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Research Article

Resistant Level of Wheat Lines with Known Genetic Stock to Stem Rust in Central and South Eastern Parts of Ethiopia

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Abstract

Wheat rusts caused by *Puccinia graminea* f.sp Triticiare are among the major biotic constraints of wheat all over the world. Nowadays different stem rust races have evolved and threaten wheat production worldwide, among which Ethiopia is found the most vulnerable. In view of the above facts, the present investigation was carried out to evaluate and identify resistant wheat lines and genes against stem rust in the central and southeastern parts of the country. An inventory of 93 wheat lines, checks, and 17 differential lines was evaluated for stem rust resistance under field conditions of Sinana, Kulumsa, and Debrezeit during the 2015 cropping season. The experiments were laid out in augmented design. Each plot consisted of two rows of 2 m long with 0.2 m between rows. The terminal severity of stem rust varied from 0 to 90S at Kulumsa and it was as high as 60S on the susceptible check cultivar Kekeba at Debrezeit. At Sinana 92% of wheat lines tested exhibited resistance to moderately resistant to stem rust, with a coefficient of infection ranging from 0 to 30. At Debrezeit 49% of wheat lines were resistant and moderately resistant to stem rust. Wheat lines, PavonSr 24+ Sr 31+Sr 50, WHEAT (westonia+ Sr B, WestoniaSr 50+ Sr 26, WestoniaSr 24+ Sr 26, AngasSr 32, Shorima, Sr 50+Sr 45 # 28, Sr 22/CO 1213, PavonSr 24+ SR 26+Sr 31, Sr 45/Kulin, Sr 33+Sr 45 #23, Sr 33+Sr 45 #36, PavonSr 26+ SR 31, WestoniaSr 24+ Sr 31, PavonSr 24+ Sr 31, GatoSr 50, Sr 50+Sr 45 # 5, WestoniaSr 24+ Sr 50, PavonSr 24+ Sr 50 and SA 8123 (Sr B) were resistant to stem rust at field condition of all locations and hence, they could be exploited in wheat improvement programs at national level.

Keywords

Infection Type, Coefficient of Infection, Resistant Gene, Severity

1. Introduction

Stem rust is a dangerous disease that affects essential grasses such perennial ryegrass, tall fescue, and timothy, as well as grains like wheat, barley, oats, and rye [10]. In the 2013 cropping season, 100 samples of wheat stem rust were collected in the Oromia, Amhara, and Tigray region. These samples allowed the identification of nine races: TTKSK

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(Ug99), TTKTF, TTKTK, JRCQC, TKTTF, TTKSC, TRTTF, SRKSC, and RRKSF [7].

The Puccinia graminis tritici fungus race Ug99, which causes stem or black rust disease on wheat, was discovered for the first time in Uganda in 1998 [19]. According to [20] seven races that are descended from the Ug99 lineage are now recognized and have expanded to several wheat-growing nations in the highlands of eastern Africa, as well as Zimbabwe, South Africa, Sudan, Yemen, and Iran. The Ug99 group of races was identified as a significant danger to wheat production and food security due to the susceptibility of 90% of the wheat varieties farmed globally [20]. Its expansion, whether caused by the wind or human intervention, to other nations in Africa, Asia, and beyond is obvious [20].

Wheat plants that have stem rust show elliptical blisters or pustules called uredia that grow parallel to the long axis of the stem, leaf, or leaf sheath. The wheat spike's neck and glumes may also develop blisters. Later, an uneven rupture and pushing back of the epidermis covering the pustules reveals a powdery mass of brick-red uredospores. The uredia are 1 to 3 millimeters wide and 10 millimeters long, depending on the species. As the plant matures later in the season, the pustules turn black because the fungus develops teliospores rather than uredospores, and the uredia are changed into black telia. Telia may occasionally grow apart from uredia. On wheat plants, uredia and telia may be seen in such large quantities.

The principal hosts for the telial/uredinial stage of Puccinia graminis, the cause of stem rust on wheat, barley, and oat, are plants in the Triticeae [10]. Stem rust can have alternative hosts, such as Berberis vulgaris [16]. Wheat stem rust was found to have secondary hosts in the plants Lolium temulentum and Setaria pumila [4].

