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Research Article

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Characterizing Bread Wheat Germplasms for Slow Rusting To Stem Rust through Field and Seedling Phenotyping Approaches at Kulumsa, South Eastern Ethiopia

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Abstract

Wheat is a principal food produce in the world including Ethiopia. It gets the paramount attention of the Ethiopian government among the top 10 cereal crops to assure food security and start export. However, biotic stress especially yellow and stem rusts remained a constant production bottlenecks posing yearly economic losses of 10s of millions USD. Stem rust is being threat to wheat production due to the evolution of the virulent race of the *Puccinia graminis* f. sp *tritici*, races like Ug99 (TTKS) and its variants with rapid dispersal towards major growing areas of the country. This experiment was conducted with the aim of evaluating thirty-three advanced bread wheat genotypes and twelve commercially released varieties for both field and seedling resistance to stem rust. Treatments were grown in augmented design at field conditions and completely randomized in green house at Kulumsa agricultural research center in 2023/2024 offseason growing season respectively. Accordingly, 25(75.75%) advanced genotypes and 2(16.66%) of varieties had shown low diseases severities (FRS and ACI below 30 and 20) respectively at field conditions. However, only 11(33.33%) advanced genotypes namely; EBW170072, EBW160066, EBW170172, EBW170051, EBW160002, EBW222680, EBW170056, EBW170059, EBW170058, EBW160065 and EBW224096; and 2(16.66%) varieties Abay and Boru showed higher diseases infection type at seedling stage revealing their true slow rusting to stem rust. Therefore, the aforementioned lines can directly be exploited as resistant parental lines in the regional wheat breeding program, or suggested to be forward-looking to regional yield trials for the development of high yielding and stem rust resistant cultivars to battle with the continually changing races of stem rust and avert further wheat yield declines.

Keywords: Slow Rusting; Seedling Resistance; Final Rust Severity; Coefficient of Infections and Wheat Genotypes

Introduction

Common wheat (*Triticum aestivum* L.) occupied an important position amongst the grain crops in the world which provides food resources to more than 2.5billions of people globally [1]. It is cultivated on an estimated 217 million ha, making it the most widely grown crop in the world, and in terms of production it accounts for 752 M tones [2]. In the last eight decades, the production of wheat showed dramatic increment (from 222.4 M t in 1960 to 760.9 M t in 2020). This has ensued through yield widening associated with the development of high-yielding cultivars, use of large amount of agrochemicals and better agronomic management practices [3]. In Africa, Ethiopia is the second largest producer of wheat next to Egypt and produces 6.7 Mt. This is equivalent to 21.7% of grain produced and 18.3% wheat area covered in Africa [4]. According to (CSA, 2021) Ethiopian Statistics Services wheat accounts for about 17% total grains harvested being grown on area (2.1 million ha), 21 % total production and employment for close to 5million subsistence smallholder farmers.

Unlike areal expansion and improvement in volume of grain production, wheat production and productivity in Ethiopia is quite low compared with the country's potential [5]. This low productivity is attributed to numerous aspects including high price of fertilizers, deficiency of improved seeds, high seed prices and diseases such as the rusts [6]. Among many production bottlenecks, fungal pathogens especially stem rust caused by the fungus *Puccinia graminis* f. sp. *tritici,* stripe (yellow) caused by *Puccinia striiformis* f.sp*. tritici* and fusarium head blight caused by Fusarium spp. comes forefront challenges [7,8].

Under favorable environmental conditions, stem rust can cause yield losses of up to 100% on susceptible wheat varieties. In Ethiopia, an epidemic of stem rust of wheat occurred regularly for instance in 1972, due to the loss of resistance in cultivar Lakech, which was grown on large area. Since then, recurrent epidemics have occurred during the last decades; including a major wheat stem rust outbreak caused by race TKTTF in 2013 resulting yield losses up to 100% and average losses of approximately 50%.

Ailment regulator is likely with fungicides; nevertheless they are affordable for resource poor farmers in unindustrialized countries [9]. Growing of wheat varieties with resilient resistance genes also known as slow rusting, adult plant resistance or partial resistance is the most cost-effective, effective, and environmentally sustainable method of stem rust control. Since the start of development of improved wheat varieties, more than 80 bread wheat varieties have been released in Ethiopia with the aim of increasing yields and improving disease resistance [10]. Most of these varieties were carried race-specific genes and function in a gene-for-gene fashion [11]. Genetic fingerprinting has revealed that newly released rust resistant bread wheat varieties have been largely adopted through Ethiopia [12]. Regardless of large cultivar choice, a few numbers of varieties cover most of the cultivated area. Virulence in the pathogen population has been evolving rapidly following the deployment of race-specific resistance genes, often associated with a boom and-bust cycle.

