

RESEARCH ARTICLE

Characterization of bread wheat germplasm for slow rusting resistance to stem rust using field and seedling phenotyping at Kulumsa, Southeastern Ethiopia

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Wheat (*Triticum aestivum* L.) is a principal staple crop worldwide, including in Ethiopia, where it receives top governmental priority among major cereals to ensure food security and promote export potential. However, biotic stresses, particularly yellow and stem rusts caused by *Puccinia striiformis* f. sp. *tritici* and *Puccinia graminis* f. sp. *tritici*, respectively, continue to pose serious threats to sustainable wheat production, leading to annual yield losses worth tens of millions of USD. The emergence and spread of highly virulent stem rust races such as Ug99 (TTKSK) and its variants have further exacerbated the problem by rapidly overcoming existing host resistance genes across major wheat-growing regions of Ethiopia. This study aimed to evaluate thirty-three advanced bread wheat genotypes and twelve released commercial varieties for field-based slow rusting and seedling-stage resistance to stem rust. The field experiment was conducted under an augmented design, while the seedling test was arranged in a Completely Randomized Design (CRD) under greenhouse conditions at Kulumsa agricultural research center during the 2023/2024 off-season. Results indicated that 25 (75.75%) advanced genotypes and 2 (16.66%) released varieties exhibited low final rust severity (FRS<30%) and average coefficient of infection (ACI<20%) under 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%) released varieties (Abay and Boru) demonstrated higher infection types at the seedling stage, confirming their partial or slow rusting resistance. These promising genotypes can serve as valuable parental sources for resistance breeding in regional wheat improvement programs or be advanced to regional yield trials for the development of high-yielding, stem rust-resistant cultivars. Such efforts are essential to counter the continually evolving stem rust races and safeguard national wheat productivity.

Keywords: Slow rusting, Seedling resistance, Final rust severity, Coefficient of infection, Wheat genotypes.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple cereal crops globally, providing the primary source of calories and proteins for over 2.5 billion people (Bentley et al., 2022). It is cultivated on approximately 217 million ha worldwide, making it the most extensively grown crop on Earth, with an estimated annual production of 752 million tons (FAOSTAT, 2020). Global wheat production has increased remarkably over the past eight decades—from 222 million tons in 1960 to about 761 million tons in 2020—primarily due to the introduction of high-yielding cultivars, improved agronomic management, and enhanced use of fertilizers and pesticides (Falcon et al., 2022; Ray et al., 2023).

In Africa, Ethiopia stands as the second-largest wheat producer after Egypt, contributing about 6.7 million tons, equivalent to 21.7 % of the continent's total grain production and 18.3% of its wheat-growing area (Crumpler, 2021). According to the Central Statistical Agency (CSA, 2021), wheat accounts for nearly 17% of the total grain harvest, covering more than 2.1 million ha and providing income and employment for nearly 5 million smallholder farmers. Despite substantial expansion in cultivated area and the government's strong policy commitment to achieve wheat self-sufficiency and initiate export, national productivity (≈ 3.3 t ha⁻¹) remains below the potential (>5 t ha⁻¹) under optimal conditions (Solomon et al., 2024).

The low productivity of Ethiopian wheat is attributed to multiple constraints, including limited access to quality seed, rising fertilizer costs, suboptimal agronomic practices, and recurrent disease epidemics (Abate et al., 2023). Among biotic stresses, fungal diseases especially the rusts (stem rust, *Puccinia graminis* f. sp. *tritici*; stripe rust, *Puccinia striiformis* f. sp. *tritici*; and leaf rust, *Puccinia triticina*)-represent the most serious yield-reducing factors (Abebele et al., 2021; Muche et al., 2022; Hodson et al., 2021). Under conducive environmental conditions, stem rust can cause up to 100% yield loss in susceptible cultivars (Singh et al., 2016).