Stem rust caused yield losses of 29% on the Sirbo variety and 21.1% on the Maddawalabu variety in 2005 at Agarfa. Sirbo and Maddawalabu had grain yield reductions in 2006 of 25.7% and 18.6%, respectively. According to [9], the disease decreased grain yield in 2006 by 18.1% on Maddawalabu and 22.1% on Sirbo. Enkoy yields were decreased by 65 to 100% as a result of the epidemic, which emerged in December 1993 and quickly spread throughout the districts where it was produced [17]. The outbreak is predicted to have lowered yields in the highland wheat regions by 42%. The following year, at the Kulumsa Agricultural Research Center, a fungicide trial on Enkoy revealed complete susceptibility and a complete yield loss due to stem rust [2].

While no one cultural practice is always beneficial, using a variety of them considerably strengthens the already present resistances. For epidemics that would be caused by endogenous inoculums, gene deployment (which can be obtained by a grower if more than one cultivar are used that differ in resistance and from those grown by immediate neighbors), control of timing, frequency, and amount of irrigation and fertilization applications, removing the green bridge with tillage or herbicides is an effective control measure [16]. Rust management techniques include altering planting dates, eradicating alternative host plants, and employing several lines or varietal combinations [22].

Stem rust can be effectively controlled using foliar fungicides. The higher leaves, which supply the majority of the energy needed to make grain, should be protected by the fungicides when they are applied when the crop is in the boot stage of development. To treat stem rust in Kenya, Nativo 300 SC (trifloxystrobin 100 g L^{-1} + tebuconazole 200 g L^{-1}) and Prosaro 250 EC (prothioconazole 125 g L^{-1} + tebuconazole 125 g L^{-1}) were advised [8].

Genetic resistance is the main method for controlling obligate parasites. However, effective disease control requires that durable, race-nonspecific resistance is incorporated into high-yielding genotypes. In some areas, a shift in breeding strategies towards this durable type of resistance, based on minor additive genes, is required to avoid the 'boom and bust' cycles that are frequently observed. This is particularly true for areas where a single genotype is sown and the risk of mutation to new virulent races increases under selection pressure [6]. Two types of genetic resistance to rusts are known: a) seedling resistance and b) adult plant resistance. Seedling resistance, which is controlled by a single gene, is highly effective and lasts throughout the wheat life cycle [11].

A total of 11 stem rust resistance genes (Sr5, Sr7a, Sr7b, Sr8a, Sr9e, Sr11, Sr21, Sr27, Sr29, Sr30, and Sr37) were postulated to be present either singly or in combination in the durum and bread wheat cultivars and breeding lines in Ethiopia [3]. Differential lines that carry resistant genes Sr36, SrTmp, and Sr24, were effective against the most dominant stem rust race TTKSK (UG99) whereas only Sr11, Sr24, and Sr31 were effective against the most virulence race TKTTF [7]. Emmer wheat and durum wheat are good sources of resistance to wheat rust [13].

2. Materials and Methods

The study accommodates the phenotyping activity both at green house and field conditions. It contains 93- spring bread wheat lines from Australia, with known gene stocks. Seven checks, and 17 stem rust differential lines, respectively, were included.

2.1. Description of the Study Areas

The study was conducted at three locations in Central and Southeast of Ethiopia, namely Sinana, Debrezeit and Kulumsa. The three places have different agro ecological zones which favor the development of rusts at various degrees. Sinana is well known hot spot area for yellow rust. Kulumsa is hot spot for the three rusts while Debrezeit is hot spot for leaf and stem rusts. Sinana is located at 7°7'N, 40°10'E and at 2450masl. It receives mean annual rainfall of 808 mm. The monthly mean minimum and maximum temperatures are 9.3 and 20.9℃, respectively. The dominant soil type is pellic vertisol which is slightly acidic. Debrezeit research center is located at

08°44'N and 38°58'E and at 1900meter above sea level (masl). The area receives mean annual rain fall of 851mm. The monthly mean minimum and maximum temperatures are 8.9 and 28.3 ℃, respectively. The dominant soil type is vertisoil. Kulumsa is located at 39°09'East 08°01' North and at 2200m above sea level. It receives 820mm of rainfall annually. The monthly mean minimum and maximum temperatures are 10.5 ℃ and 22.8 ℃, respectively. The dominant soil type is Clay soil (Luvisols).

2.2. Field Tests

2.2.1. Planting

Wheat lines were planted in June, 2015 at Kulumsa, in July, 2015 at Debrezeit and in August, 2015 at Sinana using augumented design, along with various differentials and checks. In addition to the aformentioned wheat lines, about 380 wheat lines including checks were planted at Kulumsa. The wheat lines were planted in four blocks with two rows that are 0.2m apart and 2m long using a seeding rate of 100 kg/ha. Fertilizers Urea and DAP were applied at the rate of 50kg/ha and 100kg/ha, respectively. Weed managment and intercultivation was carried out according to the recommendation in each location.

