



Research article

Multivariate analysis of phenotypic diversity elite bread wheat (*Triticum aestivum* L.) genotypes from ICARDA in Ethiopia

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ABSTRACT

Wheat is an important crop for food security, providing a source of protein and energy for the growing population in Ethiopia. However, both biotic and abiotic factors limit national wheat productivity. The availability of genetically diverse wheat genotypes is crucial for developing new wheat varieties that are both high-yielding and resilient to stress. Therefore, this field trial aimed to assess phenotypic variation and relationship among ICARDA-derived bread wheat genotypes using multivariate analysis techniques. The trial was conducted at three locations: Enewari, Wogere, and Kulumsa using an alpha lattice design with two replications during the main cropping seasons of 2022 and 2023. Phenotypic data on eight agronomic traits and the severity of yellow rust were collected and R programming was used for data analysis. Individual and combined location data analysis of variance showed significant differences ($p \leq 0.05$) among genotypes for most of the studied traits. The highest heritability and genetic advance as a percentage of the mean were observed in days to heading (90.8, 21.29), plant height (72.4, 28.6), seeds per spike (61.7, 28), thousand kernel weight (61.9, 12), and area under the disease progress curve (67, 39.8), suggesting a predominance of additive gene action. Grain yield showed a strong positive correlation with days to maturity, plant height, spike length, spikelet per spike, and thousand kernel weight for each location. Dendrogram and phylogenetic tree methods were used to group genotypes into four genetically distinct clusters. Cluster II and III had the greatest inter-cluster distance, indicating higher diversity among their genotypes. This study identified new candidate genotypes with superior agronomic performance, high grain yield traits, and robust resistance to yellow rust, making them valuable for both current and future wheat breeding programs. Additionally, the comprehensive dataset produced in this study could facilitate the identification of genetic variations influencing desirable traits through genome-wide association analysis.

1. Introduction

The world's crop production is dominated by three cereals: maize, rice, and wheat. Among them, wheat (*Triticum* species) is one of

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the most well-known staple food crops, representing 26 % of world cereal production. The crop is a staple for the poor and rich alike [1]. It is estimated to be consumed by more than 2.5 billion people [2]. Wheat-based diets provide 20 % of the total protein, compared to 12 % for rice and only 5 % for maize, and they are good sources of various micronutrients [2–4]. Due to its special role in global food security, almost 25 % of world wheat production is consumed outside of the country of production [5]. However, only about 0.4 % of rice is traded globally [2]. The top three largest wheat producers in the world are China, India, and Russia, and in Sub-Saharan Africa (SSA), Ethiopia is the primary wheat producer [6]. In Ethiopia, out of the total grain crop area, 71.45 % was under the four cereals: teff, maize, wheat, and sorghum, with wheat alone occupying 15.31 % of the crop grain area, ranking third among others [7]. According to the same report, in terms of production, wheat contributes 18 % of the total grain crops following maize.

World wheat production and productivity are currently affected by the combined effects of biotic and abiotic factors, particularly in the SSA [8,9]. Climate change is the primary factor influencing wheat growth and productivity due to its direct impact on plant physiological processes. A recent study suggests that future climate change could lead to a decline in wheat yield by 2–19 % and 9–30 % under irrigated and rainfed conditions, respectively [10]. In Ethiopia climate change has exacerbated fungal diseases affecting wheat, resulting in significant financial losses. Specifically, fungal diseases like stem and yellow rust pose a threat to the livelihoods of Ethiopian smallholder farmers [11]. In Ethiopia, biotic and abiotic problems limit wheat production by up to 25 %, with rust diseases accounting for the majority of the loss. Every year, fungal diseases reduce 15–20 % of wheat yield, with rust disease accounting for the majority of the losses estimated to be up to \$10 million per year [12]. Generally, the challenge to control diseases in Ethiopia is tough as new disease pathotypes are developed continuously because of the high rate of mutation of the pathogens. As a result, Ethiopia's wheat productivity, at 3.3 t/ha is significantly lower than that of Ireland, which stands at 10.4 t/ha [6].

Therefore, in Ethiopia, there is a pressing need to raise wheat productivity through an appropriate breeding program, mainly by targeting complex traits related to yield potential and climate resilience. For Ethiopia's national wheat improvement program, the most important sources of wheat germplasm are CIMMYT and ICARDA. In collaboration with such international agricultural research centers and from other sources, Ethiopian national and regional-based research centers have released and registered over 140 varieties, both bread and durum wheat [12,13]. For developing and deploying improved wheat varieties, it is pertinent to accelerate the rate of genetic gain for grain yield with other key traits [1]. Moreover, economical disease control and defensive breeding strategies such as pyramiding of genes using markers to develop varieties with several resistance genes are highly important. To do so, the presence of significant genetic variability in any source population is very important. The existence of an adequate amount of genetic variability helps to integrate novel traits that cover biotic and abiotic factors. Several statistical techniques have been used to estimate genetic variability among different genotypes, of which multivariate analysis is the most effective and frequently used [14]. It is expected that diversity analysis of wheat germplasm currently obtained from ICARDA will provide fertile ground to develop wheat varieties by exploring specific genes for different agronomic and resistance traits. The main objective of the present study was to determine the extent of phenotypic diversity and relationship among ICARDA-origin wheat genotypes using multivariate analysis and cataloging morpho-agronomic attributes that could be further manipulated in the forthcoming wheat breeding program.

2. Materials and methods

2.1. Plant materials, study locations, experimental design, and crop management

The study was conducted at Kulumsa Agricultural Research Center (Kulumsa; 8°01'13.7" E; 39°07'45.6" N; 2400 masl) and Debre Birhan Agricultural Research Center subsites: Enewari (9° 52' 10.7" N and 39° 10 '46.5" E; 2650 masl) and Wogere (10°03'94.1" N and 39°26'50.8" E; 2700 masl) under rain-fed conditions during the main cropping seasons of 2022 and 2023. Enewari and Wogere have a soil classification of black vertisol with a pH of 6.8, while the predominant soil type at Kulumsa is nitosol soil with a pH of 6.7. The climatic descriptions of the three test locations are shown in Table 1.

A total of 150 elite bread wheat genotypes were used in this study, including two standard check varieties, Denede'a and Daka

Table 1
Annually and monthly climatic conditions of the study areas.

| Year | Enewari | | | | Kulumsa | | | Wogere | | |
|------|-----------|------|------------|--------|---------|------------|--------|--------|------------|--------|
| | month | RH | Range (T°) | RF | RH | Range (T°) | RF | RH | Range (T°) | RF |
| 2022 | August | 83.2 | 9.1–21.7 | 303 | 82.7 | 9.6–21.5 | 220.1 | 76.8 | 11.7–22.5 | 254.6 |
| | September | 81.9 | 9.1–21.7 | 143 | 83.6 | 9.7–21.5 | 208.9 | 75.1 | 11.7–21.4 | 149.1 |
| | October | 75.8 | 4.5–21.6 | 52.8 | 78.6 | 5.7–21.5 | 117.7 | 72.3 | 6.5–20.6 | 67.2 |
| | November | 66.7 | 3.5–21.1 | 65 | 70.8 | 5–22.2 | 192.3 | 62.2 | 5.4–22.5 | 134.8 |
| | December | 65.8 | 1.4–24 | 54 | 71.1 | 2.5–22.4 | 84.1 | 67.2 | 3.8–22.5 | 133.3 |
| | Annual | 66.3 | 1.4–29.2 | 1268.3 | 71.5 | 2.5–27.4 | 1628.7 | 63.5 | 3.8–28 | 1363 |
| 2023 | August | 86.2 | 9.7–21.1 | 220.2 | 84.8 | 10.3–20.8 | 173.7 | 82.9 | 11.3–20.9 | 283.9 |
| | September | 82.5 | 9.1–21.3 | 103.1 | 82.4 | 9.8–21.6 | 109.4 | 77.1 | 11.6–21.9 | 96 |
| | October | 76.9 | 4.8–21.4 | 63.6 | 78.6 | 5.7–21.7 | 83.2 | 74.5 | 7.6–20.4 | 101.1 |
| | November | 67.5 | 4.6–21.7 | 7.5 | 67.5 | 5.7–22.3 | 24.7 | 63.8 | 7–21.4 | 11 |
| | December | 65.3 | 3.4–23.5 | 22.7 | 62.4 | 5–23.7 | 20.2 | 64.9 | 6.5–23.1 | 22.3 |
| | Annual | 67.7 | 3.4–29.3 | 1054.6 | 70.3 | 4.7–27.5 | 982.5 | 66.8 | 5.9–27.9 | 1196.7 |

RF: sum/average rain fall, T°: Temperature, RH: relative humidity.

(Table S1). These elite bread wheat lines were developed using the shuttle breeding strategies of ICARDA to release desirable new varieties mainly for Central and West Asia and North Africa (CWANA) and Sub-Saharan African countries [15]. The checks are released varieties that are widely cultivated in Ethiopia. The field trial was laid out using an alpha lattice design with two replications. The plot size is 1.2 m in width and 1 m in length, with 0.20m inter-row spacing. The spacing between plots and replications were 0.40m and 1m, respectively. The recommended seed rate of 150 kg per hectare was used, along with 275 kg per hectare of Urea, and 273 kg per hectare of NPS (Nitrogen Phosphorus, and Sulfur) fertilizer. Urea was applied in two stages: half at 20 days after planting, and the remaining half at the booting stage. All other management practices were implemented uniformly according to previous recommendations of the areas. Artificial inoculation with *Puccinia striiformis f.sp. tritici* was not employed to induce yellow rust. Instead, a highly susceptible variety, Ogolcho, was planted in the rows to facilitate the spread of the disease among the genotypes. During the trial, the average monthly temperatures throughout both cropping seasons and across all test locations were favorable for pathogen development (Table 1). Yellow rust thrives in cool temperatures, with germination and penetration occurring best between 9 and 13 °C and growth and development optimal between 12 and 15 °C [16]. Furthermore, yellow rust can typically thrive in cooler environments (2–20°C) at higher altitudes [17]. The trial sites are hotspot areas for yellow rust, showing that yellow rust occurred naturally in the field.

