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Evaluation of Bread Wheat (*Triticum aestivum* **L.) Germplasm for Wheat Rust Diseases Resistance and Yield Performance**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The most important biotic constraints for wheat production in Ethiopia are Wheat rust diseases, insects, and weeds. Among wheat rust diseases, stem rust and yellow rust are the more limiting factors for yield. A study was undertaken at Kulumsa and Melkasa in alpha lattice design with two replications from July to December 2023 to evaluate the performance of introduced bread wheat materials from CIMMYT for wheat rust diseases and yield. Data were collected for stem rust, yellow rust, and agronomic traits, including grain yield. The ANOVA showed high and significant genotypic variation (p<0.05) on Days to heading (DTH), Hectoliter weight (HLW), Thousand kernels weight (TKW), and Yield (YLD). Then, the result from LSD mean comparision revealed genotypes: EBW222102 YLD=7.7 t ha-1, EBW222106 YLD= 7.61 t ha-1, EBW222108 YLD=7.66 tha-1, EBW222136 YLD= 7.76 t ha-1 to be significantly higher than the check variety Daka YLD= 6.22 t ha-1 (p<0.05) at Kulumsa. Genotypes: EBW222108, EBW222109, EBW222111, EBW222112, EBW222114, EBW222125, EBW222142, and EBW222143 showed resistance to moderate resistance for yellow rust and stem rust across both locations. About eighteen genotypes were susceptible to stem rust diseases with the coefficient of infection YRCI≥30 at Melkasa. Genotypes EBW222111, EBW222126, EBW222129, EBW222134, and EBW222142 delivered high yield, greater than or equal to 2 t ha-1 at Melkasa. Generally, selection for disease resistance and highyielding genotypes enable the development and release of noble varieties for wheat-producing farmers.

Keywords: CI; CIMMYT' genotype; SAWYT; pipelines; resistance; YLD; stem rust.

1. INTRODUCTION

Wheat productivity and production have increased in Ethiopia over the past two decades. The productivity increased from 2.4tha-1 in 2013 to 3.1 tha-1 in 2023 [1,2]. The wheat production in 2022/2023 is about 5.8 million tons [1]. Still, the country has a higher potential to enhance productivity and production. However, wheat in Ethiopia is lagging the production potential due to biotic, abiotic, and post-harvest losses [3,4,5].

The major biotic constraints for wheat production in Ethiopia are Wheat rust diseases, insects, and weeds. Among the biotic constraints, wheat rust diseases (mainly stem rust and yellow rust) are more limiting factors [6,7]. Similarly, the most important abiotic stresses are terminal drought, soil acidity, erosion, poor soil fertility, prolonged high rainfall, water-logging and pre-harvest sprouting.

The occurrences and outbreaks of stem rust and yellow rust diseases in Ethiopia varied from year to year in severity [8]. The prevalence of wheat rusts also differed significantly amongst bread wheat varieties under production [9-11]. Some variety shows resistance (Balcha for yellow rust) and moderate resistance (Daka for yellow rust). Others show susceptibility (Hidasse for yellow and stem rusts) and highly susceptible (Kubsa for yellow and stem rusts). The country experiences recurrent epidemics of stem rust

due to the evolution of new stem rust races [8,12]. Stem rust caused by the basidiomycete Puccinia graminis f.sp. tritici (Pgt) causes a total crop loss during epidemic years [12]. Yellow rust caused by the basidiomycete fungus Puccinia striiformis f. sp. tritici (Pst) causes significant yield loss. In 2010, the outbreak of this disease seriously damaged popular adapted varieties: Kubsa and Galema. It affected more than 600,000ha of wheat growing areas and reduced up to 20% production [7,13,14]. Due to these rusts, farmers in the area expended additional expenses for fungicides, which they spray three to four times per cropping season.

