



Multi-Environment Trial Analysis Using Linear Mixed Model for the Bread Wheat Genotypes in Ethiopia

Solomon T*, Duga R, Sime B, Zewedu D, Geleta N, Alemu G, Dabi A, Delesa A, Zegeye H, Asefa B, Negash T, Kasa D, Asnake D, Getamesay A, Bayisa M, Muche G, Ayele A and Degefa N

Ethiopian Institute of Agricultural Research, Kulumsa Agricultural Research Center, Ethiopia

*Corresponding author: Tafesse Solomon Bekele, Ethiopian Institute of Agricultural Research, Kulumsa Agricultural Research Center, National Wheat Research Program, Ethiopia, Email: tafessesolomon@gmail.com

Received Date: October 04, 2024; Published Date: October 21, 2024

Abstract

The primary source of variation between genotypes evaluated across different environments is Genotype by Environment GXE interaction. Not all genotypes interact equally with the environment. Multi Environment Trial (MET) data are required, for estimating genetic variance, phenotypic variance, and heritability. A total of ninety bread wheat genotypes including eighty-eight genotypes from the national wheat breeding pipelines and two bread wheat released varieties namely, Lemu and Wane fixed as, the National Variety Trial for mid to high-altitude and evaluated across eleven locations in 2020 and 2021, main cropping season. The experimental design was a Row-Column design with two replications across all locations in both years. The plots comprise six rows of 1.2m width by 2.5m length. The space between plots and between columns was 0.2m and 1m respectively. The study showed the heritability for grain yield across the environments ranges from 56.21 for 20BWNL1AA to 99.95 for 20BWNL1DZ. Among tested genotypes, EBW192346 and EBW192347 have both resistance to moderate resistance for yellow and stem rust diseases and are stable. Developing and releasing stable and wheat-rust disease-resistant bread wheat varieties for wheat society increases productivity and wheat production. Thus, the above genotypes are proposed for release.

Keywords: GXE; MET; Heritability; Stable; Yellow Rust; Stem Rust

Abbreviations

MET: Multi Environment Trial; GXE: Genotype by Environment.

Introduction

Wheat is one of the staple food for millions of people in Ethiopia. It is produced by over 4.8 million smallholders on about 1.89 million hectares of land with an annual total production of 5.7 million tons in 2024 [1]. Wheat also

accounts for 14 % of the total national calorie intake and it is one of the major food crops it comprises about 18 percent of the country's total cereal production in Ethiopia [2].

Several biotic and abiotic factors constrain wheat production in Ethiopia. Among the biotic factors, wheat rust diseases are among the most important biotic constraints threatening wheat production [3]. The country encountered several times the outbreak of these diseases and as a result received significant economic losses [4-9]. The Yellow Rust disease epidemic in 2010 on the widely adopted variety "Kubsa"

caused huge losses in wheat production across main wheat-producing areas, Arsi and Bale [10]. Also, the stem rust disease outbreak by race TKTTF in 2013 causing total crop damage on the widely adopted bread wheat variety, Digelu in major wheat-producing areas [11].

The primary source of variation between genotypes evaluated across different environments is Genotype by Environment GXE interaction. Not all genotypes interact equally with the environment. The rank of grain yield performance changes from one environment to another. The Multi Environment Trial MET approach is a good strategy for examining the existing interaction and selecting stable genotypes [12].

Multi-environment trials (METs) are experiments carried out in multiple environments or contexts. It is a standard research tool in crop breeding that ease selection. Multi Environment Trial (MET) data are required, for estimating genetic variance, phenotypic variance, and heritability. Heritability is the extent to which a given trait transfers from one generation to another generation, from parent to offspring. It is an important tool in predicting genetic gain in wheat due to the selection of the character of interest in the breeding program [13-15]. High heritability specifies the possibility of the improvement of specific traits through selection. Also, indicate the low impact of the environments on the phenotypic expression of the trait. In other words, it

tells us the environment's suitability to the specific trait.

The objective of this study was to evaluate bread wheat genotypes across multiple environments and select promising genotypes for releases of commercial variety for wheat farmers.

Material and Methods

Material, Design, and Description of Experimental Sites

A total of ninety bread wheat genotypes including eighty-eight genotypes from the national wheat breeding pipelines and two bread wheat released varieties namely, Lemu and Wane fixed as, the National Variety Trial for mid to high-altitude and evaluated across eleven locations in 2020 and 2021, main cropping season. The experimental design was a Row-Column design with two replications across all locations in both year's plots consisting of six rows of 1.2m width by 2.5m length. The space between plots and between columns was 0.2m and 1m respectively. All the six rows were harvested from 3m² areas. Urea and NPS fertilizers were applied as per area recommendations and all other management practices were uniformly applied for each trial Table 1.

Location	Altitude (m)	Representing Agro-ecology	Soil type	Annual Rainfall	Temperature	
	2				Max	Min
Kulumsa	2200	Mid altitude	Clay soil (luisols)	820mm	23	11
Asasa	2340	Terminal drought stress	Clay loam soil(gleysols)	620	24	5.8
Holeta	2400	Tepid to cool moist mountains and plateau	Nitosols and Vertisols	1144	22	6.2
Sinana	2400	Wet high land	clayey soils	812	21	9.5
Arsi robe	2420	Waterlogged vertisoil	Heavy clay soil(Vertisoil)	890	22	6
Bokoji	2780	High land/high rainfall	Clay soil(Nitosols)	1020	19	7.9
Debreziet	1900	Tepid to cool sub-moist	Alfisols/Mollisols and	851	28	8.9
		highlands	Vertisols			

Table 1: Agro-ecology and weather description of the study areas.

Data Collection and Analysis

Data Collection: Data collected includes Days to heading, Days to maturity, plant height, Yellow rust, Stem rust, Hectoliter weight, Thousand kernel weight, and Grain yield. For this study, Yellow rust, stem rust, and grain yield data were used. The description of the data used in this study has been shown as follows Wheat rust diseases: Stem rust and yellow rust diseases were collected using a modified Cobb scale, a combination of severity and reaction. The severity

of the rust was taken in percent using 5% 10% 20% 30% ... 100%. The reaction of the host for the pathogen designates using the English letters where: 0= No visible infection on the plant; R= Resistant: visible chlorosis or necrosis, no uredia are present; MR= Moderately Resistant: small uredia are present and surrounded by either chlorotic or necrotic areas; M=Intermediate: variable-sized uredia are present; some with chlorosis, necrosis, or both; MS= Moderately Susceptible: medium-sized uredia are present and possible

surrounded by chlorotic areas; S= Susceptible: Large uredia are present, generally with little or no chlorosis and no necrosis [16] Grain yield data collected from 3m² harvested area in grams per harvested area, and then, converted to tone per hectare

