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Research Article

EVALUATION OF BREAD WHEAT GERMPLASM FOR ADULT PLANT RESISTANCE TO STEM RUST USING ARTIFICIAL INOCULATION

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ABSTRACT

Wheat stem rust, caused by *Puccinia graminis* f.sp. *tritici*, is a major biotic threat to wheat (*Triticum aestivum* L.) in many wheat-producing countries worldwide, including Ethiopia. The pathogen is capable of continuously evolving new fungal races with high reproductive potential. It can be easily dispersed by wind over large geographic areas, allowing it to attack resistant varieties. Under optimal environmental conditions, it can cause epidemics leading to severe yield losses (up to 100%). A total of 91 germplasms, comprising 81 advanced bread wheat lines and 10 varieties, including the susceptible check Morocco, were planted using an augmented design. The experiment was conducted with artificial inoculation on spreaders to evaluate their slow rusting response against four virulent stem rust races viz. TTKTF, TKKTF, TTKTT, and TTTTF under field conditions at the Kulumsa Agricultural Research Center during the 2022-2023 offseason. Slow rusting resistance at the adult-plant stage was assessed by measuring the final rust severity (FRS) and the average coefficient of infection (ACI). Accordingly, 50 (61.7%) of the advanced lines and 6 (60%) of the released varieties namely Boru, Abay, Shaki, Kakaba, Deka, and Dursa exhibited low levels of FRS and ACI. In contrast, 31 (48.3%) of the advanced lines and 4 (40%) of the varieties showed high FRS and ACI values. The germplasms with low FRS and ACI values were considered to exhibit good slow rusting resistance and could be recommended for release into production and/or used in breeding programs aimed at achieving durable stem rust resistance in Ethiopia.

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INTRODUCTION

Wheat is the most widely cultivated cereal in the world, with over 200 million hectares planted annually (Tubiello, 2023). It is also the most crucial crop for sustaining global food security (Shiferaw et al., 2013). In East Africa, Ethiopia is the leading wheat producer, accounting for about 65% of the total wheat production in sub-Saharan Africa. The area under wheat cultivation in Ethiopia expanded from approximately 1.5 million hectares in 2010 to 2.5 million hectares in 2023. More importantly, productivity increased from 1.8 tons per hectare to about 3 tons per hectare over the same period, reflecting an annual productivity growth rate of about 5% (CIMMYT, 2023; CSA, 2021). Wheat and wheat products contribute 14 percent of the total caloric intake in Ethiopia, making it the second most important food staple, following maize

(19 percent) and ahead of teff, sorghum, and enset (each contributing 10-12 percent) (FAO, 2018). However, increasing wheat production in the face of a changing climate necessitates protection against biotic stresses, which can cause significant yield losses (Singh et al., 2008, 2011; Mukhtar et al., 2018; Mukhtar and Saeed, 2024). Among various biotic challenges, three rust diseases, stem rust, leaf rust, and stripe rust, pose the most significant threats to global wheat production (Murray and Brennan, 2009). All three rust pathogens belong to the genus *Puccinia* and are highly host-specific: *P. graminis* f.sp. *tritici* Erik. & E Henn causes stem rust, *P. triticina* Erik causes leaf rust, and *P. striiformis* West causes stripe rust. Of these, stem rust and stripe rust are the most devastating in Ethiopia (Olivera et al., 2015).

Under suitable weather conditions, stem rust can cause yield losses of up to 100% in susceptible wheat varieties (Roelfs, 1985). The impact of this disease is typically most severe if it becomes widespread before the grain is fully formed. However, yield losses are generally influenced by the resistance level of the cultivar, the prevailing weather conditions, and the timing of the disease onset (Luig, 1985). In East Africa, including Ethiopia, yield losses due to stem rust have been reported to range from 61% to 100%, depending on the susceptibility of the variety and environmental conditions (Eshetu, 1985; Shank, 1994).

In Ethiopia, the first significant stem rust epidemic was recorded in 1972, caused by the loss of resistance in the widely cultivated variety Lakech. Similarly, the second major epidemic and associated yield losses occurred in 1995 when the resistance gene Sr36 was overcome in the cultivar Enkoy. More recently, severe stem rust damage was reported in southern and central Ethiopia between November 2013 and January 2014, with yield losses approaching 100% in the most widely grown wheat variety, “Digalu”, leading to severe food insecurity among farmers dependent on wheat production (Olivera et al., 2015).