Different wheat rust disease-controlling options are available for wheat farmers, such as cultural practices, fungicides, and growing resistance varieties [15,16]. Due to the high pressure of yellow and stem rust pressure, farmers in the country use fungicides extensively to control wheat rust diseases [17,18]. However, due to the cost of the chemicals, the lack of skill when and how to use the fungicides, and the poor quality of the chemicals to control the diseases effectively, the farmers still lose their crops. A sustainable management option could be growing resistance varieties for wheat rust diseases. It is an environmentally safe and economically viable option for the farmers. However, the lack of availability of the desired resistance varieties forced the farmers to grow obsolete varieties.

To overcome the problem, the continuous development and release of wheat rust diseaseresistant noble bread wheat varieties is crucial.

The generation and release of novel bread wheat varieties for the wheat-producing community goes through different breeding procedures. Getting germplasm from diverse sources, evaluating the genotypes on the field, and selecting among the lines for better performances are the activities practiced by the wheat research program. The national wheat research program has introduced germplasm from CGIAR centers, as well as its crossing materials. Consequently, the program has released widely adopted bread wheat varieties [19]. Therefore, the objective of this study was to evaluate the performance of introduced bread wheat materials from CIMMYT for wheat rust diseases and yield.

2. MATERIALS AND METHODS

2.1 Material, Design, and Description of the Study Area:

A study was undertaken at Kulumsa and Melkasa in alpha lattice design with two replications from July to December 2023. MARC is located at 8°24′N 39°12′E latitude and longitude with an Altitude of 550 m.a.s.l. The minimum and maximum temperatures are 14oc and 280c respectively. The area received an annual rainfall of about 763mm. KARK is located at 8°02′N 39°10′E latitude and longitude with an Altitude of 2200 m.a.s.l. The minimum and maximum temperatures are 10° c and 22° c respectively. Annual rainfall in kulumsa is about 840mm.

In 2022, the national wheat research program introduced the 29 Semi-Arid Wheat Yield Trials (29SAWYT) along with other trials from CIMMYT, Mexico. A total of fifty genotypes, including one local check variety, Daka planted in alpha lattice design with two replications. The rep/block had five sub-blocks, and a sub-block had ten plots. The plot size was 1.2m in width and 2.5m in length. A plot had six rows with a total area of 3m² . All agronomic practices applied equally to all plots.

2.2 Data Collection and Analysis

Wheat rust diseases: Stem rust and yellow rust diseases were collected in two rounds. The first score was taken a week after the symptom had seen on the plot. The second was taken at the pick time of the disease's pressure on the trial. 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 [20]. Severity and field response readings are usually combined. For example, tR = Trace severity with a resistant field response. 10MS= 10% severity with moderately susceptible field response. For analysis, the disease data changed to the Coefficient of infection (CI) as outlined by [21,22] in which the host reaction changed to numeric and then multiplied by severity. Immunity (O) = 0.05 , resistant (R) = 0.1 , moderately resistant $(MR) = 0.2$, intermediate $(M) = 0.4$, moderately susceptible $(MS) = 0.6$, and susceptible (S) =1. For example CI for 10MS= $10*0.6= 6$; CI=6.

The analysis of variance, ANOVA is computed to compare the variance related to genotypes to that of variance environmentally occurring between plots [23].

The broad-sense heritability of a given trait in an individual environment is calculated as [24]

H2=(δ^2 g)/(δ^2 g+δ^2 E/nRep)

where σg2 and σε2 are the genotype and error variance components, respectively, and nRep is the number of replicates.

The LSD at 5% of significance is calculated as LSD = $t(1-0.05, dfErr)$ × ASED, where t is the cumulative Student's t distribution, 0.05 is the selected α level (5%), dfErr is the degrees of freedom for error in the linear mixed model, and ASED is the average standard error of the differences of the means [17,18].

All the analyses in this study were computed using R software 3.6.0 and META-R [1,6, 25].