Data Analysis: The Data analysis was done with the collaboration of MERCI project teams from the University of Queensland, Australia, and Biometricians from the Ethiopian Institute Agricultural Research headquarters, in Addis Ababa, Ethiopia

Linear Mixed Model

Consider a MET dataset collected from t-trials (environments can be used instead) in which m varieties are grown (all varieties may not be grown in all trials). The jth trial, $j = 1, \dots, t$, consists of n_j plots arranged in a rectangular array with c_j columns by r_j rows ($n_j = c_j r_j$). Let y_j be the $(n_j \times 1)$ data vector for trial j, ordered as rows within columns, and let $y = (y'_1, y'_2, \dots, y'_t)$ be the $(n \times 1)$ data vector combined across the t trials, where $n = \sum_{j=1}^t n_j$. The linear mixed model for y can then be written as

$$Y = X\alpha + ZgYg + ZpYp + \epsilon$$

where α is the vector of fixed effects (including terms for the grand mean, the environment's main effects, global spatial trends at each trial, and other trial-specific fixed effects) with an associated design matrix X (assumed to be full column rank), Yg is the $mt \times 1$ vector of random genetic (or variety by trial) effects with associated design matrix Zg , Yp is a vector of non-genetic (or peripheral) random effects (including terms associated with the blocking structure at each trial, and other trial-specific random effects), with associated design matrix Zp , and ϵ is the $n \times 1$ vector of residual errors across all trials.

Environment	Row	Column	Genotype	Missing	Mean	Genetic	Error	Heritability
				plots	GYLD	Variance	Variance	
20BWNL1AA	18	10	90	0	5.64	3.02	0.29	56.21
20BWNL1BE	18	10	90	0	2.13	1.33	0.27	82.05
20BWNL1DM	18	10	90	0	2.77	0.13	0.37	97.6
20BWNL1DZ	18	10	90	12	1.58	0.01	0.11	99.95
20BWNL1HL	18	10	90	1	2.54	1.06	0.17	90.99
20BWNL1KU	18	10	90	1	3.18	0.5	0.21	94.75
20BWNL1RA	18	10	90	1	1.56	0.3	0.11	98.35
20BWNL1SN	18	10	90	3	1.77	0.78	0.12	95.32
20BWNL2AA	18	10	90	1	3.42	0.81	0.12	95.14
20BWNL2BE	18	10	90	0	1.27	0.8	0.07	97.2
20BWNL2DM	18	10	90	0	5.16	0.12	0.22	98.68

Heritability

Practically, multi-environment trials have unbalanced data due to when plot data is lost or when the number of replicates at each location varies between genotypes [17]. In this sense, our trials have missed plots in some environments (Table 2). Therefore, we used the heritability formula which was proposed by Cellis [18] that used to account for the unbalanced scenario in multi-environment trials [18] In this case, the genetic terms fitted as a random effect (BLUP). The heritability is calculated as follows

$$Hj^2 = 1 - \frac{Aj}{2\delta gj^2}$$

Where Aj is the average pairwise prediction error variance of genetic effects for the jth environment and δgj^2 is the genetic variance at environment j The licensed ASReml-R was used to fit all models in this study [19].

Result and Discussion

The genetic variance, error variance, and heritability (H^2) for each trial/environment and grain yield from the final fitted Spatial+FA models are presented in Table 2. The heritability for grain yield across the environments ranges from 56.21 for 20BWNL1AA to 99.95 for 20BWNL1DZ. Unpredictably, the highest heritability was observed at Debreziet; the area where the stem rust disease infection is very high. In this area, the environmental influence is high due to this disease. So, the heritability is expected to be low. This may be due to the twelve missed plots in the test environment (Table 2). Overall, high heritability is shown in most environments for the grain yield. This showed that the trait is predominantly genetic preponderance than environmental influences, therefore selection in these genotypes would be advantageous to develop high-yielding varieties.

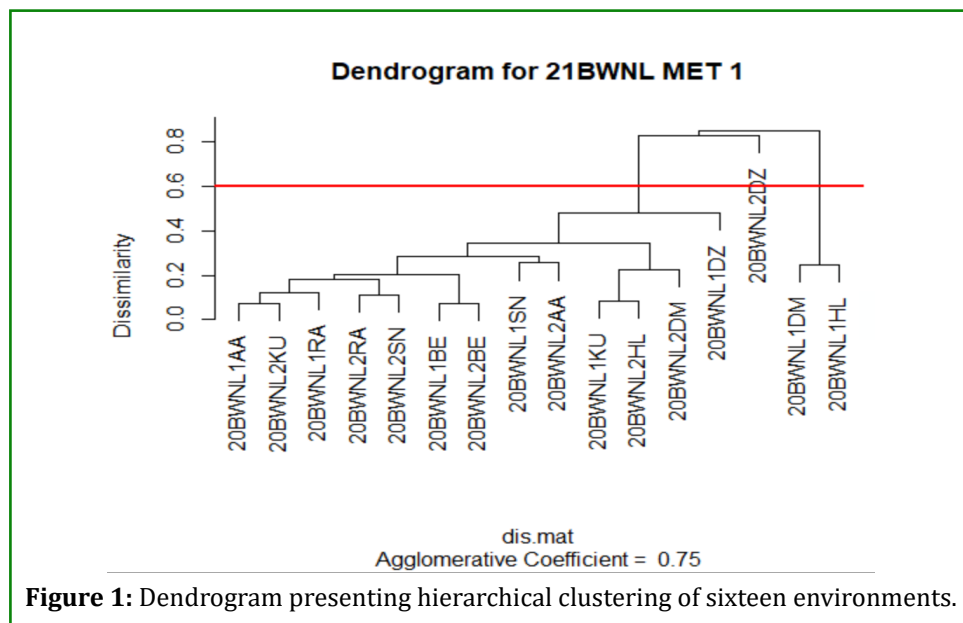
20BWNL2DZ	18	10	90	2	2.19	0.13	0.18	98.83
20BWNL2HL	18	10	90	0	2.4	0.77	0.11	95.77
20BWNL2KU	18	10	90	2	4.76	1.49	0.19	85.85
20BWNL2RA	18	10	90	0	3.01	0.85	0.25	89.38
20BWNL2SN	18	10	90	0	3.41	1.32	0.17	88.78

Table 2: Genetic variance, error variance, Mean Grain yield, and heritability (H²) of bread wheat genotypes tested across sixteen environment.

