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Adaptability, Genotype Ranking and Environmental Discrimination of 25 Wheat Lines Across Five Multilocation Environments

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Title. Adaptability, Genotype Ranking and Environmental Discrimination of 25 Wheat Lines Across Five Multilocation Environments

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Abstract

Background: Genotypic selection needs integrated consideration of performance, stability, site-specific adaptation, and the usefulness of test environments. The present study aimed to evaluate 25 wheat lines across five multilocation environments including Attock, Chakwal, Swat, Bahawalpur and Multan by using complementary GGE biplot for genotype comparison, ranking, which-won-where patterns, environmental discriminativeness and representativeness, and environmental ranking. **Results:** The genotypes were evaluated on the basis of physiological, biochemical and agronomic traits: chlorophyll (Chl), photosynthetic rate (Pn), stomatal conductance (Gs), proline, malondialdehyde (MDA), antioxidant enzyme activity (AEA), grain yield (GY), biological yield (BY), and harvest index (HI). The study was conducted in field during two seasons 2023-24 and 1024-25. The graphical results demonstrated significant differences among genotypes in association to environment and explained why broad adaptation and specific adaptation should be considered separately. Moreover, the analysis identified the high impacting environment and the most promising genotypes for further assessments. The results further illustrated that an environment closer to ideal impact on GGE is considered as more representative and discriminating.

Conclusion: Nevertheless, graphical position is not the only criteria to prove the statistical superiority of one environment over other. Besides, the integrated analysis from present study identified the genotypes L-08 (Akbar-19), L-11 (TD-1), and L-21 (Azam-06) with broad adaptability, while the genotypes L-20 (Ujala-19), L-03 (Faisalabad-08), and L-13 (Imdad-2005) showing specific adaptability or trait combinations.

Keywords: wheat; GGE biplot; genotype ranking; which-won-where; discriminativeness; representativeness; environmental evaluation; adaptability; multilocation trial

1. Introduction

Multilocation trial are intended to test the adaptability of genotypes in broader or specific environment. When wheat is exposed to heterogenous environmental conditions then breeder must assess whether the genotype is broadly adapted or specifically adapted, and whether the test environments adequately represent the intended target population. Studies conducted in recent past have concluded that genotype, environment, and genotype x environment interaction (GEI) can all contribute substantially to yield variation and that distinct environmental region can favor different genotypes [1-3]. In this context GGE biplot analysis was devised to concentrate on genotype plus genotype × environment effects after removal of the environment main effect. The graphical prospects of GGE analysis have some unique prospects including genotype ranking, mean versus stability, which-won-where, genotype comparison, and environmental discriminativeness versus representativeness [4]. Besides, the ranking feature of GGE evaluate the genotypes in comparison an idealized target, while which-won-where feature identifies the genotype performing exceptionally in specific meteorological regions [5]. Correspondingly, the environment perspective of GGE provides knowledge on the tendency of environments to differentiate among genotypes and to represent overall targeted production environments [6]. Similarly, distinction between discriminativeness and representativeness is another important aspect of multi-location evaluation using GGE [7]. In this context a highly discriminating environment can substantially differentiate among genotypes, but it may not adequately identify their target production meteorological region. On the other hand, a representative environment may represent the target production environment for wheat genotypes, whereas it provides minimal genotypic differentiation [8]. Currently various multi-environment studies have highlighted the significance of combining the environmental attributes with GGE for identifying the mega-environments [7]. The present study aimed to assess 25 wheat lines and five environments (used in the companion AMMI analysis) based on their physiological, biochemical and agronomic traits using GGE biplot analysis. In general, the analysis intended to provide a practical assessment of genotype performance, environment-specific adaptation, and the usefulness of the testing environments. In particular, the study performed with objectives to compare genotypes performance across multiple environments, rank genotype using GGE biplot analysis, identify which genotype-won-where, assess environmental discriminativeness and representativeness, and to compare the relative positioning of the five environments for each trait. Besides, various studies from the recent past found that the integration of GGE analysis with environmental aspects provide a more comprehensive basis for understanding genotype adaptation and selecting suitable testing environments [2,3,7-9].

2. Materials and Methods

2.1 Plant Material and Pedigree Structure

A multi-environment-based field trial was performed in two turns, 2023-24 and 2024-25, at five different locations, including Attock, Chakwal, Bahawalpur, Multan, and Swat. The adaptability, genotypic ranking and environmental discrimination of 25 wheat genotypes (Table 1) were tested under varying environmental conditions. The plant material was collected from National Agricultural Research Center (NARC), Islamabad, Pakistan. The tri-replicated experiment was executed in Randomized Complete Block Design (RCBD), while each genotype was allocated randomly within each replicate. The averaged two-season physiological, biochemical and agronomic data obtained from all locations were passed through pooled ANOVA (Table 2) for further analysis.

2.2 Soil, Environment and Crop Husbandry

All experimental regions illustrated varying soil and environment features. The soil attributes such as electrical conductivity (EC), pH and texture recoded during the experiment are indicated in Figure 1. In the same way, meteorological variables including average temperature, total rainfall, average relative humidity, and wind speed were also quantified during wheat growing season (Figure 2). For sowing field was prepared by conventional tillage and leveling practices. The potassium and phosphorus fertilizers were applied during land preparation. On the other hand, nitrogen was applied during crop growth in split doses. Irrigation was planned according to crop requirement and regional conditions, while weeds were controlled using manual and chemical methods. The illustrative schedule of crop husbandry is illustrated in table 3.

2.3 DATA Collection and Quantification

The data for physiological and biochemical traits were collected at the 50% anthesis stage (Feekes 10.5.1) during both wheat growth season and averaged before analysis. The traits were quantified when approximately half of the plants in each plot had reached anthesis. For the estimation of physiological and biochemical traits fully expanded flag leaves were selected from representative plants in each plot. For minimizing the variation associated with plant growth stage, the data for all traits were collected at the same developmental stage across all regions.

2.4. Estimation of Physiological Traits

The photosynthesis rate (Pn) and stomatal conductance (Gs) were measured from intact leaf using portable infrared gas analyser (IRGA; ADC BioScientific Ltd., UK) provided with leaf chamber. The Pn was expressed as μmol CO_2 m^{-2} s^{-1}, whereas Gs was expressed as mol H_2O m^{-2} s^{-1}.

The total chlorophyll (Chl) content was measured from freshly collected leaf tissue using spectrophotometric based solvent extraction method. The crushed fresh leaf samples were homogenized in acetone (80% v/v) under dim-light. The extract was cleared, absorbance was recorded at 663 and 645 nm, and total chl were estimated by the standard Arnon equations [10].

2.5. Quantification Biochemical parameters

The ninhydrin-based colorimetric method [11] was used to estimate proline content. The crushed fresh leaf samples were homogenized in extraction medium and afterwards mixed with ninhydrin. The chromophore obtained was partitioned into organic phase to perform quantification against standard curve using spectrophotometer. The thiobarbituric-acid-reactive-substances method was used to quantify malondialdehyde (MDA). The plant extract was reacted with thiobarbituric acid under hot acidic environment to attain chromophore for spectrophotometric quantification. For MDA quantification, established relationship between lipid peroxidation and MDA accumulation under oxidative stress was used [12].