2.2. Data collected

A total of eight quantitative traits were collected in this field trial. Phenological traits, such as days to heading (HD) and days to maturity (MD), were collected at the plot level. For other traits, five plants per genotype were randomly selected from the central three rows of each plot within each replication. The average measurement of five plants was used to determine traits such as plant height (PH in cm), spike length (SL in cm), spikelet per spike (SLPS), and seeds per spike (SPS). The one thousand kernel weights (TKW in grams) were determined by weighing a sample of one thousand kernel weights. Grain yield (GY) for the entire plot was calculated by converting the total grams of grain produced per plot into tons per hectare. Yellow rust severity was visually assessed using a modified Cobb scale [18], expressed as a percentage of diseased leaf area ranging from 0 to 100. Yellow rust score was recorded during the tillering (YR1), booting (YR2), and heading (YR3) growth stages. Yellow rust infection types were categorized and recorded as follows: immune (0), with no uredia or other visible sign of infection, resistant (R), with small uredia surrounded by necrosis, mid-resistant (MR), with small to medium uredia surrounded by chlorosis or necrosis, mid-susceptible (MS), with medium-sized uredia that may be associated with chlorosis, and susceptible (S), with large uredia without chlorosis or necrosis [19]. The area under the disease progression curve (AUDPC) was calculated using the method provided by Ref. [20].

$$AUDPC = \sum_{i=1}^{n-1} 0.5(X_{i+1} + X_i)(t_{i+1} - t_i)$$

where x_i is an assessment of sickness at the i th observation, t_i is the time (in days, hours, etc.) at the i th observation, and n is the total number of observations.

The coefficient of infection (CI) was calculated by multiplying the terminal yellow rust severity value by the genotype resistance at field response. The field constants are resistance (R) = 0.2, moderate resistance (MR) = 0.4, moderately susceptible = 0.6, moderately susceptible to susceptible = 0.8, and susceptible = 1.

2.3. Statistical analysis

All collected phenotypic data were checked for normality using Shapiro Wilks test and for homogeneity of variance using the Bartlett test, both performed with R programming. The data were then subjected to analysis of variance using R-programming to detect the differences among the genotypes. Variance [variance-covariance] functions in R, such as `var["genotype", "vcov"]`, `var["Residual", "vcov"]`, `var["genotype by location", "vcov"]`, `var["genotype by year", "vcov"]`, and `var["genotype by location by year", "vcov"]`, are used to calculate various variance components, coefficient of variance, heritability, genetic advance, and genetic advance as percent of the mean. To analyze the variance and mean performance of genotypes in R, the following individual and combined location models were utilized [21].

Single location ANOVA model;

$$y_{ijk} = \mu + g_i + r_j + bl(j) + e_{ijk}$$

Where:

y_{ijk} = the observed value of the trait Y for the genotype in replication j; μ = the general mean of trait Y; r_j = the effect of replication; g_i = the effect of genotypes and $bl(j)$ = block within replicate effect; e_{ijk} = the experimental error associated with the trait y for the genotype in the i th block within replication and replication.

Combined ANOVA model;

$$y_{ijkl} = \mu + Gen_i + rep_j (Lock) + lock + Lock * Gen_i + block_l(Lock : rep_j) + e_{ijkl}$$

Where;

y_{ijkl} = observed value of genotype i in block l of replication j in location k; μ = grand mean; gen_i = effect of genotype i; $Lock$ =

Location effect; $Lock * Genl$ = the effect of genotypes (i) and location (k) interaction; $blockl(Lockl : repj)$ = effect of block l in location k; ϵ_{ijkl} = random error or residual effect of genotype i in block l of location k.

The phenotypic and genotypic coefficients of variation were estimated according to the method given by Ref. [22] as follows:

$$\sigma_e^2 = MS_e$$

$$\sigma^2g = \left[\frac{MS_g - MS_e}{RL} \right] = (\sigma_e^2 + R\sigma^2gL + RL\sigma^2g) - (\sigma^2e + R\sigma^2gL) / RL$$

$$\sigma^2p = \sigma^2g + \sigma_e^2 = \sigma^2g + \frac{\sigma^2gL}{L} + \sigma^2e / RL$$

$$PCV = \sigma p / x * 100$$

$$GCV = \sigma g / x * 100$$

Where, x = grand mean of a character, R= Replications, L = Location. Broad sense heritability (H^2) was computed using the following formula as described by Ref. [21].

$$\text{single location } H2 = \frac{\sigma^2g}{\sigma^2g + \frac{\sigma^2e}{Rep.}}$$

$$\text{combined location } H2 = \frac{\sigma^2g}{\sigma^2g + \frac{\sigma^2ge}{No. env.} + \frac{\sigma^2e}{No. env.*Rep.}}$$

$$GA = K * \sigma^2p * H^2; \text{ GAM} = \{GA / x\} * 100$$

Where: k = standardized selection differential (K = 2.063 at 5 % selection intensity), X = grand mean of the respective trait, σ_{ge}^2 = Genotypes*environment variance, No. env. = the number of environments, Rep. = the number of replications. GAM is divided as low (0-10), moderate (10–20), and high (>20) as suggested by Ref. [23]. Similarly, H2 estimate can be grouped as low (<40 %), medium (40–59 %), moderately high (60–79 %), and very high (≥ 80) [24].

All data visualizations were performed using R programming with various functions. The bar plot function was used to create a bar plot diagram. A box plot and a t-test value, produced with the compare means function, were used to evaluate the significance of the

Table 2

Analysis of variance for measured traits of bread wheat genotypes tested at Enewari, Kulumsa and Wogere in the 2022 and 2023 main cropping season.

| Location | Traits | Genotype (149) | Year (1) | Rep (1) | G*Y (149) | Blk (29) | Residuals (285) | Range | Mean | CV (%) | |
|----------|--------|----------------|----------|-----------|-----------|----------|-----------------|------------|-------------|--------|------|
| Enwari | DH | 36.6*** | 187** | 3.4 | 15.2 | 8.2 | 14.7 | 64.8–84.8 | 70.24 | 4.31 | |
| | DM | 31*** | 12141*** | 98** | 18 | 28* | 14 | 129–144.8 | 136.7 | 2.05 | |
| | PH | 82*** | 475*** | 1034*** | 36.9*** | 35.6 | 23.3 | 80.7–101.5 | 92.74 | 6.29 | |
| | SL | 1.4*** | 499.8*** | 1 | 0.8 | 1.1 | 0.6 | 8.1–14.7 | 9.98 | 7.1 | |
| | SLPS | 2.3 | 20.5** | 2.4 | 1.9 | 2.7 | 2.1 | 15.9–20.7 | 18.3 | 4.38 | |
| | SPS | 101* | 4146*** | 440* | 86 | 75 | 77 | 33–67.1 | 50.49 | 11.92 | |
| | TKW | 50*** | 5708*** | 60 | 28*** | 13 | 18 | 34.8–55.8 | 45.6 | 7.82 | |
| | GY | 1.5*** | 12.9*** | 2.2 | 1.3*** | 0.69 | 0.69 | 3.4–6.5 | 4.9 | 12.44 | |
| | Wogere | DH | 47*** | 5539*** | 1 | 5*** | 7** | 3 | 73.4–90.9 | 79.92 | 4.28 |
| | | DM | 26 | 66024*** | 826*** | 24 | 19 | 22 | 132.8–150.3 | 143.98 | 2.11 |
| PH | | 102*** | 1330*** | 1155.6*** | 61** | 56.5 | 40.6 | 71–99.8 | 85.84 | 5.88 | |
| SL | | 2.1*** | 130.4*** | 0.41 | 1.5*** | 0.73 | 0.74 | 8.5–13.2 | 10.15 | 7.61 | |
| SLPS | | 3.6* | 484*** | 115.6*** | 3.5 | 4.1 | 2.8 | 17.2–25.6 | 19.51 | 8.61 | |
| SPS | | 153*** | 5020*** | 1951*** | 32 | 141** | 64 | 38.4–70.5 | 57.9 | 14.54 | |
| TKW | | 51*** | 4573*** | 214*** | 25*** | 12 | 16 | 36.5–58.5 | 47.43 | 7.76 | |
| GY | | 2.3*** | 311.2*** | 20.9*** | 1.5 | 1.6 | 1.4 | 3.1–8.1 | 5.09 | 15.29 | |
| Kulumsa | | DH | 41.4*** | 109.2*** | 11 | 2.8 | 7.4 | 4.2 | 63–80 | 70.18 | 4.58 |
| | | DM | 56.3*** | 90.4*** | 7.4 | 5.2 | 16.9** | 7.8 | 91.5–138.5 | 119.54 | 3.87 |
| | PH | 132*** | 18 | 9 | 58 | 59.6 | 49 | 67.5–97.5 | 83.28 | 7.1 | |
| | SL | 2.3 | 214*** | 23.9*** | 1.7 | 2.3 | 2 | 9–15.3 | 11.41 | 9.58 | |
| | SLPS | 6.4*** | 3.6 | 4.3 | 1.9 | 2.4 | 3 | 14.5–21.3 | 17.64 | 7.15 | |
| | SPS | 37*** | 41722*** | 239** | 30* | 28 | 24 | 39.8–62 | 52.25 | 8.82 | |
| | TKW | 45*** | 21117*** | 0.01 | 28 | 24 | 22 | 26.6–43.4 | 37.09 | 8.61 | |
| | GY | 0.56*** | 117.6*** | 2.2** | 0.56*** | 0.08 | 0.22 | 2.2–4.9 | 3.15 | 16.59 | |

Significant levels (***: Very high significant; **: High significant; and *: Significant); Gen: Genotype; Rep: Replication; CV: Coefficient of variation; HD: Days to heading; MD: Days to maturity; PH: Plant height; SL: Spike length; SPS: Seeds per spike; SLPS: Spikelet per spike; TKW: Thousand kernel weight; GY: Grain yield.

difference between top and low-performing genotypes for agronomic traits. The function `fviz_dend` was employed to visualize a circular dendrogram and phylogenetic tree using the `ggplot2` package. `fviz_pca_var` was used to display variable graphs from the principal component. The `ggcorrplot` function generated correlation plots for the measured traits of genotypes.

