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RESEARCH ARTICLE

Haplotype analysis of molecular markers linked to stem rust resistance genes in Ethiopian improved durum wheat varieties and tetraploid wheat landraces

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Abstract The recent emergence of wheat stem rust race Ug99 (TTKSK) and related strains threaten Ethiopian as well as world wheat production because they overcome widely used resistance genes that had been effective for many years. The major cause which aggravates the ineffectiveness of Ethiopian wheat varieties against stem rust is the narrow genetic base on which the breeding for resistance has been founded, however, little is known about the resistance genotypes of Ethiopian durum wheat varieties and tetraploid wheat landraces. The objective of the study was to identify stem rust resistance genes that are present in the Ethiopian tetraploid wheat landraces and improved durum wheat varieties using molecular markers and assess which genes are effective for current Ethiopian stem rust races of *Puccinia graminis* f. sp. *tritici* including Ug99. The investigated 58 tetraploid wheat accessions consisted of 32 (*Triticum durum s.l. incl. Triticum aethiopicum* Jakubz., *Triticum polonicum*) landraces and 22 registered *T. durum* varieties released in Ethiopia between 1966 and 2009 and four *T. durum* varieties from ICARDA. A total of 17 molecular markers (SSR, EST and InDel) linked or diagnostic for stem rust resistance genes *Sr2*, *Sr13*, *Sr22* and *Sr35* were used for genotyping. Haplotype analysis indicated that only few of the Ethiopian durum wheat varieties carried *Sr13*. The resistant variety 'Sebatel' showed a haplotype for *Sr2* and *Sr22* and variety 'Boohai' for *Sr22*, however further evaluation is needed for the diagnostic value of these haplotypes. This study is the first report on the presence of stem rust resistance (*Sr*) genes in Ethiopian durum wheat varieties and tetraploid wheat landraces based on linked or associated molecular markers. Thus it might help in the identification of varieties carrying resistant alleles that provide valuable genetic material for the development of new improved varieties in further breeding programmes.

Keywords Diagnostic molecular markers · Haplotype · *Sr* gene · Stem rust · Tetraploid wheats

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Abbreviations

BARC	Beltsville Agriculture Research Center
bp	Base pair
CI	Coefficient of infection
CIMMYT	International Maize and Wheat Improvement Center
cM	CentiMorgan
DN	Denbi
DZ	Debre-Zeit
EST	Expressed sequence tags
GS	Growth stage
GWM	Gatersleben wheat microsatellite
ICARDA	International Center for Agricultural Research in the Dry Areas
InDel	Insertion–deletion
IR	Infection response
IT	Infection type
LR	Landrace
MAS	Marker-assisted selection
MS	Main season
OS	Off season
PCR	Polymerase chain reaction
Pgt	*Puccinia graminis* f. sp. *tritici*
Sev	Field severity
Sr	Stem rust resistance
SSR	Simple sequence repeat
WMC	Wheat microsatellite consortium

Introduction

Wheat is one of the most important cereals cultivated in Ethiopia. It ranks fourth after Teff [*Eragrostis tef* (Zucc.) Trotter), Maize (*Zea mays* L.) and Sorghum (*Sorghum bicolor* (L.) Moench] in area coverage and third in total production (CSA 2009). The average per capital consumption of wheat in Ethiopia was estimated to be 39 kg/year during 1994–1997 and 331,000 t of wheat imported to meet the national wheat requirements during 1995–1997 (CIMMYT 2000). In the country, more than 70 bread and 30 durum wheat varieties have been released for production since 1940s. However, the national average yield of wheat is still 1.4 tons/ha (FAOSTAT 2003). Demand of wheat has steadily increased in the last decades in Ethiopia particularly due to the emergence of many food processing industries. Wheat in Ethiopia is represented by hexaploid ($2n = 6X = 42$) and tetraploid ($2n = 4X = 28$) species. Bread wheat is widely grown hexaploid wheat (*Triticum aestivum* L.), while durum wheat (*Triticum durum* s.l. incl. *Triticum aethiopicum*.) and emmer wheat (*Triticum dicoccon* Schrank) are the two cultivated tetraploid wheats.

The enormous genetic variability of the cultivated tetraploid wheats makes Ethiopia the Center of diversity for cultivated tetraploid wheats (Vavilov 1929). In Ethiopia, there are numerous accessions of wheat germplasm (about 12,000 accessions) that has been collected and maintained mainly in the Institute of Biodiversity Conservation (Addis Ababa, Ethiopia). These wild and cultivated relatives of wheat offer a tremendous potential to be used as a source of stem rust resistance, and to broaden the genetic basis of wheat cultivars. Landraces have priority, as they may be used as starting population for cultivar development (Lakew et al. 1997; Teklu and Hammer 2009), specific adaptation to the different environmental conditions in their regions of growth, and as sources for the introgression of genes and quantitative trait loci conferring resistance to biotic (Huang et al. 1997; Mujeeb-Kati and Rajarm 2000) and abiotic stresses (Forster et al. 2000). Despite these valuable features, the use of landraces has been discouraged in many developing countries on the basis that they have low yield potential (Teklu and Hammer 2009).