To mitigate the damage caused by wheat stem rust, growing tolerant varieties is an environmentally friendly and cost-effective approach. However, breeding for wheat rust resistance requires continuous exploration for new resistance genes due to the emergence of new virulent pathogen races (Singh et al., 2011). In most countries, breeding programs frequently replace older wheat varieties with new, resistant cultivars (Admassu et al., 2009).

The most effective way to prevent crop yield losses due to stem rust, apart from using fungicides, is by planting wheat varieties that are resistant to stem rust. Therefore, the objective of this research was to evaluate different bread wheat germplasms from various origins against four selected virulent stem rust races at the Kulumsa Agricultural Research Center, under field and irrigation conditions, to identify resistant genotypes for inclusion in wheat breeding programs.

MATERIALS AND METHODS

Description of study area

Arsi, characterized by its diverse agro-ecological zones, is a key wheat belt in East Africa and a hotspot for various pathogens. This region provides an excellent opportunity to screen wheat germplasm across several disease hotspots on a large scale. The Kulumsa Agricultural Research Center is located at 08° 01' 10'' N, 39° 09' 11'' E, at an elevation of 2200 meters above sea level. The area receives an average annual rainfall of 820 mm, indicative of its highland and high-rainfall agro-ecology. The average monthly minimum and maximum temperatures are 10.5°C and 22.8°C, respectively. The predominant soil type in the area is loam, known for its fertility. This screening site is particularly significant as a hotspot for stem rusts and serves as a natural niche for the early inoculum of these rusts.

Field layout and experimental design

To assess the level of adult plant stem rust resistance in the field, 91 genotypes, including susceptible checks, were evaluated using an augmented design during the 2022-2023 off-season under irrigation conditions. The entries were planted in plots consisting of double rows, each 1 meter long, with a spacing of 0.2 meters within rows, 0.5 meters between blocks, and 0.5 meters between plots. The plots were sown with a seed rate of 150 kg/ha, and DAP and urea fertilizers were applied based on the recommended rates for the area. Weeds were controlled manually through hand weeding. To ensure uniform inoculum distribution and adequate disease development during the trial period, a mixture of susceptible wheat cultivars, ‘Morocco’ and ‘Kubsa’, was planted as infector rows between blocks, perpendicular to the entries, one week prior to the main planting.

Planting materials

The study involved a total of 91 bread wheat germplasms (Figure 1), including 9 Ethiopian bread wheat cultivars, one universal susceptible check, and 81 advanced bread wheat breeding lines. These lines comprised crosses developed by the Kulumsa Agricultural Research Center, as well as advanced lines from the International Maize and Wheat Improvement Center (CIMMYT) and the International Center for Agricultural Research in the Dry Areas (ICARDA). Seeds for the bread wheat cultivars and breeding lines were sourced from the Kulumsa Agricultural Research Center. The Ethiopian wheat cultivar 'Ogolcho' and the universal susceptible variety 'Morocco' were used as repeated checks in the field nursery. These check cultivars are known to be susceptible to most of the stem rust races prevalent in Ethiopia.

All advanced bread wheat genotypes from various sources (local crosses, CIMMYT, and ICARDA) were previously field-tested in dedicated nurseries under artificial epidemics of both yellow rust and stem rust diseases during the main growing season in 2022. These genotypes were selected from over one thousand germplasms based on their tolerant response to both yellow and stem rust diseases, having been tested for yellow rust at Meraro and for stem rust at Debre Zeit.

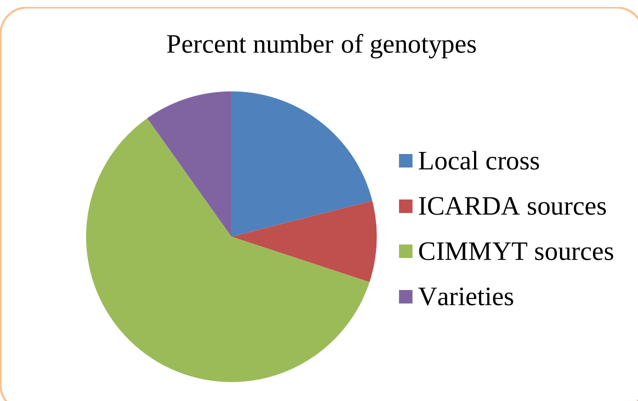

Figure 1. Bread wheat germplasms employed in the study by their source.