3. Results and discussion

3.1. Analysis of variance

Significant variation was observed for phenological, agronomic, and yield traits among 150 genotypes of bread wheat at each testing location in Enewari, Wogere, and Kulumsa (Table 2). This wide diversity within ICARDA bread wheat genotypes could aid in developing and improving new bread wheat varieties. Previous studies also noted phenotypic variations in valuable agronomic traits among bread wheat genotypes [25–28]. Indeed, the evaluated materials originated from a segregating population created through hybridization efforts between parents with desirable traits such as adaptation, resistance to biotic and abiotic stresses, Rht genes, physiological traits, and other traits at both the molecular and phenotypic levels [15]. Previous research has shown that variability in agronomic and disease resistance traits among ICARDA and CIMMYT breeding wheat lines has contributed to the development of high-yielding varieties that were tolerant to various environmental conditions [15,29,30].

The combined analysis of variance indicated a significant difference ($p < 0.01$) between genotypes for all studied traits (Table 3). The interaction between genotype and location (GEI) and genotype and year was highly significant for most traits. This finding is consistent with the results of [31,32], which reported a significant GEI for yield-related traits in bread wheat genotypes. The significant GEI suggests that a breeding strategy should focus on developing genotypes specifically adapted genotypes in homogeneously clustered environments [33]. This strategy could benefit smallholder farmers by providing improved varieties tailored to specific locations, addressing the diverse agroecological conditions in Ethiopia [34]. In this study, GEI may be influenced by climate as well as by biotic (such as disease) and abiotic (including soil fertility, moisture availability, and soil type) factors, all of which vary between testing locations and seasons. This is supported by variations in temperature, precipitation, and humidity data across testing locations and seasons (Table 1). Therefore, crop breeders should consider climate variables, including long-term data [35], when selecting genotypes that are stable across environments to avoid promoting inferior genetic materials or rejecting promising breeding lines [36].

3.2. Genotypic mean performance

The genotypic mean performance of 150 bread wheat genotypes for phenological, agronomic, and grain yield traits are shown in Table 2. The mean days to heading (DH) ranged from 64.8 to 84.8 days at Enewari, 73.7–90.9 days at Wogere, and 63–80 days at Kulumsa, with mean values of 70.2, 79.9, and 70.2 days, respectively. For days to maturity (DM), the range was 129–144.8 days at Enewari, 132.8–150.3 days at Wogere, and 91.5–138.5 days at Kulumsa, with average values of 136.7, 143.9, and 119.5 days, respectively. At Enewari, Wogere, and Kulumsa, 54 %, 10.6 %, and 78 % of the distinct genotypes mature earlier than the standard check varieties, respectively. One of our breeding objectives is to identify traits that enable crops to mature early helping them escape frost and terminal moisture stress at the end of the growing season. In the combined data, the mean values for DH and DM ranged from 60 to 94 days and 112–150.4 days, respectively (Table 3). Among the 150 bread wheat genotypes, 58.6 % were headed earlier, and 64 % matured earlier than the standard check variety (Fig. 4 and Table S1). This suggests that there are numerous opportunities to select genotypes of early-maturing bread wheat varieties that may escape frost and the terminal moisture stress in the study locations. However, early maturity trait alone is not desirable because smallholder farmers also consider other useful traits, such as grain yield, seed color, and feed value, when selecting which varieties are suited for production. Some genotypes of early maturing bread wheat were found to have average to greater grain yield (Table S1), which is surprising because it means that these genotypes integrated two important traits, making them desirable. Early-maturing, high-yielding, heat-tolerant wheat genotypes with good adaptability to various environments that integrate vernalization, photoperiod, and dwarfing genes have been developed during recent decades by ICARDA, CIMMYT, and other international breeding programs [37]. This might be the possible reason for obtaining genotypes with these desirable traits from such wheat genotype sources.

Table 3
Analysis of variance for measured traits of bread wheat for combined data.

| Traits | G (149) | L (2) | Y (1) | Rep (1) | Block (14) | G*L (298) | G*Y (149) | Residuals (885) | Mean | Range | CV |
|--------|---------|----------|----------|---------|------------|-----------|-----------|-----------------|-------|----------|----|
| DH | 101*** | 18828*** | 843*** | 6 | 7 | 12*** | 13*** | 7 | 73.59 | 60–94 | 8 |
| DM | 66*** | 93765*** | 7610*** | 559*** | 27 | 29*** | 23* | 18 | 131 | 112–150 | 9 |
| PH | 198*** | 13114*** | 1303*** | 1595*** | 51 | 59*** | 76*** | 39 | 87.19 | 61–101.5 | 10 |
| SL | 3.2*** | 102.2*** | 119.1*** | 6.2* | 1.5 | 1.3 | 1.4 | 1.2 | 10.14 | 6–15.3 | 12 |
| SLPS | 6.4*** | 464*** | 113*** | 76*** | 4.6 | 3.2 | 2.9 | 3 | 18.34 | 13–25.6 | 9 |
| NSPS | 128*** | 28033*** | 38420*** | 498** | 101* | 82*** | 69 | 58 | 52.94 | 30–70.5 | 15 |
| TKW | 79*** | 529*** | 2 | 160*** | 14 | 34*** | 38*** | 19 | 44.34 | 28–58.5 | 14 |
| GY | 2.2*** | 692*** | 165*** | 36*** | 0.8 | 1.3*** | 1.2*** | 0.9 | 4.68 | 1.4–8.1 | 26 |

Significant levels (***: Very high significant; **: High significant and *: Significant); G: Genotype; R: Replication; CV: Coefficient of variation; HD: Days to heading; MD: Days to maturity; PH: Plant height; SL: Spike length; SPS: Seeds per spike; SLPS: Spikelet per spike; TKW: Thousand kernel weight; GY: Grain yield ton/ha.

The plant height (PH) ranged from 80 to 101.5 cm at Enewari, 71–99.8 cm at Wogere, and 67.5–97.5 cm at Kulumsa, with mean values of 92.74, 85.84, and 83.28 cm, respectively. The lowest mean PH readings were observed at Kulumsa. In combined data, PH ranged from 61 to 101.5 cm. About 40 % of bread wheat genotypes were shorter than the mean performance of the standard check variety (Table S1). A previous report showed that by combining the dwarfing genes *Rht4* and *Rht8*, plants might reduce plant heights to optimal levels while still exhibiting enhanced yield-related traits and grain yield [38]. The agricultural sector is crucial for meeting food and feed requirements, especially cereal-livestock farming systems where grains are used for food, and crop residues serve as the main sources of animal feed. This is the reason why biomass is an important feed trait for smallholder farmers.

The ICARDA-derived bread wheat genotypes showed notable phenotypic variation in terms of the spike-related traits, showing mean values greater than the standard check variety for SL, SLPS, and NSPS, respectively, at 35.5 %, 9.3 %, and 16.6 % (Table S1). Further, SL, SLPS, and NSPS ranged between 6 and 15.3 cm, 13–25.5 cm, and 30–70.5 cm, respectively. A previous report revealed many spike-related traits in wheat, including large spikes (high assimilate partitioning to spike), large viable florets per spikelet, and a high number of spikelets. Overall, the most desirable traits for wheat breeding are available with sufficient diversity at ICARDA and CIMMYT [37].

TKW ranged from 34.8 to 55.8 g at Enewari, 36.5–58.5 g at Wogere, and 36.5–43.4 g at Kulumsa, with mean values of 45.6, 43.34, and 37.07 g, respectively (Table 2). In combined data, TKW ranged from 34 to 60.7 g, with a mean value of 44.3 g (Table 3). This is a substantial amount above the ISO 520 minimum requirements for TKW bread wheat grain, which is 30 g for producing flour for bread baking [39]. The current wheat genotypes originating from ICARDA displayed that 20 genotypes (13.3 %) have TKW higher than those of the standard check varieties (49g), implying that these wheat genotypes with higher thousand kernel weights produce more white flour [40]. Smallholder farmers generally prioritize grain productivity, while bakers focus on both the quantity and quality of bread, and millers are concerned with flour production. Thus, further evaluation of quality traits following global grain quality standards is required before releasing a new variety from the studied genotypes.