20BWNL1AA= 1st year Bread Wheat National Variety Trial late maturity tested at Asasa in 2020; 20BWNL1BE= 1st year Bread Wheat National Variety Trial late maturity tested at Bokoji in 2020; 20BWNL1DM= 1st year Bread Wheat National Variety Trial late maturity tested at Debremarkos in 2020; 20BWNL1DZ= 1st year Bread Wheat National Variety Trial late maturity tested at Debreziet in 2020; 20BWNL1HL=1st year Bread Wheat National Variety Trial late maturity tested at Holeta in 2020; 20BWNL1KU=1st year Bread Wheat National Variety Trial late maturity tested at Kulumsa in 2020; 20BWNL1RA=1st year Bread Wheat National Variety Trial late maturity tested at Robe Arsi in 2020; 20BWNL1SN= 1st year Bread Wheat National Variety Trial late maturity tested at Sinana in 2020; 20BWNL2AA=2nd year Bread Wheat National Variety Trial late maturity tested at Asasa in 2021; 20BWNL2BE=2nd year Bread Wheat National Variety Trial late maturity tested at Bokoji in 2021; 20BWNL2DM=2nd year Bread Wheat National Variety Trial late maturity tested at Debremarkos in 2021; 20BWNL2DZ=2nd year Bread Wheat National Variety Trial late maturity tested at Debreziet in 2021; 20BWNL2HL=2nd year Bread Wheat National Variety Trial late maturity tested at Holeta in 2021; 20BWNL2KU=2nd year Bread Wheat National Variety Trial late maturity tested

at Kulumsa in 2021; 20BWNL2RA=2nd year Bread Wheat National Variety Trial late maturity tested at Robe Arsi in 2021; 20BWNL2SN=2nd year Bread Wheat National Variety Trial late maturity tested at Sinana in 2021

The dendrogram clusters the environments based on similarity measures using total effect. It is used to interpret the genetic correlation among trials/ environments. Based on Cullis's suggestion on the dissimilarity cut-off (approximately below 0.6) groups are formed [18]. The dendrogram from hierarchical clustering of the given environments based on grain yield data groups the environments into three groups. Most of the environments/trials fall under Group 1 which consists of 20BWNL1AA, 20BWNL2KU, 20BWNL1RA, 20BWNL2RA, 20BWNL2SN, 20BWNL1BE, 20BWNL2BE, 20BWNL1SN, 20BWNL2AA, 20BWNL1KU, 20BWNL2HL, 20BWNL2DM, and 20BWNL1DZ. Under Group two, only 20BWNL2DZ is found. In Group three, 20BWNL1DM and 20BWNL1HL are clustered (Figure 1). Genotypes that show better performances for grain yield in group 1 are more stable and widely adopted because thirteen of sixteen tested environments/ trials were found in this group.



In addition to the dendrogram, other typical summaries from the MET analysis include a heat map of the genetic correlations between all environments for grain yield. The heat map gives a further visual illustration of the genetic correlation between environments. Results in Figure 2 show different correlation patterns for grain yield. Most of the environments/trials are highly correlated. Also, weak genetic

correlation was observed in a few trials/environments. A strong positive correlation was observed between 20BWNL1BE and 20BWNL1AA; between 20BWNL2KU and 20BWNL1AA; between 20BWNL2BE and 20BWNL1BE; 20BWNL2HL and 20BWNL1KU. On the other hand, a weak negative genetic correlation is shown between 20BWNL2SN and 20BWNL1HL.

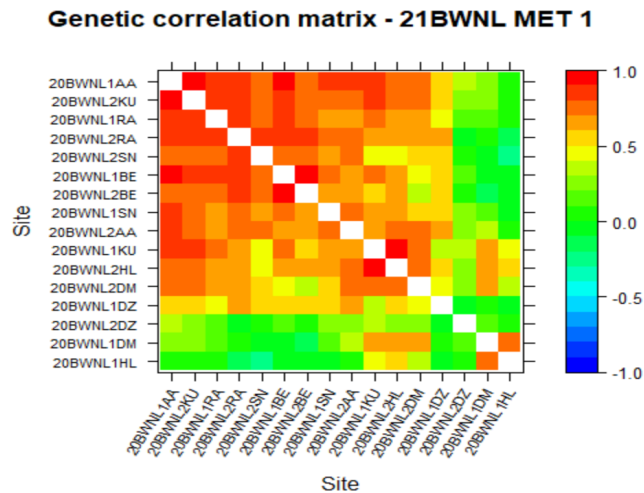


Figure 2: Heat map representation of the genetic correlation matrix of bread wheat genotypes tested across sixteen environments for grain yield.

Clustering the Multi Environment Trials MET into groups allows us to select elite genotypes from our population based on their adaptability. Selecting genotypes for their specific adaptability or broad adaptability. The BLUP values allow increased analysis accuracy to detect the differences between genotypes [20]. Based on the BLUP value, genotypes ranked in each group; the higher the BLUP value, the better the genotype's performance for the grain yield. Those genotypes perform better in group 1 selected as broadly adapted genotypes since group 1 consists of thirteen environments out of sixteen tested environments. Genotypes EBW173353, EBW192346, EBW192347, EBW172093, and EBW172088 were selected as the top five stable genotypes (Table 3). On the other hand, genotypes EBW173366, EBW120002, EBW192336, and EBW192337 are considered better

genotypes for specific adaptation.

Like broad adaptability, wheat rust disease resistance is a key trait for selection genotypes to develop and release a variety for wheat farmers. Because wheat rust, specifically yellow and stem rust diseases limit wheat production in the country. A released variety is accepted by farmers only if resistant to these rusts and has high-yielding potential. From the above stable genotypes, EBW192346 and EBW192347 have both qualities, resistance to moderate resistance for yellow and stem rust diseases and stability (Table 3). Therefore, these genotypes were selected and proposed in 2022 for release and registration to the National Variety Releasing Committee in the Ministry of Agriculture.

Genotype	BLUP	Rank	BLUP	Rank	BLUP	Rank	KU20YR	KU20SR	BE20YR	RA20YR	KU21SR	RA21YR	RA21SR	BE21YR
Danda'a	-0.05	51	-0.23	63	-0.17	50	30MSS	5MRMS	20MRMS	50S	1MS	70S	50S	50S
EBW120002	0.07	48	0.61	5	0.99	3	5MR	10MRMS	10MRMS	60S	1MR	30S	50S	50MSS
EBW120004	-0.4	64	0.35	18	0.61	14	50S	10MRMS	40S	70S	0	60S	40S	50S
EBW120011	-1.12	77	0.17	28	0.38	23	60S	15MSS	5MRMS	10MSS	5MS	60S	30S	60S
EBW120014	-0.08	54	0.26	24	-0.04	43	40S	10S	20MS	70S	5MS	70S	30S	60S
EBW120039	-1.02	75	-0.18	58	-0.32	64	50S	5MRMS	20MSS	60S	0	80S	10S	60S
EBW120041	-2.57	88	-0.11	49	0.64	13	10MSMR	10MRMS	5MRMS	50S	0	70S	15S	60S