Stem rust races employed

Four dominant *Puccinia graminis* f.sp. *tritici* races viz.TTKTF, TKKTF, TTKTT, and TTTTF identified at the Ambo Agricultural Research Center from isolates collected during 2021-2022 from various wheat-growing regions in Ethiopia were used in this experiment. These pathotypes were the most prevalent among the 15 wheat stem rust races reported to attack major wheat cultivars in Ethiopia.

Field inoculation

Urediniospores of the pure races TTKTF, TKKTF, TTKTT, and TTTTF, obtained from the Ambo Agricultural Research Center, were multiplied in isolation chambers within a plastic enclosure at the Kulumsa greenhouse. This was done using seedlings of the susceptible wheat line McNair 701 (CItr 15288), grown in plastic pots at a density of 20 plants per pot, following standard procedures (Woldeab et al., 2017). A homogenous mixture of fresh, active urediniospores from these stem rust races was prepared by suspending 1 g of spores in 1 L of lightweight mineral oil (Soltrol 70; ConocoPhillips Inc.). This mixture was field-inoculated onto spreaders in the trials using two methods: an ultralow-volume sprayer with a single nozzle (ULVA+; Micron Sprayers) and syringe (point) inoculation. The inoculation was conducted at the stem elongation stage at a dedicated screening site.

Disease assessment and data analysis

The final assessment of stem rust disease severity began as soon as the first symptoms appeared on the susceptible check, with evaluations carried out every 14 days until the early dough stage (Large, 1954). Final rust severity was assessed using two components: disease severity, based on a modified Cobb's scale (Peterson et al., 1948), where Tr = less than 5% and 100 = 100%, and host response (infection type), using the scale described by Stakman et al. (1962), which categorizes responses as immune (0), resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S). The coefficient of infection (CI) was calculated by multiplying rust severity by fixed values corresponding to infection type (IT). These fixed values were as follows: R = 0.2, MR = 0.4, MS = 0.6, and S = 1 (Stubbs et al., 1986). The mean coefficient of infection (ACI) was derived by summing the CI values for each line and dividing by the number of recording times.

RESULTS AND DISCUSSION

Final rust severity

The field trial at the Kulumsa Research Center, conducted during the 2022-2023 growing season, revealed diverse rust severities among the genotypes, ranging from 0 to 95%. The responses varied from immune to fully susceptible after artificial inoculation. The susceptible check, Morocco, exhibited the highest disease severity of 95%, with a completely susceptible response, indicating that sufficient epidemic pressure was established during the season for the field experiment.

According to Parlevliet and Ommeren (1975), terminal rust severity reflects the cumulative effect of all resistance factors during the development of an epidemic. Among the tested germplasms, 8 (38.1%) of the local crosses, 36 (60%) of the CIMMYT lines, 3 (33.33%) of the ICARDA lines, and 6 (60%) of the cultivars showed final rust severity below 30% and were categorized as resistant. Similarly, 7 (33.3%) of the local crosses, 21 (35.0%) of the CIMMYT lines, 4 (44.4%) of the ICARDA lines, and 2 (20%) of the cultivars exhibited final rust severity ranging from 31% to 50%, classifying them as moderately resistant. On the other hand, 6 (28.6%) of the local crosses, 3 (5%) of the CIMMYT lines, 2 (22.22%) of the ICARDA lines, and 2 (20%) of the cultivars showed heavy disease infestation and were classified as susceptible (Figure 2). Notably, four lines from CIMMYT exhibited complete resistance (zero infection) at the adult plant stage.

The findings from this study indicate that a significant percentage of the germplasms, regardless of their origin, demonstrated resistant reactions with low final rust severity. A similar trend was observed for the moderately resistant group across all germplasms. Based on the sources of origin, the number of bread wheat germplasms identified as either resistant or moderately resistant was notably higher among the CIMMYT lines.