Even if over 30 fungal diseases of wheat have been identified in Ethiopia, stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici* (*Pgt*) is a major production constraint in most wheat growing areas of the country and causes up to 100 % yield losses in epidemic outbreaks (Admassu et al. 2004). The country also considered as one of the hot spot areas for the development of the present wheat stem rust complex (Leppik 1970). The disease has become a major threat of wheat production after the epidemics of 1974 and 1993 that drove two bread wheat varieties, 'Lacketch' and 'Enkoy', out of production (Badebo 2002; Beteselassie et al. 2007). A new virulent stem rust race, Ug99, was first identified in Uganda in 1999 (Pretorius et al. 2000), then it spred to Kenya in 2001 and to Ethiopia in 2003 following the migration path suggested by Singh et al. (2006). Due to Ug99 and its variants widely used major stem rust resistance genes became ineffective (Singh et al. 2006; Jin et al. 2007, Yu et al. 2011). Therefore, from the identified 50 stem rust resistance genes, only a few are effective against

Ug99. *Sr2*, *13*, *22*, *25*, *26*, *35*, *39* and *Sr40* were reported genes to be effective against Ug99 (Singh et al. 2006, 2008; Yu et al. 2010, 2011).

The major cause for the ineffectiveness of wheat varieties against stem rust is the narrow genetic base on which the breeding for resistance has been founded (Beteselassie et al. 2007). Earlier works on stem rust in Ethiopia concentrated on occurrence of *Pgt* physiologic races on hexaploid wheat (Temam 1984; Maresha 1996). Badebo et al. (1990) postulated yellow rust resistance genes (*Yr*) in hexaploid wheat varieties. However, little work has been done on gene postulations on Ethiopian tetraploid wheat accessions. Dawit (2008) postulated *Yr* genes in Ethiopian hexaploid and durum wheat varieties. Beteselassie et al. (2007) postulated the stem rust (*Sr*) genes of Ethiopian tetraploid and emmer wheat accessions through multipathotype testing. The basis for genetic analysis and gene postulation for the past studies is resistance-specificity of the host, as expressed by distinct qualitative disease reactions on seedlings, i.e. infection types (ITs), when challenged by a series of pathogen isolates.

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 (Vanzetti et al. 2011). In recent times there have been advances in development and mapping of molecular markers that are diagnostic for major *Sr* genes (Saal and Wricke 1999; Spielmeyer et al. 2003; Hayden et al. 2004; Mago et al. 2005, 2011; Tsilo et al. 2008; Babiker et al. 2009; Wu et al. 2009; Hiebert et al. 2010; Olson et al. 2010; Liu et al. 2010; Yu et al. 2010; Zhang et al. 2010; Admassu et al. 2011; Simons et al. 2011). However, there are no reports on identification of stem rust resistance genes in Ethiopian durum wheat varieties and tetraploid wheat landraces by reported linked or diagnostic molecular markers.

Objectives of this work were therefore (1) to identify stem rust resistance genes that are present in the durum wheat varieties and tetraploid wheat landraces using molecular markers, (2) to assess which *Sr* genes are effective for current Ethiopian stem rust races of *Pgt* including Ug99 based on the response of the accessions against field stem rust evaluation.

Materials and methods

Plant materials

A set of 58 tetraploid wheat accessions were used in this study. The materials consisted of 22 durum wheat (*T. durum* Desf.) varieties that were released in Ethiopia between 1966 and 2009 and 32 Ethiopian tetraploid wheat landraces (*T. durum* Desf. s.l., incl. *T. aethiopicum*, *T. turgidum* L. and *T. polonicum* L.). Additionally four durum varieties from ICARDA were included in the study. These accessions were obtained from Debre–Zeit Agricultural Research Center, Ethiopia, which also provided the taxonomical classification based on morphological characters. Lists of varieties and landraces with their stem rust response are presented in Supplemental Tables 1 and 2. More information about the varieties were presented in Haile et al. (2012b). *Sr* gene carrying differentials W2691SR13 (*Sr13*), SWSR22TB (*Sr22*), W3763–SR35 (*Sr35*) and Kingbird#1 (stem rust resistant line carrying the *Sr2* complex and other unknown genes based on phenotype, Singh et al. 2009) were used as reference lines for molecular markers analysis.