Developing and use of varieties having race non-specific resistance genes is the best strategy for breeding to long-lasting stem rust resistance. Such custom of resistance is effective against a wide array of stem rust races with an ideal level of expression at the adult plant stages [11,13]. Numerous wheat varieties grown in Ethiopia that were inherited with major genes for stem rust resistance have been more recurrently surrendered to stem rust epiphytotic and frequently fallen out of production even with sustained desired agronomic qualities [14]. Hence, examining and ascertaining new sources of resistance to stem rust is a continual course in stem rust breeding programs. Thus, the present study was conducted to distinguish the occurrence of adult plant resistance to stem rust in advanced bread wheat genotypes and varieties through seedling and field phenotyping approaches.

Materials and Methods

Descriptions of the study site

To examine 33 advanced bread wheat genotypes and 12 released varieties (Figure 1) for their slow rusting resistance to stem rust, a field experiment was executed during 2023/2024 irrigation cropping seasons (December to April) at Kulumsa agricultural research center. Kulumsa agricultural research center is situated at 08o 01' 10'' N, 39o 09' 11'' E and at 2200 meters above sea level (m.a.s.l). The center receives mean annual rainfall of 820 mm signifying highland and high rainfall agro ecology. The monthly average minimum and maximum temperature is 10.5 and 22.8°C in that order. The sites dominant soil type is loam, which is fertile [15].

Planting materials

A total of 33 elite bread wheat lines that were nominated and advanced from intercontinental nurseries as well as 12 commercially released varieties, including susceptible and resistant checks, were used in the study. The advanced lines included in the study had different origins viz; three (ICARD); sixteen (CIMMYT); fourteen (local crosses), and twelve released varieties developed from the national bread wheat breeding and improvement program including susceptible checks.

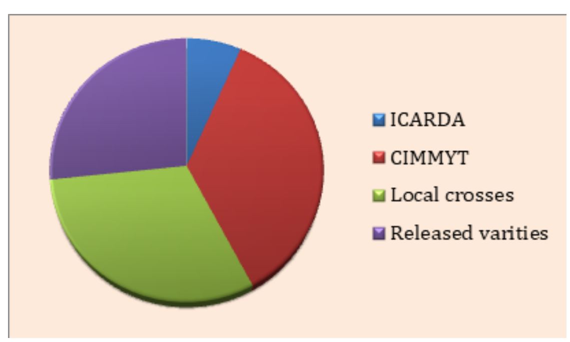

Figure 1: Sources of wheat germplasms examined in the study.

Employed Stem rust race

One dominant P. *graminis* f. sp. *tritici* race namely; TKKTF, identified at Ambo agricultural research center from isolates collected in2021/2022 from different parts of wheat growing parts of Ethiopia was engaged both field and seedling assay of this experiment. This race is identified as virulent to Sr genes of 5,21,9e,7b,6,8a,9g, ,9b,30,17,9a,9d,10,Tmp, 38, and McNeil [16]; and is common to East African region. It broken most of the resistant genes carried in Ethiopian bread wheat varieties.

Field phenotyping and diseases scoring

Stem rust disease severity notes were taken by estimating the approximate percentage of plsnt area affected using modified Cobb scale [17]. Note taking was started from the first appearance of stem rust on the susceptible check and continued every 14 days from all plants until the early dough stage. Readings of disease severity and reaction were recorded together with severity first followed by host response. The numerical values of final rust severity are multiplied with fixed values of response to compute coefficient of infections.

Seedling phenotyping and data assessments

The seedling phenotyping in the greenhouse for determining seedling resistance was executed at Kulumsa agricultural research center during the 2024 under controlled environment. The spores were increased by inoculating onto a totally stem rust susceptible host McNair (without Sr gene) to get enough inoculum. Seedlings of the wheat germplasms along with the susceptible check, McNair, were grown in 7 cm × 7 cm × 6 cm plastic pots filled with compost, light soil and sand at a 1:1:1 ratio (v/v/v). McNair was applied to establish the viability of spores inoculated to the lines. Spores of each race were suspended in light weight mineral oil, Soltrol 170 and adjusted to 1 × 105 sporesml^{-1}. The spore suspension of the pathogen race was then inoculated on 7 day-old seedlings using atomized inoculators. Seedlings were moistened with fine droplets of distilled water 30 min after inoculation and transferred into a dew chamber for 18 h darkness at 18 to 22°C followed by disclosure to light for 4hrs to deliver promising situations for infection. After ventilation for 2hrs, the seedlings were transferred to glass compartments in the greenhouse where conditions were regulated at 12hrs photoperiod; at a temperature of 18 to 25 °C and relative humidity: 60 to 70%. Seedling infection types (IT) were took 14 days post inoculations, based on a 0–4 scale [18]. Infection types readings of 3 (medium-size uredinia with/without chlorosis) and 4 (large uredinia without chlorosis or necrosis) had considered as compatible reactions, while readings 0 (immune or fleck), 1 (small active spores with necrosis), and 2 (small to medium uredinia with discoloration) were assumed as discordant. The differences were refined by adjusting characters as follows: uredinia somewhat smaller than normal for the infection type; +, uredinia somewhat larger than usual for the infection type.