Historically, Ethiopia has experienced several devastating stem rust outbreaks, including the 1972 epidemic that destroyed the popular cultivar Lakech and the more recent outbreak of the virulent race TKTTF ("Digelu race") in 2013, which caused localized yield losses approaching 100% and average national losses of around 50% (Meyer et al., 2021; Wolde et al., 2023). The continuing evolution and rapid spread of the Ug99 lineage (TTKSK and its variants) across East Africa further threaten regional wheat production (Pretorius et al., 2017; Bhavani et al., 2022).

While fungicides can provide temporary control, their cost and limited accessibility make them impractical for resource-poor smallholders in developing nations (Rehman et al., 2019). Therefore, the development and deployment of resistant cultivars remain the most sustainable, environmentally sound, and economically viable solution. Most improved wheat cultivars released in Ethiopia to date carry race-specific resistance genes that operate in a gene-for-gene manner (McIntosh et al., 2017). However, these genes often confer short-lived protection because new virulent pathotypes rapidly emerge, resulting in the well-known "boom-and-bust" cycles of resistance breakdown (Hodson et al., 2020; Bhavani et al., 2022).

To achieve durable protection, emphasis has shifted toward identifying and incorporating slow rusting or Adult-Plant Resistance (APR) genes, which provide partial, non-race-specific resistance that is more stable across diverse environments and pathogen populations (Singh et al., 2016; Chen, 2020). Such resistance minimizes disease development and delays epidemic onset without imposing strong selection pressure on pathogen populations, thereby enhancing the longevity of cultivar resistance. Nevertheless, the frequent emergence of new races in Ethiopia has rendered several previously resistant varieties obsolete despite their otherwise desirable agronomic traits (Worku et al., 2016; Bekele et al., 2019).

Consequently, continuous evaluation and identification of new sources of slow rusting resistance remain critical components of national wheat improvement programs. The present study was undertaken to assess thirty-three advanced bread wheat genotypes and twelve released varieties for their seedling and adult-plant resistance to stem rust under both field and greenhouse conditions. The ultimate goal is to identify promising genotypes that can serve as parental sources or candidate cultivars for developing high-yielding, stem rust-resistant wheat suited to Ethiopian agro-ecologies.

Materials and Methods

Description of the study site

The field experiment was conducted during the 2023/2024 irrigation cropping season (December to April) at the Kulumsa Agricultural Research Center (KARC), located in the Arsi Zone of Oromia Regional State, Southeastern Ethiopia. Geographically, the site lies at 08°01'10" N latitude and 39°09'11" E longitude, at an elevation of 2200 meters above sea level (m.a.s.l.). The area represents a highland, high-rainfall wheat-growing agro-ecology, characterized by a mean annual precipitation of approximately 820 mm. The mean monthly minimum and maximum temperatures are 10.5°C and 22.8°C, respectively. The predominant soil type is a fertile loam, classified as a Eutric Nitisol, with good structure, moderate organic matter content, and slightly acidic pH, making it highly favorable for bread wheat cultivation (Abdulkadir, 2011; Tadesse et al., 2019).

Kulumsa is recognized as one of the national hotspot sites for wheat rust evaluation due to its conducive environmental conditions for the development and natural proliferation of *Puccinia graminis* f. sp. *tritici* (Hodson et al., 2020; Wolde et al., 2023). Standard agronomic practices were employed before sowing, including plowing, harrowing, and leveling. Land preparation and management practices followed the Ethiopian national wheat production recommendations (Solomon et al., 2024).

Planting materials

The experimental materials consisted of 33 advanced bread wheat genotypes and 12 released commercial varieties (Fig. 1), including both resistant and susceptible checks. The advanced genotypes were sourced from international wheat breeding nurseries—specifically, three from the International Center for Agricultural Research in the Dry Areas (ICARDA), sixteen from the International Maize and Wheat Improvement Center (CIMMYT), and fourteen developed through local crosses under the Ethiopian National Wheat Improvement Program. The 12 released varieties were selected based on their known field performance, resistance background, and national importance in Ethiopian wheat production systems.