2.2.2. Data Collection and Analysis

Field severity data collection was done on the basis of whole plot. Disease severity as a percentage of leaf area covered with the disease was assessed following a modified Cobb's scale [15]. Field response was recorded three times at 10 days interval from tillering to soft-dough stage for yellow rust and until late maturity for leaf and stem rusts. The data on disease severity and host reaction was combined to calculate the coefficient of infection (CI) by multiplying the severity value by constant values of 0, 0.2, 0.4, 0.6, 0.8, or 1.0. After the last disease score when the disease progress ceased, according to [21]: [14] the field severity data was converted to Coefficient of Infection (CI) by multiplying with constant values of response. Wheat lines with coefficient of infections ranging from 0 to 20 were considered as resistant while 20 to 30, 30 to 40, 40 to 60 and 60 to 100 were moderately resistant, moderately susceptible, moderately susceptible to susceptible, and susceptible, respectively.

3. Results

3.1. Detection of Stem Rust Races Prevailing in Southeastern and Central Ethiopia at Field Condition

In order to determine which races were dominant, stem rust differentials were investigated at Kulumsa, Sinana, and Debrezeit field conditions. At Sinana, stem rust attacked every studied host differential with the exception of those that carried the stem rust resistance genes Sr22 and Sr30 (Table 1). As a result, it is possible that Sinana does not include the stem rust races Sr22 and Sr30. All known races of stem rust were present during the 2015 cropping season in the area, with the exception of Sr17 and Sr22, which were discovered at Kulumsa and Debrezeit, respectively.

Table 1. *Stem rust differential lines field response.*

No	Host differentials	Sr-genes	Field response		
			Sinana	Debrezeit	Kulumsa
1	ISr6-Ra	6	30S	20SMS	15MS
2	ISr8-Ra	8	15MS	40SMS	15MSS
3	W261Sr9b	9b	40S	50S	30S
4	ISr9d-Ra	9d	10S	20RMR	10S
5	CnsSr9g	9g	60S	70S	70S
6	W261Sr10	10	30S	30SMS	15MS
7	St64Sr13	13	60S	60S	30S
8	Combination VII	17	5MS	25MRMS	0
9	Sr22TB	22	0	10MRMS	0
10	W2691Sr28Kt	28	40S	50SMS	80S
11	BtSr30Wst	30	0	30MSS	5MR
12	Sr31 (Benno)/6*LMPG	31	50S	30MSS	80S

No	Host differentials	Sr-genes	Sinana	Field response	
				Debrezeit	Kulumsa
13	CnsSR32 AS	32	20S	15MSMR	20S
14	RL6082	39	20S	30SMS	20S
15	RL6088	40	60S	50S	50S
16	Taf-2	44	5S	20MS	5MS
17	CnSSrTmp	Tmp	10S	10MRMS	5S

Source: Kulumsa Agricultural Research Center
Note: S= Susceptible MS= Moderately Susceptible MSS = moderately susceptible and susceptible MR= moderately resistance SMS=Susceptible and moderately susceptible MSMR=moderately susceptible moderately resistance

3.2. Frequency Distribution of Wheat Lines Reaction to Stem Rust Under Field Conditions

3.2.1. Field Response of Wheat Lines at Sinana

In the current experiment, 100 wheat lines were evaluated, and 86, were shown to be resistant to stem rust, (Figure 1). The wheat lines' coefficient of infection ranged from 0 (immune) to 20 (moderately resistant), indicating significant differences in the lines' resistance to stem rust. Six wheat lines displayed a moderately resistant response to stem rust at the same area. Eight wheat lines showed susceptible reaction for stem rust at field condition of Sinana research station during 2015 cropping season.