Out of 81 advanced lines screened against stem rust at Kulumsa, 22 (27.1%) germplasms exhibited moderately resistant (MR) to moderately resistant-susceptible (MRMS) reaction (Figure 3 and Table 1). However, a huge segment 59 (72.8%) of advanced lines showed moderately susceptible to susceptible infection types against virulent stem rust races. On the other hand, most of the commercially released varieties except “Abay” showed moderately susceptible to susceptible reaction.

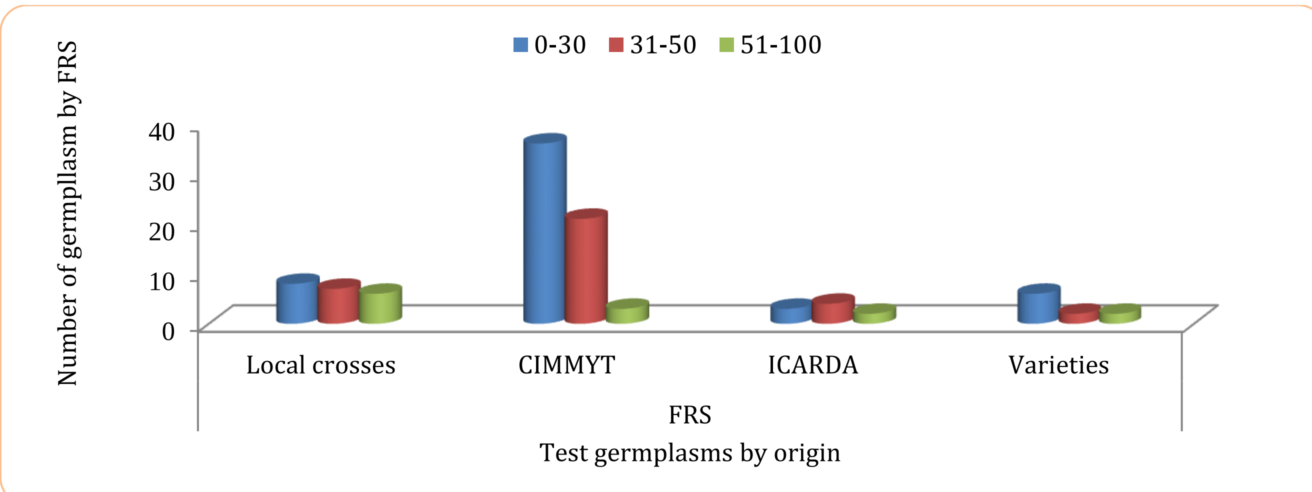

Figure 2. Final rust severity values of test bread wheat germplasms from different origin including susceptible check tested at Kulumsa during offseason in 2022-2023.