These genotypes represented diverse genetic backgrounds, aiming to capture variability in resistance mechanisms such as race-specific (seedling) resistance and race non-specific (adult plant or slow rusting) resistance (Singh et al., 2016; Bhavani et al., 2022). All seeds were obtained from the Wheat Breeding Division of KARC and verified for purity and viability before planting.

Seeds were surface sterilized using 1% sodium hypochlorite for 2 minutes and rinsed thoroughly with sterile distilled water to remove potential contaminants before sowing (Chen, 2020).

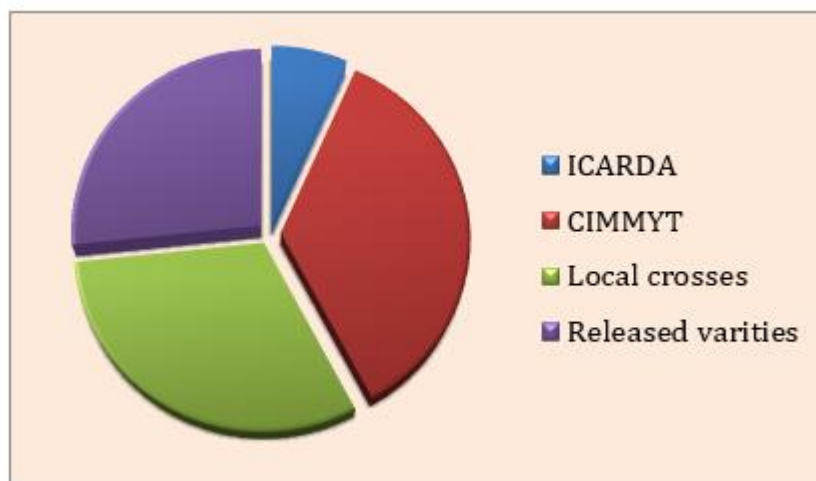


Fig. 1. Sources and genetic origins of bread wheat germplasms examined in the study.

Employed stem rust race

A dominant *Puccinia graminis* f. sp. *tritici* race, TKKTF, identified by the Ambo Agricultural Research Center from isolates collected during the 2021/2022 cropping season across major Ethiopian wheat-growing regions, was used for both field and seedling evaluations. This race is recognized as one of the most virulent and prevalent races in East Africa (Moges et al., 2023), exhibiting virulence to several major stem rust resistance genes (Sr genes) including *Sr5*, *Sr21*, *Sr9e*, *Sr7b*, *Sr6*, *Sr8a*, *Sr9g*, *Sr9b*, *Sr30*, *Sr17*, *Sr9a*, *Sr9d*, *Sr10*, *SrTnp*, *Sr38*, and *SrMcN*. The TKKTF race has been responsible for breaking resistance in many Ethiopian bread wheat varieties and remains a major constraint to stable wheat production (Bhavani et al., 2022; Wolde et al., 2023).

Field phenotyping and disease scoring

The field experiment was conducted using an augmented design to accommodate a large number of test entries with replicated standard checks (Federer et al., 1975). Each genotype was planted in two 1-meter rows with a 20 cm inter-row spacing and 5 cm intra-row spacing. The recommended fertilizer rate of 100 kg ha⁻¹ NPS and 50 kg ha⁻¹ urea was applied in two splits: Half at planting and half at tillering. Irrigation was applied every 7–10 days to maintain adequate soil moisture.

To ensure uniform disease pressure, the field was artificially inoculated with a urediniospore suspension of race TKKTF at the tillering stage (Zadoks 25–30) using a hand sprayer. The inoculum was prepared by suspending freshly collected urediniospores in lightweight mineral oil (Soltrol 170) at a concentration of approximately 1×10^6 spores mL⁻¹ (Hodson et al., 2020). Inoculations were conducted in the late afternoon to maximize humidity and facilitate spore germination.