Phenotyping

For the varieties, seven field trials were carried out during three consecutive years (2008, 2009 and 2010) at two wheat growing locations (Debre-Zeit, 2000 m a.s.l. and black soil; and Denbi, 1800 m a.s.l. with light sandy soil, abbreviated as DZ and DN, respectively) of Ethiopia. DZ is one of the hot spot locations and an internationally selected site for stem rust evaluation. At this location we have evaluated the materials twice a year, i.e. main season (July–October, rain-fed) and off-season (January–April, irrigated). So it was possible to expose the tested varieties to all the year round available stem rust races of Ethiopia. But the landraces were tested only in 2009 off-season at DZ.

The accessions were sown in two rows of 1 m length and 0.20 m spacing between rows. To facilitate and optimize the natural infection, the nursery was enclosed by spreader rows comprising ‘PBW343’ (bread wheat with the gene *Sr31*) (Das et al. 2006), ‘Morocco’ (susceptible bread wheat), ‘local red’ (susceptible durum wheat) and ‘Arendeto’ (susceptible Ethiopian tetraploid wheat variety) in 2:1:1:1 ratio, respectively. In addition to the natural infection, the

trial was also artificially inoculated with *Pgt* urediniospores. Urediniospores from Ug99, bread and durum bulks were mixed in 1:1:1 ratio and about 2 mg/ml of spores was suspended in distilled water and then a drop of Tween 20 was applied per 10 ml of suspension and inoculated using a syringe. Inoculation started at stem elongation growth stage and was repeated 2–3 times every week.

For scoring stem rust severity in the field, the modified Cobb Scale (Peterson et al. 1948) was used to determine the percentage of tissue infected with rust. The host response to infection in the field was scored using "R" or resistant (small uredinia surrounded by chlorosis or necrosis); "MR" or moderately resistant (medium sized uredinia surrounded by chlorosis or necrosis); "MS" or moderately susceptible (medium-large compatible uredinia without chlorosis and necrosis); and "S" or susceptible (large, compatible uredinia without chlorosis and necrosis). Disease severity and host response data were combined in a single value called the coefficient of infection (CI). The average coefficient of infection (ACI) and CI for the improved varieties and the landraces was calculated by multiplying the mean (seven environments) and one season severity %, respectively times a constant for host response: immune = 0.0, R = 0.2, MR = 0.4, MS = 0.8 and S = 1.0.

Marker analyses

Genomic DNA was extracted from 2 weeks old fresh leaves that were harvested and pooled from five seedlings of each accession and stored at −80 °C. Extraction from frozen leaves was performed using the modified CTAB method described by Doyle and Doyle (1990).

A total of 17 PCR markers [SSRs (simple sequence repeats), InDels (insertion–deletion polymorphisms) and EST (expressed sequence tags)] that are linked/associated with four reported major *Sr* genes (*Sr2*, *Sr13*, *Sr22* and *Sr35*) were included in this study. Primer names, forward and reverse primer sequences and references from *Sr* genes associated markers are detailed in Supplemental Table 3. PCR reactions and amplifications of these markers were performed using procedures described at UCDavis website (http://maswheat.ucdavis.edu/protocols/stemrust/) and Yu et al. (2010).

PCR reactions contained 50–100 ng template DNA, 250 nM Cy5-labelled forward primer, 250 nM unlabelled reverse primer, 0.2 mM dNTPs, 2.5 µL PCR buffer (10x), 1.5 mM $MgCl_2$ and 1 U *Taq* DNA Polymerase in a total volume of 25 µL. Fragment detection was performed as described by Röder et al. (1998). For SSR markers, fragments were detected by an automated laser fluorescence (ALF express) sequencer (Amersham Biosciences Europe GmbH, Freiburg, Germany) using a short gel cassette. Fragment sizes were calculated using the computer program Fragment Analyzer Version 1.02 (Amersham Biosciences) by comparison with the internal and external size standards. The EST and InDel markers were resolved in 2.0 % agarose gels for amplification and the amplified fragments were stained with ethidium bromide and photographed. To clearly detect the fragment sizes for these InDel and EST markers, the analysis of fragment sizes was repeated on an AdvanCE FS96 microcapillary fragment analyzer system by loading 25 µL PCR products.