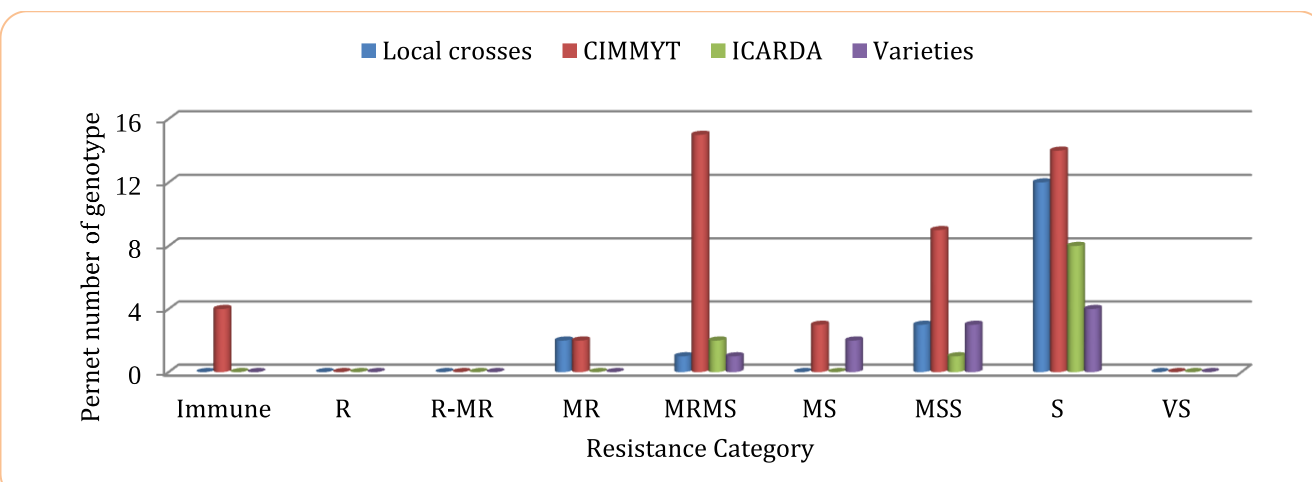

Figure 3. Response of bread wheat genotypes to stem rust tested during offseason at Kulumsa in 2022-2023

Table 1. Severity and reaction of 81 advanced bread wheat genotypes and 9 cultivars to stem rust at adult stage under field conditions during 2022-2023 offseason growing season.

No.	Genotypes	Sources	FRS	ACI	No.	Genotypes	Sources	FRS	ACI	No.	Genotypes	Sources	FRS	ACI
1	EBW160086	local Cross	70 s	70	31	EBW192154	CIMMYT	30 s	30	61	EBW160061	local Cross	30 mss	27
2	EBW160095	local Cross	60 s	60	32	EBW182767	CIMMYT	60 s	60	62	EBW214162	ICARDA	50 s	50
3	EBW212789	CIMMYT	5 mrms	3	33	EBW202370	CIMMYT	70 s	70	63	EBW202213	CIMMYT	tmr	0.4
4	EBW202471	CIMMYT	5 mrms	3	34	EBW160065	local Cross	10 mr	4	64	EBW213016	CIMMYT	10 mrms	6
5	EBW182463	CIMMYT	5 mrms	3	35	EBW160069	local Cross	10 mr	4	65	EBW160103	local Cross	40 s	40
6	EBW212570	CIMMYT	0	0	36	EBW202245	CIMMYT	10 mrms	6	66	EBW214012	ICARDA	40 s	40
7	EBW212350	CIMMYT	5 mrms	3	37	EBW212371	CIMMYT	20 mss	18	67	EBW212274	CIMMYT	40 s	40
8	EBW202005	CIMMYT	0	0	38	EBW212571	CIMMYT	30 s	30	68	EBW160063	local Cross	60 s	60
9	EBW213003	CIMMYT	30 sss	27	39	EBW192347	CIMMYT	20 mss	18	69	EBW160100	local Cross	15 mrms	9
10	EBW212577	CIMMYT	5 mrms	3	40	EBW202243	CIMMYT	80 s	80	70	EBW202216	CIMMYT	20 mss	18
11	EBW192345	CIMMYT	5 mrms	3	41	EBW204020	ICARDA	10 mrms	6	71	EBW192255	CIMMYT	20 mss	18
12	EBW212354	CIMMYT	0	0	42	EBW212575	CIMMYT	10 mss	6	72	EBW204023	ICARDA	70 s	70
13	EBW202473	CIMMYT	5 mss	4.5	43	EBW212448	CIMMYT	5 mr	2	73	EBW160038	local Cross	50 s	50