EBW120042	-1.1	76	0.3	20	0.86	8	40S	15MSS	30MS	70S	1S	70S	50S	50S
EBW120044	-1.97	84	-0.26	69	0.42	20	70S	5MR	60S	90S	0	70S	15S	60S
EBW120052	-3.07	90	-0.59	90	-0.44	70	10MSMR	15MSS	25MS	10MRMS	5MS	30MSS	60S	40S
EBW120053	0.67	25	0.55	8	0.73	10	5MR	20S	30MRMS	20MSS	20MSS	20MSMR	50S	30S
EBW120054	0.42	33	0.45	13	0.87	6	70S	5MRMS	50S	60S	0	70S	50S	60S
EBW120056	-2.78	89	-0.53	87	-0.09	44	20MSS	10MSS	25MS	60S	5S	60S	50S	40S
EBW120060	-0.55	69	0.35	19	0.73	11	5MSMR	10MSS	50S	70S	0	50S	40S	40S
EBW120063	-0.31	62	-0.31	75	-0.48	74	50S	15S	40MS	90S	0	80S	20S	80S
EBW172056	-1.22	79	-0.29	72	-0.29	59	20MSMR	5MRMS	30MRMS	60S	0	60S	30S	50S
EBW172082	-0.45	66	-0.15	55	-0.46	71	0	5MRMS	1MRMS	1010MSMR	1MS	5MSMR	40S	5MSMR
EBW172088	1.78	5	0.27	22	0.15	34	15MSS	30S	5MRMS	30S	5MSS	30MSS	60S	30MSS
EBW172093	1.8	4	-0.18	59	-0.12	45	20S	15S	5MR	70S	1MS	30MSS	40S	40S
EBW172105	1	14	0.42	14	0.26	28	30S	15MSS	10MRMS	60S	5MS	70S	30S	50S
EBW172319	0.01	49	0.45	12	0.41	21	40S	10MSS	50S	60S	5MSS	60S	30S	70S
EBW172393	-1.22	78	0.03	38	-0.14	46	1MR	5MR	1MR	10MRMS	5MSMR	40S	50S	20MSMR
EBW172440	0.6	27	-0.11	48	-0.25	56	5MR	5MSS	5MRMS	20MSS	20MSS	30S	60S	10MSS
EBW172474	1.18	13	-0.11	50	0.36	25	15MSMR	20MSS	5MRMS	10MRMS	1MS	20MSMR	50S	15MSS
EBW172862	1.59	6	-0.28	70	-0.47	72	5MRMS	40S	5MR	5MSS	1MRMS	10MSMR	50S	20MSMR
EBW172864	1.58	7	-0.34	77	-0.38	67	20MSS	10S	15MS	50S	10MSS	40S	50S	50MSS
EBW172872	-0.28	60	-0.12	51	-0.41	69	1MR	5MS	1MR	60S	0	10MSMR	60S	10MSMR
EBW172936	0.82	20	-0.44	83	-0.36	66	60S	15MSS	80S	70S	5MSS	60S	60S	60S
EBW172996	-2.31	87	-0.28	71	-0.68	84	5MSMR	10MS	5MRMS	70S	5S	30MSS	30S	40MSS
EBW173001	0.55	31	0.11	33	0.07	38	40S	15MSS	80S	60S	5MS	40S	50S	40MSS
EBW173004	-1.38	80	-0.35	80	-0.77	86	60S	30S	70S	60S	20S	60S	70S	60S
EBW173006	-2.08	85	-0.39	82	-0.97	90	30S	20S	20MR	20MSS	15MSS	20MSS	70S	60S
EBW173031	0.35	37	-0.33	76	-0.82	87	5MR	5MRMS	20MRMS	60S	5S	70S	40S	60S
EBW173207	0.14	44	-0.14	54	-0.31	61	60S	1MR	90S	90S	1MR	70S	5MRMS	70S
EBW173261	-1.81	82	-0.35	81	-0.61	81	1MR	5MSS	50S	40S	0	60S	40S	60S
EBW173263	-0.46	67	-0.13	53	-0.4	68	30MSS	0	40MS	60S	5MSMR	70S	50S	40S
EBW173270	-0.08	53	0.21	25	0.25	30	5MRMS	10MSS	1MR	20MSS	20MSS	30MSS	60S	40S
EBW173288	0.93	15	0.63	4	0.85	9	5MR	10S	5MRMS	5MSMR	1MS	20MSS	40S	30MSS
EBW173292	1.25	9	0.46	11	0.69	12	50S	20S	40MRMS	90S	10MSMR	50S	50S	40MSS
EBW173332	0.09	45	-0.46	84	-0.82	88	5MSMR	10MSS	1MS	60S	1MS	20MSS	40S	20MSMR
EBW173353	2.23	1	0.18	26	0.32	26	30S	5MR	20MS	50S	1MR	60S	50S	60S
EBW173366	0.19	41	0.72	1	1.69	1	40S	20MSS	20MRMS	40S	10MSS	50S	30S	40S
EBW173378	0.18	42	-0.24	67	-0.63	82	30S	30S	10MR	40S	5MSS	40S	50S	40S
EBW173380	0.8	22	0.69	2	0.52	17	20MSS	5MRMS	5MRMS	70S	15MSS	50S	70S	40S
EBW174116	0.89	16	-0.34	79	-0.16	49	50S	15MRMS	30S	60S	1MS	70S	50S	70S
EBW174170	-2.15	86	-0.52	86	-0.73	85	30MSS	10S	20MS	60S	1MS	40S	50S	40S
EBW174187	-0.66	71	-0.3	73	-0.28	58	40S	30S	50S	80S	10MSS	50S	60S	80S
EBW174456	-0.97	73	-0.18	57	-0.15	47	1MR	10MRMS	1MR	10MSS	0	5MSMR	20MSS	10MSMR
EBW182052	1.2	11	-0.34	78	-0.5	75	20MSMR	10MSMR	50S	40S	1MS	70S	30S	50S
EBW182122	-0.52	68	-0.09	45	-0.31	62	20MSMR	20MSS	30MS	20MSS	15MSS	40S	70S	40S
EBW182146	0.27	40	-0.55	88	-0.95	89	20MSMR	20S	30MS	70S	1MR	30MSMR	50S	40S
EBW192318	0.58	29	-0.24	65	-0.33	65	5MSMR	5S	15MRMS	50S	0	50S	50S	50MSS
EBW192319	0.7	24	0.18	27	0.3	27	5MR	10MS	10MRMS	30MSS	1MR	50S	30S	60S