Furthermore, overall antioxidant enzyme activity (AEA) index was measured from the combined activities of peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD). The flag-leaf samples collected at approximately 50% anthesis (Feekes 10.5.1) were homogenized in cold phosphate buffer and centrifuged at 4°C. Afterwards, the resulting supernatant was used for enzyme assays. SOD activity was recorded following Beauchamp and Fridovich [13] based on inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), CAT activity was recorded by observing the dissociation of H_2O_2 at 240 nm according to set protocol of Aebi [14], and POD activity was measured following Maehly [15] procedure, by measuring H_2O_2-dependent guaiacol oxidation at 470 nm. Moreover, the enzymatic activities were measured with respect to change in absorbance per unit time and expressed on a common protein basis. The enzyme activity was standardized using the Z-score equation; $Z = (X - \mu)/\sigma$, where X indicates the observed enzyme activity, μ represents overall mean activity, and σ represents standard deviation. At the end, AEA index was measured **as** $AEA = (Z_{SOD} + Z_{CAT} + Z_{POD})/3$

2.6 Quantification of Agronomic Traits:

After manual harvesting, plants were bundled and sun dried till constant weight. Besides, grain yield (GY) and biological yield (BY) were measured from the plants harvested from the net plot area of each experimental unit at maturity. For the GY, the harvested plants from each plot were threshed separately. The cleaned grain weight was measured with the help of electronic balance as kg ha^{-1}. Besides, BY was measured as the total dry biomass present above ground that included grain

and straw, and expressed as kg ha^{-1}. The harvest index (HI) was measured as the ratio of GY to total above-ground BY and expressed as a percentage using the formula: HI (%) = **[GY / BY]** × 100**.**

2.7 ***Statistical Analysis:***

The GGE biplot analysis was performed in "metan package" of RStudio. The analysis was based on the G + GE model, in which the main effect of environment was removed and the remaining genotype and G×E effects were decomposed using singular value decomposition (SVD). The statistical model was expressed as $Yij - \mu - Ej = \Sigma(k=1 \text{ to } n)\ \lambda k \xi ik \eta jk + \varepsilon ij$,

Whereas, Yij, mean performance of genotype i in environment j; μ, overall mean;' Ej, environment effect; λ, singular value of the kth principal component; ξik, genotypic score; ηjk, environment scores; n, number of principal components retained; εij, residual error.

The first two principal components (PC1 and PC2) were used to construct the GGE biplots for genotype comparison and ranking, which-won-where analysis, and assessment of environmental discrimination and representativeness.

3. Results

3.1 Pairwise Genotype Comparison Across Environments

The GGE biplots compared selected pairs of wheat genotypes for nine traits across Multan, Bahawalpur, Swat, Attock, and Chakwal, with PC1 and PC2 jointly accounting 53.1–61.2% of the variation, depending on the trait as illustrated in figure 3. For Chl, PC1 and PC2 explained 29.8% and 28.3% of the variation, respectively, and the comparison between L-02 (Galaxy-2013) and L-03 (Faisalabad-08) showed different relative responses across environments, particularly between Multan and Swat versus Attock and Chakwal (Figure 3). For Pn, PC1 (29.6%) and PC2 (23.5%) together showed 53.1% of the variation, with L-10 (Chakwal-50) and L-02 (Galaxy-2013) showing contrasting responses among Chakwal, Bahawalpur, Attock, and Multan (Figure 3). For Gs, PC1 (28.7%) and PC2 (25.5%) combinedly explained 54.2% of the variation, and L-02 (Galaxy-2013) and L-03 (Faisalabad-08) differed in their relative responses, particularly between Chakwal and Attock (Figure 3). The proline (Pro) biplot explained 54.3% of the variation through PC1 (30.0%) and PC2 (24.3%); the comparison of L-02 (Galaxy-2013) and L-03 (Faisalabad-08) showed contrasting responses of the two lines across Swat, Multan, Bahawalpur, and Chakwal (Figure 3). Correspondingly, For MDA, PC1 (33.8%) and PC2 (23.1%) explained 56.9% of the variation, with L-06 (Pak-81) and L-08 (Akbar-19) showing different associations with the five environments (Figure 3). Besides, AEA showed 59.3% of the variation explained by PC1 (32.4%) and PC2 (26.9%), where L-10 (Chakwal-50) and L-03 (Faisalabad-08) exhibited contrasting environmental responses (Figure 3). For GY, PC1 (31.9%) and PC2 (26.4%) jointly indicated 58.3% of the

variation, and L-02 (Galaxy-2013) and L-24 (Sky-2017) showed different associations with Swat, Chakwal, and Multan (Figure 3). For BY, PC1 (31.0%) and PC2 (30.2%) collectively explained the highest proportion of variation (61.2%), with L-02 (Galaxy-2013) and L-03 (Faisalabad-08) showing environment-dependent responses (Figure 3). Finally, for HI, PC1 contributed 39.6% and PC2 18.6%, explaining 58.2% of the variation, while L-23 (Anmol-91) and L-03 (Faisalabad-08) showed contrasting responses across the five environments (Figure 3). Overall, the PC1–PC2 distributions demonstrate that the relative performance of each genotype pair changed across environments and traits, confirming substantial G × E interaction. Environments farther from the equality line represented greater differences between the two compared genotypes, whereas environments closer to the equality line indicated more similar relative performance as illustrated in Figure 3.

3.2 Ranking genotypes relative to the GGE ideal

The GGE biplots revealed clear environment-specific differences in the ranking and performance of the 25 wheat lines across Multan, Bahawalpur, Swat, Attock, and Chakwal as indicated in Figure 4. In this analysis, an environment positioned close to a group of genotypes indicates that the environmental conditions at that location were more favorable for the expression of the respective trait in those genotypes, resulting in a closer GE relationship (Figure 4). For Chl, Multan and Swat were positioned toward L-14 (Koh-e-Safaid), L-20 (Ujala-19), and L-09 (NARC-02), whereas Attock and Chakwal were positioned toward L-25 (NARC-2011), L-03 (Faisalabad-08), and L-04 (Inqilab-91) as shown in Figure 4. For Pn, Chakwal was associated with L-07 (Chenab-70), while Bahawalpur was positioned toward L-02 (Galaxy-2013), L-09 (NARC-02), and L-13 (Imdad-2005). Attock and Multan showed closer relationships with L-15 (Bhittai), L-19 (Patron-2014), and L-24 (Sky-2017) as represented in figure 4. A similar environment-dependent pattern was observed for Gs, where Chakwal was positioned toward L-04 (Inqilab-91), L-01 (Punjab-2018), and L-07 (Chenab-70), whereas Attock showed a different relationship with L-09 (NARC-02), L-03 (Faisalabad-08), and L-10 (Chakwal-50) as explained in figure 4. The same principle was observed for Pro, MDA, AEA, GY, BY, and HI, although the genotypes associated with each environment changed according to the trait (Figure 4). Thus, the environmental positions indicated that Multan, Bahawalpur, Swat, Attock, and Chakwal provided different conditions for the expression of the measured traits, resulting in different genotype rankings across locations. Besides, PC1 and PC2 together explained approximately 51–61% of the G+GE variation, capturing the major patterns of genotype × environment interaction (Figure 4). The concentric circles further show the overall ranking of genotypes based on their distance from the ideal-genotype position, with a shorter distance

indicating a more desirable combination of performance and stability (Figure 4). Overall, the results demonstrate that genotype performance and ranking were strongly dependent on both the trait and the environmental conditions, and that a line closely associated with one environment was not necessarily similarly positioned in another environment.

