed the manuscript.

Funding

This research received no external funding.

Conflict of Interest

The authors declare that they have no conflict of interest.

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