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Exploring Bread Wheat (*Triticum aestivum L.*) Accessions Conserved in the Ethiopian Biodiversity Institute Gene Bank to Identify New Sources of Adult Plant Resistance to Yellow (*Puccinia striiformis f.sp. tritici*) and Stem Rust (*Puccinia graminis f.sp. tritici*) Diseases

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Abstract

Wheat is a key food security grain crop in the world including Ethiopia. The government of Ethiopia is dedicated to self-reliance through initiatives such as wheat area extension, irrigation enlargement and yield gap cessation. However, the stem rust and yellow rust currently poses the greatest threat to wheat production due to the emergence of the virulent race of the Puccinia graminis f.sp. tritici, and warm temperature adapted yellow rust (*Puccinia striiformis f.sp. tritici*), races leading to about to 100% yield losses. This study was executed aiming at evaluating one hundred thirty nine bread wheat accessions and six check varieties on dedicated nurseries under natural infections for both stem and yellow rust diseases. Entries were field established in augmented design across two stripe rust hot-spot locations(Meraro and Bekoji) and one stem rust hot spot location for three ensuing growing season 2020-2022 for their slow rusting characteristics. Slow rusting resistance at the adult-plant stage was evaluated through the determination of final rust severity (FRS) and average coefficient of infection (ACI). Based on the summarized information's of final rust severity and average coefficient of infections, most of the tested accessions and all checks displayed higher diseases severity. But five accessions with identification code 31346, 31405, 31643, 34209 and 34797 had low values of FRS and ACI across locations and over years were regarded as good slow rusting accessions. These accessions with high stem rust and yellow rust resistance are suggested for yield trials and release and/or could be backcrossed to the adapted and

high yielding but susceptible Ethiopian wheat varieties to prevent further wheat yield declines as well as to broaden the genetic diversity of the available wheat germplasms.

Keywords: Bread Wheat; Accessions; Stem Rust; Yellow Rust; Diseases Severity; Yellow Rust

Introduction

Bread wheat (*Triticum aestivum L.*) is one of the staple food crops and a major diet that is consumed by more than 2.5 billion people globally [1]. It provides more than 21% of the calories and 20% of the protein and its demand in the developing world is growing year after year [2]. It is grown on a predictable 217 million ha, making it the most broadly cultivated crop in the world, and in terms of production, it accounts for 752 M tonnes [3]. In Ethiopia, bread wheat (*Triticum aestivum L.*) is one of the leading grain crops in terms of production and consumption; becoming a major crop for improving food security in the country [4]. Nationally, bread wheat is projected to give 12% to the daily per capita calorie intake, making it the third most key contributor to nationwide calorie intake, after maize (Zea mays) and sorghum (Sorghum bicolor) [5]. It is cultivated under rain fed, irrigation and belg season production conditions of which rainfed takes the largest percentage. According to CSA [6] data, more than 5 million farmers grow wheat on a total area of 2.1 million hectares with a volume of 6.7 million tons of yearly grain production and average productivity of 3.0 and 4.0 t/ha under rain-fed and irrigated conditions respectively.

The universal wheat breeding efforts have made notable contributions to the advancement of wheat yield potential. Nevertheless, the yearly growth rate of wheat yield has been static in the recent decade [7]. In Ethiopia, the national average productivity is estimated to be 3 and 4tha^{-1} under rain-fed and irrigated production conditions respectively which is by far below experimental yields of above 5.1 tons ha^{-1} [8,9]. The sustainable production and supply of wheat for upcoming generations including Ethiopia is threatened and challenged by quick population growth, climate changes, and various biotic and abiotic stresses [7]. Among biotic stress, yellow and stem rusts are the most destructive ailments [10] resulting in yield losses estimated at 45 million tons of wheat (valued at $9 billion due to wheat diseases and other pests annually [11].

Likewise, wheat stem rust and wheat stripe rust are the major biotic serious threats to wheat production in Ethiopia. The yearly loss due to wheat rusts in Ethiopia is estimated at 10s of millions of USD [12]. Frequent epidemics have arisen during the last decades, including a major wheat yellow rust epidemic in 2010 [13] and a large wheat stem rust plague caused by race TKTTF in 2013 with yield losses up to 100% and average losses of approximately 50% [14]. This is largely due to the breakdown of prevailing resistance genes and gradual alteration of new strains in warmer regions, the pathogen's ability to mutate, multiply rapidly, and use its air-borne dispersal mechanism from one field to another and even over long distances [15].