3.3. The "Which Won Where" GGE biplots

The "Which Won Where" GGE biplots showed that the winning genotype differed among environments and traits, demonstrating clear genotype × environment interaction as demonstrated in figure 5. For Chl, L-14 (Koh-e-Safaid) was the winning genotype for Multan, L-22 (Sahar-03) for Swat, L-03 (Faisalabad-08) for Attock and Chakwal, and L-17 (Mehr-11) for Bahawalpur (Figure 5). For Pn, L-07 (Chenab-70) was associated with Chakwal and Bahawalpur, L-24 (Sky-2017) with Multan and Attock, and L-01 (Punjab-2018) with Swat (Figure 5). For Gs, L-01 (Punjab-2018) was associated with Chakwal, L-23 (Anmol-91) with Multan and Bahawalpur, and L-10 (Chakwal-50) with Swat and Attock (Figure 5). For proline (Pro), L-19 (Patron-2014) was associated with Bahawalpur and Swat, L-12 (Zardana) with Multan and Chakwal, and L-05 (Seher-06) with Attock (Figure 5). For MDA, the closest GE associations were observed between Anmol-91 (L-23) and Bahawalpur, TD-1 (L-11) and Chakwal, and Inqilab-91 (L-04) and both Multan and Attock. In contrast, Patron-2014 (L-19) showed a closer association with Swat (Figure 5). The pattern for AEA was different: Azam-06 (L-21) was associated with Multan, while Koh-e-Safaid (L-14) showed a close relationship with Bahawalpur (Figure 5). Besides, NARC-2011 (L-25) was associated with Attock and Chakwal, whereas Pak-81 (L-06) was more closely related to Swat (Figure 5). For GY, TD-1 (L-11) showed the strongest association with Chakwal, while Sky-2017 (L-24) was linked with Multan and Attock (Figure 5). On the other hand, Bahawalpur was more closely associated with Patron-2014 (L-19), whereas Mehr-11 (L-17) showed a closer relationship with Swat (Figure 5). A different pattern emerged for BY, with Akbar-19 (L-08) associated with Swat and Punjab-2018 (L-01) with Multan (Figure 5). Meanwhile, Sahar-03 (L-22) showed an association with Attock and Chakwal, and Mehr-11 (L-17) was linked with Bahawalpur. For HI, Punjab-2018 (L-01) was associated with Chakwal, whereas Chenab-70 (L-07) showed a closer relationship with Multan (Figure 5). Faisalabad-08 (L-03) was associated with both Swat and Bahawalpur, while Mehr-11 (L-17) showed a closer association with Attock (Figure 5). Overall, the GE association changed considerably among traits, indicating that no single genotype consistently showed the strongest association across all five locations and traits as illustrated in Figure 5. This variation provides clear evidence of trait-specific G×E interaction and differential adaptation among the wheat genotypes.

3.4. Discriminating ability and Representativeness

The GGE biplots showed clear differences among the five environments in their discriminating ability and representativeness for the evaluated traits as explained in figure 6. In this analysis, a longer environmental vector indicates that the environment was more effective in distinguishing differences among the 25 wheat lines, whereas an environment located closer to the average-environment direction was more representative of the overall testing conditions (Figure 6). For Chl, Multan and Bahawalpur showed relatively strong discriminating ability, while Multan was comparatively closer to the overall environmental direction (Figure 6). For Pn, Attock and Multan were highly discriminating, whereas Bahawalpur and Chakwal were more representative of the general environmental pattern (Figure 6). In the case of Gs, Attock and Swat showed strong discrimination among the wheat lines, while Multan was relatively representative (Figure 6). For proline (Pro), Bahawalpur, Swat, and Chakwal showed greater discriminating ability, whereas Multan was closer to the overall environmental direction (Figure 6). The MDA biplot showed strong discrimination by Attock and Multan, while Swat, Bahawalpur, and Chakwal showed different degrees of environmental differentiation (Figure 6). For AEA, Swat and Chakwal were highly discriminating, whereas Multan was relatively representative (Figure 6). Similarly, for GY, Swat and Bahawalpur showed strong discriminating ability, while Multan and Chakwal were closer to the average environmental direction (Figure 6). For BY, Bahawalpur and Swat were strongly discriminating, whereas Multan and Chakwal were comparatively more representative (Figure 6). For HI, Bahawalpur and Swat showed strong discrimination, while the remaining environments showed lower or more moderate discrimination (Figure 6). Overall, the results indicated that the ability of an environment to distinguish among wheat lines differed according to the trait, and no single environment was consistently the most discriminating or most representative for all traits as explained in Figure 6. Therefore, environments should be selected according to both their ability to differentiate genotypes and their similarity to the overall target environment.

3.5 The Ranking of Environments

The GGE biplots showed that the ranking of the five environments varied considerably among the studied traits, indicating that environmental suitability depended on the trait being evaluated as depicted in Figure 7. For Chl, Attock ranked first, followed by Chakwal, Swat, Multan, and Bahawalpur, while for Pn, Attock ranked first, followed by Chakwal, Multan, Bahawalpur, and Swat (Figure 7). In contrast, Gs showed Multan as the highest-ranked environment, followed by Bahawalpur, Attock, Swat, and Chakwal (Figure 7). For proline (Pro), Chakwal ranked first, followed by Attock, Multan, Bahawalpur, and Swat, whereas MDA showed Multan, Attock, Chakwal,

Bahawalpur, and Swat in descending order of ranking (Figure 7). Besides for AEA, Chakwal ranked first, followed by Attock, Multan, Swat, and Bahawalpur (Figure 7). Among the yield traits, Bahawalpur ranked first for GY, followed by Attock, Chakwal, Multan, and Swat, while for BY, Chakwal ranked first, followed by Multan, Attock, Bahawalpur, and Swat (Figure 7). On the other hand, HI showed Multan as the highest-ranked environment, followed by Swat, Attock, Chakwal, and Bahawalpur (Figure 7). Overall, the ranking varied among traits, demonstrating that no single environment was consistently closest to the ideal environment for all traits as explicated in Figure 7. This variation indicates that the suitability of an environment for wheat evaluation depended on the specific trait being considered.

4. Discussion

The present study recorded significant G × E interaction among the 25 wheat lines evaluated across multi-locations such as Multan, Bahawalpur, Swat, Attock, and Chakwal. The GGE biplots showed that the relative performance of genotypes changed considerably among environments and traits (Figures 3 to 7). For all combined physiological, biochemical and agronomic traits, PC1 and PC2 collectively accounted 53.1–61.2% of the variation, illustrating that the two-dimensional biplots captured the major patterns of genotype and G × E variation. Such environmental variation is expected in multi-location wheat trials as genotypes may respond variably to changing soil and meteorological factors. Correspondingly, Saeidnia et al. [16], Mullualem et al. [8], Hnizil et al. [17] and Mohammadi et al. [2] reported significant genotype, environment, and G × E effects in wheat and recorded significant changes in genotype ranking across testing environments. Likewise present study they emphasized on the importance of evaluating wheat genotypes across contrasting environments rather than relying on performance at a single location.

The pairwise genotype comparisons provided further evidence of changing G × E interaction based on their physiological, biochemical and agronomic performance (Figure 3). For example, L-02 (Galaxy-2013) and L-03 (Faisalabad-08) showed different relative responses for Chl, Gs, and proline across the five environments (Figure 3). Similarly, L-06 (Pak-81) and L-08 (Akbar-19) differed in their environmental associations for MDA, while L-02 (Galaxy-2013) and L-24 (Sky-2017) showed contrasting relationships with environments for GY as illustrated in figure 3. These results proved that the superiority of one genotype over another is determined by the environment in which the genotypes were evaluated. Our results were further rectified by Saeidnia et al. [16], Mullualem et al. [8] and Taherian et al. [18] as they reported comparable changes in genotype ranking in wheat multi-

environment studies, where changing GE interaction resulted in different genotype responses across locations. Similarly, Hnizil et al. [17] also noticed considerable environmental effects on yield and other agronomic traits across Moroccan wheat meteorological regions, supporting the significance of environment-specific evaluation.