GY ranged from 3.4 to 6.5 tons/ha at Enwari, 3.1–8.1 tons/ha at Wogere, and 2.2–4.9 tons/ha at Kulumsa, with averages of 4.9, 5.1, and 3.2 tons/ha, respectively. As displayed in the box plot (Fig. 1), genotype mean performance is lower at Kulumsa than at Enwari and Wogere. In combined data, GY ranged from 1.4 to 8.1 tons/ha, with an average of 4.7 tons/ha (Fig. 1, Table 3). Grain yield is a genetically complex trait and is a result of the combined effect of several agro-morphological and physiological traits [41]. Thus, variations in agronomic and yield traits evaluated in this study may assist the breeding program in developing superior bread wheat varieties for Ethiopian agriculture with either broad or targeted adaptation. GY of up to 8.1 tons/ha was produced in bread wheat genotypes of ICARDA origin (Table 3), suggesting the potential to generate high-yielding varieties that could enhance smallholder farmers' incomes in Ethiopia's largest wheat-producing regions and beyond. This grain yield potential in ICARDA-origin bread wheat genotypes is more than twice the national average of 3.1 tons/ha for wheat, which is grown on 1.9 million hectares of land [7]. Further, sixteen ICARDA-origin bread wheat genotypes outperformed the standard check varieties (Fig. 4). Therefore, selecting the most promising genotypes and conducting additional multi-location trials through participatory varietal selection is crucial for developing modern varieties that farmers prefer and suitable for diverse locations (Fig. 4).

3.3. Variation in resistance to yellow rust

3.3.1. Final yellow rust severity

The severity of yellow rust and responses of genotypes were examined, along with their yield and agronomic traits (Table S2). Yellow rust, caused by the fungus *Puccinia striiformis*, seriously affects wheat production in Ethiopia [42]. Therefore, generating and deploying genetically resistant varieties adapted to specific locations is the most economical and environmentally friendly method for controlling wheat rust infections, especially for smallholder farmers [43]. For both combined and individual location datasets, there was a highly significant variation in final disease severity due to year, location, and genotypes by location interaction (Table 4). This indicates that both year and location significantly affect yellow rust incidence. In our multi-location trial, final yellow rust severity ranged from 0 % to 95 % at different stages across two cropping seasons and three locations (Table S2). This variability provided considerable disease pressure on the evaluated bread wheat genotypes, offering a valuable opportunity to identify and select resistant

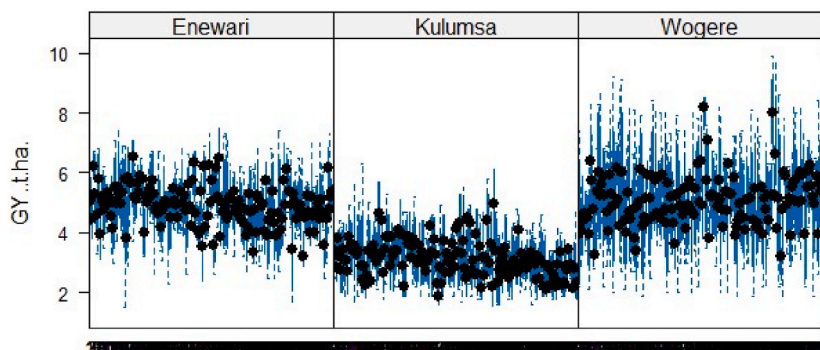


Fig. 1. Graphical representation of grain yield men performance of genotypes across location.

Table 4

Analysis of variance for yellow rust scores of bread wheat genotypes tested at Enewari, Kulumsa and Wogere in the 2022 and 2023 main cropping season.

| Location | Traits | Genotype (149) | Year (1) | Replication (1) | Genotype by Year (149) | Block (29) | Residuals (285) |
|----------|---------------|----------------|--------------|-----------------|------------------------|------------------|------------------------|
| Enwari | FDS | 1256*** | 209216*** | 2158* | 642* | 224 | 190 |
| | CI | 1174*** | 246005*** | 1129* | 660*** | 260 | 190 |
| | AUDPC | 2.4*** | 126.7*** | 3* | 0.7*** | 0.76 | 0.53 |
| Wogere | FDS | 26.6*** | 678.4*** | 0.2 | 12.8 | 15.2 | 12 |
| | CI | 18.2*** | 806.9*** | 0.01 | 9.1*** | 10.8 | 9 |
| | AUDPC | 1.87*** | 188.9*** | 3.2** | 0.85*** | 0.71* | 0.44 |
| Kulumsa | FDS | 732*** | 43129*** | 1710*** | 645*** | 125 | 94 |
| | CI | 526*** | 10634*** | 1210** | 444*** | 100 | 83 |
| | AUDPC | 1.7*** | 37.4*** | 10*** | 1.14*** | 0.63 | 0.36 |
| Combined | Traits | G (149) | Y (1) | L (2) | G*L (298) | G*Y (149) | Residuals (885) |
| | FDS | 1087*** | 25350*** | 129589*** | 463*** | 407*** | 101 |
| | CI | 891*** | 1957*** | 113446*** | 413*** | 400*** | 95 |
| | AUDPC | 4.32*** | 118.9*** | 67.5*** | 0.83*** | 1.28*** | 0.47 |

FDS= Final disease severity (%), CI = Coefficient of infection, AUDPC = Area under the disease progress curve, Significant levels (***: Very high significant; **: High significant and *: Significant); G: Genotype; R: Replication, L = Location, Y = Year, G*L = Genotype by location interaction, G*Y = Genotype by year interaction.

varieties to mitigate yellow rust and improve wheat production in Ethiopia. As shown in the bar graph below, 8, 39, and 6 genotypes at Enewari, Wegere, and Kulumsa were free of yellow rust during the 2022 cropping season. In 2023, 8, 11, and 9 genotypes at Enewari, Wegere, and kulumsa, respectively, remained free of yellow rust. Among these, six genotypes were free of yellow rust at all locations during both cropping seasons. These yellow rust-resistant materials could be used as parent stock for crossing programs to develop new varieties that are both resistant and high-yielding. In the 2022 cropping season, 70, 97, and 36 genotypes at Enewari, Wogere, and Kulumsa exhibited a low level of severity (<20 %), whereas in 2023, 4, 137, and 49 genotypes at Enewari, Wogere, and Kulumsa showed a low level of severity (Fig. 2). The result indicates yellow rust disease impacts wheat production and other yield components, making it critical to take precautions to prevent the disease and introduce new disease-resistant varieties. Previous findings have reported that rust is the most devastating disease across all wheat-producing districts in Ethiopia [44]. As a result, wheat researchers should focus on screening for disease-resistant wheat varieties and exploring other management methods, including evaluating chemical efficacy. Interestingly, this study identified six bread wheat genotypes (Tables S1 and S2, Fig. 4) with excellent yield potential and free of yellow rust. These genotypes show great promise for developing disease-resistant wheat varieties and could serve as valuable parents in hybridization breeding programs. This would enable the wheat breeding program to address disease pressure and enhance wheat production in the largest wheat-producing regions of Ethiopia and beyond. Furthermore, it is crucial to continue characterizing and identifying disease-resistant genes in wild relatives, landraces, and elite lines, as well as promoting the use of these disease-resistant varieties for sustainable production.

3.3.2. Area under disease progress curve (AUDPC)

The analysis of the variance of AUDPC showed highly significant differences among the genotypes, influenced by the growing season, testing location, and genotype-by-location interactions. This indicates that the occurrence and prevalence of wheat yellow rust differ from year to year and across different locations. The highest AUDPC values were recorded for genotypes 99 (Daka the check variety), 144, 137,91 and 113. Conversely, the lowest AUDPC values (a score of 0) were observed in genotypes 3, 70, 80, 84, and 147 (Table S2). The lowest AUDPC values likely indicate that these genotypes possess resistant genes (R) to yellow rust disease. Previously, several Yr-genes that confer resistance to yellow rust have been identified and incorporated into commercial wheat varieties

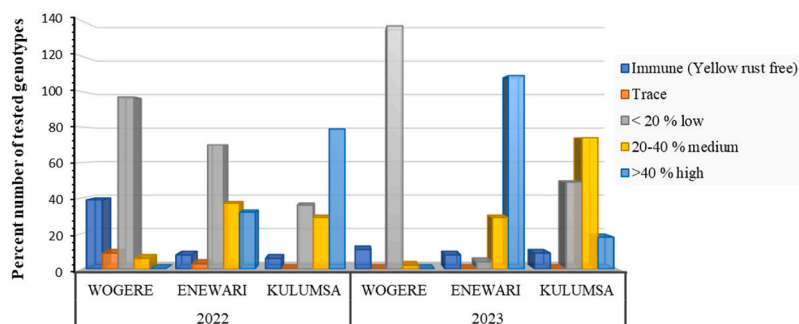


Fig. 2. Final yellow rust disease severity. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

worldwide [45]. However, the wheat varieties previously released in Ethiopia are no longer in production due to the emergence of new local races [46]. As a result, yellow rust has spread to all provinces of Ethiopia and has become a significant economic concern in all wheat-growing regions [47]. This indicates the need for continuous screening of novel resistant wheat varieties against yellow rust, and research into the variability of emerging rust races to effectively manage the disease and protect wheat production. The availability of resistant varieties in this study implies substantial diversity within the breeding germplasm for developing resistant varieties, which is a highly effective strategy for disease control. This finding aligns with [48], which revealed that resistant wheat varieties are the most effective method for managing yellow rust. Furthermore, adopting resistant varieties in combination with recommended fungicide treatments contributes to reducing the impact of yellow rust on wheat production [49].

