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Seedling Resistance to Stem Rust (*Puccinia graminis* f.sp.*tritici*) and Molecular Marker Analysis of Resistance Genes in Some Wheat Cultivars

Tesfaye Letta

Oromia Agricultural Research Institute, Addis Ababa, Ethiopia

Email address:
tesfayeletta@gmail.com

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Abstract: Stem rust caused by Puccinia graminis Pers.f.sp.*tritici* Eriks.and E.Henn.(Pgt) is one of the most destructive diseases of wheat which causing considerable yield losses in wheat growing areas worldwide. It has become a renewed threat to global wheat production after the emergence and spread of race TTKSK (also known as Ug99) and related races from Africa. Races of the pathogen in the "Ug99 lineages" are of international concern due to their virulence for widely used stem rust resistance genes and their spread throughout Africa. Disease resistant cultivars provide one of the best means for controlling stem rust. Bale zone, located on the Southeast part of Ethiopia, is one of the main wheat growing regions, playing a pivotal role in the wheat stem rust epidemic in Ethiopia. This study investigated levels of resistance in key wheat cultivars (lines) grown in Bale zone using seedling resistance evaluation and marker aided selection. Twenty wheat cultivars were evaluated for their response to stem rust infection at seedling stage under green house condition. Wheat cultivars were challenged with four stem rust races *viz* TTKSK, TRTTF, TTTTF and JRQCQ. A high level of phenotypic variation was observed in response to these races in the test entries, allowing for selection in these germplasm as a pre-breeding work. Out of the tested cultivars, three wheat cultivars exhibited low infection types (0–2) response to all the four races and hence selected as a source of resistance to stem rust. In addition, the existence of *Sr2, Sr22, Sr24, Sr25, Sr26, Sr35* and *Sr36* genes in wheat cultivars were assessed using specific DNA markers. Using molecular markers, resistance gene *Sr2* was identified in 2 cultivars and *Sr24* in five cultivars. However, no *Sr25, Sr26, Sr35* and *Sr36* were identified in any cultivars tested using DNA markers. The results of both seedling evaluation and marker based resistance gene identification will enable to breed wheat varieties with durable resistance to stem rust disease.

Keywords: Cultivars, DNA Markers, Puccinia Graminis, Seedling Resistance

1. Introduction

Stem rust (caused by *Puccinia graminis* Pers.f.sp. *Tritici* Eriks & E. Henn.) is one of the most serious diseases of wheat, worldwide [26, 21]. In Ethiopia, it has been effectively controlled through the development of resistant cultivars and deployment of effective resistance genes, especially1B/1R translocation gene *Sr31* [28]. However, in 1998, a new race of wheat stem rust pathogen designated as Ug99 (TTKSK), expressing virulence to *Sr31*,was first identified in Uganda [23, 3, 17]. It has spread through out the major wheat growing regions of Africa such as Ethiopia, Zimbabwe, Mozambique, Kenya, Sudan, Egypt, and Tanzania [27, 22].The variants exhibited stronger virulence and could rapidly spread worldwide. For example, variants with virulence against common stem rust-resistance genes *Sr24*, *Sr38*, and *Sr36* have also been detected [10]. According to the Food and Agriculture Organization (FAO) forecasts, this disease may spread east ward from Iran into countries of Central Asia [5]. TTKSK has been detected in Iran [19] and may soon threaten wheat production in the Indian sub-continent [26, 31].

Bale zone which is located in the Southeastern part of Ethiopia is one of the major wheat growing zone in the country where wheat is growing throughout the year and it is also close to Kenya, which increasing the risk of Ug99 and

its variants as well as the spread of other emerging races of stem rust from East Africa. Consequently, the resistance level of wheat cultivars growing in Bale zone has a direct impact on epidemiology of stem rust in the country. Therefore, due to the imminent risk in Ethiopian wheat production posed by Ug99 and other variants of stem rust races, analysis of resistance against stem rust and delineation of the resistance genes in the cultivars (lines) locally grown are of great significance in evaluation of the risk. It also raises the possibility of development of new rust-resistant sources.

Wheat protection and breeding of resistant cultivars using conventional methods are time-consuming, intricate, slow, and are influenced by the environment. Currently, plant breeding effort is supported using molecular markers to enhance variety development effort [32].