Various management options have been recommended like chemical, biological, cultural and other management approaches. The most effective strategy to control yellow and stem rust diseases is breeding and growing resistant cultivars, as this approach has no additional cost to farmers and is environmentally desirable [16]. In the last six decades, more than 102 bread wheat varieties have been released in Ethiopia to increase yields and improve disease resistance [17,]. However, the promising varieties developed were susceptible to breakdown and regularly short-lived. Therefore, searching for a new source of resistance to yellow and stem rust diseases from new bread wheat genotypes under field conditions in different locations is requisite to cope with the developing virulent races of the pathogen.

Materials and Methods

Descriptions of the Study Area

Field experiments were implemented at two yellow rust dedicated locations viz; Meraro and Bekoji (Research Stations) and one stem rust hotspot location; Kulumsa (Main Research Center) of Arsi Zone South Eastern Ethiopia. Meraro substation is positioned at 07°24'27''N, 39°14'56''E and 2990 m asl. The site's mean annual rainfall is 1196 mm suggesting great highland and frostiness agroecology. The lowermost and utmost temperature is 5.7°C and 18.1°C, in their order. Bekoji station is located at latitude 07°32'37''N and longitude 39°15'21''E with an altitude of 2780 meters above sea level. The extreme and least weather hotness was 3.8°C and 20.4°C respectively with yearly rainfall 939 mm. Both Meraro and Bekoji test sites were perfect for conducting yellow rust phenotyping works. Thus, enables to choice of auspicious germplasm against yellow rust under natural infection. Kulumsa's test site is situated on 08°01'10''N, 39°09'11''E and on 2200 meters above mean sea level. The site gets a mean yearly rainfall of 820 mm demonstrating optimum midland and high rainfall agroecology. The regular mean least and supreme hotness are 10.5°C and 22.8°C, respectively. The place's prominent soil is loam type, which is fertile. The center's farmland has very appropriate weather to conduct wheat germplasm evaluation against stem rust in the presence of spreader rows. With the aforementioned spatial climatic information, Meraro and Bekoji in the testing years periods of 2020 to 2022 were dedicated sites for screening yellow rust while Kulumsa's main site was a dedicated site for screening to stem rust.

Planting Materials

In this study, 139 germplasm accessions of cultivated wheat species Triticum aestivum (bread wheat), were conserved in the National Genebank of Ethiopian Biodiversity Institute along with six commercial cultivars acquisition from Ethiopia national bread wheat breeding and improvement center, Kulumsa that served as susceptible checks were employed in the evaluation process. The majority of cultivars were previously mega varieties in the areas where the experiment was conducted. However, become susceptible either to stem or yellow rust thus used as local susceptible checks in field phenotyping screening works.

Number	Accesion ID	Number	Accesion ID	Number	Accesion ID	Number	Accesion ID	Number	Accesion ID	Number	Accesion ID
1	5011	26	31247	51	31595	76	34225	101	34480	126	34754
2	5200	27	31257	52	31596	77	34228	102	34608	127	34786
3	5380	28	31258	53	31600	78	34231	103	34621	128	34797
4	5548	29	31261	54	31605	79	34233	104	34626	129	34873
5	5585	30	31270	55	31619	80	34236	105	34633	130	34900
6	5661	31	31273	56	31621	81	34239	106	34640	131	34902
7	5694	32	31301	57	31623	82	34245	107	34642	132	34906
8	5774	33	31346	58	31627	83	34248	108	34655	133	34909
9	6873	34	31351	59	31630	84	34255	109	34658	134	34942
10	6883	35	31355	60	31632	85	34267	110	34667	135	206691
11	6930	36	31357	61	31635	86	34270	111	34676	136	206693
12	7917	37	31386	62	31638	87	34280	112	34688	137	206697
13	8390	38	31401	63	31643	88	34284	113	34698	138	213209
14	8393	39	31405	64	31792	89	34291	114	34704	139	214716
15	31130	40	31406	65	31813	90	34309	115	34706	Check varieties	
16	31134	41	31412	66	31816	91	34311	116	34710	140	Kubsa
17	31147	42	31438	67	31818	92	34329	117	34712	141	Morocco
18	31161	43	43 31443	68	68 31832	93	34332	118	34714	142	Diga
19	31164	44	31510	69	31836	94	34335	119	34718	143	PbW343
20	31186	45	31543	70	31841	95	34341	120	34720	144	Kingbird
21	31196	46	31554	71	31848	96	34349	121	34722	145	Wane
22	31204	47	31573	72	31856	97	34362	122	34730		
23	31224	48	31583	73	34209	98	34405	123	34742		
24	31233	49	31585	74	34218	99	34419	124	34745		
25	31244	50	31593	75	34220	100	34435	125	34752		