EBW192320	0.83	18	0.57	7	0.61	15	20MSS	10MSS	15MRMS	50S	1MS	50S	15MSS	60S
EBW192321	0.5	32	0.17	29	0.18	32	10MSMR	15MSS	20MRMS	60S	15S	30S	40S	50S
EBW192322	0.59	28	0.48	10	0.37	24	15MSS	10MSS	10MRMS	30MSS	5MSMR	30MSS	40S	30MSS
EBW192323	0.63	26	-0.05	42	-0.21	52	20MSS	10MSS	10MRMS	80S	0	40S	20MSS	40S
EBW192324	-0.01	50	-0.04	41	-0.58	79	15MRMS	10MS	25MRMS	50S	5MSS	40S	30S	25MSS
EBW192325	-0.13	55	-0.25	68	-0.51	76	20MSS	10MSS	20MS	10MSMR	0	50S	40S	40S
EBW192326	-0.06	52	-0.16	56	-0.29	60	5MR	5MS	15MRMS	40S	0	30MSS	30S	30S
EBW192327	0.31	39	-0.58	89	-0.66	83	20MSMR	10MSS	5MRMS	30MSS	5MSS	20MSS	30S	25MSMR
EBW192328	0.38	36	-0.2	62	-0.47	73	5MSMR	20S	5MR	60S	1MSS	30S	50S	30MSS
EBW192330	0.58	30	0.51	9	0.86	7	30MSS	10MSS	1MRMS	20MSS	20MSS	30MSS	30MSS	40S
EBW192331	0.4	34	-0.07	44	-0.17	51	15MSS	10S	1MR	10MRMS	1MR	30MSS	15MSS	30MSS
EBW192332	0.39	35	0.16	30	-0.04	42	10MSMR	10MSS	15MRMS	10MSS	0	30MSS	10MSS	40S
EBW192333	0.09	46	0.09	36	-0.24	55	5S	15MSS	25MRMS	60S	1MS	30MSS	20MSS	50S
EBW192335	-0.79	72	-0.03	40	-0.31	63	30MSMR	10MSS	70S	30S	1MS	60S	30S	50S
EBW192336	-0.23	58	0.58	6	0.98	4	50S	10MSS	10MS	60S	0	80S	5MSMR	60S
EBW192337	-0.55	70	0.67	3	0.95	5	50S	20MSS	50S	70S	5MSS	70S	20S	70S
EBW192339	-1.59	81	-0.02	39	-0.16	48	60S	10MRMS	40MS	70S	0	80S	10MSMR	40S
EBW192341	-0.4	63	0.14	31	0.43	18	10MSMR	15MSS	20MRMS	30S	15MSS	60S	40S	50S
EBW192343	1.55	8	-0.23	64	-0.26	57	1MR	15MSS	1MR	40S	5MRMS	10MSMR	20MSS	10MSMR
EBW192346	2.22	2	-0.1	47	0.2	31	15MSMR	10MSMR	5MR	5S	0	20MSS	30S	10MSMR
EBW192347	2.06	3	-0.2	61	0.43	19	1MR	5MS	1MS	10MSS	1MR	10MSMR	20MSS	5MSMR
EBW192348	0.16	43	0.4	17	0.1	36	15MSMR	5MS	50S	20MRMS	1MR	30MSS	30MSS	60S
EBW192991	0.84	17	0.27	21	0.1	35	25MSS	20S	1MRMS	10MSMR	0	40S	15MSS	40S
EBW192992	0.81	21	0.11	32	0.25	29	15MSMR	5MS	10MR	30S	0	60S	40S	40S
ETBW9077	1.19	12	-0.49	85	-0.55	78	1MR	5MRMS	0	5MRMS	0	5MSMR	15MSMR	1MR
ETBW9080	0.82	19	0.4	16	-0.02	41	5MSMR	5MSS	1MR	70S	0	40S	30S	50S
ETBW9128	-0.44	65	-0.1	46	-0.21	53	60S	20S	50S	70S	1MS	60S	20MSS	70S
ETBW9136	1.25	10	0.1	35	0	40	5MSMR	20MSS	5MRMS	60S	10MSS	50S	15MSS	50S
ETBW9396	0.8	23	-0.19	60	0.05	39	40S	10MSS	10MRMS	50S	20MSS	60S	60S	40S
ETBW9452	-0.2	57	0.11	34	0.1	37	5MSMR	10MS	10MR	70S	0	40S	20MSMR	80S
ETBW9642	-0.28	61	0.41	15	0.58	16	40S	40S	15MRMS	80S	15MSS	70S	50S	80S
ETBW9647	-0.13	56	-0.06	43	-0.52	77	10MSMR	30S	20MRMS	70S	30S	60S	50S	40S
ETBW9648	-1.94	83	-0.24	66	0.4	22	60S	0	40S	90S	0	80S	20S	60S
ETBW9650	0.31	38	-0.12	52	-0.22	54	50S	20S	20MS	50S	10MSS	70S	50S	40S
ETBW9654	-0.27	59	-0.3	74	-0.6	80	30MSS	50S	30MS	60S	15MSS	50S	70S	60S
Lemmu	-0.99	74	0.26	23	1.04	2	50S	20MRMS	50S	50S	1MR	50S	60S	50S
Wane	0.08	47	0.08	37	0.17	33	40MSS	5MSS	15MRMS	60S	15MSMR	50S	50S	50S

KU20YR = Yellow rust disease score in 2020 at Kulumsa; KU20SR= Stem rust disease score in 2020 at Kulumsa; BE20YR= Yellow rust disease score in 2020 at Bekoji; RA20YR= Yellow rust disease score in 2020 at Arsirobe; KU21SR= Stem rust disease score in 2021 at Kulumsa; RA21YR= Yellow rust disease score in 2021 at Arsirobe; RA21SR= Stem rust disease score in 2021 at Arsirobe; BE21YR= Yellow rust disease score in 2021 at Bekoji

Note: The occurrence of yellow and stem rust disease varies from season to season in one location; in some seasons both diseases occur, and in some seasons only one of the two diseases occur.

Table 3: BLUP-based ranking and wheat rust diseases performance of 90 bread wheat genotypes and evaluated under sixteen environments during the main seasons of 2020 and 2021.