Host resistance is likely to be more durable when several stem rust resistance genes are pyramided in a single wheat variety; however, little is known about the resistance level of genotypes widely used in Ethiopian wheat germplasm. To date, a number of stem rust resistance genes have been identified against different races of stem rust fungus. The use of cultivars with single-gene resistance permits the selection of mutations at a single locus to render the effectiveness of resistance in a relatively short time. Hence, the use of combinations of resistance genes has been suggested as the best method for genetic control of rusts. This can be achieved by pyramiding effective resistance genes, but expression of individual resistance gene is difficult to monitor in the field.

With the advent of molecular marker technology now it seems to be possible to solve such complex problems. Molecular markers can be used to tag rust resistance genes and further ,they can serve for improvement of the efficiency of selection in plant breeding by so called, marker-assisted selection (MAS). As an alternative to gene postulation, presence of resistance genes can be determined by testing host cultivars with molecular markers linked to resistance genes. This approach over comes gene interactions and plant stage depending gene expression problems associated with traditional gene postulation [33].

There is limited information on the presence/absence of major stem rust resistance genes by reported linked or diagnostic molecular markers in Ethiopian-adapted wheat cultivars particularly those dominantly grown in Bale zone. In this study, on the basis of resistance levels to Ethiopian stem rust in wheat cultivars growing in Bale zone, the reported molecular markers closely linked to major stem rust resistance genes *Sr2, Sr22, Sr24, Sr2, Sr26, Sr35* and *Sr36,* were used to assess the prevalence of stem rust resistance genes in wheat cultivars grown in the zone. Besides, breeders may use seedling resistance information to genetically engineer new and potentially durable combinations of stem rust resistance cultivars. The development of rust-resistant wheat cultivars using seedling reaction type as a predictor of adult-plant resistance has been conducted globally, with different countries placing emphasis on those rust species of economic concern to them.

In the present study, major wheat cultivars that are growing in Bale zone were evaluated for resistance to stem rust races such as TTKSK, TRTTF, TTTTF and JRCQC under the controlled conditions of a green house at seedling stage. Hence, this study was carried out with the aim of evaluating wheat cultivars to help the breeder in identifying the best parents to be used in the breeding program in fight against stem rust by employing both seedling resistance evaluation results and marker based profiling of wheat cultivars.

2. Materials and Methods

2.1. Plant Materials

A total of 20 tested wheat cultivars (both bread and durum) which are dominantly grown in Bale zone were included in this study. Among these, nine cultivars were durum wheat and the remaining were bread wheat cultivars and advanced lines. Details of wheat cultivars, their code, and pedigree are presented inTable1.

Table 1. *Wheat cultivars used in this study along with their pedigrees and year of release.*

SN	Code	Pedigree/selection history	Name of the cultivar/genotype	Year of release
1	G1	KIRITATI//2*PBW65/2*SERI.1B	Danda'a	2010
2	G2	14F/HAR1685	Sanate	2014
3	G3	HAR1889	Sofumar	1999
4	G4	WORRAKATTA/PASTOR	Mandoyu	2014
5	G5	ETBW5513	Advanced line	-
6	G6	HAR1480	Maddawalabu	1999
7	G7	UTQUE96/3/PYN/BAU//MILLAN	Advanced line	
8	G8	NS732/HER//MILLAN/SHA7	Advanced line	
9	G9	LABUD/NIGERIS3/GAN(CD98206)	Ejersa	2005
10	G10	98OSNGEDIRAF/GWEROU#15)	Bakalcha	2005
11	G11	DZ2234	Ilani	2004
12	G12	DZ1605	Leliso	2002
13	G13	CD94523	Tate	2009
14	G14	ALTAR84ALTO-1/AJAYA	Obsa	2006

SN	Code	Pedigree/selection history	Name of the cultivar/genotype	Year of release
15	G15	DZ2227	Oda	2004
16	G16	4/B/R9096#221001(980SNpatho)	Toltu	2010
17	G17	CHEN/TE3/BUSHEN4/3/AC089CDSS92B1ZOZ	Dire	2012
18	G18	ETBW5484	Buluk	2015
19	G19	ETBW5653	Liben	2015
20	G20	ETBW115	Digalu	2005