3.3.3. Coefficient of infection (CI)

The result of the current study revealed a range of genotypic diversity in terms of the coefficient of infection, varying from zero (free of infection) to a very high level of severity (>40 %) (Table S2, Fig. 3). The genotypes showed a spectrum of reactions to yellow rust, with some showing high resistance and others showing no disease symptoms. According to Ref. [50], the genotypes in this study were grouped into low (<20 %), medium (20–40 %), and high (>40 %) levels of severity to yellow rust (Fig. 3). In both cropping seasons, all genotypes at Wogere showed low coefficients of infection (<20 %), and none were severely affected by yellow rust. In contrast, 50 genotypes in Enewari and 39 genotypes in Kulumsa showed high coefficients of infection (>40 %), indicating that yellow rust is widespread in these wheat-growing regions and poses a serious threat to wheat production. The variations in yellow rust pressure and spread across the studied locations may be attributed to somatic recombination, which can lead to the evolution of new races with a combination of previously existing pathogenic traits [51]. Yellow rust is widespread across all study locations with varying severity and response levels indicating that it has become a major pathogen and a significant threat to wheat production in the highland areas of Ethiopia. To address this challenge, it is essential to gain a thorough understanding of the genetic basis of yellow rust and breeding methods for the introgression of resistance genes into locally adapted, high-yielding bread wheat varieties.

3.4. Genotypic comparison and promising genotypes

The *t*-test was used to evaluate the mean performance of the top and low-performing genotypes for the quantitative traits under evaluation (Fig. 5). The results showed a significant difference between the top and low-performing genotypes for traits such as DM, GY, PH, SL, and TKW. However, no significant difference was observed among the genotypes for SLPS and NSPS. The *t*-test analysis revealed a significant difference between genotypes with strong agronomic performance and those with weaker performance. The bar plot (Fig. 4) displays the number of new promising genotypes for measured agronomic traits and their resistance to yellow rust. The results revealed the following distribution of genotypes: 89 had early heading, 96 were early maturing, 80 had both early heading and early maturing traits., Additionally, 60 genotypes had short PH, 65 exhibited a high NSPS, 41 had long SL, 57 revealed large NSPS, 20 had high TKW, 16 were top-yielding, and 6 were resistant to yellow rust. These genotypes show excellent agronomic traits, GY, and strong resistance to yellow rust, making them promising candidates for developing novel bread wheat varieties (Table 5; Fig. 4). Furthermore, they can serve as valuable donors of beneficial traits in wheat breeding programs, helping to diversify and enhance the parental material used.

3.5. Estimates of genetic parameters

The estimated genetic parameters for the evaluated bread wheat genotypes are shown in Tables S3, S4, and S5. The phenotypic

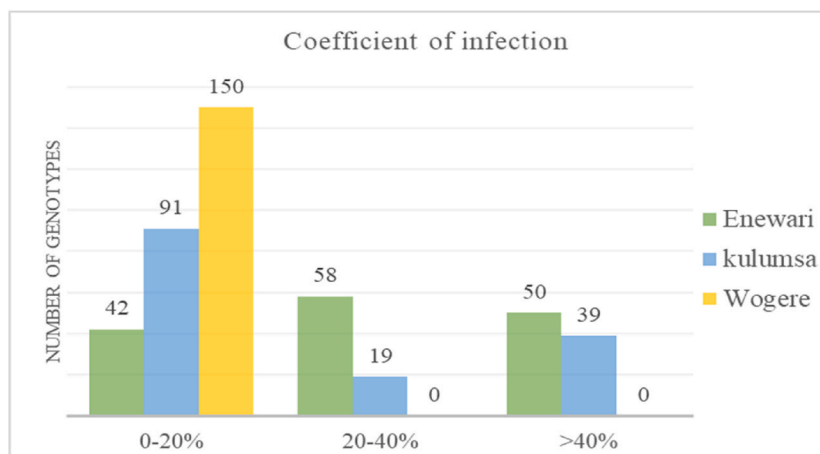


Fig. 3. Yellow rust coefficient of infection. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

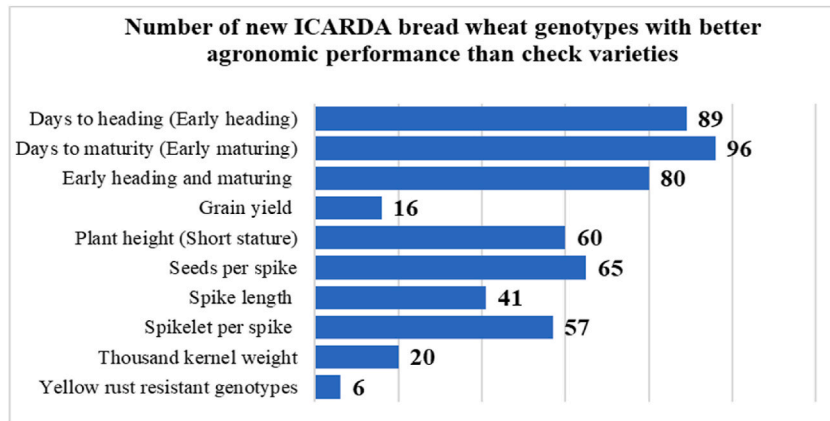


Fig. 4. Bar plot displaying new genotypes with superb agronomic performance and better yellow rust resistance than recently released standard check varieties. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

coefficients of variation (PCV) ranged from 3.58 % (DM) to 34.25 % (GY) at Kulumsa, from 1.62 % (DM) to 22.17 % (GY) at Wogere, and from 3.28 % (DM) to 58.2 % (GY) at Enewari. The genotypic coefficients of variation (GCV) varied from 3.19 % (DM) to 32.35 % (GY) at Kulumsa, from 1.17 % (DM) to 13.18 % (GY) at Wogere, and from 2.51 % (DM) to 42.18 % (GY) at Enewari. These coefficients were used to assess the extent of genetic variation relative to environmental and genetic factors. Higher GCV values suggest that genetic factors predominantly influence population variance, while lower GCV values indicate a larger environmental effect on trait expression [36].

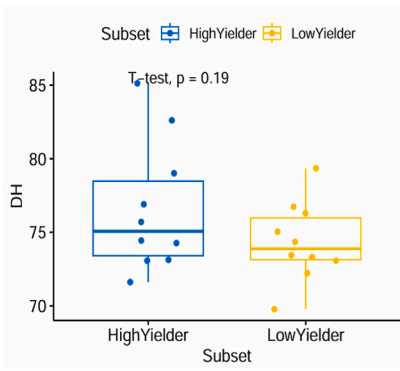
In the combined data, the results showed that DH, PH, NSPS, and yellow rust scores exhibited the highest GCV and PCV (Table 6). The broad sense heritability estimates (H^2) ranged from 36.35 % for SLPS to 90.8 % for DH. High H^2 estimates were observed for DM (65.9 %), PH (72.4 %), NSPS (61.7 %), TKW (61.9 %), and AUDPC (67 %), with DH showing the highest H^2 estimate. The GAM ranged from 1.48 for SLPS to 65.5 for final rust disease severity. Notably high GAM was observed for DH (21.29 %), PH (28.63 %), NSPS (28.03 %), final rust disease severity (65.5 %), coefficient of infection (49.2 %), and area under the disease progress curve (39.8 %). Traits with high heritability and genetic advance expressed as a percentage of the mean are particularly valuable for selecting superior genotypes [23]. Measured traits such as DH (90.8 %, 21.93 %), PH (72.4 %, 28.63 %), NSPS (61.7 %, 28.03 %), and TKW (61.9 %, 12.46 %) showed high heritability coupled with high GAM. This suggests that these traits are likely hereditary and that selection may be more successful because heritability is most likely the result of additive gene effects. Thus, various selection methods can be utilized for these traits to maximize the benefit of additive gene action and develop varieties that are widely adopted. The present results are consistent with previous studies [40–42], which reported high heritability estimates along with high GAM for PH and DH. For traits like SL and NSPP, we observed low heritability and low GAM. This suggests a significant influence of non-additive gene effects on the expression of these traits. As a result, heterosis breeding may be more effective than selection for enhancing these traits.

3.6. Correlation among measured traits

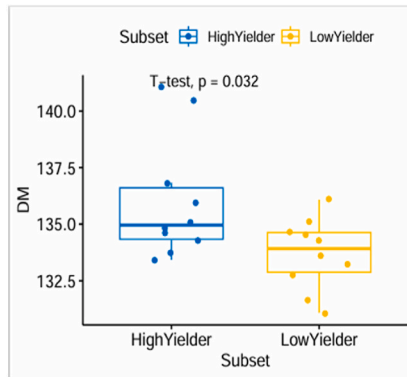
GY exhibited a positive correlation with DM, PH, SL, NSPS, and TKW at each location (Fig. 6 B, C, and D). This suggests that these traits are important for determining the yield potential of wheat and highlighting their significance in breeding processes aimed at enhancing grain yield. Supporting this, high-yielding genotypes had significantly higher mean values for DM, PH, SL, and TKW, compared to low-yielding genotypes (Table S1). Additionally, DM, PH NSPS, and TKW were positively correlated with GY (Fig. 6 A), consistent with findings from previous studies [19,43,52,53]. However, the results also suggest that yield-related traits and yield are negatively impacted by yellow rust, as evidenced by the significant negative association between GY, TKW, NSPS, and yellow rust scores such as final disease severity, area under the disease progress curve and coefficient of infection, except at Wegere (Fig D). This is supported by the yellow rust score data (Table S2), which indicated higher susceptibility at Kulumsa and Enewari compared to Wogere. Previous studies also support the negative impact of yellow rust on yield and yield components [53–55].