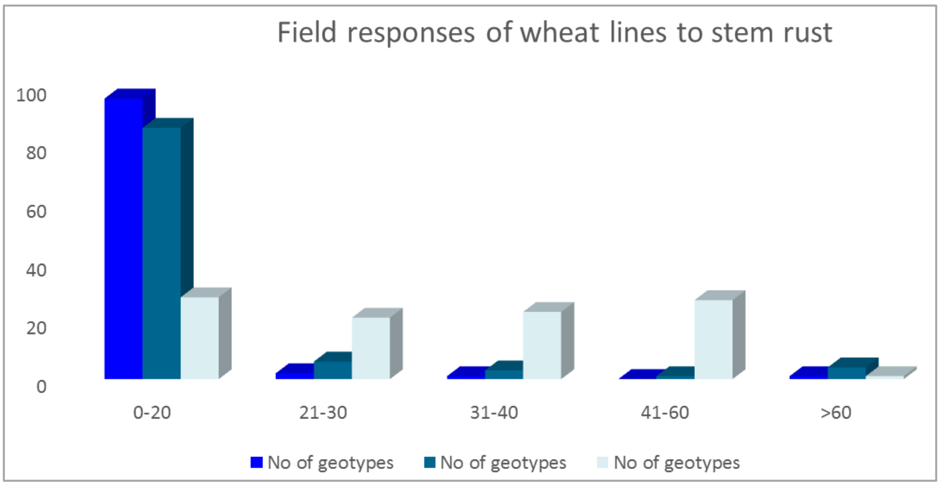

Figure 1. Reaction of wheat lines and checks to stem rust at all locatios.

3.2.2. Field Response of Wheat Lines at Debrezeit

Among tested wheat lines at Debrezeit 28 gave resistance type of reaction for stem rust at field condition. Twenty one wheat lines showed moderately resistant types of reaction to stem rust and the rest were susceptible, respectively, on the basis of coefficient of infection as shown above (Figure 1).

3.2.3. Field Response of Wheat Lines at Kulumsa

At Kulumsa field condition most of the genotypes tested showed low level of infection to stem rust. As revealed by current results 96, wheat lines including checks have coefficient of infection below 20 to stem rust, and hence could be considered resistant to the respective rust (Figure 1). In addition, 2 wheat lines were categorized as moderately resistant to stem rust, as their coefficient of infection varied between 20 and 30. Further 2 had CI in excess of 30 and hence could be categorized as susceptible to stem rust at Kulumsa field condition.

3.3. Field Reaction of Wheat Lines to Stem Rust

Stem rust was observed at three of test locations, Debrezeit, Sinana and Kulumsa. Bigger-sized pustules that expand with virtually no limit were predominant on susceptible wheat lines (Figure 2). This led to breaking down of plants before maturity and/or harvest.

CO1 NS 766, AEG 91586 WHEA (westonia+ Sr B), Westonia Sr 24+ Sr 26, Co 1 NS 765, Sr 50+Sr 45 # 33, Pavon Sr 24+ Sr 31, Pavon Sr 24+ Sr 50, SA 8123 (Sr B), Sr 45/Cs #21, Sr 45+Sr 2/Cs #29, Sr 22 CO/1213, Sr 33+Sr45#23, ND Sr 1 and others were found resistant to stem rust at field conditions of Kulumsa, Deberezeit and Sinana. The lowest stem rust infection at Debrezeit was recorded on the wheat line Coins 766. On the other hand, the highest level of stem rust was recorded on wheat lines Westonia across all locations. Of the seven checks tested in the current experiment, Kekeba was the most susceptible while Shorima appeared to have the lowest level of stem rust regardless of location.