2.2. Stem rust Evaluation

2.2.1. Pathogen Races and Their Virulence

All wheat cultivars were evaluated for seedling resistance to four *Pgt* races:TTKSK,TRTTF,TTTTF and JRCQC in a green house at the USDA Cereal Disease Laboratory in St. Paul, MN.The race designation is based on the letter code nomenclature system [24, 25], modified to further delineate races in the TTKS lineage [10]. These races were selected based on their differential virulence pattern. Race TTKSK (Ug99) has a wide virulence spectrum and is rapidly evolving in East Africa. Race TTTTF is the most widely virulent race known in the United States, producing high infection types (ITs) on the majority of stem rust differential lines [9]. Races TRTTF and JRCQC present in Ethiopia, possess a virulence combination that over comes both the resistance genes *Sr13* and *Sr9e*, two genes present at high frequency in durum wheat [13]. All isolates were derived from single pustules, increased in isolation, and stored at-80^{o}C. Information about the stem rust isolates used in the disease phenotyping test is summarized in Table2.

Table 2. *Origin and virulence properties of the Puccinia graminis f.sp.tritici races used to evaluate the wheat cultivars.*

Race	Isolate	Origin	Virulence/avirulence formula
TTKSK(Ug99)	04KEN156/04	Kenya	*Sr5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,31,38,McN/Sr24,36,Tmp*
TRTTF	06YEM34-1	Yemen	*Sr5,6,7b,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,McN/Sr8a,24,31*
TTTTF	01MN84A-1-2	United States	*Sr5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,McN/Sr24,31*
JRCQC	09ETH08-3	Ethiopia	*Sr21,9a,9d,9e,9g,11,6,17,McN/Sr5,7b,8a,36,9b,30,Tmp,24,31,38*

2.2.2. Inoculation, Incubation, and Disease Assessment

The wheat cultivars were evaluated under controlled conditions using a Completely Randomized Design and were repeated once over time for each of the four races. Five to six seedlings per line were inoculated on the fully expanded primary leaves 8 to 9days after planting. This work was conducted at the Cereal Disease Laboratory, St.Paul, MN, and the experimental procedures in inoculation and disease assessment were performed [9]. Wheat cultivar Mc Nair 701(CItr15288) was used as susceptible control in all evaluations to monitor the virulence of the race. Plants were evaluated for their Infection Types (ITs) 14 days post inoculation using the 0 to 4 scale [30], where ITs of 0,1,2,or X are considered as incompatible (low ITs), whereas ITs 3or higher were considered as compatible (high ITs).When IT = 0 (immune reaction) occurred, the test was repeated to exclude the possibility of disease escape. Lines giving variable reactions between experiments were repeated again to confirm the most likely reactions.

2.3. Genomic DNA Extraction and Genotyping

Eight to ten seeds of each wheat cultivars were sown in pots in a green house, and leaf tissue from three to four plants of each cultivar were harvested after 2 to 4 wks of growth for genomic DNA extraction. Genomic DNA extraction and other molecular procedures were performed [15]. PCR products were analyzed using either APAGE using Licor and/or ABI3730 DNA Analyzer. A 25-bp DNA ladder was used for size standard and the sizes of PCR amplicons were recorded accordingly. Genotype alleles of the 20 wheat cultivars were scored using standard lines for each *Sr* locus as an allele reference set.

2.4. Marker Primers Used

High quality molecular markers that are closely linked, co-dominant and high throughput markers (combination of microsatellite and sequence tagged site (STS) markers that are linked/associated with seven reported major *Sr* genes (*Sr2 ,Sr22, Sr24, Sr25, Sr26, Sr35* and *Sr36*) were included in this study and were used for haplotyping major *Sr* loci.These selected linked markers for major stem rust resistance genes were collected from related web sites and literatures. Accordingly, seven *Sr* genes were selected: *Sr2, Sr22, Sr24*, *Sr25, Sr26, Sr35* and *Sr36* and for each locus, diagnostic and closely linked markers were identified and used for marker analysis in each wheat cultivar. Details of the primers used in this study are presented in Table 3.The wheat lines carrying interested specific resistance gene were included as a positive control for each *Sr* locus (Table 4).Comparisons were made between each wheat cultivars and positive control for each *Sr* locus to know whether a particular wheat cultivar has specific resistance gene or not. Comparisons were also made one gene at a time and done for each *Sr* locus.