3.7. Genetic divergence and cluster analysis

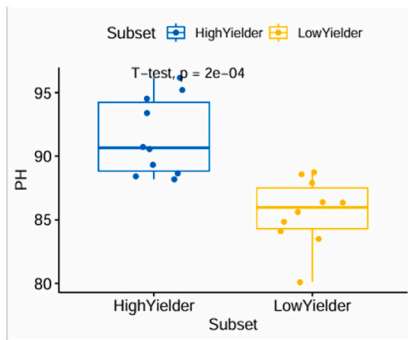
The study analyzed genetic diversity among bread wheat genotypes using multivariate analysis techniques (Table 7, Fig. 7A and B). Understanding genetic diversity is crucial for advancing wheat breeding efforts [34,35,56–58]. This includes analyzing the current genetic variability in varieties, identifying diverse parental combinations to generate segregating progenies with maximum genetic variability for subsequent selection, and incorporating desirable genes from diverse germplasm into the available genetic base. In this study, 150 bread wheat genotypes were classified into four genetically distinct groups using both the dendrogram and phylogenetic tree techniques (Fig. 7), suggesting that genes from the ICARDA origin material could enhance genetic diversity in wheat breeding. All bread wheat genotypes evaluated in this study came from ICARDA, developed through continuous hybridization and selection for



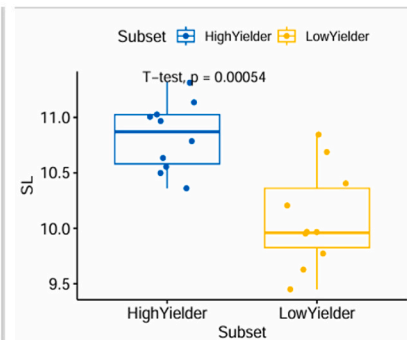
A



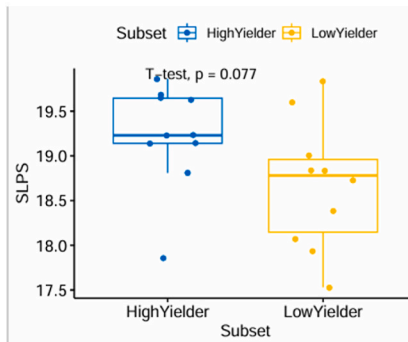
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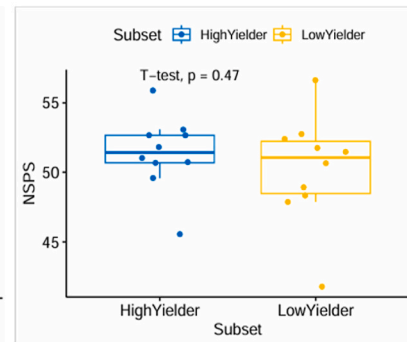
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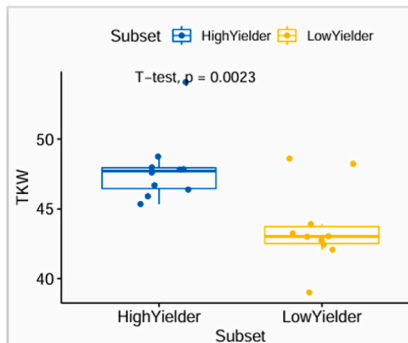
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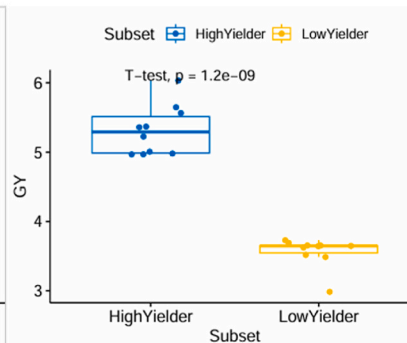
E



F



G



H

(caption on next page)

Fig. 5. Comparing specific quantitative traits in combined data between the subset of genotypes with high and low yields. According to the legend, the bread wheat genotypes are grouped into high-yielding and low-yielding genotypes on the X-axis. On the Y axis, the estimated quantitative traits of days to heading (Fig. 5A, DH in days), days to maturity (Fig. 5B, DM in days), plant height (Fig. 5C, PH in cm), spike length (Fig. 5D, SL in cm), spikelet per spike (Fig. 5E), number of seeds per spike (Fig. 5F), thousand kernel weight (Fig. 5G, TKW in gm), and grain yield (Fig. 5H, GY in t/ha).

Table 5
Top five promising genotypes for the listed traits relative to check varieties.

| Traits | Genotypes | Mean value | Compared to top-performing check |
|--|-----------|-------------|----------------------------------|
| Days to physiological maturity (earliness) | 5 | 128.08 Days | Dende'a (133 days) |
| | 72 | 128.7 Days | |
| | 58 | 129 Days | |
| | 136 | 129.8 days | |
| | 61 | 129.9 Days | |
| Thousand kernel weight | 78 | 54.08 Gram | Dende'a (49.03 g) |
| | 112 | 53.16 g | |
| | 16 | 53.06 g | |
| | 21 | 52.31 g | |
| | 6 | 52.23 g | |
| Grain yield (t/ha) | 78 | 6.03 t/ha | Dende'a (4.94 t/ha) |
| | 120 | 5.65 t/ha | |
| | 80 | 5.56 t/ha | |
| | 76 | 5.37 t/ha | |
| | 122 | 5.36 t/ha | |
| Yellow rust resistance | 80 | free | Dende'a (10 ms and Daka 40 ms) |
| | 3 | free | |
| | 84 | free | |
| | 147 | free | |
| | 70 | free | |

Table 6
Estimates of genetic parameters, Heritability(H²), Genetic advance (GA), and Genetic advance as percent of the mean (GAM) of measured traits of bread wheat genotypes.

| Traits | σ ² g | σ ² gL | σ ² gy | σ ² e | σ ² p | PCV | GCV | H ² (%) | GA | GAM (%) |
|--------|------------------|-------------------|-------------------|------------------|------------------|-------|-------|--------------------|-------|---------|
| DH | 7.58 | 0.85 | 1.1 | 7.46 | 8.34 | 11.36 | 10.32 | 90.84 | 15.63 | 21.29 |
| DM | 3.65 | 2.51 | 1.1 | 17.59 | 5.53 | 4.21 | 2.78 | 65.95 | 7.53 | 5.73 |
| PH | 12.10 | 8.46 | 6.1 | 38.37 | 16.70 | 19.17 | 13.88 | 72.41 | 24.95 | 28.63 |
| SL | 0.11 | 0.23 | 0.03 | 0.91 | 0.22 | 2.20 | 1.08 | 39.21 | 0.18 | 1.78 |
| SLPS | 0.17 | 0.19 | 1.9 | 1.99 | 0.37 | 1.98 | 0.91 | 36.35 | 0.27 | 1.48 |
| NSPS | 7.67 | 3.19 | 3 | 41.21 | 11.64 | 22.02 | 14.51 | 61.72 | 14.82 | 28.03 |
| TKW | 2.68 | 4.26 | 2.7 | 11.25 | 4.33 | 9.76 | 6.04 | 61.91 | 5.53 | 12.46 |
| GY | 0.05 | 0.16 | 0.03 | 0.58 | 0.13 | 2.86 | 1.20 | 42.11 | 0.11 | 2.48 |
| FDS | 52 | 0.03 | 0.09 | 97 | 28.7 | 24.85 | 0.86 | 57 | 13.8 | 65.5 |
| CI | 36 | 14 | 7.14 | 93 | 74.2 | 42.1 | 20.5 | 48 | 8.7 | 49.2 |
| AUDPC | 0.24 | 0.03 | 0.09 | 0.45 | 0.36 | 17.2 | 11.6 | 67 | 0.83 | 39.8 |

σ²g = genotypic variance, σ²gL = Genotype-environment interaction variance, σ²gy = Genotypic-year interaction variance, σ²e = Environmental variance, σ²p = phenotypic variance, PCV=Phenotypic coefficient variance and GCV=Genotypic coefficient variance.

traits like adaptability, resistance to biotic and abiotic stresses, and other useful traits [43]. The largest cluster contained 55 genotypes (Cluster III) followed by Cluster II with 47 genotypes, Cluster IV with 26 genotypes), and Cluster I with 22 genotypes). Cluster II and III showed the greatest inter-cluster distance (D2 = 46.5), followed by Cluster II and IV (33.5) and Cluster I and III (29.2), indicating higher genetic diversity within these clusters. The highest inter-cluster distance was observed in cluster II and cluster III (D2 = 46.5), suggesting greater genetic divergence among the genotypes in these clusters (Table 7). This implies that that crosses between distantly related parents can provides significant opportunities for selecting desirable genotypes and that a large parental distance can reveal a broad spectrum of alternative alleles at the targeted loci [59,60]. Additionally, traits that contribute significantly to the genetic divergence should be considered carefully when selecting parents for hybridization [61]. Thus, the crossing genotypes from cluster II and cluster III are expected to result in high genetic recombination and segregation in their progeny.