Genotype	20BWNL1AA	20BWNL1BE	20BWNL1DM	20BWNL1DZ	20BWNL1HL	20BWNL1KU	20BWNL1RA	20BWNL1SN	20BWNL2AA	20BWNL2BE	20BWNL2DM	20BWNL2DZ	20BWNL2HL	20BWNL2KU	20BWNL2RA	20BWNL2SN
Danda'a	5.357	2.268	2.572	1.585	2.556	2.963	1.607	1.058	3.039	1.088	4.928	1.837	2.116	4.874	3.093	3.469
EBW120002	5.603	1.894	3.408	1.49	4.433	3.924	1.528	1.414	3.313	1.162	5.338	2.271	3.359	5.084	2.629	2.349
EBW120004	5.191	1.601	3.171	1.462	3.985	3.486	1.316	1.842	3.045	0.653	5.257	1.982	2.688	4.309	2.638	2.274
EBW120011	3.973	1.23	2.921	1.578	3.658	2.751	1.063	1.532	3.144	1.022	5.128	1.809	2.382	3.525	2.501	2.356
EBW120014	5.383	1.569	3.055	1.549	2.555	3.229	1.53	1.929	3.487	0.593	5.344	2.092	2.489	4.833	3.016	3.095
EBW120039	4.311	1.254	2.588	1.539	2.058	2.433	1.179	1.304	3.367	0.545	4.919	2.056	1.741	3.376	2.477	2.902
EBW120041	1.929	0.287	2.647	1.398	3.837	2.2	0.432	0.466	1.735	0.247	4.499	2.136	1.398	1.889	0.779	0.73
EBW120042	4.049	1.698	3.005	1.54	4.308	3.211	1.011	1.372	3.126	1.034	4.915	2.172	3.053	3.501	1.698	1.437
EBW120044	2.503	0.899	2.527	1.484	3.75	2.207	0.757	0.516	1.65	0.763	4.628	1.766	1.41	2.828	1.807	2.191
EBW120052	1.248	0.019	2.15	1.425	1.843	1.364	0.229	0.33	1.539	-0.02	4.294	2.01	0.389	1.255	0.867	1.217
EBW120053	6.51	2.443	3.334	1.588	3.941	4.039	1.698	1.474	3.881	1.376	5.51	2.25	3.56	5.796	3.212	3.314
EBW120054	6.188	2.807	3.243	1.585	4.324	4.065	1.808	1.293	3.514	1.648	5.315	2.14	3.754	5.245	3.075	2.888
EBW120056	1.222	0.261	2.224	1.407	2.583	1.633	0.314	-0.111	0.898	0.209	4.229	1.94	0.532	2.115	0.894	1.261
EBW120060	4.556	1.198	3.134	1.498	3.916	3.239	1.347	1.62	2.714	0.711	5.127	2.22	2.365	4.426	2.436	3.139
EBW120063	5.263	2.087	2.48	1.549	1.659	2.738	1.492	1.655	3.062	1.326	4.955	2.087	1.797	4.316	3.214	3.663
EBW172056	3.966	0.98	2.506	1.426	1.691	2.49	1.286	0.838	2.26	0.269	4.822	2.476	1.105	3.027	1.867	1.947
EBW172082	5.135	1.516	2.635	1.546	1.694	2.617	1.452	1.401	3.561	0.929	5.171	2.102	1.767	4.045	3.14	4.581
EBW172088	8.186	3.723	3.111	1.656	3.037	4.247	2.566	3.381	4.207	2.631	5.688	1.951	3.703	6.645	4.834	5.532
EBW172093	8.374	4.088	2.646	1.584	2.046	4.071	2.853	2.302	3.716	2.887	5.414	2.405	2.99	6.138	4.404	4.406
EBW172105	7.224	2.581	3.185	1.643	2.952	3.915	1.847	2.238	4.311	1.35	5.657	2.305	3.245	5.897	3.73	3.96
EBW172319	5.598	1.332	3.269	1.411	3.046	3.714	1.652	1.498	2.76	0.47	5.376	2.514	2.36	4.738	2.456	3.097
EBW172393	3.741	0.459	2.767	1.623	2.205	2.136	0.926	1.124	3.718	0.288	5.186	2.199	1.631	3.622	2.552	3.339
EBW172440	6.411	2.825	2.638	1.565	2.023	3.375	1.715	1.592	3.516	1.958	5.197	2.214	2.533	5.575	3.34	3.636
EBW172474	7.574	3.93	2.655	1.619	2.944	4.062	2.216	1.938	3.408	2.386	5.117	2.506	3.144	5.674	3.533	4.149
EBW172862	7.739	3.759	2.541	1.625	1.764	3.513	2.547	2.594	4.141	2.566	5.353	1.995	2.838	6.498	4.731	6.018
EBW172864	7.978	4.536	2.413	1.718	2.236	3.688	2.001	2.174	4.421	3.621	5.196	1.765	3.405	6.719	4.532	4.677
EBW172872	5.309	1.968	2.641	1.513	1.643	3.037	1.426	1.361	2.972	0.951	5.03	2.3	1.989	4.356	2.668	2.654
EBW172936	6.921	3.26	2.321	1.613	1.593	3.265	2.154	2.769	3.56	2.07	5.068	2.423	2.181	5.344	3.442	4.276
EBW172996	2.153	0.172	2.536	1.497	2.077	1.732	0.835	0.071	2.163	0.279	4.736	1.319	1.009	2.037	2.037	2.584
EBW173001	6.372	2.091	2.859	1.54	2.356	3.376	1.698	2.121	3.864	1.05	5.32	2.537	2.396	5.566	3.048	4.037
EBW173004	3.2	0.75	2.421	1.539	1.461	1.836	0.789	0.498	3.02	1.154	4.766	1.729	1.237	3.858	2.446	2.616
EBW173006	2.42	0.219	2.382	1.461	1.212	1.728	0.675	0.532	2.122	0.175	4.698	1.628	0.689	2.607	1.703	1.939
EBW173031	6.168	2.729	2.491	1.593	1.408	3.148	1.895	0.731	3.095	1.539	5.079	1.727	2.084	4.952	3.487	4.223
EBW173207	5.924	2.34	2.606	1.558	1.794	3.11	1.745	2.256	3.546	1.185	5.1	2.349	2.231	4.678	3.008	3.771
EBW173261	3.046	0.469	2.375	1.563	1.383	1.749	0.742	0.984	3.01	0.295	4.752	2.12	0.98	2.673	1.998	3.203
EBW173263	4.91	1.261	2.63	1.58	1.683	2.476	1.248	1.62	3.686	0.666	5.09	2.233	1.655	4.427	2.989	4.55
EBW173270	5.554	1.78	2.937	1.625	2.987	3.193	1.329	2.346	3.815	0.958	5.258	2.262	2.555	4.786	2.834	3.633