Results

Phenotyping

Stem rust severity (%), infection response and ACI for the varieties tested at DZ and DN during 2008–2010 and for landraces tested at DZ during 2009 are presented in Supplemental Tables 1 and 2, respectively. Of the 26 tested varieties, only 'Sebatel' showed a moderately resistant (MR) type of response with an ACI of 2. Varieties 'Yerer', 'Ude', 'Boohai', 'Leliso', 'Ld-357, 'Ginchi', 'Robe', 'Bichena', 'Gerardo', 'Foka', 'Oda', 'Quamy', 'Assassa' and 'Cham-1' showed a MS type of response with an ACI of 8–28 whereas the rest of varieties showed a susceptible (S) type of reaction with 30–55 ACI (Supplemental Table 1). Landraces LR2, LR3, LR4, LR8, LR10, LR19, LR25, LR28, LR29 and LR32 showed a MS reaction with 8–40 ACI values. For the rest of the tested landraces a S type of reaction with up to 70 ACI was recorded (Supplemental Table 2).

Identification of stem rust resistance genes using molecular markers

Initially we screened 25 molecular markers that are associated with *Sr2*, *Sr13*, *Sr22* and *Sr35*. But we used

only 17 of the markers which showed polymorphism and clear fragments for haplotyping the genes in the present study (Tables 1, 2). Haplotypes were sorted for each stem rust resistance gene by the size of their fragments. Similar haplotypes for each gene were grouped together and compared to the original source of the gene based on the reference lines.

Sr2 is the only catalogued adult plant stem rust resistance gene in wheat (McIntosh et al. 2003). It is located on the short arm of chromosome 3B (Hare and McIntosh 1979). Spielmeyer et al. (2003) reported that the SSR marker *GWM533* was linked to *Sr2* on chromosome 3B with a map distance of approximately 2 cM. Spielmeyer et al. (2003) also showed that a 120 bp PCR fragment was amplified in most lines carrying *Sr2*. The diagnostic PCR fragment for *GWM533*—120 bp was detected in 'Sebatel', 'Hitosa', LR25, LR10, LR26, LR27, LR28, LR32, LRW and in the *Sr2* containing line 'KINGBIRD#1'. *BARC133*, the other marker associated with *Sr2*, amplified a fragment size of 122 bp in 'Sebatel', 'Hitosa', LR25, LR3, LR7 and in the *Sr2* containing line 'KINGBIRD#1'. These markers amplified various sizes of PCR fragments in the rest of the varieties and landraces. Some varieties produced similar haplotypes as reported by Yu et al. (2010) for *Sr2* positive lines i.e. 117 bp (for *GWM533*) and 120 bp (for *BARC133*). Thus, the haplotype *GWM533*—120 bp and *BARC133*—122 bp was considered to be indicative for *Sr2* positive lines (Table 1).

Sr13 is a stem rust resistance gene present in several *T. durum* cultivars. Its main sources are the Ethiopian land race ST464 and the *T. dicoccon* (emmer wheat) germplasm Khapli (Klindworth et al. 2007). It is located on the long arm of chromosome 6A. EST marker BE403950; and SSRs *DUPW167*, *WMC580*, *BARC104b* and *BARC104c* were used for haplotyping *Sr13* in this study. These markers showed null alleles and also produced different fragment sizes in the tested varieties and landraces. *BE403950*, *DUPW167*, *BARC104b*, *WMC580* and *BARC104c* amplified fragment sizes of 691 bp, 243 bp, 273 bp, 316 bp and 175 bp, respectively, in varieties 'Sebatel', 'Quamy' and 'Boohai'. The same fragment sizes amplified also in 'Cocorit-71' and 'Cham-1' by markers *BE403950*, *DUPW167* and *BARC104b*. 'Tob-66' revealed fragment sizes of 243 bp, 273 bp and 316 bp for *DUPW167*, *BARC104b*, *WMC580*, respectively. 'Robe' and 'Bichena' revealed similar fragment sizes for *DUPW167* and *BARC104b*, and 'Yerer' for *BARC104b* and *BARC104c*. Therefore, a five-marker combination with fragment sizes of "691-243-273-316-175" was considered as a haplotype for *Sr13* in this study based on the reference line W2691SR13 (Table 1).

Sr22 was mapped on the long arm of chromosome 7A (Khan et al. 2005). Three linked markers, *CFA2019*, *CFA2123* and *BARC121*, were used for haplotyping this locus by Yu et al. (2010). Olson et al. (2010) produced a new set of lines with reduced alien fragments and found that the closest markers flanking *Sr22* in these lines are *WMC633* and *CFA2123*. In this study we have used *CFA2019*, *CFA2123*, *WMC633* and *BARC121* to haplotype this locus. Markers *CFA2019*, *CFA2123* and *WMC633* produced 168, 234 and 119 bp fragments and *BARC121* 170 and 197 bp fragments in the *Sr22* carrying line SESR22TB. *CFA2019* and *WMC633* produced a haplotype of 168 bp and 119 bp fragment sizes in LR1. Additionally, *CFA2019* amplified a fragment size of 168 bp in 'Sebatel', 'Boohai', 'Mamouri', 'JennahKhetifa', LR12 and LR25. *CFA2123* produced a fragment size of 234 bp in LR14, LR15 and LR30. But *BARC121* and WMC633 did not amplify the fragment sizes that have been amplified in the *Sr22* carrying line (170 + 197 bp and 119 bp). A fragment size of 215 bp, reported in a similar study by Yu et al. (2010) was amplified in LR25, LR22 and LR24 (Table 2).