The percentage contribution of each trait towards genetic divergence is outlined in Table 8. Cluster I displayed moderate values for PH (88.6 cm), GY (4.9 t/ha), and TKW (45.1 g). Cluster II contained genotypes with the lowest values for days for DM, SL, and NSPS. Cluster III was characterized by late maturity (135), tall PH (96 cm), long SL (10.5 cm), high NSPS (54.3), a significant amount of TKW (49 g), and high GY (5.3 tons/ha). Cluster IV included genotypes with the lowest GY (4 tons/ha) and TKW (41.6 g). Cluster V exhibited late heading (81.2 days), late maturing (137.3 days), and moderate GY (5.2 tons/ha). Additionally, the cluster analysis results assist in identifying superior genotypes with enhanced grain yield and agronomic traits (Fig. 5 and Table 8). For instance, genotypes in Cluster III were the highest yielders, followed by those in Cluster IV (Table 5). The earliest and shortest genotypes were found in Cluster II,

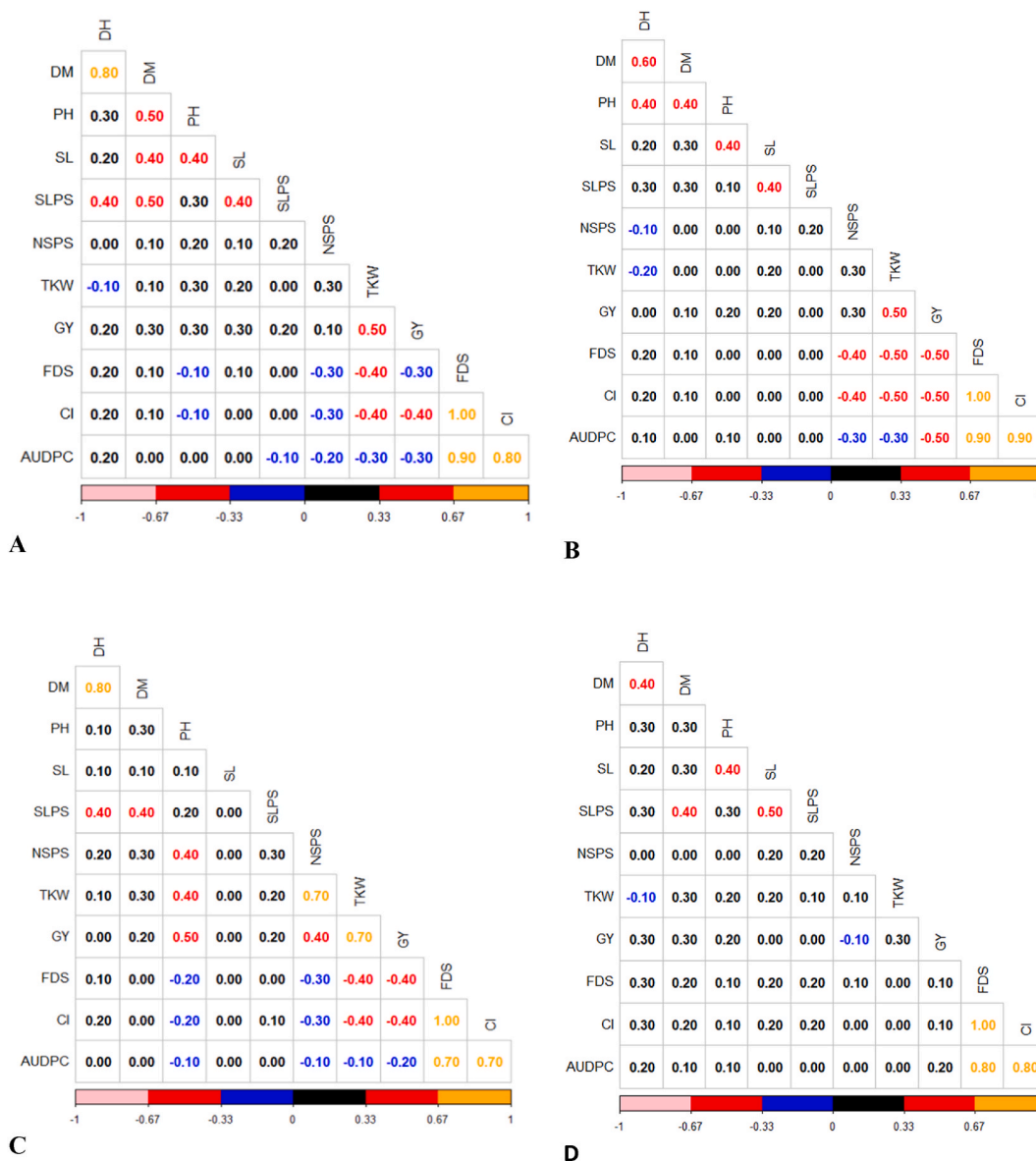


Fig. 6. Correlation coefficient of bread wheat genotypes at each location and combined over locations. Fig. 6A combined over locations, Fig. 6B. Enewari, Fig. 6C. Kulumsa, Fig. 6D Wogere, HD: Days to heading; MD: Days to maturity; PH: Plant height; SL: Spike length; SPS: Seeds per spike; SLPS: Spikelet per spike; TKW: Thousand kernel weight; GY: Grain yield; FDS: Final yellow rust disease severity; AUDPC: Area under the disease progress curve, and CI: Yellow rust coefficient of infection. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 7

Average inter-cluster and intra-cluster distance of measured traits of bread wheat genotypes for combined data.

| Cluster | I | II | III | IV | Number of genotypes |
|---------|------|-------|--------|--------|---------------------|
| I | 11.6 | 21.7* | 29.2* | 17.4** | 47 |
| II | | 14.7 | 46.5** | 33.5** | 22 |
| III | | | 11 | 17.5* | 55 |
| IV | | | | 11.5 | 26 |

Significant levels (*: significant, *: high significant and **: very high significant).

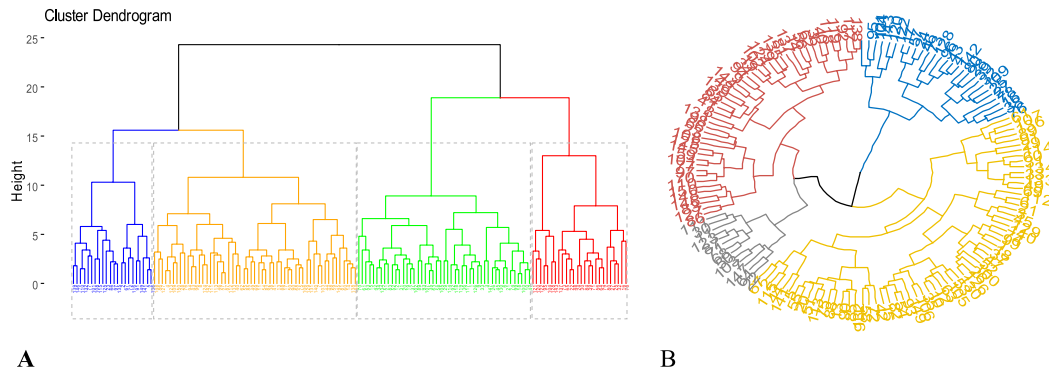


Fig. 7. Variation and relationship in the ICARDA-origin bread wheat genotypes. A. Cluster dendrogram; B. Phylogenetic tree data using squared distance (D 2).

Table 8

Mean values of clusters and contribution of traits towards genetic divergence of genotypes.

| | DH | DM | PH | SL | SLPS | NSPS | TKW | GY |
|-------------|------|-------|------|------|------|------|------|-----|
| Cluster I | 74.0 | 131.6 | 88.6 | 10.4 | 18.3 | 52.9 | 45.1 | 4.9 |
| Cluster II | 72.4 | 129.8 | 85.9 | 9.9 | 17.8 | 52.7 | 44.0 | 4.6 |
| Cluster III | 77.5 | 135.0 | 96.6 | 10.5 | 18.4 | 54.3 | 49.0 | 5.3 |
| Cluster IV | 76.1 | 131.9 | 85.8 | 9.8 | 17.8 | 49.7 | 41.6 | 4.0 |

DH = Days to heading, DM = Days to maturity, PH = Plant height, SL = Spike length, NSPS = Seeds per spike, SLPS = Spikelet per spike, TKW = Thousand kernel weight, and GY = Grain yield.

while late and tall genotypes were in Cluster V and Cluster III, respectively. Recent study, such as that by Ref. [60], has highlighted varying grouping patterns within the wheat population at the ICARDA gene bank.

3.8. Principal Component Analysis (PCA) of various traits

Principal component analysis (PCA) was also used to visualize variation among 150 bread wheat genotypes across 8 quantitative traits and 3 yellow rust traits (Table 9 and Fig. 8). This analysis helps refine and reduce the number of selection criteria into a few meaningful and practical traits. Data from all locations were pooled for the PCA (Table 9). The first three principal components (PC1, PC2, and PC3) together explained 67.65 % of the total variation among the genotypes (Table 6). This is consistent with findings from Ref. [62], which reported that the first three PCs captured a significant portion of the variability in bread wheat genotypes from the ICARDA source. Similar results were also observed in studies by Refs. [58,60,63], where the first three PCs accounted for the largest share of the total variation, with subsequent PCs explaining progressively less. PC1 exhibited a strong positive association with HD, MD, PH, SL, SPS, and GY. This indicates that improving wheat production could benefit from wheat breeding that integrates these key agronomic and phenological traits, which are important for yield enhancement and commonly used in selection criteria. In contrast, GY showed a negative correlation with the FDS, AUDPC, and CI, likely due to the wide angular separation of these traits. PC2 was positively associated with TKW, NSPS, and GY, but negatively correlated with HD (Fig. 8). These findings align with previous studies [36] that found these traits contributed significantly to the evaluation of genetic variation in wheat.

Genotype-by-trait biplot analysis is employed to identify genotypes with desirable traits and to evaluate the interrelationship among wheat genotypes (Fig. 9). Beyond genotype-by-trait data analysis, the biplot also accommodates diallel cross and genotype-by-marker data analysis [60,64,65], making it a powerful tool in quantitative genetics and breeding. The biplot analysis showed that genotypes located on the left side of the ordinate had low values for GY, TKW, NSPS, and SL. In contrast, genotypes on the right side of the plot showed higher values for these traits. The highest-yielding genotypes identified were 78, 120, 80, 76, and 122, while the lowest-yielding genotypes, positioned at the bottom left of the biplot, included 125, 108, 67, and 98. Most traits, including DM, PH, SL, SPS, and TKW, showed a strong correlation with GY. There was also a slight positive relationship between GY and DH. These results align with [36], which showed that genotype by trait biplot analysis effectively identifies genotypes with multiple useful traits and high yield potential. Additionally, agronomic traits and GY were negatively associated with FDS, AUDPC, and CI, as shown in the trait association graph (Fig. 7). This indicates that yellow rust negatively impacts genotype performance.