The environment-dependent responses of Chl, Pn, and Gs are particularly important because these traits are directly associated with photosynthetic activity and plant responses to environmental stress. In the present study, different wheat lines showed closer associations with different environments for these physiological traits, indicating that physiological performance was not uniform across the testing locations (Figure 4). These findings support the present observation that physiological traits can contribute to the differentiation of wheat genotypes under contrasting environmental conditions. Tiryakioglu et al. [19] further demonstrated relationships among Pn, Gs, water-use efficiency, and yield-related traits in wheat grown under contrasting stress conditions. The variation observed for proline and MDA also indicated differences among genotypes in their biochemical responses to environmental conditions (Figure 4). In the present study, different genotypes were associated with Swat, Multan, Bahawalpur, Attock, and Chakwal for proline and MDA, rather than one genotype showing the same association across all environments (Figure 4). Recent work has also shown considerable variation in proline, chlorophyll, and MDA among durum wheat genotypes under water deficit and rewatering conditions [19]. Thus, the different GGE associations observed for proline and MDA in the present study are consistent with the existence of genotype-specific biochemical responses to environmental variables. The results for GY, BY, and HI provided additional evidence that genotype performance was strongly dependent on the environment. GY showed different genotype associations with Swat, Chakwal, Multan, Bahawalpur, and Attock, while BY and HI also showed distinct patterns of G x E relationships (Figure 4). It proved; a genotype associated showing a desirable response for one yield-related trait was not necessarily showingsimilar response for another trait. These findings are in line with recent wheat studies in which environmental effects and G × E interaction substantially influenced agronomic performance and genotype ranking. For instance, Mullualem et al. [8] reported significant genotype, environment, and G × E effects for bread wheat GY, while Mohammadi et al. [2] reported considerable G × E interaction across durum wheat environments and revealed different mega-environments using GGE biplot analysis. Consistently, Al-Ghumaiz et al. [9] found significant genotype, environment, and interaction effects in wheat genotypes evaluated over multiple years in different production systems.

Furthermore, the "Which-Won-Where" analysis provided additional information about specific adaptation of the wheat lines as different genotypes were associated with different environments for each trait. For example, L-14 (Koh-e-Safaid) was associated with Multan for Chl, L-07 (Chenab-70) with Chakwal for Pn, L-23 (Anmol-91) with Multan and Bahawalpur for Gs, L-19 (Patron-2014) with Bahawalpur and Swat for proline, and L-11 (TD-1) with Chakwal for GY (Figure 5). It indicates that winning genotype changed according to both the trait and environment. In the same pattern environment-specific winners have been reported in recent wheat GGE studies. For instance, Ogutu et al. [20] demonstrated environment-dependent genotype performance in durum wheat across seasons and Saeidnia et al. [16] identified different genotype responses across sowing dates, while. Mohammadi et al. [2] reported distinct mega-environments and genotype adaptation patterns in durum wheat using combined GGE and environmental analyses. These findings substantiate that the "Which-Won-Where" approach is useful for characterizing specific genotype–environment associations, particularly when the breeding objective is targeted adaptation.

The discriminating ability and representativeness of the testing environments also varied among traits. Attock and Multan showed strong discrimination for Pn, Attock and Swat were strongly discriminating for Gs, and Bahawalpur, Swat, and Chakwal showed greater discrimination for proline (Figure 6). For yield traits, Swat and Bahawalpur were strongly discriminating for GY, BY and for HI (Figure 6). Conversely, Multan was relatively representative for physiological (Chl, Gs, Pn) and biochemical (proline, MDA) traits, whereas Chakwal was comparatively representative for some yield-related traits (Figure 6). This suggests, an environment that reveals differences among genotypes is not necessarily a target environment as a whole. Therefore, this distinction is really important while selecting testing sites for future breeding trials. In the present context, Hnizil et al. [17] and Mohammadi et al. [2] reported substantial environmental impacts on wheat performance across contrasting agro-climatic zones and explained that climatic variables could explain a substantial proportion of G × E variation. Similarly, Yue et al. [7] further substantiated that integration of G × E analysis with environmental assessment can elucidate the differences among testing environments and improve interpretation of genotype adaptation.

The variation in environmental ranking among traits provides another important finding of this study. Attock ranked first for Chl and Pn, Multan ranked first for Gs and HI, Chakwal ranked first for proline and AEA, while Bahawalpur ranked first for GY (Figure 7). BY showed Chakwal as the highest-ranked environment (Figure 7). These results indicated that environmental suitability was trait dependent and that no single location provided the same conditions for optimal expression of all

traits. Besides, the present study revealed environment specific responses of traits as physiological, biochemical and agronomic traits respond to environment through multiple biological processes. In line with these findings Wang et al. [21] and Fenta et al. [22] respectively reported genotype-specific responses in physiological and agronomic traits under drought, and varying Pn, Gs, and yield-related traits according to environmental conditions.

The results from present study demonstrated the significance of integration of physiological, biochemical, and agronomic traits while interpreting G × E interaction. Correspondingly recent multi-environment investigations have emphasized on the significance of combining agronomic and physiological findings while understanding the wheat adaptability. For instance, Brković et al. [23] integrated GGE and AMMI analyses with antioxidant measurements while evaluating the genotypic adaptability and found consistent relationships between stress-related biochemical characteristics and yield traits. In line with these findings, Kumar et al. [24] reported significant G × E effects for GY and micronutrient traits during multilocation trials, which further endorsed our results that different traits may exhibit different patterns of environmental response.

The present results illustrated that genotypic performances varied in context of environment and traits, explicating the significance of simultaneously considering broad and specific adaptability in wheat breeding. The genotypes associated with specific environment are important candidate for targeted breeding, whereas stable genotypes are more useful for broad adaptation as concluded by different wheat multi-location studies [2, 9, 20, 25]. Besides, no environment was consistently the most discriminating or representative across all traits, indicating that testing environments should be selected according to breeding objectives and target traits. Present study also highlighted the importance of environmental characterization for understanding G × E interaction and improving environment selection. As a whole, the GGE analyses showed that genotype responses are determined collectively by environment and trait that provide useful information for identifying broadly adapted and specifically adapted wheat genotypes. However, validation across additional seasons and integration of soil and meteorological variables would strengthen interpretation of the environmental factors underlying G × E interaction.

5. Conclusion

In conclusion present study recorded significant GE interaction among 25 wheat genotypes evaluated across Attock, Chakwal, Swat, Bahawalpur and Multan. The genotypic performance and ranking varied according to trait and environment. Besides, the GGE biplot analysis revealed remarkable difference in physiological, biochemical and agronomic responses of wheat genotypes, and illus-

trated that specific adaptation took place across the five environmental regions. The "Which-Won-Where" analysis further showed that different genotypes were associated with different environments for individual traits, while the discrimination and representativeness analyses confirmed that the usefulness of testing environments also varied among traits. Overall, the findings demonstrate that no single genotype or environment showed consistently favorable performance across all traits, emphasizing the importance of multi-environment testing and trait-specific selection in wheat breeding. The identified GE relationships provide a useful basis for selecting lines for specific production conditions, while further multi-season evaluation combined with soil and meteorological data is recommended to validate the observed adaptation patterns and better explain the environmental factors underlying G × E interaction.

Declarations:

Abbreviation: Not Applicable

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Table 1. The 25 wheat lines evaluated in the multilocation trial, with cultivar names and pedigrees.

Line ID	Cultivar	Pedigree
Line-1	Punjab-2018	Kalyansona/Bluebird × Pasban-90
Line-2	Galaxy-2013	Pavon 76//Frontana /3/Kharchia-65
Line-3	Faisalabad-08	CNO79-75/Ae.Sq × Kalyansona
Line-4	Inqilab-91	Kharchia-65 × Bavi-87
Line-5	Seher-06	Kalyansona / Mumtaz-19
Line-6	Pak-81	(Kalyansona × Bluebird) / Frontana
Line-7	Chenab-70	Kalyansona × Gabo
Line-8	Akbar-19	Faisalabad-08 Selection × Galaxy-13
Line-9	NARC-02	Atta-2002 / Galaxy-13
Line-10	Chakwal-50	Kharchia-65 / SARC-5
Line-11	TD-1	P-18 × Pak-81
Line-12	Zardana	Pak-81 / Galaxy-13 × Faisalabad-08
Line-13	Imdad-2005	C-273 / Seher-06
Line-14	Koh-e-Safaid	Nil-2 / Pak-81
Line-15	Bhittai	Galaxy-13 × NARC-02
Line-16	Moomal-02	Galaxy-13 / Inqilab-91
Line-17	Mehr-11	Faisalabad-08 × Imdad-2005
Line-18	Bakhtawar-19	Faisalabad-08 / Galaxy-13
Line-19	Patron-2014	Faisalabad-08 × Galaxy-13
Line-20	Ujala-19	Inqilab-91 × Seher-06
Line-21	Azam-06	Faisalabad-08 × Pak-81
Line-22	Sahar-03	Galaxy-13 / Kalyansona
Line-23	Anmol-91	Kalyansona × Pasban-90
Line-24	Sky-2017	Faisalabad-08 × NARC-02
Line-25	NARC-2011	Inqilab-91 / Faisalabad-08