EBW173288	6.738	2.439	3.457	1.549	4.187	4.317	2.031	1.096	3.573	1.419	5.59	2.261	3.59	6.036	3.125	3.039
EBW173292	7.443	3.244	3.231	1.631	3.847	4.196	1.932	1.838	4.462	2.135	5.522	2.26	3.946	6.342	3.853	4.45
EBW173332	5.657	2.797	2.366	1.6	1.5	2.717	1.921	1.272	3.306	2.314	5.08	1.548	2.131	4.625	4.068	4.53
EBW173353	8.671	4.609	2.96	1.709	3.293	4.451	2.594	4.393	4.394	3.824	5.547	2.053	4.171	7.39	4.917	4.661
EBW173366	5.712	1.953	3.55	1.534	5.83	4.126	1.741	1.945	3.178	0.965	5.439	2.23	3.57	5.094	2.79	3.149
EBW173378	5.969	2.893	2.525	1.568	1.892	3.252	1.615	1.58	3.097	1.857	5.002	1.677	2.419	4.867	3.203	3.321
EBW173380	6.799	1.888	3.544	1.546	3.739	4.109	2.087	1.469	3.753	0.803	5.78	2.037	3.198	5.5	3.603	3.563
EBW174116	7.229	3.553	2.355	1.573	1.729	3.581	1.789	2.634	3.458	2.162	5.103	2.708	2.413	5.543	3.105	3.155
EBW174170	2.614	0.699	2.279	1.362	1.415	1.965	0.78	0.562	1.707	0.331	4.426	1.918	0.774	2.126	1.404	1.27
EBW174187	4.795	2.034	2.438	1.494	1.911	2.742	1.152	1.966	2.857	0.988	4.834	2.275	1.852	3.958	2.504	2.749
EBW174456	3.997	0.942	2.605	1.451	1.971	2.606	1.111	0.84	2.124	0.33	4.824	2.435	1.179	3.984	1.946	2.037
EBW182052	7.569	3.559	2.458	1.493	1.343	3.614	2.236	3.115	3.39	1.929	5.21	2.393	2.357	5.805	3.961	5.536
EBW182122	4.928	1.446	2.705	1.558	1.986	2.664	1.468	1.768	3.308	0.841	5.11	2.09	1.813	4.02	3.135	4.049
EBW182146	5.77	2.754	2.277	1.607	1.26	2.766	1.815	0.924	2.907	1.844	4.936	1.571	1.762	5.184	3.788	3.732
EBW192318	6.46	2.555	2.518	1.611	1.554	3.123	1.771	1.925	3.576	1.506	5.218	2.501	2.033	5.463	3.454	3.793
EBW192319	6.718	2.923	2.944	1.624	3.21	3.69	1.753	1.924	4.141	1.943	5.398	2.136	3.292	5.592	3.522	3.982
EBW192320	6.992	2.231	3.314	1.703	3.743	3.752	1.727	2.654	5.164	1.208	5.718	2.217	3.56	5.725	3.628	3.744
EBW192321	6.435	2.327	2.894	1.584	2.668	3.455	1.569	2.117	4.077	1.298	5.245	2.424	2.739	5.386	3.131	3.475
EBW192322	6.472	2.253	3.225	1.679	3.482	3.66	1.732	3.04	4.421	1.508	5.494	2.01	3.346	5.501	3.575	3.889
EBW192323	6.609	2.851	2.714	1.693	2.487	3.296	1.692	2.507	4.369	1.718	5.279	1.867	2.904	5.568	3.67	4.231
EBW192324	5.711	2.171	2.662	1.669	1.637	2.875	1.37	1.973	4.388	1.739	5.186	1.983	2.549	4.752	3.152	3.038
EBW192325	5.502	2.241	2.472	1.635	1.75	2.694	1.289	1.948	3.877	1.563	4.985	1.976	2.146	4.731	3.24	3.825
EBW192326	5.749	2.226	2.573	1.625	1.968	2.91	1.56	2.004	3.814	1.249	5.119	2.198	2.211	4.373	3.131	3.747
EBW192327	6.271	3.055	2.165	1.594	1.19	2.828	1.615	2.525	3.542	1.87	4.876	2.215	1.927	5.028	3.495	4.969
EBW192328	6.395	2.453	2.55	1.579	1.561	3.086	1.671	2.244	3.898	1.182	5.098	2.272	2.155	5.004	3.289	4.211
EBW192330	6.512	2.522	3.241	1.656	4.265	3.883	1.784	2.102	4.437	1.431	5.439	2.218	3.685	5.291	3.101	3.381
EBW192331	6.378	2.698	2.636	1.679	2.092	3.156	1.411	2.01	4.414	1.446	5.177	2.316	2.75	5.382	3.349	4.501
EBW192332	6.275	2.141	2.877	1.643	2.179	3.212	1.506	1.931	4.589	1.27	5.406	2.481	2.766	5.331	3.242	4.017
EBW192333	5.767	1.981	2.823	1.656	2.112	3.016	1.48	2.178	4.25	0.986	5.23	2.163	2.576	4.953	3.248	3.851
EBW192335	4.413	0.944	2.707	1.528	1.742	2.615	1.275	1.068	3.156	0.337	5.041	2.391	1.577	3.831	2.118	2.069
EBW192336	5.662	1.495	3.302	1.499	3.75	3.746	1.301	0.916	3.897	0.459	5.407	2.953	3.045	4.253	1.897	1.718
EBW192337	5.12	1.064	3.423	1.485	4.088	3.688	1.146	0.884	3.316	0.523	5.411	2.569	2.832	3.997	1.93	2.381
EBW192339	3.442	0.521	2.732	1.452	2.27	2.47	0.828	0.688	2.36	0.104	4.982	2.165	1.331	2.869	1.707	1.753
EBW192341	5.118	1.753	2.877	1.559	3.271	3.115	1.254	1.749	3.581	0.949	5.025	2.331	2.537	4.375	2.487	2.654
EBW192343	8.069	3.838	2.575	1.654	2.176	3.704	2.607	2.661	4.238	2.485	5.439	2.029	2.913	5.884	4.505	5.226
EBW192346	9.196	5.072	2.597	1.803	2.691	4.119	2.508	3.747	5.713	4.254	5.406	2.422	4.256	6.873	4.813	5.397
EBW192347	9.162	5.133	2.528	1.705	3.029	4.299	2.386	3.253	5.045	3.854	5.347	2.557	4.013	6.652	4.246	5.245
EBW192348	6.043	1.391	3.099	1.581	2.177	3.227	1.279	2.791	4.373	0.672	5.576	2.732	2.463	5.052	3.086	4.309
EBW192991	6.78	2.177	3.061	1.614	2.523	3.499	1.956	2.554	4.252	0.955	5.468	2.374	2.754	5.764	3.846	4.564
EBW192992	6.933	2.733	2.877	1.53	2.611	3.858	1.972	2.361	3.359	1.365	5.367	2.647	2.657	5.46	3.048	3.657
ETBW9077	7.778	3.451	2.284	1.499	0.924	3.439	1.91	3.088	3.426	1.673	5.184	2.692	1.865	5.89	3.91	5.055
ETBW9080	7.152	2.626	3.102	1.714	2.322	3.758	1.815	2.994	4.953	1.397	5.57	2.424	3.434	5.337	3.471	3.891
ETBW9128	4.908	1.721	2.694	1.584	2.473	2.803	1.329	1.533	3.149	0.702	5.088	1.839	2.032	4.405	2.976	3.183