Results and Discussions

Final rust severity

There was broad variation in the final stem rust severity ranging from zero to 100% during the 2023/2024 offseason growing season at Kulumsa agricultural research center. Diverse field reactions ranging from immune to susceptible (S) responses were displayed (Table 1). The susceptible check Morocco, displayed the highest disease severity of 100% with completely susceptible (S) responses indicating that an adequate epidemic pressure was established over the season for field experiment.

According to Parlevliet, et al. [13] final rust severity signifies the collective result of all resistance factors during the progress of epidemics. Accordingly, among tested germplasms 2 ICARDA crosses, 14 CIMMYT crosses; 10 local crosses and 1 variety showed a final rust severity below 30%. While 1 ICARDA cross; 1 CIMMYT cross; 3 local crosses and 2 varieties were showed 31-50% diseases severities which signifies moderately resistant reaction. On contrary, none of ICARDA crosses; 1 CIMMYT cross; 1 local cross and 9 varieties were displayed heavy diseases infestation and identified as susceptible.

Number	Genotype	Field response				Seedling response
		Origin	Reaction	FRS	ACI	Infection type
1	EBW214061	ICARDA	S	40	40	3
2	EBW170072	Local cross	TMR	2	0.4	3+
3	EBW160066	Local cross	MS	10	9	3
4	EBW202406	CIMMYT	MR	2	0.4	3
5	EBW222923	CIMMYT	MSS	30	27	;
6	EBW170172	Local cross	MR	2	0.4	3

7	EBW222059	CIMMYT	MR	2	0.4	;
8	EBW202211	CIMMYT	MSS	10	9	2
9	EBW170051	Local cross	MSS	20	18	3
10	EBW160002	Local cross	S	40	40	3-
12	EBW160017	Local cross	S	60	50	3
13	EBW222680	CIMMYT	MR	10	4	3+
14	EBW202471	CIMMYT	S	60	50	2
15	EBW214045	ICARDA	MR	5	2	1
16	EBW212354	CIMMYT	Immune	0	0	1
17	EBW192345	CIMMYT	MSS	20	18	2
18	EBW170179	Local cross	S	40	40	3
19	EBW170056	Local cross	MSS	30	27	3
20	EBW170391	Local cross	MR	10	4	2
21	EBW170390	Local cross	MSS	30	27	2-
22	EBW170059	Local cross	MSS	20	18	3+
23	EBW202276	CIMMYT	S	40	40	1
24	EBW160058	Local cross	S	40	40	2
25	EBW212106	CIMMYT	MS	20	16	;
26	EBW222895	CIMMYT	MSS	30	27	2-
27	EBW170058	Local cross	MSS	30	27	3+
28	EBW222252	CIMMYT	MR	2	0.4	;
29	EBW212705	CIMMYT	MS	20	18	;
30	EBW222241	CIMMYT	MR	2	0.4	1
31	EBW202020	CIMMYT	MR	2	0.4	;
32	EBW160065	Local cross	MSS	30	27	3-
33	EBW224096	ICARDA	MSS	30	27	3-
	Varieties	**Center of release**				
1	Lemu	Kulumsa, EIAR	S	90	90	3
2	Deka	Kulumsa, EIAR	S	70	70	3
3	Abay	Kulumsa, EIAR	MS	5	4	3
4	Shaki	Kulumsa, EIAR	S	40	40	3
5	Boru	Kulumsa, EIAR	MSS	30	27	3
6	Balcha	Kulumsa, EIAR	S	80	80	3
7	Kingbird	Kulumsa, EIAR	S	90	90	3
8	Wane	Kulumsa, EIAR	S	90	90	3-
9	Ogolcho	Kulumsa, EIAR	S	90	90	3+
10	Hidassie	Kulumsa, EIAR	S	90	90	3
11	Morocco	Kulumsa, EIAR	S	100	100	4
12	Danda	Kulumsa, EIAR	S	90	100	3
13	McNair	-	-	-	-	4

Table 1: Sources, field and seedling responses of bread wheat genotypes against virulent stem rust race (TKKTF)