The stem rust resistance gene *Sr35* was originally transferred from *Triticum monococcum* to hexaploid wheat (McIntosh et al. 1984) and is effective against the TTKSK (Ug99) race of *P. graminis*. f. sp. *tritici* (Jin et al. 2007) and its variants, TTKST and TTTSK. It is mapped on the long arm of chromosome 3A between markers *BF483299* and *CJ656351* in a region of 2.2–3.1 cM, depending on the population (Zhang et al. 2010) and is located 41.5 cM from the centromere (McIntosh et al. 1995). Some of the markers that were found on the *T. monococcum* fragment containing *Sr35* which are useful for marker assisted selection are *CFA2193*, *BE423242*, *WMC559*, *BF485004*, *CFA2170*, *AK335187*, *CFA2076*, *BE405552*, *WMC169* and G*WM480* (http://maswheat.ucdavis.edu/protocols/Sr35/index.htm).

We have employed these 10 SSR/EST-derived molecular markers to test for the presence of this gene in our accessions. But *WMC559*, *WMC169*, *AK335187* and *CFA2076* produced monomorphic fragments.

Table 1 Haplotype diversity of stem rust resistance genes *Sr2* and *Sr13* using linked molecular markers in Ethiopian durum wheat varieties and tetraploid wheat landraces. Numeric values are the fragment sizes (bp) of PCR amplicons for the respective marker and wheat line. *NA* null allele. Amplicons with same size were sorted together as a haplotype group and coded as follows: *gray* highlight the haplotypes similar to the known gene resources, numeric values in *bold* indicate fragment size reported by Yu et al. (2010) in a similar study and on UCDavis website (http://maswheat.ucdavis.edu/protocols/stemrust/)