Table 1: List of planting bread wheat accessions and check varieties.

A mixture of three bread wheat cultivars namely; Morocco (universal susceptible cultivar both to stem and yellow rust races) and; PBW343 and Digalu which are susceptible for most of the stem and yellow rust races prevalent in Ethiopia were used as spreader rows in the field nurseries. The spreader cultivars were planted 10 days earlier than the treatments; thus helping to develop uniform disease pressure and is ideal to evaluate intended accessions.

Field Plan and Disease Assessment

To evaluate the intensity of slow rusting of wheat genotypes in the field, test accessions and checks were prepared in augmented design. The entries were customary in plots comprising double rows of 1m long with the spacing of 0.2m intra-row, 1m between blocks and 0.5 m between plots. Entries of treatments were planted with a seed rate of

150 kgha^{-1}, DAP and urea fertilizers were applied based on the suggested rate to the area. Weeds were coped by hand weeding. Disease severity notes were taken by estimating the approximate percentage of leaf area exaggerated using a modified Cobb scale [19]. Disease data capturing was underway from the first appearance of yellow and stem rust on the susceptible checks and continued every 14 days from all plants awaiting the early dough stage. Scorings of disease severity and response were noted together with severity head ensued by infection type.

Results and Discussions

Phenotyping of (139) bread wheat accessions and (6) check varieties for yellow and stem rust under field conditions was done at Kulumsa Agricultural Research Center; Bekoji and Meraro stations of Kulumsa Research Center for three subsequent cropping seasons (2020-2022). The growing seasons had mostly consistent weather conditions. Thus, the over-years disease data; terminal rust severity and coefficient of infections - are summarized in their means form.

There was a wide variation among test bread wheat accessions to yellow rust 0 to 90% at Meraro and Bekoji and stem rust 0 to 80 at Kulumsa. There was heavy disease pressure during the seasons of testing as indicated by the susceptible checks in Kubsa and Morocco which had 90% susceptibility at both locations. Out of 139 accessions 3(2.1%), 0, 9(6.4%), 2(1.4%), 7(5%), 20(14.4%), 42(30.2%) and 56(40.1%) accessions; and 7(5.1%), 0, 11(7.9%), 5(3.6), 4(2.8), 18(12.9%), 22(15.8%) and 72(51.8%) bread wheat accessions showed immune, resistant, resistant to moderately resistant, moderately resistant, moderately resistant-moderately susceptible reaction, moderately susceptible, moderately susceptible to susceptible and susceptible reactions to yellow rust at Meraro and Bekoji in their order (Figure 1). Similarly at Kulumsa, 7(5.2%), 4(2.8%), 14(10.1%), 3(2.1%),7(5.2%),23(16.5%), 35(25.2%) and 46(33.1) accessions showed immune, resistant, resistant to moderately resistant, moderately resistant, moderately resistant to susceptible, moderately susceptible to susceptible and susceptible reaction to the prevalent stem rust races over years.