Stem rust severity was recorded using the modified Cobb's scale (Peterson et al., 1948), which estimates the proportion of plant tissue affected by rust pustules. Disease observations commenced at the first appearance of symptoms on the susceptible check and continued at 14-day intervals until the early dough stage (Zadoks 85–87). Each reading included both disease severity (%) and host reaction type (R: Resistant, MR: Moderately Resistant, MS: Moderately Susceptible, S: Susceptible). The Coefficient of Infection (CI) was calculated by multiplying the Final Rust Severity (FRS) by a constant value corresponding to the host response (Roelfs et al., 1992).

Seedling phenotyping and data assessment

Seedling tests were conducted under controlled greenhouse conditions at KARC in 2024 to assess race-specific (seedling) resistance against the TKKTF race. Urediniospores of the race were multiplied on a highly susceptible differential line, McNair (without Sr genes), to obtain sufficient inoculum.

Seedlings of the 45 test genotypes and the susceptible check were grown in 7 cm × 7 cm × 6 cm plastic pots filled with a 1:1:1 mixture (v/v/v) of sterilized light soil, sand, and compost. Inoculation was performed at the two-leaf stage (7-day-old seedlings) using an atomizer to spray a spore suspension (1×10^5 spores mL⁻¹ in Soltrol 170). Thirty minutes after inoculation, plants were lightly misted with distilled water and placed in a dark dew chamber at 18–22°C and 100% relative humidity for 18 hours, followed by 4 hours of light exposure to promote infection. After aeration for 2 hours, seedlings were transferred to the greenhouse maintained at 18–25°C, 60–70% relative humidity, and a 12-hour photoperiod (Chen, 2020; Wolde et al., 2023).

Infection Types (ITs) were recorded 14 days post-inoculation using the 0–4 scale (Stakman et al., 1962). Infection types of 0

(immune), 1 (small uredinia with necrosis), and 2 (small to medium uredinia with chlorosis or necrosis) were considered incompatible (resistant) reactions, while ITs 3 (medium uredinia with chlorosis) and 4 (large uredinia without chlorosis or necrosis) were regarded as compatible (susceptible) reactions. Intermediate reactions were refined with “+” or “–” symbols to denote slightly larger or smaller pustule sizes than the standard class.

Results and Discussion

Final rust severity

Substantial variation in Final Stem Rust Severity (FRS) was observed among the 45 bread wheat genotypes tested under field conditions at Kulumsa Agricultural Research Center during the 2023/2024 off-season. Disease severity ranged from 0% to 100%, indicating broad genetic diversity in host response to *Puccinia graminis* f. sp. *tritici* infection. The susceptible check, Morocco, exhibited 100% severity with a fully susceptible(S) reaction, confirming that the epidemic pressure and environmental conditions were adequate for disease development and reliable differentiation among genotypes.

As indicated by Parlevliet and van Ommeren (1975), the final rust severity is a key integrative measure of all resistance components expressed throughout an epidemic, and thus serves as a reliable indicator for assessing adult plant or slow rusting resistance. In the present study, two ICARDA lines, fourteen CIMMYT lines, ten local crosses, and one released variety exhibited FRS values below 30%, signifying a moderately to highly resistant reaction. Similarly, one ICARDA line, one CIMMYT line, three local crosses, and two released varieties recorded FRS values between 31% and 50%, representing moderate resistance. Conversely, one CIMMYT line, one local cross, and nine released varieties showed high disease severity (>60%), indicating susceptibility (Table 1).

These results demonstrate that a substantial proportion of the advanced breeding lines, irrespective of their origin, expressed durable or partial resistance under natural field conditions. Conversely, most of the commercially released varieties, including widely cultivated cultivars in Ethiopia, were highly susceptible, suggesting erosion of race-specific resistance genes over time due to pathogen evolution (Hodson et al., 2020; Mayer et al., 2021).

Table 1. Origin, field performance, and seedling reactions of bread wheat genotypes to the virulent stem rust race TKKTF.