		Sr2				***Sr13***				
Wheat line	**SR**	**GWM533**	**BARC133**	**Wheat line**	**SR**	**BE403950**	**DUPW167**	**BARC104b**	**WMC580**	**BARC104c**
KINGBIRD#1	**5MR-MS**	**120**	**122**	**W2691SR13**	*Sr13*	**691**	**243**	**273**	**316**	**175**
Sebatel	5MR	**120**	**122**	Sebatel	5MR	**691**	**243**	**273**	**316**	**175**
Hitosa	40S	**120**	**122**	Quamy	20MS	**691**	**243**	**273**	**316**	**175**
LR25	40MS	**120**	**122**	Boohai	20MS	**691**	**243**	**273**	**316**	**177**
LR3	10MS	132	**122**	Cocorit-71	30S	**691**	**243**	**273**	290	177
LR7	60S	132	**122**	Cham-1	20MS	**691**	**243**	**273**	290	177
LR10	30MS	**120**	NA	Tob-66	35S	NA	**243**	**273**	**316**	177
LR26	40S	**120**	NA	Robe	30MS	737	**243**	**273**	**293**	177
LR27	40S	**120**	NA	Bichena	25MS	NA	**243**	**273**	290	177
LR28	20MS	**120**	NA	Yerer	10MS	737	227	**273**	**293**	**175**
LR32	50MS	**120**	NA	Oda	30MS	**691**	247	271	312	NA
LRW	40S	**120**	120	Mamouri	50S	**691**	247	271	312	177
Boohai	20MS	**117**	**120**	LR2	40MS	**691**	247	271	**293**	177
Robe	30MS	**117**	**120**	LR14	50S	**691**	247	253	**293**	177
Mamouri	50S	**117**	**120**	LR16	50S	**691**	247	253	**293**	177
Ejersa	40S	**117**	124	LR17	60S	**691**	247	NA	**293**	NA
Ude	15MS	**117**	124	LR19	20MS	**691**	253	271	290	177
Foka	30MS	**117**	126	LR31	40S	**691**	**249**	NA	**293**	NA
Denbi	40S	**117**	126	Leliso	20MS	NA	**243**	NA	290	NA
Oda	30MS	**117**	NA	Ginchi	25MS	NA	**243**	267	**293**	NA
Klinto	40S	**117**	118	Gerardo	15MS	NA	**243**	271	314	177
Assassa	35MS	**117**	126	LR1	50S	737	**245**	**273**	**293**	177
Cocorit-71	30S	**117**	126	LR25	40MS	739	**245**	**273**	**293**	177
LR1	50S	**117**	NA	LR33	30S	NA	**245**	**273**	**293**	177
LR2	40MS	**117**	NA	Ld-357	25MS	737	247	265	**316**	177
LR4	20MS	**117**	NA	JennahKhetifa	40S	737	Null	NA	**316**	177
LR6	40S	**117**	NA	Assassa	35MS	737	227	NA	290	**175**
LR8	30MS	**117**	NA	LR30	20S	NA	227	NA	293	**175**
LR16	50S	**117**	NA	LRW	40S	NA	227	NA	**293**	**175**
LR18	60S	**117**	NA	LR3	10MS	661	**245**	NA	**293**	177
LR19	20MS	**117**	NA	LR22	60S	737	**245**	NA	**293**	NA
LR20	40S	**117**	NA	DZ04	50S	737	**249**	NA	314	177
LR24	50S	**117**	NA	LR24	50S	NA	**249**	271	290	177
LR31	40S	**117**	NA	Kristal	55S	737	227	NA	**293**	177
LR33	30S	**117**	118	Denbi	40S	NA	247	269	**293**	NA
Bakalcha	30S	132	**120**	LR4	20MS	687	247	NA	**293**	177
Tob-66	35S	132	**120**	LR6	40S	737	247	NA	**293**	177
Gerardo	15MS	132	**120**	LR8	30MS	797	247	NA	**293**	177
LR12	70S	132	**120**	LR10	30MS	659	253	NA	**293**	177
LR13	60S	132	**120**	LR12	70S	689	247	253	**293**	177
LR14	50S	132	**120**	LR13	60S	NA	247	253	**293**	177
LR15	40S	132	**120**	LR15	40S	791	247	253	**293**	177
LR17	60S	132	**120**	LR18	60S	NA	247	NA	**293**	NA
LR21	60S	132	**120**	LR21	60S	689	247	NA	**293**	NA
LR23	60S	132	**120**	LR23	60S	NA	247	253	**293**	177
				LR26	40S	NA	251	253	**293**	177
				LR27	40S	NA	251	269	**293**	NA
				LR29	20MS	689	251	253	**293**	NA
				LR32	50MS	NA	247	271	**293**	177
				LRP	50S	NA	241	NA	**293**	NA

Table 2 Haplotype diversity of stem rust resistance genes *Sr22* and *Sr35* using linked molecular markers in Ethiopian durum wheat varieties and tetraploid wheat landraces. Numeric values are the fragment sizes (bp) of PCR amplicons for the respective marker and wheat line. *NA* null allele. Amplicons with same size were sorted together as a haplotype group and coded as follows: *gray* highlight the haplotypes similar to the known gene resources, numeric values in *bold* indicate fragment size reported by Yu et al. (2010) in a similar study