14	EBW160067	local Cross	40 s	40	44	EBW212517	CIMMYT	15 mrms	9	74	EBW202211	CIMMYT	10 mss	9
15	EBW160009	local Cross	50 s	50	45	EBW160037	local Cross	70 s	70	75	EBW204044	ICARDA	80 s	80
16	EBW214009	ICARDA	50 s	50	46	EBW212676	CIMMYT	10 mrms	6	76	EBW223001	CIMMYT	20 mss	18
17	EBW212574	CIMMYT	5 s	5	47	EBW212777	CIMMYT	30 s	30	77	EBW214031	ICARDA	50 s	50
18	EBW214017	ICARDA	50 s	50	48	EBW160008	local Cross	40 s	40	78	EBW160026	local Cross	40 s	40
19	EBW160066	local Cross	10 s	10	49	EBW160058	local Cross	50 s	50	79	EBW160022	local Cross	40 s	40
20	EBW160002	local Cross	20 mss	18	50	EBW214061	ICARDA	5 mrms	3	80	EBW212229	CIMMYT	40 s	40
21	EBW212572	CIMMYT	5 ms	4	51	EBW202610	CIMMYT	5 mrms	3	81	EBW213196	CIMMYT	40 s	40
22	EBW160062	local Cross	10 s	10	52	EBW214029	ICARDA	20 mss	18		**Varieties**			
23	EBW212106	CIMMYT	5 mrms	3	53	EBW213275	CIMMYT	5 mrms	3	82	Wane	Variety	40 s	40
24	EBW160017	local Cross	15 s	15	54	EBW212573	CIMMYT	5 mrms	3	83	Boru	Variety	25 ms	20
25	EBW202056	CIMMYT	0	0	55	EBW212277	CIMMYT	5 mrms	3	84	Dursa	Variety	5 mss	4.5
26	EBW212159	CIMMYT	20 ms	16	56	EBW160118	local Cross	5 mss	4.5	85	Shaki	Variety	5 mss	4.5
27	EBW212599	CIMMYT	20 ms	20	57	EBW212578	CIMMYT	5 mss	4.5	86	Abay	Variety	5 mrms	3
28	EBW212568	CIMMYT	70 s	70	58	EBW212059	ICARDA	70 s	70	87	Deka	Variety	20 ms	16
29	EBW202411	CIMMYT	90 s	90	59	EBW212576	CIMMYT	30 s	30	88	Kakaba	Variety	15 mss	9
30	EBW202379	CIMMYT	90 s	90	60	EBW192800	CIMMYT	5 ms	4	89	Ogolcho	Variety	90 s	90
										90	Kingbird	Variety	50 s	50
										91	Morocco	Check/Variety	95 s	95