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
Note: EBW: Ethiopian Bread Wheat; EIAR: Ethiopian Institute of Agricultural Research						

Comparable trends have been reported in several recent studies. Worku et al. (2017) and Bekele et al. (2019) identified genotypes with slow rusting resistance based on low FRS and ACI values under Ethiopian field conditions, while Alemu et al. (2022) confirmed that lines with $FRS \leq 30\%$ are likely to carry Adult Plant Resistance (APR) genes effective against a wide range of virulent races. Moreover, Bacha et al. (2020) evaluated 62 durum and bread wheat genotypes under both field and greenhouse assays and identified five genotypes exhibiting consistent slow rusting resistance, supporting the present findings. Similarly, Hunde et al. (2019) screened over 100 wheat genotypes against multiple virulent stem rust races in Ethiopia and reported that only four exhibited durable resistance, reflecting the rarity yet value of such traits in breeding programs.

The predominance of moderate to low FRS values among CIMMYT and locally derived lines in this study implies successful introgression of non-race-specific resistance genes, such as *Sr2*, *Sr55* (*Lr67*), *Sr57* (*Lr34*), and *Sr58* (*Lr46*), which have been widely used in global breeding programs to confer slow rusting resistance (Singh et al., 2015; Pretorius et al., 2020). The low FRS observed in several of these lines aligns with earlier findings that genotypes carrying combinations of these minor, additive genes display reduced epidemic development and delayed disease onset, even under high inoculum pressure (Kolmer et al., 2019).

The current study's outcomes reaffirm that slow rusting resistance remains the most sustainable defense mechanism against rapidly evolving rust populations. Similar results have been documented by Nzuve et al. (2012), who evaluated 25 bread wheat genotypes in Kenya and identified five lines with effective adult plant resistance. Furthermore, Abebe et al. (2021) reported that advanced Ethiopian bread wheat genotypes exhibiting low FRS values under hotspot conditions were superior candidates for stem rust breeding pipelines.

The susceptibility observed among older released varieties suggests the breakdown of major, race-specific genes (e.g., *SrTnp*, *Sr24*, *Sr31*) due to the emergence of virulent races such as TTKTF and TTKSK (Ug99), both of which are prevalent in East Africa (Mogen's et al., 2023; Hodson et al., 2021). This underscores the urgent need to replace obsolete varieties with new cultivars possessing polygenic, slow rusting resistance, which offers broad-spectrum and long-lasting protection (Singh et al., 2020).

Overall, the study confirmed that several of the tested germplasms—particularly CIMMYT-derived and locally adapted lines—exhibited incompatible host–pathogen interactions, characterized by low FRS and resistant field reactions. These genotypes represent valuable parental sources for the development of high-yielding, stem rust-resistant cultivars in Ethiopia and other regions threatened by evolving rust populations.

Coefficient of Infection (CI)

The Coefficient of Infection (CI), which integrates disease severity with host response, was computed for all genotypes (Table 1) to quantify their slow rusting potential. According to Ali et al. (2009), genotypes with CI values of 0–20, 21–40, and 41–60 are classified as highly, moderately, and lowly slow-rusting, respectively.

In the present study, 1 ICARDA line, 12 CIMMYT lines, 6 local crosses, and 1 released variety exhibited CI values below 20, indicating strong slow rusting resistance and confirming the presence of durable, Adult-Plant Resistance (APR) genes. A further 2 ICARDA lines, 2 CIMMYT lines, 7 local crosses, and 2 released varieties displayed CI values in the 21–40 range, corresponding to moderate slow rusting. Conversely, none of the ICARDA lines, 2 CIMMYT lines, 1 local cross, and 9 released varieties showed CI values exceeding 41, which signals high susceptibility.

These results indicate that a majority of the advanced breeding lines (57.7%), regardless of their origin, possess desirable slow rusting characteristics, whereas most commercial cultivars including widely grown varieties such as Deka and Shaki—exhibited CI values exceeding 41, reflecting the erosion of major stem rust resistance genes. This highlights the urgent need for continuous screening and identification of new sources of durable resistance to safeguard wheat production in Ethiopia (Hodson et al., 2020; Wolde et al., 2023).