EBW, Ethiopian bread wheat; EIAR, Ethiopian Institute of Agricultural Research

The results of the current finding revealed that reasonable percentage of advanced lines regardless of their origin have showed noble diseases resistant types of reaction with a lower disease severity. Conversely, most of the released wheat varieties including widely grown cultivars in areas of the study disclosed highly susceptible reaction. Formerly, scholars such as Worku, et al. [14] used final rust severity to assess slow rusting behavior of wheat lines. Overall, most of the tested germplasms included in this study showed incompatible host to pathogen reaction revealing that germplasms possessing slow rusting genes. According to Singh, et al. [19] bread wheat lines that show high and moderate level of FRS and ACI could have durable resistance, which can serve as good parents for breeding. The current finding were agreed with findings by Bacha, et al. [20] that tested thirty two durum and thirty bread wheat genotypes against stem rust at field on hotspots and seedling stage under greenhouse conditions of Ethiopia and found five resistant lines. Similarly Hunde, et al. evaluated ninety seven bread wheat and fourteen durum wheat genotypes against four virulent stem rust races both at field and seedling condition and found four genotype with adult plant resistance. Moreover, Nzuve, et al. [21-26] had evaluated twenty five bread wheat genotypes for slow rusting resistance at seedling and field conditions and discovered five genotypes with adult plant resistance.

Coefficient of infection

The records on disease severity and fixed values of host response were combined to compute CI (Table 1). As expressed by Ali, et al., genotypes with coefficient of infections values of 0-20, 21-40, 41-60 were considered as having high, moderate and low levels of slow rusting resistance.

Accordingly, among tested germplasms 1 ICARDA Crosse, 12 CIMMYT crosses; 6 local crosses and 1 variety had displayed coefficients of infections below 0-20 and are regarded as promising lines carried slow rusting. Whereas 2 ICARDA crosses; 2 CIMMYT crosses; 7 local crosses and 2 commercially released varieties were manifested 21-40 coefficients of infections. On contrary, none of ICARDA crosses; 2 CIMMYT crosses; 1 local cross and 9 varieties displayed coefficients of infections exceeding 41. The present study revealed that majority of the advanced genotypes 57.7% in regardless of their sources showed higher slow rusting characteristics to stem rust. However, most of commercial wheat cultivars including Deka and Shaki had CI values exceeding 41 indicating the broken of their stem rust resistant genes and going to dropping out of production in the absence of fungicides. Therefore, regular searching of new sources of resistant genes is becoming mandatory. Formerly, several scholars for instance [14] assessed thousands of germplasms for adult plant rusting resistance to wheat stem rust using coefficient of infection and reported the presence of different polygenic resistance in the test lines. This study also agreed with findings of Worku, et al. [14] who examined three hundred and six elite breeding lines against stem rust at hot spots of East Africa and discovered significant percentage of lines were adult plant resistant.

Seedling reaction of wheat genotypes to stem rust race

The universal stem rust susceptible check varieties MacNair and Morocco were displayed the highest infection types; 4 which are completely susceptible (S) responses indicating that an adequate epidemic pressure was established in green house. Under such favorable conditions neither advanced bread wheat genotypes nor the commercial released varieties had showed immune response at seedling stage in green house. However, 2 ICARDA crosses, 2 CIMMYT crosses, 11 local crosses lines had showed fleck to 2 which signifies incompatible reaction among hosts and pathogen race. All the commercially released wheat varieties displayed host pathogen compatible reaction or susceptible response. As conveyed by Nzuve, et al. [21], wheat plants showing host pathogen compatible reaction at seedling stage and lower disease severities at adult stage are considered polygenic. Moreover Hunde, et al., had demonstrated 843 bread wheat genotypes and cultivars under field conditions and seedling response in green house and identified 52 lines were identified as slow rusting resistant. More recently, Bacha, et al. [20, 27-29] had evaluated bread 32 durum wheat and 30 advanced bread wheat lines for their slow rusting under field and greenhouse conditions and four and two durum and bread wheat germplasms were identified as true slow rusting.

Conclusion

Unlike commercially released varieties, most of the appraised advanced genotypes exhibited better field resistance in high disease stress presented by susceptible check. However, few genotypes were identified as true slow rusting confirmed by seedling assay. Thus, eleven advanced genotypes namely; EBW170072, EBW160066, EBW170172, EBW170051, EBW160002, EBW222680, EBW170056, EBW170059, EBW170058, EBW160065 and EBW224096; and two commercially released varieties Abay and Boru showed true slow rusting. Therefore, the aforementioned lines can directly be exploited as resistant parental lines in the regional wheat breeding programme, or suggested to be forward-looking to regional yield trials for the development of high yielding and stem rust resistant cultivars to battle with the continually changing races of stem rust.

- **Author's contribution:** All the authors have equally involved in execution of field and greenhouse

experiments; while the corresponding author wrote the paper.

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