3. RESULTS AND DISCUSSION

In the field experiment, there are many potential source of variation between experimental units or plots. The variation arises from genotype and a group of all the unexplained variance (error). The presence of variation among tested genotypes is an opportunity for breeders to do selection. The data from the two locations combined, and the analysis of variance ANOVA computed. High and significant genotypic variation (p<0.05) observed on days to heading (DTH), Hectoliter weight (HLW), Thousand kernels weight (TKW), and Yield (YLD). Hence, sufficient variability existed in the present genotypes in the experiment. Therefore, selection is effective for the crop improvement [26]. Similarly, very high significant differences (p<0.05) and the highest magnitude of mean square of error observed on test sites (Site) for all traits (Table 1). This explains the importance of environments contribution to the total variations.

Since, variation among genotypes, environments, genotypes by environments was observed, other statistics broad sense heritability. Least Significant Difference LSD. Coefficient of Variance CV were computed.

Heritability is an important quantitative parameter that evaluates how genetics and environment interact to determine a trait's expression. Broad sense heritability, which accounts for total genetic variance (additive, dominant/recessive, and epistatic) computed based on BLUP values for each environment separately to see the responses of the locations in heritability of the traits and for the combined environments to see how heritability of the traits responses across the two locations. Result from the experiment showed different responses from very low heritability 2.3% for yield to high heritability 79.2% for plant height across locations (Table 2). Grain yield is a complex quantitative trait attributed by many other traits or yield components. In very low heritability for yield scenario, indirect selection uses for some yield components that are more heritable than the yield and more stable in relation to genetic and environmental factors affecting them. At kulumsa, high heritability gained for all traits in the study: YLD, DTH, TKW, HLW, DTM and PHT (Table 3). Similar result were reported by [27]. This showed that the genotypes were able to phenotypically express the traits due to their genetic makeup.

Wheat rust diseases resistance is the most important trait to evaluate genotypes at this stage of breeding pipelines in Ethiopia. The trial is at nursery stage; it is early to look for yield and

stability across different environment. But, later, genotypes selected here will be advanced to yield trial and evaluated at different stages of breeding pipelines before released as a variety. Hence, considering the yield performance helps to select better genotypes. Subsequently, the main target of the wheat breeding program is to develop and release high yielding variety with better wheat rust resistance. For these two important traits, wheat rust resistant and grain yield better to see the responses across location and for each location separately.

The check variety Deka used to mean yield comparison. The average yield across locations for Deka was 3.2 t ha-1. The least significant difference LSD across location was 0.277 (Table 2). About thirty three genotypes delivered higher average grain yield than the check, Deka variety across the locations. Moreover, thirty one out of thirty three genotypes were significantly different in grain yield than the check variety Deka at (P<0.005) (Tables 2 and 3).

The least significant difference LSD for yield at Kulumsa was 1.37 (Table 2). The average yield of the check variety Daka was 6.22 t ha-1 at Kulumsa. Yield at Kulumsa for Genotypes: EBW222102 YLD=7.7 t ha-1, EBW222106 YLD= 7.61t ha-1, EBW222108 YLD=7.66 t ha-1, EBW222136 YLD= 7.76t ha-1 significantly higher than the check variety Deka at (p<0.05) (Table 3). The check variety Deka delivered the lowest yield, 0.17 t ha-1 at Melkasa. The least significant difference at melkasa was 0.577. Unexpectedly, all genotypes but EBW222119 are significantly different than Deka for yield at (p<0.05) (Table 3).