Similar patterns have been reported in recent studies. Worku et al. (2016) evaluated 306 elite bread wheat lines under East African hotspot conditions and reported that a significant proportion of the lines exhibited adult-plant resistance, demonstrating the efficacy of CI as a reliable measure for slow rusting. Likewise, Alemu et al. (2023) assessed thousands of wheat germplasms and confirmed that CI values accurately distinguish polygenic resistance levels among advanced lines. These findings corroborate the current study, confirming that CI is an effective tool for identifying genotypes with durable, non-race-specific resistance.

Seedling reaction of wheat genotypes to stem rust race

Seedling evaluation under controlled greenhouse conditions revealed that the universally susceptible checks, McNair and Morocco, displayed Infection Type (IT) 4, indicating fully compatible and susceptible reactions. This confirms that adequate inoculum pressure was achieved during greenhouse assays. Under the same conditions, none of the advanced or released genotypes exhibited a completely immune (IT 0) response, suggesting that all tested lines rely on adult-plant, polygenic resistance rather than race-specific seedling resistance.

Among the advanced genotypes, 2 ICARDA lines, 2 CIMMYT lines, and 11 local crosses exhibited ITs ranging from fleck (0) to 2, representing incompatible reactions and indicative of partial resistance at the seedling stage. In contrast, all commercially released varieties showed compatible reactions (IT 3–4), demonstrating that their resistance genes were either race-specific or broken by the TKKTF race or entirely absent.

These results are consistent with previous findings that genotypes displaying susceptible seedling reactions but reduced adult-plant severity typically carry polygenic, race-nonspecific resistance (Nzuve et al., 2012; Hunde et al., 2018). In Ethiopia, similar screening of 843 bread wheat genotypes identified 52 lines exhibiting slow rusting characteristics, showing low disease severity in the field despite susceptible seedling reactions (Hunde et al., 2018). Likewise, Bacha et al. (2020) evaluated 62 wheat genotypes (32 durum and 30 bread wheat) under field and greenhouse conditions and identified six lines (four durum and two bread wheat) as true slow-rusting genotypes.

The present study confirms that several advanced CIMMYT, ICARDA, and locally developed lines exhibit true slow rusting resistance, characterized by low CI values and partial resistance at the adult plant stage, making them promising candidates for incorporation into Ethiopian wheat breeding programs aimed at durable, broad-spectrum stem rust resistance. The absence of immune reactions at the seedling stage further emphasizes that slow rusting resistance is predominantly adult plant-mediated and relies on the additive effects of multiple minor genes, rather than single major genes (Singh et al., 2015; Pretorius et al., 2020).

Conclusions

In contrast to the commercially released wheat varieties, the majority of the evaluated advanced genotypes exhibited superior field resistance under high disease pressure, as indicated by the response of the susceptible check. However, true slow rusting, confirmed through seedling assays, was observed in only a subset of genotypes. Specifically, eleven advanced genotypes (EBW170072, EBW160066, EBW170172, EBW170051, EBW160002, EBW222680, EBW170056, EBW170059, EBW170058, EBW160065, and EBW224096) and two released varieties (Abay and Boru) demonstrated genuine slow rusting behavior. These genotypes represent valuable genetic resources and can be directly utilized as resistant parental lines in regional wheat breeding programs. Moreover, they are suitable candidates for inclusion in regional yield trials, aimed at developing high-yielding, stem rust-resistant cultivars capable of withstanding the continual evolution of virulent *Puccinia graminis* f. sp. *tritici* races.

Authors Contributions

GMA conceptualized and designed the study, prepared the experimental materials, and drafted the manuscript. AAZ and HTD conducted the fieldwork, including data collection and disease assessments. All authors critically reviewed, revised, and approved the final version of the manuscript for submission.

Conflict of Interest

The authors declare no conflict of interest.

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