4. Conclusion

Multivariate analysis is a valuable technique for identifying genotypes with superior agronomic performance, selecting potential parents for hybridization programs, and visually representing the relationship between quantitative traits. In the current study, 150

Table 9
Eigenvalue and percentage of the variance of each trait of bread wheat genotypes to PCA dimensions.

| | PC 1 | PC 2 | PC 3 | PC 4 |
|-----------------------------------|-------|-------|-------|-------|
| Days to heading | -0.01 | 0.79 | -0.35 | -0.21 |
| Days to maturity | 0.23 | 0.84 | -0.20 | -0.16 |
| Plant height | 0.35 | 0.59 | 0.24 | -0.05 |
| Spike length | 0.24 | 0.61 | 0.27 | 0.11 |
| Spikelet per spike | 0.22 | 0.64 | -0.36 | 0.22 |
| Number of seeds per spike | 0.45 | 0.09 | -0.12 | 0.80 |
| Thousand kernel weight | 0.62 | 0.07 | 0.55 | 0.12 |
| Grain yield | 0.61 | 0.29 | 0.37 | -0.27 |
| Final disease severity | -0.88 | 0.37 | 0.18 | 0.12 |
| Coefficient of infection | -0.88 | 0.38 | 0.13 | 0.09 |
| Area under disease progress curve | -0.79 | 0.35 | 0.34 | 0.15 |
| Eigenvalue | 3.42 | 2.95 | 1.04 | 0.91 |
| Variance percent | 31.12 | 26.83 | 9.42 | 8.25 |
| Cumulative variance percent | 31.12 | 57.95 | 67.37 | 75.61 |

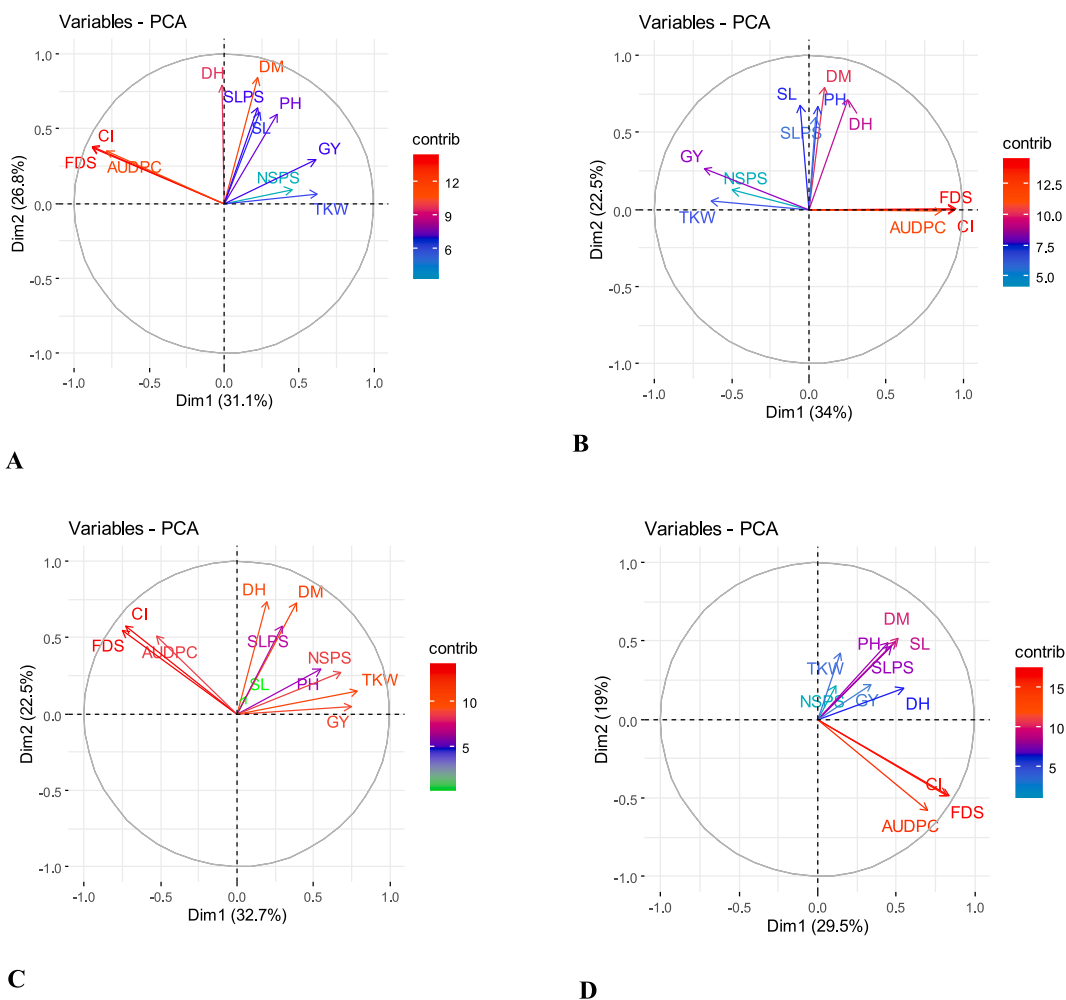


Fig. 8. Principal Component Analysis representing 11 quantitative traits of bread genotypes. Fig. 8A combined over locations, Fig. 8B at Enewari, Fig. 8C at Kulumsa, and Fig. 8D at Wogere. HD: Days to heading; MD: Days to maturity; PH: Plant height; SL: Spike length; SPS: Seeds per spike; SLPS: Spikelet per spike; TKW: Thousand kernel weight; GY: Grain yield; FDS: Final yellow rust disease severity; AUDPC: Area under the disease progress curve, and CI: Yellow rust coefficient of infection. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

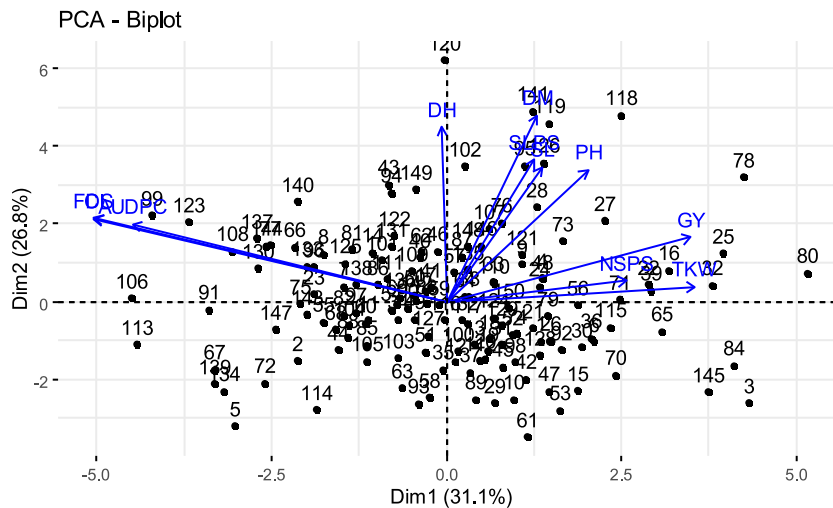


Fig. 9. Genotype by traits Biplot showing the relationship between traits and mean performance of bread wheat. The blue vector represents the agronomic and yellow rust traits in the PCA. Black dots are bread wheat genotypes. Dim1 and Dim 2 are shown on the X and Y axis, respectively, aside from their explained variance. HD: Days to heading; MD: Days to maturity; PH: Plant height; SL: Spike length; SPS: Seeds per spike; SLPS: Spikelet per spike; TKW: Thousand kernel weight; GY: Grain yield; FDS: Final yellow rust disease severity; AUDPC: Area under the disease progress curve, and CI: Yellow rust coefficient of infection. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

bread wheat genotypes from the ICARDA international nursery were evaluated for key traits in Ethiopia's major wheat-producing regions. The results showed significant variation among genotypes for useful traits such as yield, phenological, agronomic, and yellow rust resistance, which are crucial for both current and future wheat breeding programs. The genotypes were grouped into four distinct clusters, indicating notable variability among the studied genotypes. Sixteen ICARDA-origin bread wheat genotypes outperformed the standard check varieties. Additionally, another sixteen ICARDA-origin bread wheat genotypes also showed superior performance compared to standard checks. Notably, six of the ICARDA-derived bread wheat genotypes showed excellent yield potential and were free of yellow rust. These promising genotypes should be considered for advancement into national variety trials to develop new high-yielding and farmer-preferred varieties with robust yellow rust resistance and acceptable end-use quality. Furthermore, the traits dataset from the 150 bread wheat genotypes in this study can be utilized to identify marker-trait associations through genome-wide association studies.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Tesfaye Mulugeta: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alemu Abate:** Writing – review & editing, Visualization, Validation, Supervision, Methodology. **Wuletaw Tadesse:** Conceptualization, Supervision, Writing – review & editing. **Aemiro Bezabih Woldeyohannes:** Writing – review & editing, Visualization, Software, Methodology, Data curation, Conceptualization. **Neway Tefera:** Visualization, Supervision, Data curation. **Wondwosen Shiferaw:** Visualization, Supervision, Resources, Data curation. **Altaye Tiruneh:** Writing – review & editing, Visualization, Data curation.

Declaration of competing interest

The authors declare that there have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e36062>.

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