Table 3. *Markers used for profiling wheat cultivars for major stem rust resistance genes and their primer sequences.*

Sr gene	Chromosome	Resistance	Marker name	Primer sequences5'-3'
Sr2	3BS	APR[@]	gwm533F	5'-AAGGCGAATCAAACGGAATA
			Gwm533R	5'-GTTGCTTTAGGGGAAAAGCC
Sr22	7AL	race-specific	csIH81-BMF	TTCCATAAGTTCCTACAGTAC
			csIH81-BMR	TAGACAAACAAGATTTAGCAC
Sr24/Lr24	1BS,3DL	race-specific	barc71F	GCGCTTGTTCCTCACCTGCTCATA
			Barc71R	GCGTATATTCTCTCGTCTTCTTGTTGGTT
Sr25/Lr19	7DL	race-specific	GbF	CATCCTTGGGGACCTC
			GbR	CCAGCTCGCATACATCCA
Sr26	6AL	race-specific	Sr26#43F	AATCGTCCACATTGGCTTCT
			Sr26#43R	CGCAACAAAATCATGCACTA
Sr35	3AL	race-specific	barc51F	CGCATGAGCAAACAAGCCAACAACT
			barc51R	CGGCCACAGCATCGGTTCTCCAAA
	3AL	race-specific	cfa2076F	CGAAAAACCATGATCGACAG
			cfa2076R	ACCTGTCCAGCTAGCCTCCA
Sr36	2B	race-specific	wmc477F	CGTCGAAAACCGTACACTCTCC
			wmc477R	GCGAAACAGAATAGCCCTGATG

[@]APR=Adult Plant Resistance

Table 4. *Standard cultivars used for haplotyping wheat cultivars for major stem rust loci.*

S. No	Line	*Sr* gene
1	Pavon 76	2
2	Hope	2
3	SwSr22T.B.	22
4	DK14	22
5	LcSr24Ag	24
6	BtSr24 Ag	24
7	Agatha/9*LMPG	25
8	LcSr25Ars	25
9	Agrus	25
10	Eagle	26
11	Line T sel.	26
12	Federation*4/Kavkaz	31
13	Line E/Kavkaz	31
14	Mq(2)5XG2919	35
15	Prelude/SrTt-1	36

3. Results and Discussion

3.1. Wheat Seedling Evaluation

The reaction of twenty wheat cultivars growing in Bale zone to the *Pgt* races used in this study were presented in Table 5. In all of the seedling tests, the susceptible controls McNair 701 was heavily infected and exhibited the expected compatible ITs ranging from 3 to 4 to all the four races. The high levels of infection achieved in each experiment allowed for the reliable scoring of ITs on all wheat cultivars. A high level of variability was observed in response to stem rust races TTKSK, TRTTF, TTTTF and JRCQC in wheat cultivars (Figure1).

Seedling Infection Types (ITs) for each of the 20 wheat cultivars is presented in Table5.The frequencies of the cultivars categorized as resistant, susceptible, and heterogeneous in their reaction to the four races varied markedly depending on the race. The result of seedling test indicates that there was successful inoculation as shown by the susceptible infection types of the check cultivar 'McNear with IT score of 33+ and 4.The twenty wheat cultivars displayed a wide range of seedling infection types to all four races. The ITs frequency distribution presented in Figure1depicts a variability in reaction among the test cultivars for all four races used in this study with the majority of the cultivars showing resistance reaction with score of 2.Only few cultivars showed a susceptible reaction with score of 3 (Figure1).The results of test of the wheat cultivars to four races showed that, the tested entries differ in their resistance to disease. For example, seedling resistance toTTKSK (Ug99), TRTTF, JRCQC was observed in 16 (80%), 19 (95%),16 (80%), and 18 (90%) cultivars, respectively (Table 6).The ranking values of the four races based on their frequencies of avirulence/virulence interactions considering wheat cultivars collection as a whole (with TRTTF showing the highest degree of avirulent interactions, followed by JRCQC. Races TTTTF and TTKSK showed relatively the highest frequency of virulent interactions).When considering all four races together, there are only three (15%) wheat cultivars that showed resistant (IT=1 to 2) to all four races. The current results revealed that the majority of the wheat cultivars showed infection type score of 2 and 22+, particularly the durum wheat cultivars. Similar results were also reported in wild emmer wheat [2] [20].