Final Rust Severity

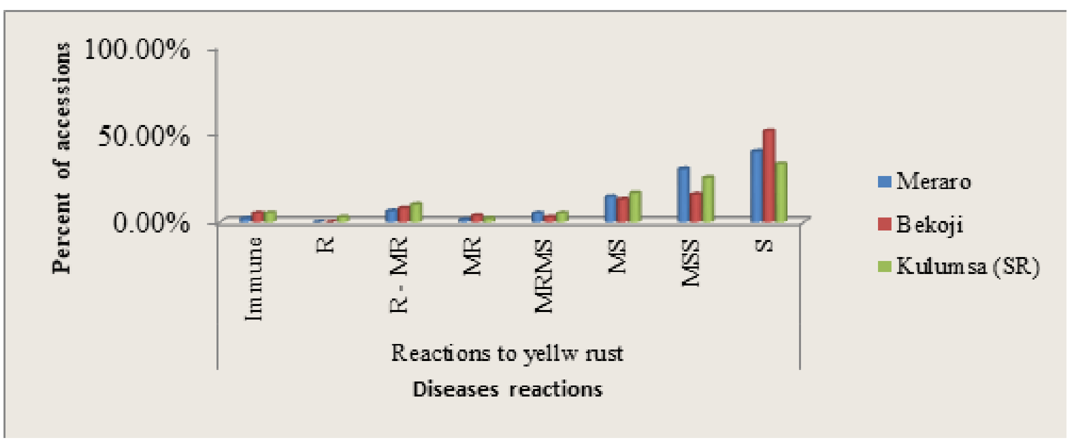

Figure 1: The three-year mean response of 139 bread-wheat accessions to yellow and stem rust diseases across locations.

Taking the year average terminal rust severity and the manifested response, the result of the present study revealed very limited proportions of the lines were promising with almost equal proportion of the accessions showing similar responses. According to Parlevliet, et al. [20]; final rust severity signifies the collective result of all resistance factors during the progress of epidemics. Formerly, many scholars Ali, et al. [21-23] also used final severity as a parameter to assess the slow rusting behavior of wheat lines. Depending on final rust severity, the test accessions bread wheat lines showed different reactions to the prevalent yellow and stem rust strains. Accessions that showed resistant to moderately resistant reactions both to yellow and stem rust diseases are suspected to carry many minor genes but are needed for seedling phenotyping for conformation [25]. Once the genes carried by those accessions are known, possible to incorporate resistance genes into high-yielding and domestically acclimatized bread wheat cultivars and release new stripe rust-resistant varieties for large-scale production by close producers.

Out of (6) commercially released cultivars used as a check in this experiment, all had exhibited susceptible reactions except Wane with different severity levels against yellow. Similarly, all the checks except Wane and Kingbird showed susceptible reactions to stem rust revealing sufficient epidemics (Table 2).

Varieties	Bekoji (yellow rust)		Meraro (Yellow rust)		Kulumsa (Stem rust)	
	FRS	CI	FRS	CI	FRS	CI
Kubsa	90s	90	90s	70	90s	90
Morocco	90s	90	90s	90	90s	90
Digalu	80s	80	70s	70	70s	70
PbW343	90s	90	80S	80	80s	80
Kingbird	60s	60	70s	70	30ms	24
Wane	40ms	36	40ms	36	20ms	16

FRS: final rust severity; CI: coefficient of infections.

Table 2: The three-year mean disease severity and coefficient of infections of check varieties to yellow rust and stem rust diseases across locations during 2020-2022.

Coefficients of Infection

The data on final disease severity was combined with the fixed values of disease response to calculate a coefficient of infection CI. There was heavy disease pressure during the seasons of testing as indicated by the susceptible checks Kubsa and Morcco, which had 90% susceptibility over three years. In the present study, at KARC, the range of coefficient of infections of test bread wheat accessions to stem rust was 0 to 90 % (Table 1).

Locations	Reactions to yellow rust and stem rust		
	Minimum	Maximum	Mean
Meraro (YR)	0	90	35.5
Bekoji (YR)	0	90	29.7
Kulumsa (SR)	0	80	26.3

YR: yellow rust; SR: stem rust.

Table 3: Three years mean coefficients of infections of 139 bread wheat accessions to yellow and stem rust diseases across locations during 2020 to 2022.

