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

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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 overcomes 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 resistance 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 in Table 1.

| SN | Code | Pedigree/selection history | Name of the cultivar/genotype | Year of release |
|---|---|---|---|---|
| 1 | G1 | KIRITATU/2*PBW65/2*SERL1B | 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 |
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 overcomes 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°C. Information about the stem rust isolates used in the disease phenotyping test is summarized in Table 2.

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

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 9 days 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 McNair 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 3 or 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 cultivar 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 

| Race        | Isolate   | Origin     | Virulence/avirulence formula |
|-------------|-----------|------------|-----------------------------|
| TTKSK(Ug99) | 04KEN156/04 | Kenya      | Sr5,6,7b,8a,9a,9b,9d,9e,10,11,17,21,30,31,38,Mc/N/Sr24,36,Tmp |
| TRTTF       | 06YEM34-1  | Yemen      | Sr5,6,7b,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,Mc/N/Sr8a,24,31 |
| TTTTF       | 01MN84A1-1-2 | United States | Sr5,6,7b,8a,9a,9b,9d,9e,9g,10,11,17,21,30,36,38,Mc/N/Sr24,31 |
| JRCQC       | 09ETH08-3  | Ethiopia   | Sr21,9a,9d,9e,9g,9l,6,17,Mc/N/Sr5,7b,8a,36,9b,30,Tmp,24,31,38 |

| Name of the cultivar/genotype | Year of release |
|------------------------------|----------------|
| Oda                          | 2004           |
| Toltu                        | 2010           |
| Dire                         | 2012           |
| Baluk                        | 2015           |
| Liben                        | 2015           |
| Digalu                       | 2005           |
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’-AAGGCGAATCAAAACGGAATA      |
|          |            |                  | Gwm533R     | 5’-GTGCTTTTGAAGGAAAGGCC       |
| Sr22     | 7AL        | race-specific    | csIH81-BMF  | TTCCATAAGTTCCACAGTAC          |
|          |            |                  | csIH81-BMR  | TAGACAAAAGGATTTAGCAC          |
| Sr24/Er24| 1BS,3DL    | race-specific    | bare71F     | GCGTTGTCTCCTCAGGTCCTATA       |
|          |            |                  | Bae71R      | GCGATATTTCCTGTCCTTTGTTGTTGTT |
| Sr25/Lr19| 7DL        | race-specific    | GbF         | CACTCTGGGGGACCTCT             |
|          |            |                  | GbR         | CACGCTTGCTACATTCCA            |
| Sr26     | 6AL        | race-specific    | Sr26#43F    | AATCGTTACATTGGCTTTCT          |
|          |            |                  | Sr26#43R    | CGCAAAAAATGATGACTA            |
|          |            |                  | bare51F     | CAGTGAGAAACCAAAAGCCAAACACT    |
|          |            |                  | bare51R     | CGGCAACAGCAGTTGCCTTCAAA       |
| Sr35     | 3AL        | race-specific    | cia2076F    | CGAAAAACCATGACGAG             |
|          |            |                  | cia2076R    | ACCTGTCCAGCTGCTCCCA           |
| Sr36     | 2B         | race-specific    | wme477F     | CGTGGAAAACGCTACCTCCTC         |
|          |            |                  | wme477R     | CGGAAACAGAATAGCCCTGATG        |

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     | LeSr24Ag       | 24      |
| 6     | BtSr24 Ag      | 24      |
| 7     | Agatha/9*LMPG  | 25      |
| 8     | LeSr25Ar       | 25      |
| 9     | Agrus          | 25      |
| 10    | Eagle          | 26      |
| 11    | Line T sel.    | 26      |
| 12    | Federation*4/Kavkaz | 31    |
| 13    | Line E/Kavkaz  | 31      |
| 14    | Mg(2)XG2919    | 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 3+ and 4. The twenty wheat cultivars displayed a wide range of seedling infection types to all four races. The ITs frequency distribution presented in Figure1 depicts 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 for four races showed that, the tested entries differ in their resistance to disease. For example, seedling resistance to TTKSK (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* /Reaction | TTKSK(Ug99) | TRTTF | TKTTF | 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 “3” | 16 | 19 | 16 | 18 | 3 | 0.15 |
| “4”           | 3           | 1     | 2     | 0     | 0         | 0             |
| Susceptible Reaction | 0 | 0 | 0 | 0 | 0 | 0 |
| Heterogeneous2 | 1           | 0     | 2     | 2     | 0         | 0             |

Notes: *IT: Reaction: 0, 1, 2, or X are considered as low infection types; 3 or 4 are considered as high IT. Cultivars that contained both resistant and susceptible reactions.

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 7 and 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 SwSr22TB and DK14 were used as 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 Thinopyrum 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 a 130bp 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 130bp 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 3A. 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    | no        | no        | no     | no     | no     |
| 2   | G2            | Sr2   | no    | no        | no        | no     | no     | no     |
| 3   | G3            | no    | no    | no        | no        | no     | no     | no     |
| 4   | G4            | no    | no    | no        | no        | no     | no     | no     |
| 5   | G5            | no    | no    | no        | no        | no     | no     | no     |
| 6   | G6            | no    | no    | no        | 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    | no        | 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) do not 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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