		Sr22				***Sr35***							
Wheat line	**SR**	**CFA2019**	**CFA2123**	**WMC633**	**BARC121**	**Wheat line**	**SR**	**GWM480**	**CFA2170**	**BF485004**	**BE405552**	**CFA2193**	**BE423242**
SWSR22TB	*Sr22*	**168**	**234**	**119**	**170, 197**	**W3763-SR35**	*Sr35*	**172**	**160**	**NA**	**355**	**148, 213**	**392**
LR1	50S	**168**	237	**119**	232	KINGBIRD#1	5MR-MS	**172**	197	**NA**	**355**	**148, 213**	**392**
Sebatel	5MR	**168**	249	223	228	Denbi	40S	**172**	**160**	**NA**	**355**	247	NA
Boohai	20MS	**168**	259	233	238	Cham-1	20MS	**172**	**160**	**NA**	404	**148, 213**	NA
Mamouri	50S	**168**	259	233	240	Bakalcha	30S	**172**	**160**	**NA**	346	148, 247	NA
JennahKhetifa	40S	**168**	253	227	234	Bichena	25MS	**172**	**160**	**NA**	418	148, 247	396
LR12	70S	**168**	237	243	234	Gerardo	15MS	**172**	**160**	**NA**	NA	233	396
LR25	40MS	**168**	237	NA	**215**	Leliso	20MS	**172**	**160**	581	**355**	148, 247	128
LR14	50S	233	**234**	223	234	Robe	30MS	**172**	**160**	583	**355**	148, 231	128
LR15	40S	237	**234**	237	234	Assassa	3OMS	**172**	**160**	581	**355**	148, 247	NA
LR30	20S	235	**234**	NA	230	JennahKhetifa	40S	**172**	**160**	546	**355**	245	358
Hitosa	40S	235	**245**	233	238	Ude	15MS	**172**	**160**	550	352	**148, 213**	362
Denbi	40S	235	**245**	233	238	Yerer	10MS	**172**	**160**	396	541	148, 245	**392**
Oda	30MS	235	**245**	233	240	Kristal	55S	**172**	**160**	552	536	**148, 213**	396
Assassa	35MS	235	**245**	233	240	Foka	30MS	**172**	197	**NA**	**355**	247	398
Yerer	10MS	235	245	255	238	Hitosa	40S	**172**	**160**	548	352	247	362
Leliso	20MS	235	261	233	238	Boohai	20MS	**172**	**160**	548	449	231	362
Bakalcha	30S	235	249	233	238	Ginchi	25MS	**172**	**160**	583	418	148, 231	NA
Ejersa	40S	235	263	233	238	Mamouri	50S	**172**	**160**	544	459	148, 247	356
Ld-357	25MS	235	265	233	238	Cocorit-71	30S	**172**	**160**	581	346	148, 247	NA
Tob-66	35S	235	263	233	240	Oda	30MS	**172**	197	**NA**	NA	247	NA
Bichena	25MS	235	261	233	238	Sebatel	5MR	**172**	195	388	543	148, 233	**392**
Gerardo	15MS	235	261	233	232	Klinto	40S	**172**	197	581	**355**	148, 247	NA
Foka	30MS	**235**	261	233	240	Ld-357	25MS	176	**160**	581	**355**	148, 231	NA
LR22	60S	**235**	237	NA	**215**	Tob-66	35S	176	**160**	583	**355**	148, 233	NA
Quamy	20MS	**235**	261	233	240	LR8	30MS	176	195	**NA**	346	**148, 213**	NA
Cocorit-71	30S	**235**	261	233	240	LR10	30MS	176	197	**NA**	NA	**148, 213**	NA
LR3	10MS	**235**	257	175	164	LR19	20MS	176	197	**NA**	346	**148, 213**	NA
LR10	30MS	**235**	231	261	234	Ejersa	40S	**172**	197	581	418	148, 247	NA
LR13	60S	**235**	237	243	232	Quamy	20MS	**172**	197	581	418	148, 247	NA
LR16	50S	**235**	237	243	234	LR2	40MS	**172**	199	143	346	213	398
LR17	60S	**235**	237	243	232	LR32	50MS	**172**	197	583	346	148, 211	396
LR18	60S	**235**	237	243	232	LR1	50S	176	197	**NA**	452	148, 211	396
LR19	20MS	**235**	253	237	234	LR6	40S	176	193	**NA**	346	211	362
LR20	40S	**235**	237	267	234	LR18	60S	176	197	**NA**	346	211	NA
LR21	60S	**235**	237	NA	232	LR23	60S	176	197	**NA**	346	148, 211	NA
LR23	60S	**235**	237	243	232	LR20	40S	176	197	583	346	**148, 213**	128
LR26	40S	**235**	237	NA	238	LR22	60S	176	197	583	346	**148, 213**	362
LR27	40S	**235**	237	NA	234								
LR28	20MS	**235**	237	NA	234								
LR29	20MS	**235**	253	NA	238								
LR31	40S	**235**	253	211	232								
LR32	50MS	**235**	229	NA	232								
LR33	30S	**235**	237	NA	234								
LRP	50S	**235**	237	NA	234								
LR24	50S	237	237	NA	**215**								

Therefore, we have employed only markers *CFA2193*, *CFA2170*, *GWM480*, *BE423242*, *BF485004* and *BE405552* to haplotype our accessions. Using these six marker combination, a haplotype of 172-160-NA-355-148/213-392 bp was detected in the *Sr35* carrying line, W3763-SR35, and in 'KINGBIRD#1' (except *CFA2170* produced a different fragment of 197 bp). Among the tested materials, no variety or landrace showed this haplotype. But 'Denbi' showed a haplotype of 172-160-NA-355 bp and 'Cham-1', 'Bakalcha' 'Bichena' and 'Gerardo' revealed a haplotype of 172-160-NA. The rest of the varieties

showed a haplotype of 172–160 bp for *GWM480* and *CFA2170*. Only our two resistant varieties, 'Sebatel' and 'Yerer', produced a fragment size of 392 bp (similar to the *Sr35* carrying line) for marker *BE423242* (Table 2). We have also tested Mq(2)5*G2919, a line carrying *Sr35*, but it produced different fragment sizes for all of the markers except for *BE423242* and *BE405552* in comparison to W3763-SR35 (data not shown).