ETBW9136	7.482	3.164	2.905	1.628	2.463	3.826	2.142	2.05	4.142	1.844	5.466	2.248	3.107	6.093	4.012	4.464
ETBW9396	7.031	3.492	2.484	1.638	2.197	3.62	1.581	2.437	3.672	2.785	5.124	2.665	2.751	5.681	3.087	3.723
ETBW9452	5.408	1.604	2.845	1.557	2.376	3.013	1.096	2.233	3.74	0.667	5.245	2.52	2.245	4.905	2.784	2.477
ETBW9642	4.8	1.04	3.215	1.518	3.623	3.22	1.385	0.904	3.283	0.384	5.413	2.241	2.466	4.953	2.541	2.381
ETBW9647	5.079	1.65	2.733	1.539	1.66	2.925	1.534	1.04	2.932	0.762	5.058	2.066	1.893	4.969	2.912	3.056
ETBW9648	1.849	0.184	2.52	1.313	3.278	2.209	0.432	-0.035	0.856	-0.048	4.471	2.262	0.788	3.922	0.544	0.627
ETBW9650	5.917	2.335	2.688	1.541	2.125	3.186	1.855	1.35	3.355	1.306	5.174	2.17	2.284	5.135	3.229	3.342
ETBW9654	5.254	1.937	2.533	1.46	1.511	2.943	1.606	1.287	2.345	1.026	4.986	2.022	1.516	4.329	2.917	2.887
Lemmu	4.083	1.461	3.09	1.462	4.884	3.205	1.272	0.986	2.505	0.708	4.987	1.886	2.659	3.66	2.34	2.657
Wane	5.615	1.704	2.841	1.46	2.459	3.284	1.714	2.239	3.025	0.472	5.172	2.613	2.016	4.805	2.535	3.076

Table 4: The mean grain yield of ninety bread wheat genotypes evaluated across sixteen genotypes.

Conclusion

Genotypes EBW192346 and EBW192347 were evaluated on the farmer's field and the research station by Variety Releasing Committee in 2022 [21]. The performance compared with the standard check variety, Boru which was recently released bread wheat variety. Then, EBW192346 performed better than the check and was released as a new variety.

References

1. USDA (2024) Ethiopia Production.
2. Senbeta AF, Worku W (2023) Ethiopia's wheat production pathways to self-sufficiency through land area expansion, irrigation advance, and yield gap closure. *Heliyon* 9(10): e20720.
3. Hodson D, CIMMYT (2018) Ethiopia.
4. Meyer M, Bacha N, Tesfaye T, Alemayehu Y, Abera E, et al. (2021) Wheat rust epidemics damage Ethiopian wheat production: A decade of field disease surveillance reveals national-scale trends in past outbreaks. *PLoS One* 16(2): e0245697.
5. Admassu B, Friedt W, Ordon F (2012) Stem rust seedling resistance genes in Ethiopian wheat cultivars and breeding lines. *African Crop Science Journal* 20: 149-162.
6. Denbel W, Badebo A, Alemu T (2013) Evaluation of Ethiopian commercial wheat cultivars for resistance to stem rust of wheat race UG99. *International Journal of Agronomy and Plant Production* 4: 15-24.
7. Alemu A, Getnet M (2019) Yield loss assessment in bread wheat varieties caused by yellow rust (*Puccinia striiformis* f. sp. *tritici*) in Arsi highlands of South Eastern Ethiopia. *American Journal of BioScience* 7(6): 104-112.
8. Abebe D, Gadisa A, Negash G, Alemu D, Habtemariyam Z, et al. (2022) Stability and performance evaluation of advanced bread wheat (*Triticum aestivum* L.) genotypes in optimum areas of Ethiopia. *International Journal of Bio-resource and Stress Management* 13(1): 69-80.
9. Tadesse W, Zegeye H, Debele T, Kassa D, Shiferaw W, et al. (2022) Wheat production and breeding in Ethiopia: Retrospect and Prospects. *Crop Breeding, Genetics Genomics* 4(3): e220003.
10. Olivera P, Newcomb M, Szabo LJ, Rouse M, Johnson J, et al. (2015) Phenotypic and genotypic characterization of race TKTF of *Puccinia graminis* f. sp. *tritici* that caused a wheat stem rust epidemic in southern Ethiopia in 2013–14. *Phytopathology* 105(7): 917-928.
11. Sanders R (2011) Strategies to reduce the emerging wheat stripe rust disease. Synthesis of a dialog between policy makers and scientists from 31 countries at: international wheat stripe rust symposium. Aleppo, Syria: International Center for Agricultural Research in the Dry Areas (ICARDA).
12. Malosetti M, Ribaut JM, van Eeuwijk FA (2013) The statistical analysis of multi-environment data: Modeling genotype-by-environment interaction and its genetic basis. *Front Physiol* 4: 44.
13. Taneva K, Bozhanova V, Petrova I (2019) Variability, heritability, and genetic advance of some grain quality traits and grain yield in durum wheat genotypes. *Bulgarian Journal of Agricultural Science* 25(2): 288-295
14. Jaiswal R, Gaur SC, Jaiswal SK, Kumar A (2020) An Estimate of Variability, Heritability and Genetic Advance for Grain Yield and Yield Components in Bread Wheat (*Triticum aestivum* L.). *Current Journal of Applied Science and Technology* 39(12): 1-6.

15. Ferreira CI, Peixoto MA, Santana PCE, Silva AR, Sales S, et al. (2020) Multiple-trait, random regression, and compound symmetry models for analyzing multi-environment trials in maize breeding. *Plos One* 15(11): e0242705.
16. Peterson RF, Campbell AG, Hannah AE (1948) A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can J Res* 26: 496-500.
17. Schmidt PJ, Hartung J, Rath, Piepho HP (2019) Estimating Broad-Sense Heritability with Unbalanced Data from Agricultural Cultivar Trials. *Crop Science* 59(2): 525-536.
18. Cullis BR, Thomson FM, Fisher JA, Gilmour AR, Thompson R (1996) The analysis of the NSW wheat variety database. I. Modelling trial error variance. *Theor Appl Genet* 92(1): 21-27.
19. Yan W, Tinker NA (2006) Biplot analysis of multi-environment trial data: Principles and applications. *Canadian journal of plant science* 86: 623-645.
20. Piepho HP, Mohring J, Melchinger AE, Büchse A (2008) BLUP for phenotypic selection in plant breeding and variety testing. *Euphytica* 161: 209-228.
21. Deon Vilela de Resende M (2016) Software Selegen-REML/BLUP: a useful tool for plant breeding. *Crop Breed Appl Biotechnol* 16: 330-339.