Table 2. **Pool ANOVA**

Source of Variance	DF	Chl	Pn	Gs	Pro	MDA	AEA	GY	BY	HI
Replication	2	58.98	185.30	19.63	349	220.38	485.49	59.16	129.05	14.35
Environments	4	52.87**	28.28**	10.06**	83.83**	80.74**	183.85**	47.85**	42.36**	28.94**
Genotypes	24	40.90**	28.30**	50.96**	33.90**	22.28**	24.7**	44.83**	35.15**	42.33**
Years	1	0.001 NS	0.05 NS	0.0003 NS	0.02 NS	0.003 NS	0.07 NS	0.0001 NS	0.04 NS	0.0001 NS
Genotype*Environment	96	30.01**	41.06**	40.28**	39.40**	43.64**	38.29**	37.46**	40.87**	46.93**
Environment*Year	4	0.003 NS	0.02 NS	0.0008 NS	0.004 NS	0.0002 NS	0.006 NS	0.006 NS	0.006 NS	0.0002 NS
Genotype*Year	24	0.003 NS	0.01 NS	0.0002 NS	0.004 NS	0.0008 NS	0.007 NS	0.0003 NS	0.006 NS	0.0002 NS
Genotype*Environment *Year	96	0.003 NS	0.01 NS	0.0002 NS	0.004 NS	0.001 NS	0.005 NS	0.0003 NS	0.005 NS	0.0002 NS
Error	498	0.01	0.02	0.01	0.04	0.03	0.07	0.008	0.01	0.005
Total	749									

Table 3. Crop Husbandry conditions provided during growth season

Environment	Location	Sowing window	Field unit	Planting geometry	Seed input	N–P_2O_5–K_2O	Irrigation	Harvest window
E1	Attock	10–20 Nov.	12.0 m²	22.5 cm rows	125 kg ha^{-1}	120–60–40 kg ha^{-1}	4 events	20–30 Apr.
E2	Chakwal, Pakistan	15–25 Nov.	12.0 m²	22.5 cm rows	125 kg ha^{-1}	120–60–40 kg ha^{-1}	4 events	25 Apr.–5 May
E3	Bahawalpur	20–30 Nov.	10.0 m²	20.0 cm rows	120 kg ha^{-1}	140–70–50 kg ha^{-1}	8–10 events	20–30 Mar.
E4	Multan	15–25 Nov.	10.0 m²	20.0 cm rows	120 kg ha^{-1}	140–70–50 kg ha^{-1}	7–9 events	25 Mar.–5 Apr.
E5	Swat	1–10 Nov.	10.0 m²	20.0 cm rows	120 kg ha^{-1}	130–65–45 kg ha^{-1}	6–8 events	10–20 Apr.

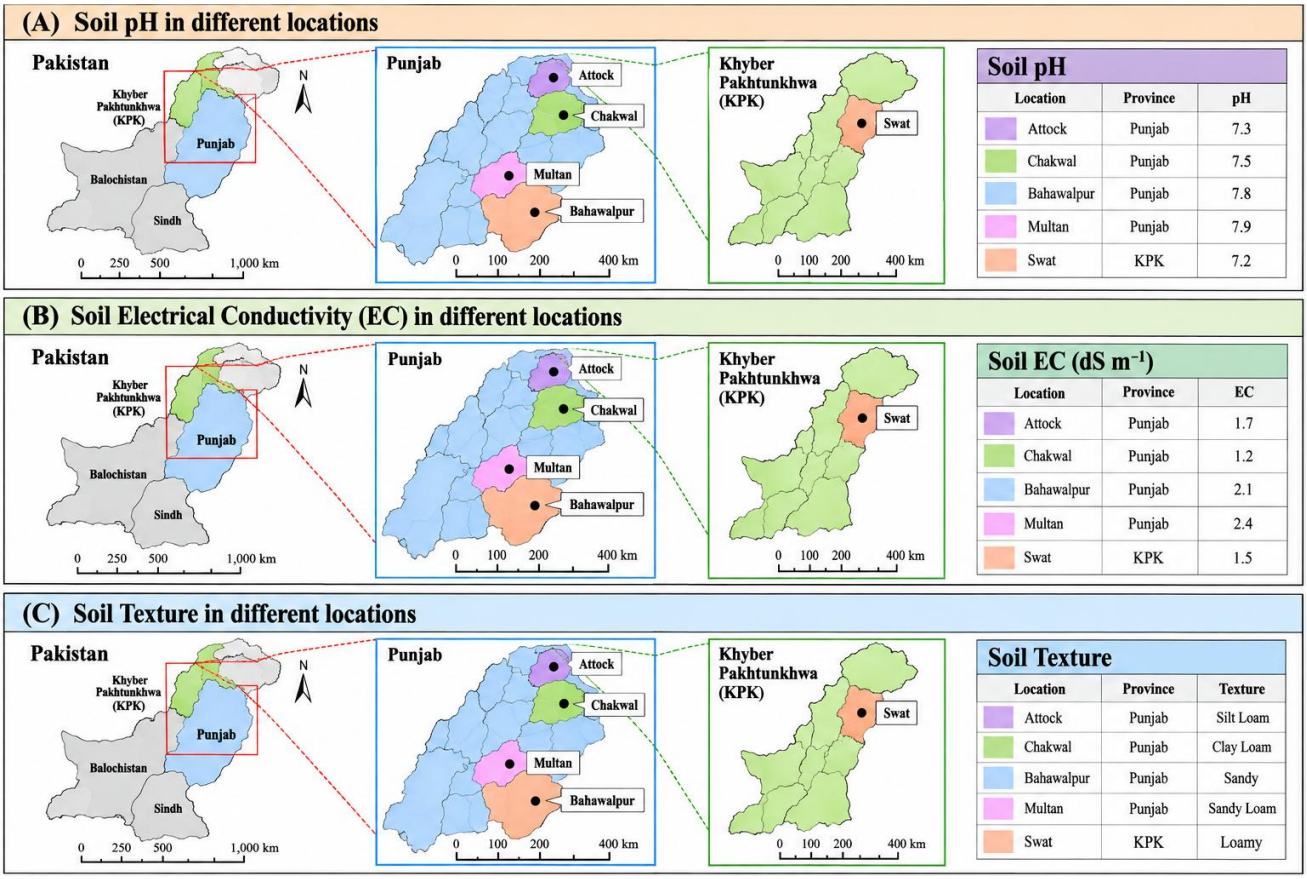


Figure 1. Soil and geographical characterization of the multilocation testing environments