The other important traits to evaluate and select better genotypes are wheat rust diseases resistance. Especially, at the early stage of breeding pipe line, performance of the genotypes for the existing important wheat rust diseases is more important than the other traits. The trial is at nursery stage; it is early to look for yield and stability across different environment. Moreover, the seed introduced from CGIAR centers is small to do multiplications trial. Genotype EBW222099 scored the highest stem rust coefficients: SRCI=55 at Kulumsa and SRCI= 60 at Melkasa. In contrast, this genotype showed very low coefficient of infection YRCI= 1.1 for yellow rust disease at Kulumsa site. Hence, this genotype is highly susceptible for stem rust but resistance for yellow rust. Genotype EBW222146 had the highest coefficient of infection YRCI=35 for

Source of variation	DF	DTH	DTM	PHT	HLW	TKW	YLD
Genotype(G)	49	$27.9***$	$6.8**$	$53*$	$24.6***$	$35.7***$	$7***$
Environment (E)		3553.2***	28962.6***	73345***	1513.44***	4608.0***	945.17***
Genotype: Environment GXE	49	$5.8***$	$6.5***$	44ns	$9.92*$	$19.0***$	1.66***
Environment: Rep		$9.6*$	0.4ns	$131*$	$30.74**$	66.6***	$3.20***$
Environment: Rep: Block		0.6 _{ns}	3.1ns	0ns	3.35ns	$25.9*$	0.65ns
Residual	96	2.1	3.5	35	6.26	71 . .	0.41

Table 1. Analysis of variance (ANOVA) of fifty bread wheat genotypes tested across Kulumsa and Melkasa during 2022 cropping season

Degree of freedom (DF), days to heading (DTH), days to maturity (DTM), Plant height (PHT), hectoliter weight (HLW), thousand kernel weight (TKW), and yield (YLD).

Table 2. Broad sense heritability and Variances for fifty genotypes evaluated across two locations , Kulumsa and Melkasa , during 2022 G.C. cropping season

*KU=Kulumsa; MK=Melkasa; DTH=Date of heading; DTM=Date of maturity; PHT=plant height;SRCI=Stem Rust Coefficient of Infection TKW= thousand kernel weight; HLW=Hectoliter weight; GYLD= Grain yield; Genotype significance for the traits at: ns =non significance, *,**, and *** significant at 5%, 1%, and 0.1% level of significance, consecutively.*

LSD= least significant differences, CV= coefficient of variation

			DTM		PHT		YRCI			TKW		HLW			
Statistics	DTH						SRCI						YLD		
	KU	ΜK	KU	ΜK	KU	MK	KU	ΚU	MK	KU	ΜK	KU	MK	KU	MK
Heritability	0.96	0.64	0.75	0.32	0.63	0.10	0.66	0.80	0.08	0.81	0.57	0.90	0.41	0.81	0.32
Genotype Variance	11.62	2.69	1.88	1.03	10.60	3.10	59.97	136.95	10.45	14.39	5.25	8.26	3.50	1.19	0.06
Residual Variance	1.02	2.97	1.27	4.46	12.25	55.17	60.57	68.97	229 .86	6.94	7.85	.94	10.05	0.57	0.25
Grand Mean	67.46	59.03	121.21	97.01	103.30	65.00	6.20	7.91	24.04	34.32	24.72	65.79	60.17	5.71	1.36
LSD	2.07	2.84	2.07	2.43	5.63	4.77	12.98	15.26	8.87	4.77	4.33	2.73	NA	1.37	0.58
CV	1.49	2.92	0.93	2.18	3.39	11.43	125.48	104.96	63.07	7.67	11.33	2.12	5.27	13.28	36.67
n Replicates	2	◠ ∠	2	2	ົ	$\overline{2}$	◠	◠	ົ	ົ	ົ	◠ ∠	ົ	ົ	ົ ∠
Genotype significance	$***$	$***$	$***$	ns	$***$	ns	$***$	$***$	ns	$***$	$***$	$***$	ns	$***$	ns

Table 3. Broad sense heritability and variances for yield and diseases of fifty genotypes evaluated at Kulumsa and Melkasa; for each site separately

*KU=Kulumsa; MK=Melkasa; DTH=Date of heading; DTM=Date of maturity; PHT=plant height; YRCI= Yellow Rust Coefficient of Infection; SRCI= Stem Rust Coefficient of Infection; TKW= thousand kernel weight; HLW=Hectoliter weight; GYLD= Grain yield; Genotype significance for the traits at: ns =non significance, *,**, and *** significant at 5%, 1%, and 0.1% level of significance, consecutively. . LSD= least significant differences, CV= coefficient of variation*