The three-year mean coefficient of infections of the test accessions to stem rust diseases at Kulumsa was 26.3%. The majority of test accessions and all the checks displayed moderate to higher average coefficients of infections exceeding 20 with the mean coefficients of infections of the test accessions. Among all eleven accessions showed (CI) values ranged from 0-20, 24 accessions showed CI values ranged from 21-40 and the remaining 104 accessions showed CI values ranged greater than 40.

As for stem rust yellow rust infection was high at both Bekoji and Meraro testing sites, allowing for clear and definite scoring of field reaction. At Bekoji, seven accessions showed CI values ranging between 0–20, twenty accessions had CI values of 21–40, and 112 accessions including susceptible check varieties displayed CI values exceeding 40. Meanwhile, at Meraro, four accessions showed CI values ranging between 0–20, seventeen accessions had CI values of 21–40, and One hundred eighteen accessions including susceptible check varieties displayed CI values exceeding 40. Despite the heavy stem and yellow rust disease pressure in the field during the years 2020, 2021 and 2022, some lines remained resistant across locations (Table 3). The trace reaction noted could be associated with hypersensitivity whereby fungal infection signals a defense mechanism leading to cell collapse which restricts further disease spread [24]. Formerly scholars like Hundie, et al. [25], Abebele, et al. [26] and Asnake, et al. [27] examined many bread wheat germplasms against stem and yellow rust diseases and found promising results that were able to replace previously mega cultivars but ignored from production due to rust diseases. In addition, the current results were consistent with findings by Worku, et al. [28] that tested three hundred and six elite breeding lines against stem rust at hot spots in Ethiopia and Kenya and 32% of lines were found resistant. However, need to know the seedling response as well. An appropriate crop improvement strategy like the use of inter-specific and remote crosses or even the direct transfer of these resistance through backcrosses could be adopted to improve the adapted but highly susceptible wheat varieties being grown widely in Ethiopia [29,30].

Number	Accession ID	Meraro (YR)		Bekoji (YR)		Kulumsa (SR)	
		MFRS	MCI	MFRS	MCI	MFRS	MCI
1	31346	20MS	16	25MS	20	25MS	20
2	31405	TMR	0.8	5MR	2	TMR	0.8
3	31643	5MR	2	TMR	0.8	TMR	0.8
4	34209	10MR	4	10MR	4	10MS	8
5	34797	25MS	20	20MS	16	5MS	4

MFRS= Mean final rust severity(three years); Mean coefficient of infections (three years).

Table 4: Promising bread wheat accessions displaying fewer infections to both stem and yellow rust diseases over three years across locations.

The genotypes that displayed resistant to moderately resistant responses are of great importance to achieving effective breeding for durable resistance of both to stem and yellow rust. While accessions showed immune reaction (vertical resistance) and host-pathogen compatible reactions are suggested to not to be for resistance breeding.

Conclusion

Ruminating the hostile impact of rusts diseases on the efficacious production of wheat in Ethiopia and to guarantee food security to the millions of resource poor Ethiopian farmers and other parts of the developing and least developed domain, it is crucial to exploration for new resistance sources for these diseases to minimize the yield losses under fluctuating climate. Appraisal of the entire cultivated wheat collection of Ethiopian genebank at various hotspots in the present study allowed selecting of potential resistance sources. Accordingly, the current study indicated that there was a wide range of variation among accessions showing diversity in diseases severities and reactions across different agro-ecological zones under natural infections in the presence of spreaders. Among 39 tested accessions 5(3.6%) namely; 31346, 31405, 31643, 34209 and 34797 showed lower diseases severities to stem and yellow rust diseases. These genotypes are suspected to carry many minor genes responsible for adult plant rust resistance and are very useful for release as a commercial variety or use as a parent for stem and yellow rust resistance.

Data Availability Statement

The datasets generated throughout and/or analyzed in the current study are available from the corresponding author on reasonable demand.

Ethics Approval and Consent to Participate

The germplasms/accessions used in the present study were bread wheat acquired from the Ethiopian Biodiversity Conservation Gene Bank and the Ethiopian National Bread Wheat Breeding and Improvement Center(Kulumsa). According to the Ethiopian national plant germplasms use policy, the corresponding author is fully authorized to collect, conserve and characterize the germplasms obtained from national plant genetic resources gene bank as well as local crop genetic resources for breeding improved varieties.

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