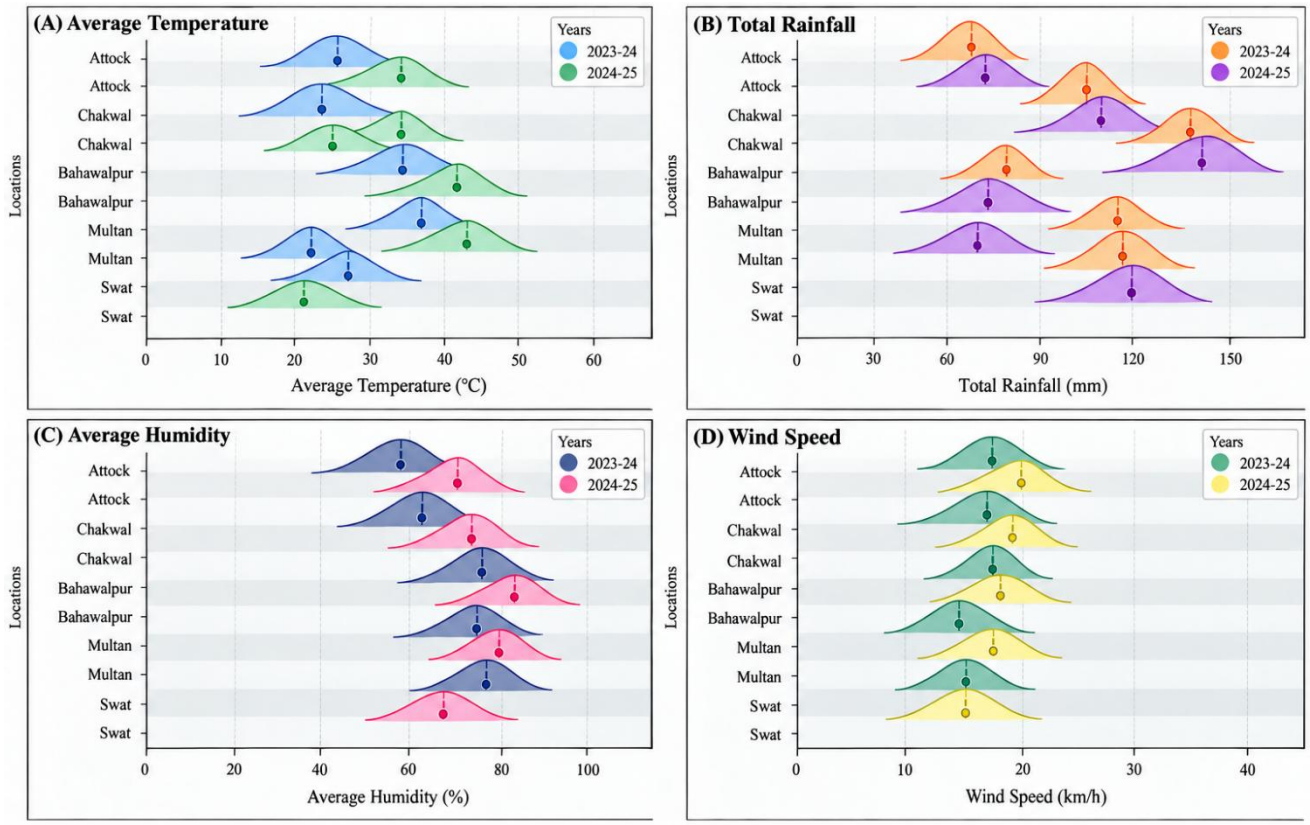


Figure 2. Meteorological characterization of the multilocation wheat trial across the recorded sites and years.

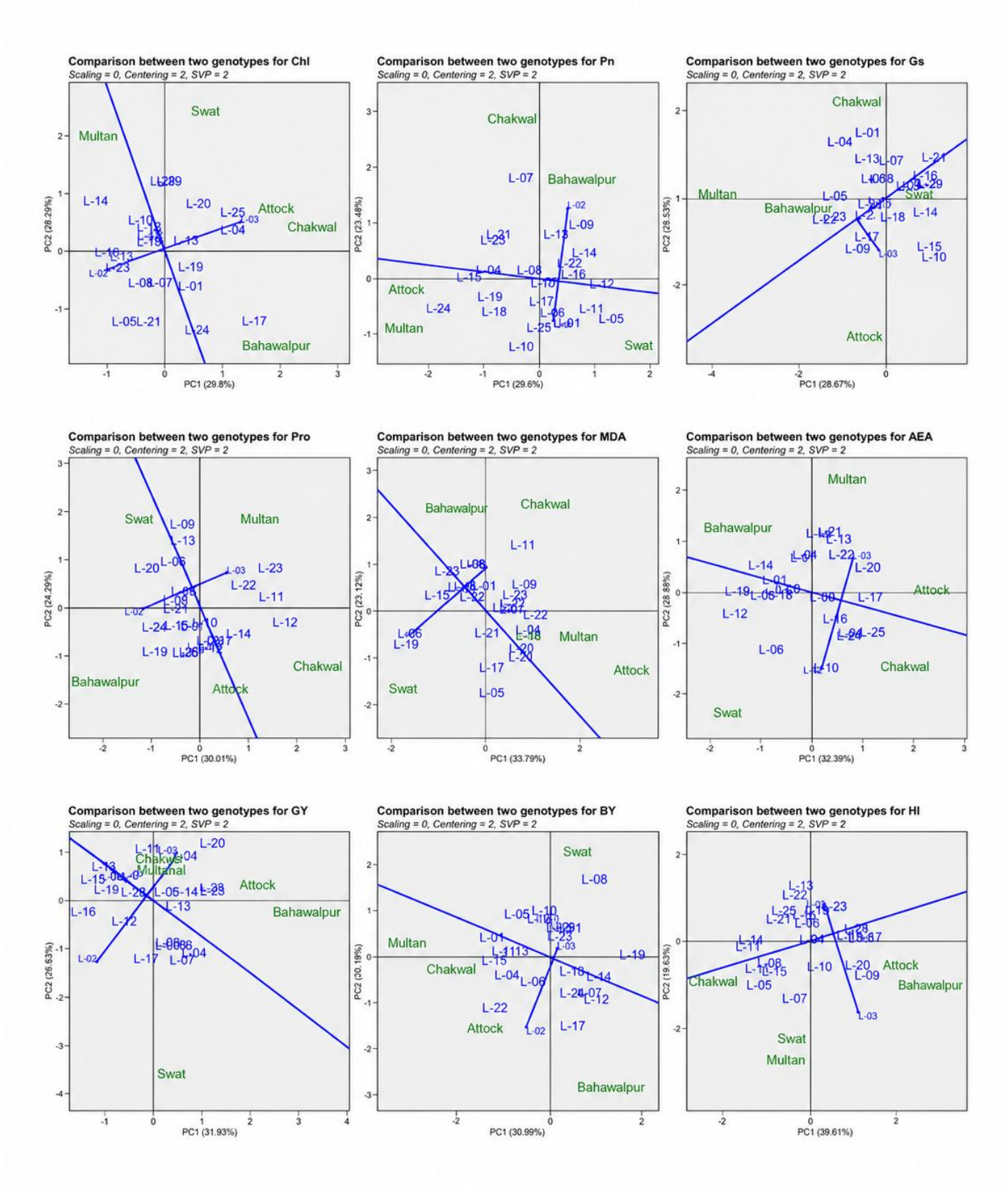


Figure 3. GGE pairwise genotype-comparison views across the five testing environments for Chl, Pn, Gs, Pro, MDA, and AEA. The comparisons identify L-03 (Faisalabad-08), L-08 (Akbar-19), L-20 (Ujala-19), L-21 (Azam-06), and L-13 (Imdad-2005) as informative candidates for contrasting adaptation patterns.