Table 4. Average grain yield, wheat rust diseases score and other agronomic traits of fifty bread wheat lines evaluated across two locations, Kulumsa and Melkasa, during 2022 cropping season

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Entry	GENOTYPE	DTH	DTM	PHT	YRCI	SRCI		TKW	HLW	YLD		Average yield
					KU	KU	МK			KU	МK	
43	EBW222140	64.25	108.50	77.00	34.00	0.13		28.50	63.18	2.81	. 48	2.14
44	EBW222141	61.00	110.50	88.50	9.00	0.05	30	33.50	61.52	6.52	0.89	3.70
45	EBW222142	64.25	109.75	91.50	4.00	1.03	8	31.00	65.85	5.77	2.37	4.07
46	EBW222143	63.50	110.25	81.00	3.50	2.15	8	25.50	62.02	3.94	1.14	2.54
47	EBW222144	60.50	78.75	81.25	10.00	6.00	19	23.00	58.96	3.54	.80	2.67
48	EBW222145	61.00	108.50	80.75	11.00	16.50	35	27.00	63.02	4.70	0.97	2.83
49	EBW222146	59.25	106.00	80.75	35.00	7.50	40	28.50	61.62	3.38	. 48. ،	2.43
50	EBW222147	60.25	109.00	87.75	.50	3.50	40	28.50	63.36	6.11	.50	3.80

KU=Kulumsa; MK=Melkasa; DTH=Date of heading; DTM=Date of maturity; PHT=plant height; YRCI= yellow rust coefficient of infection; SRCI= Stem rust coefficient of infection TKW= thousand kernel weight; HLW=Hectoliter weight; GYLD= Grain yield; Genotype significance for the traits at: ns =non significance, *,**, and *** significant at *5%, 1%, and 0.1% level of significance, consecutively*

yellow rust at Kulumsa. Also, it had higher coefficient of infection SRCI=40 for stem rust at Melkasa. These told us the genotype is highly susceptible for both diseases. Genotypes:
EBW222147. EBW222135. EBW222131. EBW222147, EBW222135, EBW222131, EBW222128, EBW222124, EBW222122, EBW222120 scored YRCI=1.5, 1, 0.6, 0.6, 0.6, 0.2, and 3 for yellow rust successively at Kulumsa. The same genotypes successively scored SRCI=3.5, 0.2, 1.03, 0.05, 0.13, 0.13, and 0.23 for stem rust at Kulumsa. So, they were resistance for both yellow rust and stem rust at kulumsa. Nonetheless, they scored high coefficient of infection from SRCI=30 to SRCI=45 for stem rust at Melkasa. Probably, it is due to the occurrence of different stem rust races between Kulumsa and Melkasa during the study [28]. TKTTF, TKKTF, TTRTF, TTTTF, TKTTF and TKKTF are the wildly distributed stem rust races that account for about 85% of the frequency of dominant races found in Ethiopia [28,29,30].