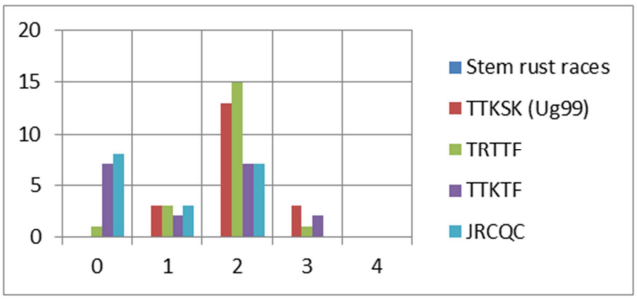

Figure 1. *Frequency distribution of infection types (ITs) of twenty wheat cultivars evaluated at the seedling stage with four stem rust races.*

Table 5. *Phenotypic response to four stem rust races (TTKSK, TRTTF, JRCQC and TKTTF) of 20 wheat cultivars /genotypes included in the study.*

S.N	Genotype code	TTKSK(Ug99)	TRTTF	JRCQC	TKTTF
1	G1	3	3	0	3-
2	G2	2-	2	;	2-
3	G3	23C	;2-	;	0;
4	G4	2-	2-	2-	0?
5	G5	2-/3	2-	;	2-
6	G6	3	2-	0	0?
7	G7	1;	2	;2-	2-
8	G8	2	2	;1	0/3
9	G9	23/2-	2	;1/2/3	3-/;1
10	G10	2-	1;	2	;2-
11	G11	;2-	;	0;	0;
12	G12	1;	;1+	2-	2-
13	G3	2/2+	2-	2	2-
14	G14	2	2-	2+/3-	;1
15	G15	1;	;1	;	;
16	G16	2	2-	2	2-
17	G17	2	2-	2	;1
18	G18	2-	2-	;1	0?
19	G19	3	2-	0	0?
20	G20	2+	22+	;1	3+

Table 6. *Numbers and frequencies of infection types (IT) and resistant, susceptible and heterogeneous reactions of the twenty wheat cultivars included in this study to four races of Puccinia graminis f.sp.tritici and the combined reaction to all races.*

IT[1]/Reaction	TTKSK(Ug99)	TRTTF	TTKTF	JRCQC	All races	% to all races
“0”or“;”	0	1	7	8	0	0
“1”	3	3	2	3	1	0.05
“2”or“23”or“X”	13	15	7	7	2	0.1
Resistant Reaction	16	19	16	18	3	0.15
“3”	3	1	2	0	0	0
“4”	0	0	0	0	0	0
Susceptible Reaction	3	1	2	0	0	0
Heterogeneous[2]	1	0	2	2	0	0

[1]Infectiontypesobservedonseedlingsat14dayspost-inoculationusinga0to4scaleaccordingto[30],whereinfectiontypesof;1,2,orXareconsideredasalowITandITsof3or4areconsideredasahighIT,[2]Cultivarsthatcontained bothresistantandsusceptibleplants.

3.2. Identification of Stem Rust Resistance Genes in Wheat Cultivars Using Molecular Markers

Molecular markers are used in wheat resistance breeding for identification of designated resistance genes in genotypes where the genetic background has not yet been clarified like most durum wheat varieties of Ethiopia[7].Closely linked markers provide a means for the selection and identification of important genes in breeding programs and, in the case of disease resistance, this can be done in the absence of pathogens [1]. PCR-based DNA markers were used to check rust resistance genes among the 20 wheat cultivars that are majorly grown in Bale zone and eight markers associated with seven stem rust resistance genes were screened. Similar work were also conducted to study the presence of *Sr* genes (*Sr2*, *Sr22*, *Sr24*, *Sr36* ,and *Sr46*), in 88 cultivars of spring soft wheat in Kazakhstan [14]. Additionally, 58 tetraploid wheat accessions of Ethiopia were screened for 30 *Sr* genes using SSR and STS markers [13]. Haplotypes were sorted for each stem rust resistance gene by the size of their PCR amplicons. Similar haplotypes for each gene were grouped together and compared to the original source of the gene (check lines). Detail results for each stem rust loci were presented in Table 7and are discussed below:

3.2.1. Sr2 Screening

Pavon76 and Hope wheat cultivars were used as a positive control for *Sr2* gene. *Sr2* is located on the short arm of the wheat chromosome 3B [8].Two closely linked microsatellite markers gwm533 and BARC133 were used for haplotyping *Sr2* but in this study, only marker gwm 533 is used. Marker *gwm* 533 amplified a 120 bp PCR fragment which is diagnostic for *Sr2* [29].Using this marker, out of 20 wheat cultivars tested, only two cultivars showed the *Sr2* haplotype.

3.2.2. Sr22 Screening

Wheat cultivars SwSr22T.B and DK14 were used aa a positive control for *Sr22*gene. *Sr22* was previously mapped on the long arm of chromosome 7A [12].Three markers,cfa2019,cfa2123 and BARC121 are linked to this gene [18], but in this study, marker IH81-BM was used in screening for this gene as it is best in screening for this gene and useful in MAS for *Sr22*. PCR amplification by this marker showed 237 bp PCR amplicon for *Sr22* source germplasm (positive control) and 355-bp for non positive cultivars. Among the test cultivars, none of them showed this

type of PCR amplicon which is similar to that of check cultivars.

3.2.3. Sr24 Screening

Wheat germplasms LcSr24Ag and BtSr24Ag were used as a positive control for *Sr24* gene. *Sr24* was previously mapped on the long arm of chromosome 3D [16]. BARC71 amplified two fragments of 88 bp and null alleles.PCR fragment with 88bp is shown in *Sr24* carrying lines such as sLcSr24Ag and BtSr24Ag.Out of the test wheat cultivars, five cultivars showed the same *Sr24* marker profile as that of the positive lines.

3.2.4. Sr25 Screening

Gene *Sr25* transferred from wheat *Thinpyrum ponticum*, and located on the long arm of chromosome 7D. It is usually closely linked to leaf rust resistance gene *Lr19*. Agatha/9*LMPG, cSr25Ars and Agrus were used as the positive control for *Sr25* gene. A dominant marker, Gb was used for haplotyping *Sr25*.This marker amplified a130bp fragment only in the *Sr25* positive lines and no PCR product was obtained in wheat lines that lack *Sr25*.This result showed that no amplification of the 130bpb and in all the 20 wheat cultivars grown in Bale zone.

3.2.5. Sr26 Screening

Eagle and Line Tsel were used as a positive control for *Sr26* gene. *Sr26* is located on the distal portion of chromosome 6AL [4].One dominant marker, Sr26#43 was used for haplotyping *Sr26* gene.Sr26#43 amplified a 207 bp PCR product in wheat lines carrying *Sr26* while no amplification product occurred in wheat lines without *Sr26*.The result indicated that, primer *Sr26*#43 amplified a 207 bp and in the positive control for *Sr26*, while no bands were amplified in the remaining wheat cultivars, indicating that the tested cultivars do not contain the resistance gene *Sr26*.

3.2.6. Sr35 Screening

Wheat line Mq (2)5XG2919 was used as a positive control for *Sr35* gene. *Sr35* is located on the long arm of chromosome 3A. Markers,BARC51 and cfa2076 were used for profiling wheat cultivars for *Sr35* gene. BARC51 amplified a 218 bp PCR product in wheat lines carrying *Sr35* while 225-307 bp PCR product in wheat lines without *Sr35*. Similarly, cfa2076 amplified 190 bp PCR product in wheat lines with positive *Sr35* while three different amplicons, null, 192 and 194 bp PCR products in wheat lines without *Sr35* gene. No test cultivars showed similar marker profile to that of *Sr35* carrying line.