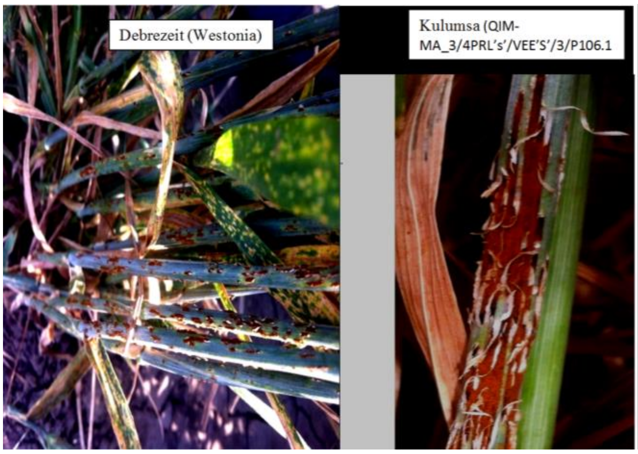

Figure 2. *Stem rust at fields of Debrezeit (left) and Kulumsa (right).*

Table 2. *Responses of wheat lines to stem rust.*

No	Genotypes	Severity KARC	KARC CI	Severity SARC	SARC CI	Severity DZARC	DZARC CI
1	CO1 NS 766	0	0	0	0	5MR	1
2	AEG 91586 WHEA (westonia+ Sr B	0	0	0	0	10MRMS	2
3	Pavon Sr 24+ Sr 31+Sr 50	5MRMS	3	0	0	10MRMS	2
4	Westonia Sr 24+ Sr 26	0	0	0	0	20MRMS	4
5	Westonia Sr 50+ Sr 26	0	0	TRMS	2.4	5MRMS	4
6	Angas Sr 32	5MSMR	3	5MS	4	20MRMS	4
7	Shorima	5MR	1	TRMS	2	7MS	5
8	Co 1 NS 765	0	0	0	0	30MRMS	6
9	Sr 50+Sr 45 # 33	0	0	0	0	10MRMS	8
10	Sr 50+Sr 45 # 28	TRS	2	0	0	10MRMS	8
11	Sr 50+Sr 45 # 35	TRSMS	2	0	0	10MRMS	8
12	Sr 50+Sr 45 # 37	TRS	2	0	0	10MSMR	8
13	CT4-NS1	5SMS	5	0	0	10MS	8
14	Pavon Sr 24+ SR 26+Sr 31	5MR	2	TRMS	2.4	10MSMR	8
15	Sr 22/CO 1213	5MSMR	3	TRMS	2.4	10MS	8
16	CTH- Ns 2	TRS	2	0	0	10SMS	10
17	CO1213	10SMS	10	0	0	10SMS	10
18	Pavon Sr 24+ Sr 31	0	0	0	0	20MSMR	16
19	Sr 45/Kulin	0	0	TRMS	2.4	20MS	16
20	Sr 33+Sr 45 #23	0	0	TRMS	2.4	20MSS	16

Note: S= Susceptible MS= Moderately Susceptible MSS =Moderately susceptible and susceptible MR= Moderately resistance SMS=Susceptible and moderately susceptible MSMR=moderately susceptible moderately resistance

Table 2. *Continued.*

No	Genotypes	Severity KARC	KARC CI	Severity SARC	SARC CI	Severity DZARC	DZARC CI
21	Sr 50+Sr 45 # 10	0	0	TRMS	2.4	20MSS	16
22	Sr 50+Sr 45 # 27	0	0	TRMS	2.4	20MSMR	16
23	Westonia Sr 24+ Sr 31	0	0	TRMS	2.4	20MSMR	16
24	Gato Sr 50	0	0	TRMS	2.4	20MS	16
25	Sr 33+Sr 45 #36	TRS	2	TRMS	2.4	20MSS	16
26	Pavon Sr 26+ SR 31	5MR	2	TRMS	2.4	20MSMR	16
27	Thatcher+Lr 67	5MR	2	TRMS	2.4	20SMS	20
28	Sr 45+Sr 2/Cs# 32	5MSMR	3	TRMS	2.4	20SMS	20
29	Honqolo	15SMS	1	TRMS	1	28MS	23
30	Pavon Sr 24+ Sr 50	0	0	0	0	30MS	24
31	SA 8123 (Sr B)	0	0	0	0	30MSS	24
32	Sr 45+Sr 2/Cs# 20	TRMR	1.6	0	0	30MSS	24
33	Westonia Sr 24+ Sr 50	5MR	2	0	0	30MS	24
34	Sr 45+Cocamba	5MSMR	3	0	0	30MSMR	24
35	Sr 50+Sr 45 # 7	0	0	TRMS	2.4	30MSS	24
36	Sr 50+Sr 45 # 5	TRMR	1.6	TRMS	2.4	30MS	24
37	Sr 33+45 # 9	TRS	2	TRMS	2.4	30MSS	24
38	Ogolcho	20MSMR	4	TRMS	3	30MSS	24
39	Kingbird	20MSS	7	5MS	3	30MSS	24
40	Av 36-29-34# 10	5MR	2	10MS	8	30MS	24

Note: S= Susceptible MS= Moderately Susceptible MSS =Moderately susceptible and susceptible MR= Moderately resistance SMS=Susceptible and moderately susceptible MSMR=moderately susceptible moderately resistance

Wheat lines with the genetic background of SrB, Sr32, Sr45, Sr50, and Sr21 in sole were effective in resisting stem rust at field conditions of all the test sites (Table 2). Similarly those which combine the following effective genes: Sr45+Sr2, Sr24+Sr50, Sr45+Sr50, Sr33+Sr45, Sr24+Sr31, Sr26+Sr31, Sr24+Sr31+Sr26, Sr24+Sr26, Sr50+Sr26 and also Sr24+Sr31+Sr50 were effective at field conditions across all the three locations (Kulumsa, Sinana and Debrezeit).