The reason for testing genotypes for their disease response across different locations is to examine their performance for the existing wheat rust races across different wheat producing agroecology. Genotypes: EBW222108 with YRCI=0.13 for yellow rust at Kulumsa, SRCI=0.4 for stem rust at Kulumsa, and SRCI=3 for stem rust at Melkasa; EBW222109 with YRCI=0.13 for yellow rust at Kulumsa, SRCI=5.5 for stem rust at Kulumsa, and SRCI=4 for stem rust at Melkasa: EBW222111 with YRCI=1.1 for yellow rust at Kulumsa, SRCI=0.05 for stem rust at Kulumsa, and SRCI=3 for stem rust at Melkasa; EBW222112 with YRCI=1.5 for yellow rust at Kulumsa, SRCI=0.05 for stem rust at Kulumsa, and SRCI=4 for stem rust at Melkasa; EBW222114 with YRCI=3 for yellow rust at Kulumsa, SRCI=4 for stem rust at Kulumsa, and SRCI=6 for stem rust at Melkasa; EBW222125 with YRCI=0.6 for yellow rust at Kulumsa, SRCI=0.05 for stem rust at Kulumsa, and SRCI=6 for stem rust at Melkasa; EBW222142 with YRCI=4 for yellow rust at Kulumsa, SRCI=1.05 for stem rust at Kulumsa, and SRCI=8 for stem rust at Melkasa; EBW222143 with YRCI=3.5 for yellow rust at Kulumsa, SRCI=2.15 for stem rust at Kulumsa, and SRCI=8 for stem rust at Melkasa showed resistance to moderately resistance for yellow rust and stem rust across both locations. The above genotypes probably had more resistance genes for more wheat rust races, which expected across different locations. Therefore, advancing these genotypes to the next stage of breeding

pipelines enable breeders to develop and release widely adapted varieties especially for diseases prone areas.

The top six high yielder genotypes, which gave seven tons per hectares and above, were
EBW222102. EBW222106. EBW222108. EBW222102, EBW222106, EBW222108, EBW222127, EBW222128, and EBW222136. Four of these genotypes: EBW222102, EBW222106, EBW222108, and EBW222128, were highly resistance for stem rust and yellow rust at Kulumsa. The remaining two genotypes, EBW222127 SRCI=40 and EBW222136SRCI=37, were resistance for yellow rust but susceptible for stem rust at Kulumsa (Table 3). Giving high yield in the presence of the disease on the genotypes is linked with adult plant resistance gen APR gene [31,32]. It is a durable rust resistance, sometime called slow rusting, in which the genotype may be susceptible at seedling stage but give high yield without significant vield loss due to the diseases [33,34,35]. All the above six genotypes were resistance for yellow rust at Kulumsa. On the other hand, the five top highly susceptible genotypes for yellow rust, EBW222115 YRCI=25, EBW222123 YRCI=25.5, EBW222134 YRCI=34, EBW222140 YRCI=34, and EBW222146 YRCI=35 delivered lower yields 3.89 t/ha, 5.3 t/ha, 4.48 2.81t/ha, and, 3.38 consecutively (Table 3). Stem Rust Coefficient of infection (SRCI) and Yellow Rust Coefficient Infection (YRCI) less than five showed that, no susceptible (S) reaction; and less than ten considered as resistant to moderately resistant for the two rusts.

Most of the time, yellow rust doesn't occur at Melkasa. Stem rust is an important wheat rust disease across low land wheat producing areas like Melkasa. About eighteen genotypes were susceptible for stem rust diseases with coefficient of infection YRCI≥30. Genotypes: EBW222111, EBW222126, EBW222129, EBW222134, and EBW222142 delivered high yield, greater than or equal to 2 t/ha at Melkasa (Table 3). Their responses for stem rust disease at Melkasa were resistance to moderately resistance except EBW222134, which was susceptible. Stem Rust Coefficients of Infection SRCI for the above genotypes were: EBW222111 SRCI=3, EBW222126 SRCI= 4, EBW222129 SRCI=16, EBW222134 SRCI=30, EBW222142 SRCI=8. Therefore, EBW222111, EBW222126, and EBW222142 considered as resistant genotypes for stem rust at Melkasa and genotype EBW222129 was moderately

resistant to moderately susceptible for stem rust disease [36-41].

4. CONCLUSION

The responss of the genotypes for wheat stem rust diseases and wheat yellow rust diseases were varying. A range of highly resistance genotypes for both disease to highly susceptible genotypes for both disease were observed in the study materials. Also, some genotypes showed resistance for either of the two wheat rust diseases. Therefore, resistance genotypes for yellow rust diseases will be advanced to the trial that will be set for yellow prone areas and genotypes resistance for stem rust diseases will be advance to trial that will be set for stem rust prone areas.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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