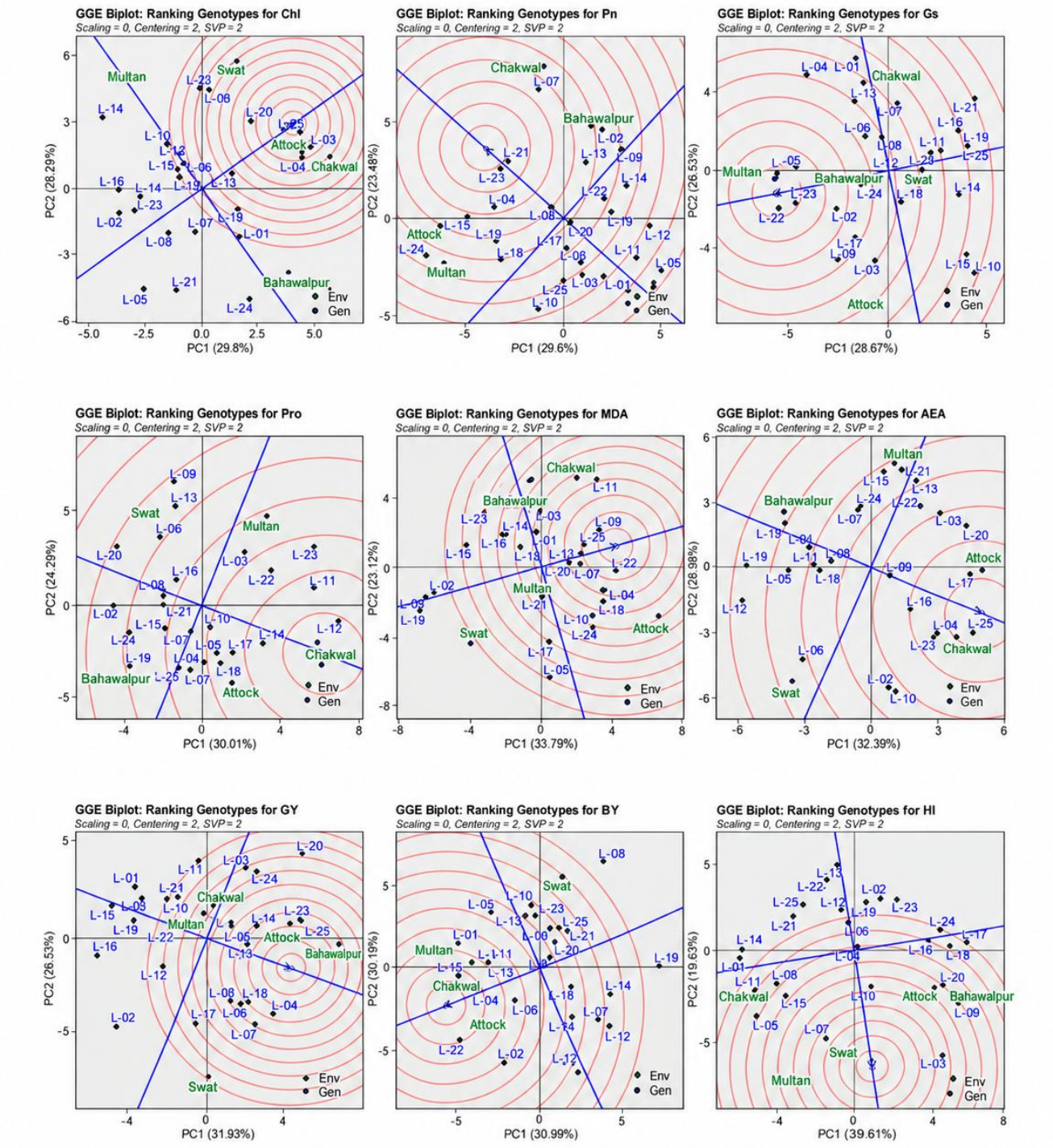


Figure 4. GGE ranking views for Chl, Pn, Gs, Pro, MDA, AEA, GY, BY, and HI relative to the ideal genotype. L-08 (Akbar-19), L-11 (TD-1), and L-13 (Imdad-2005) show favorable combined positioning across the trait set, with L-20 (Ujala-19), L-03 (Faisalabad-08), and L-21 (Azam-06) providing complementary selection candidates.

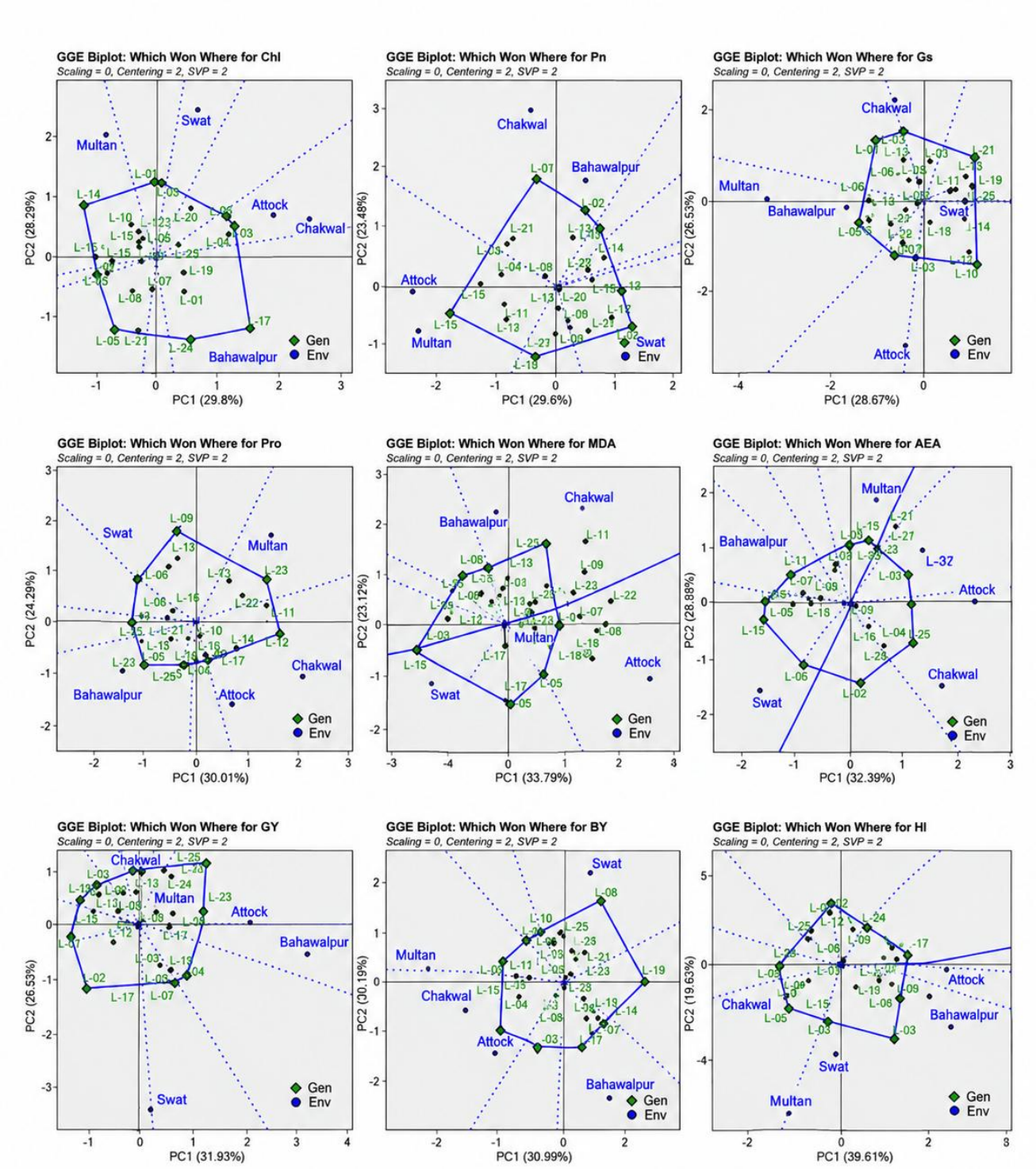


Figure 5. GGE which-won-where views identifying trait-specific genotype vertices and environmental sectors across the five locations. L-20 (Ujala-19), L-03 (Faisalabad-08), and L-13 (Imdad-2005) illustrate specific environmental responses, whereas L-08 (Akbar-19) and L-11 (TD-1) represent more broadly useful candidates.