EBW, Ethiopian bread wheat; tmr, trace response (2) with moderately resistant reaction.

Average coefficients of infections

The data on disease severity and host reactions were combined to calculate the average coefficient of infection (ACI). According to Ali et al. (2007), wheat genotypes with ACI values of 0-20, 21-40, and 41-100 are classified as having high, moderate, and low levels of slow rusting resistance, respectively. In this study, 8 (38.1%) local crosses, 37 (61.6%) CIMMYT crosses, 3 (33.33%) ICARDA crosses, and 6 (60%) cultivars exhibited ACI values between 0 and 20, indicating a high level of slow rusting resistance (Figure 4). Meanwhile, 6 (28.5%) local crosses, 20 (33.3%) CIMMYT crosses, 5 (55.5%) ICARDA crosses and 2 (20%) cultivars showed ACI values between 21 and 40, which is indicative of moderate resistance. Conversely, 7 (33.3%) local crosses, 3 (5%) CIMMYT crosses, 2 (22.22%) ICARDA crosses, and 2 (20%) cultivars, including the susceptible check, exhibited heavy disease infestation and were classified as susceptible. Notably, four lines of CIMMYT origin displayed complete resistance, showing zero infection in all tested wheat cultivars at the adult plant stage during the season.

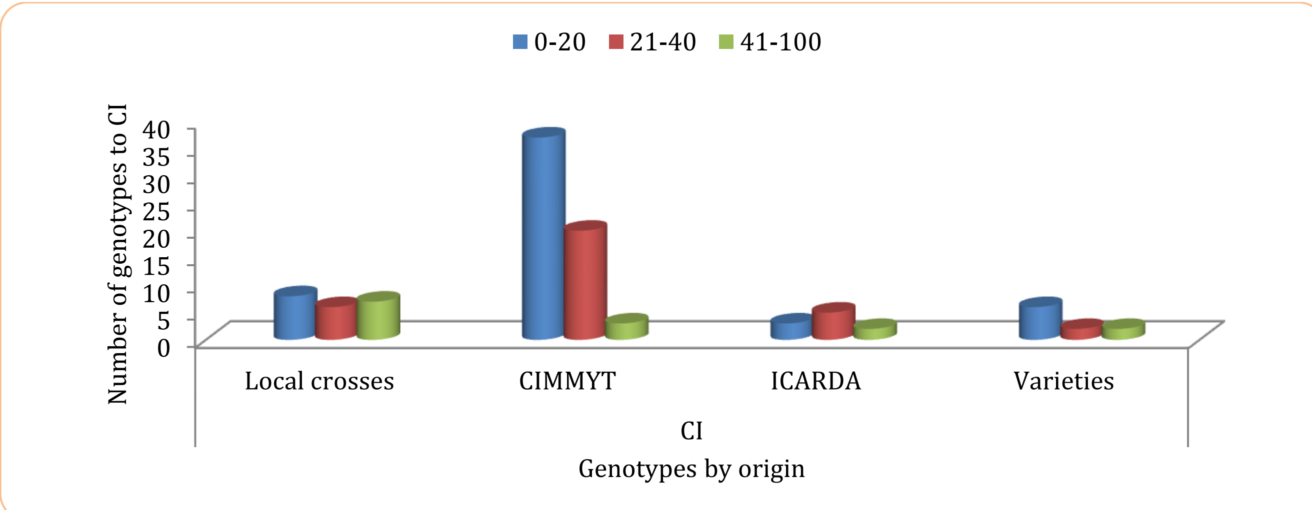

Figure 4: Coefficient of infection values of test bread wheat germplasms from different origin including susceptible check tested at Kulumsa during offseason in 2022-2023.

The results of this study align with the findings of Hundie et al. (2018, 2019) and Shiferaw et al. (2020) who evaluated numerous breeding pipelines against virulent stem rust races at the adult plant stage in stem rust hotspot sites. However, only limited percentages were identified as resistant. The current research revealed that, despite heavy stem rust disease pressure in the field during the 2022-2023 seasons, some germplasms from different origins remained resistant (Table 1). The trace reactions observed may be linked to hypersensitivity, where fungal infection triggers a defense mechanism leading to cell collapse, thereby restricting further disease spread (Rubiales and Nicks, 2000). Consequently, the germplasm identified in this study with low disease severity is recommended for seedling phenotyping to confirm the presence of true adult plant resistance genes.

Furthermore, the results of this study are consistent with the findings of Hundie et al. (2019), Getnet et al. (2021), Mitiku et al. (2018) and Nzuve et al. (2012), who reported that evaluating wheat genotypes for slow rusting in field conditions is essential for identifying new sources of resistance and effectively managing epidemics. Overall, these wheat genotypes were found to be resistant to stem rust disease and can be utilized in breeding programs to develop commercial cultivars suitable for safe production under Ethiopian conditions.

CONCLUSION

Disease-resistant wheat cultivars are considered a key focus in wheat breeding and improvement research programs, aimed at protecting wheat plants from disease epidemics and minimizing yield loss. However, the continuous emergence of new virulent stem rust races and their rapid spread to wheat-growing regions worldwide necessitates the search for new sources of resistance. Based on two major disease assessment criteria, terminal rust severity and the average coefficient of infection, 8 local crosses, 33 CIMMYT crosses, 3 ICARDA crosses, and 6 cultivars have demonstrated resistance to four virulent stem rust races. Consequently, 50 (61.7%) of the breeding pipelines, including EBW212789, EBW202471, EBW182463, EBW212350, EBW213003, EBW212577, EBW192345, EBW202473, EBW212574, EBW160066, EBW160002, EBW212572, EBW160062, EBW212106, EBW160017, EBW212159, EBW212599, EBW192154, EBW160065, EBW160069, EBW202245, EBW212371, EBW212571, EBW192347, EBW204020, EBW212575, EBW212448, EBW212517, EBW212676, EBW212777, EBW214061, EBW202610, EBW214029, EBW213275, EBW212573, EBW212277, EBW160118, EBW212578, EBW212576, EBW192800, EBW160061, EBW202213, EBW213016, EBW160100, EBW202216, and EBW192255, along with 6 (60%) released commercial varieties, were identified as resistant. These genotypes are suspected to carry multiple minor genes responsible for adult plant resistance. Therefore, they could either be introgressed into adapted but susceptible Ethiopian wheat varieties through intercrossing with other genotypes containing minor genes or suggested for release into production. Moreover, they could be utilized in breeding programs for durable stem rust resistance in Ethiopia.

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AUTHOR'S CONTRIBUTION

GMA and AAZ designed the study and conducted the experiments; GMA collected and analyzed the data and wrote the manuscript While AAZ proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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