4. Discussion

In Ethiopia, several wheat cultivars have been released since the inception of wheat breeding in the 1950s. However, most of those cultivars were abandoned from production due to their susceptibility to diseases especially the cereal rusts, yellow rust (*Puccinia striiformis Westend*.), and stem rust (*Puccinia graminis f.sp. tritici*). Screening wheat lines against triple rusts both at the field and greenhouse, an inventory of the resistance genes in the current wheat cultivars, and searching for new sources of resistance are among the major objectives of a successful wheat improvement program.

Currently, most of the wheat varieties produced in wheat belt areas of the country are whipped out due to rust within a short period. Such a problem has occurred due to the development of virulent races of major rusts in Ethiopia. The most destructive rust types are stem and yellow rusts, which threaten wheat production in the country and cause considerable yield losses, sometimes even crop failures. Screening or developing wheat lines against a specific (single) rust type does not guarantee better yield or disease resistance in any cropping season as wheat rusts often occur in combination than in isolation. As a result screening and evaluating wheat lines to triple rusts should be given due attention to minimize the loss of wheat yields and feed the ever-increasing population of the world. Given the above facts, field experiments were conducted in three rust hotbeds, namely: Kulumsa, Sinana, and Debrezeit.

The current experiment in Southeastern and Central Ethiopia indicates that among tested stem rust differential hosts, virulence races to *Sr22* and *Sr30* were not detected at Sinana. At Kulumsa virulences were not detected to *Sr22* and *Sr17*. But, at Debrezeit virulences were detected in all differential hosts tested at field conditions. A stem rust differential with resistant gene SrTmp was susceptible at all locations and the current result contradicts the finding of [7]. Of wheat lines tested at field conditions, those with the genetic background of *Sr45*, and *Sr50* in sole and in different combinations were found to be effective against the prevailing stem rust races in all study areas, and the current results are in line with the findings of [19]. Wheat lines with a genetic background of resistant genes *Sr21* and *Sr32* were proven resistant in the present study [7].

5. Summary and Conclusion

Wheat Stem rust caused by *Puccinia* spp., are major devastating fungal disease worldwide and in Ethiopia. Stem rust causes grain yield losses of 100% in susceptible common wheat cultivars during disease epidemics. Despite the frequent occurrence of stem rust, there have been no surplus studies concerning stem rust resistance wheat lines with known genetic backgrounds. In addition, the genetic makeups of the resistant varieties were not identified and stated. In the present study resistant wheat lines and effective genes were evaluated and identified in the context of wheat improvement to overcome the stem rust problem in Ethiopia.

Wheat lines Pavon Sr 24+ Sr 31+Sr 50, Angas Sr 32, Westonia Sr 50+ Sr 26, Pavon Sr 24+ SR 26+Sr 31, Gato Sr 50, Sr 50+Sr 45 # 5, Westonia Sr 24+ Sr 50 and Pavon Sr 24+ Sr 50 wheat lines were proved to be resistant to stem rust under field conditions all tested locations.

Effective resistant gene and genes combination Wheat lines with the genetic background of SrB, Sr32, Sr45, Sr50, Sr21, Lr67, and Lr34 were effective in resisting stem rust at field conditions and the combination of genes: Sr45+Sr2, Sr24+Sr50, Sr45+Sr50, Sr33+Sr45, Sr24+Sr31, Sr26+Sr31, Sr24+Sr31+Sr26, Sr24+Sr26, Sr50+Sr26 and also Sr24+Sr31+Sr50 were effective against stem rust at field conditions across all the three locations and further genotyping work recommended to identify and characterize the stem rust resistance genes in that wheat germplasm associated to overall and adult plant resistance to newly identified or characterized stem rust races.

Abbreviations

CIMMYT	International Maize and Wheat Improvement Center
CI	Coefficient of Infection
CSA	Central Statistical Agency
DAP	Die Ammonium Phosphate
DZARC	Debrezeit Agricultural Research Center
EC	Emulsified Concentrate
EIAR	Ethiopian Institute of Agricultural Research
G.C	Gregorian Calendar
ICARDA	International Centre for Agricultural Research in Dry Areas
KARC	Kulumsa Agricultural Research Center
m.a.s.l	Metter Above Sea Level
mm	Millimeter
SARC	Sinana Agricultural Research Center

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Conflicts of Interest

The authors declare no conflicts of interest.

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