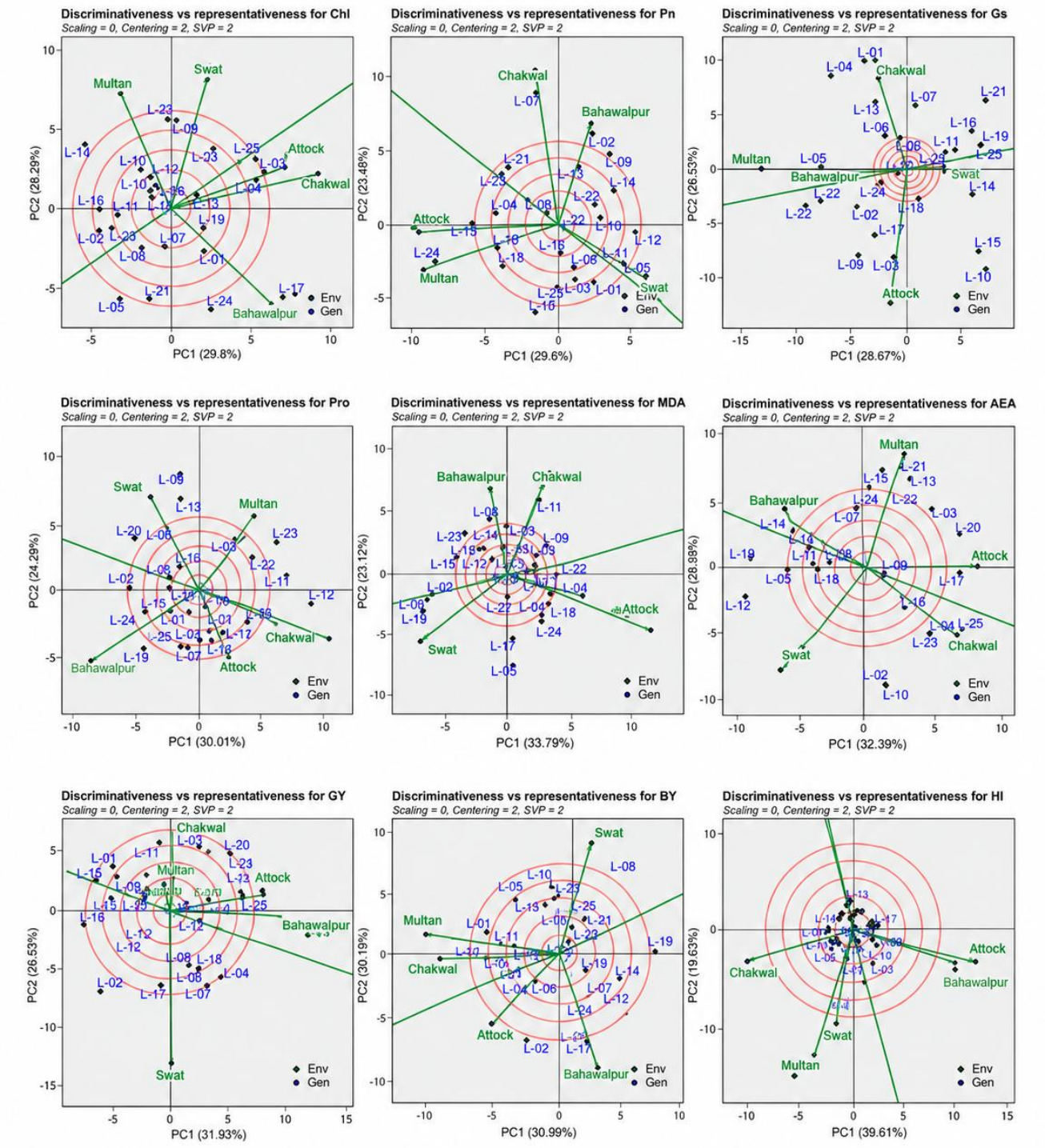


Figure 6. GGE discriminativeness-versus-representativeness views for Bahawalpur, Swat, Multan, Chakwal, and Attock. The five environments differ in their ability to discriminate among genotypes and to represent the broader target environment, providing context for selecting both broad- and specific-adaptation genotypes.

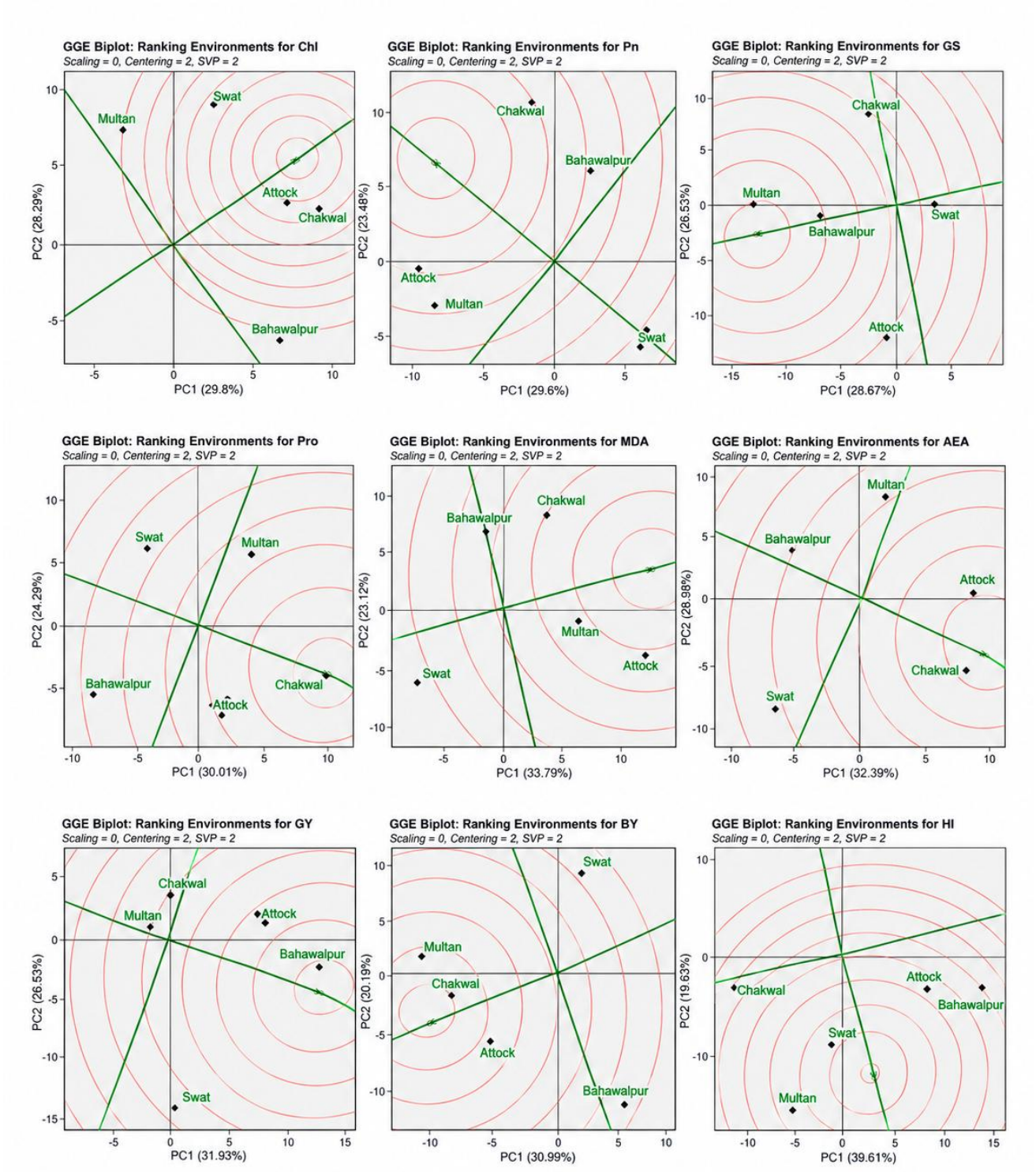


Figure 7. GGE environment-ranking views across Chl, Pn, Gs, Pro, MDA, AEA, GY, BY, and HI. The rankings identify complementary testing environments and support subsequent evaluation of broad-adaptation candidates such as L-08 (Akbar-19), L-11 (TD-1), and L-21 (Azam-06) and target-environment candidates such as L-20 (Ujala-19) and L-13 (Imdad-2005).