3.2.7. Sr36 Screening

Wheat line Prelude/SrTt-1 was used as a positive control for *Sr36* gene. *Sr36* is located on the short arm of chromosome 2B [6]. Marker wmc477 was used for profiling wheat cultivars for *Sr36* gene.Wmc477 amplified a 187 bp PCR product in wheat lines carrying *Sr36* while 162-169 bp PCR product were amplified in wheat lines without *Sr36*. .No test cultivars showed similar marker profile to that of *Sr36* carrying lines.

Table 7. *Results for wheat stem rust resistance gene detection using linked molecular markers.*

S.N	Genotype code	*Sr2*	*Sr22*	*Sr24/Lr24*	*Sr25/Lr19*	*Sr26*	*Sr35*	*Sr36*
1	G1	no	no	*Sr24/Lr24*	no	no	no	no
2	G2	*Sr2*	no	No	no	no	no	no
3	G3	no	no	*Sr24/Lr24*	no	no	no	no
4	G4	no	no	*Sr24/Lr24*	no	no	no	no
5	G5	no	no	No	no	no	no	no
6	G6	no	no	*Sr24/Lr24*	no	no	no	no
7	G7	no	no	No	no	no	no	no
8	G8	no	no	No	no	no	no	no
9	G9	no	no	No	no	no	no	no
10	G10	no	no	No	no	no	no	no
11	G11	no	no	No	no	no	no	no
12	G12	no	no	No	no	no	no	no
13	G13	no	no	No	no	no	no	no
14	G14	no	no	No	no	no	no	no
15	G15	no	no	No	no	no	no	no
16	G16	no	no	No	no	no	no	no
17	G17	no	no	*Sr24/Lr24*	no	no	no	no
18	G18	no	no	No	no	no	no	no
19	G19	no	no	No	no	no	no	no
20	G20	*Sr2*	N0	No	no	no	no	no

4. Conclusion

Ethiopia is one of the hot spot areas for the development of the present wheat stem rust complex. The disease has become a major threat to wheat production after the epidemics of 1974 and 1993, 2010 and 2014 that drove out many bread wheat (*Triticum aestivum* L.) varieties, such as Lacketch, Enkoy and Digelu of production. Achieving more durable resistance will depend on deploying diverse combinations of race-specific qualitative resistance and/or race-non specific quantitative resistance genes. The development of rust-resistant wheat cultivars using seedling reaction type as a predictor of adult-plant resistance has been conducted

globally, with different countries placing emphasis on those rust species of economic concern to them.

MAS is a powerful alternative to facilitate new gene deployment and gene pyramiding for quick release of rust-resistant cultivars. Wheat cultivars were screened for major stem rust genes to identify sources of resistance parents to combat the spread of stem rust. Similar marker profile among different genotypes facilitates hypothesis testing for resistance genes. However, marker profiling alone are in adequate to confirm the presence of a specific resistant allele in wheat cultivars. Combining marker profiling with pedigree information that allows the identification of a source of the resistance allele can greatly increase the success of the gene postulation based on markers. It is not always possible to obtain the reaction of breeding lines to specific races of rust so haplotyping with linked markers can be quite useful for strategic crossing and selection. Even though many cultivars showed resistance reaction to four major stem rust races, the used markers did not identify the resistance gene available in those cultivars because of the limitations in the currently used molecular markers. Because of these, low frequency of *Sr2* gene is identified in the studied cultivars which otherwise many of them supposed to have *Sr2* gene since most of them are derivatives of CIMMYT materials where *Sr2* is extensively deployed in CIMMYT wheat breeding. Thus, the use of multiple linked and diagnostic markers are imperative to reliably identify the resistance genes available in wheat genotypes. The present study confirms that, most wheat cultivars used in this study have showed resistance to these four races and can serve as an alternative source of resistance in wheat improvement program.

Recommendation

The current Ethiopian wheat cultivars (except few varieties) donot have an adequate level of resistance to stem rust races populations prevailing in the region, indicating the need for incorporating more effective resistance genes in to the local wheat cultivars. Cultivars carrying resistant genes against the four races should be tested against a collection of other different stem rust isolates in the green house to determine whether they possess abroad-based resistance. Since most molecular markers used in this study are not diagnostic and also few in number, more closely linked stem rust resistance genes and more number of markers should be used to identify resistance genes available in the studied wheat cultivars as most of wheat cultivars did not show resistance genes that they are supposed to have based on pedigree information.

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