Discussion

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. Closely linked markers provide a means for the selection and identification of important genes in breeding programs and, in the case of diseases resistance, this can be done in the absence of pathogens (Babiker et al. 2009).

Resistance gene *Sr2*, in addition to other unknown minor genes derived from variety 'Hope' commonly known as the '*Sr2*-complex' (McIntosh 1988; Singh et al. 2006) is the basis for the effectiveness of *Sr2* (Singh et al. 2006). This stem rust resistance gene has provided durable, broad-spectrum resistance and has been used as an effective control measure against wheat stem rust in modern wheat breeding. The use of *Sr2* in CIMMYT wheat improvement program resulted in the release of several popular varieties worldwide carrying this gene (Singh et al. 2009). This resistance gene is currently effective against all isolates of *Pgt* throughout wheat-growing regions of the world (Sunderlund and Roelfs 1980).

Even if Spielmeyer et al. (2003) reported a 120 bp PCR fragment amplified in most lines carrying *Sr2*, there are some exceptions as reported by Mago et al. (2011) where the 120 bp allele also occurred in many North American and CIMMYT lines which are considered not to have *Sr2*. Thus *GWM533* is complicated to use because there are two different *GWM533* loci on 3BS. But Spielmeyer et al. (2003) showed by DNA sequence that the two 120 bp PCR fragments amplified by the microsatellite marker *GWM533* from wheat lines known to carry *Sr2*, and those without the resistance gene differed by the number of dinucleotide repeat units that formed the compound microsatellite motif. Based on this report, it is difficult to conclude that all the accessions that showed a 120 bp fragment size for this marker carry *Sr2*. Therefore it is important to apply the pair of STM markers developed by Mago et al. (2011) to exploit the DNA sequence variation within the microsatellite repeat.

Some varieties and landraces also showed the haplotype fragments 117 bp (*GWM533*) and 120 (*BARC133*). Similar fragment sizes were reported by Yu et al. (2010) as haplotypes for *Sr2* positive lines for these markers. But it is difficult to conclude whether the lines carrying this haplotype in our study also possessed *Sr2*, since it is a different genetic background. A major QTL for resistance to stem rust including Ug99 was reported for chromosome 3BS close to the genomic region of *Sr2* in a mapping population derived from 'Sebatel' as resistance source (Haile et al. 2012a). This observation supports the conclusion that *Sr2* is present as effective resistance gene in 'Sebatel'.

Sr13 is present in several *T. durum* varieties. Despite being a frequent gene in durum varieties, *Sr13* was not detected in most of the Ethiopian durum wheat varieties in the present study. But this might be the reason that most of the markers we have used to haplotype this locus are not diagnostic in all the genetic backgrounds. These markers can be used to follow the *Sr13* resistant alleles in segregating populations including some of the parental lines with known *Sr13* sources, but the markers may fail to predict the presence of *Sr13* in an unknown set of germplasm for example in landraces. The resistance in some of the durum wheat varieties that showed the haplotype for this gene, such as 'Sebatel', 'Quamy', 'Boohai', 'Cocorit-71' and 'Cham-1' might be due to other *Sr* genes. Using a mapping population developed from 'Kristal' and 'Sebatel', in our previous study, we have identified QTL for resistance to race Ug99 about 17.4 cM from *Sr13* flanking markers (Haile et al. 2012a). Therefore, the resistance in these varieties could be due to the action of an allele of *Sr13* since the Ethiopian stem rust pathotype is high on *Sr13*.

Admassu et al. (2011) reported that *Sr13* is the only known gene effective against race TTKSK (Ug99) and its variants (TTKST and TTTSK) and other Ethiopian wheat stem rust races of *Pgt*. However, this result is based on a study of hexaploid wheat. In another study Admassu et al. (2009) also showed that the

effectiveness of *Sr13* in Ethiopia is regional. Thus, it is important to note that some virulent races other than Ug99 are reported to overcome *Sr13* in some countries (Huerta-Espino 1992; McIntosh et al. 1995) and Ethiopia particularly on durum wheat (Olivera et al. 2011). Olivera et al. (2011) identified race JRCQC from 38 single-pustule isolates at Debre-Zeit from a 2009 durum screening nursery of Ethiopia that possesses a virulence overcoming the resistance gene *Sr13*. Therefore, it can best be used in combination with other genes through gene pyramiding particularly in Ethiopia where there is a current virulent *Pgt* race on durum wheat for this gene.

Stem rust resistance gene *Sr22* was originally identified in the diploid wheat species *Triticum boeoticum* Boiss. accession G-21 (Gerechter-Amitai et al. 1971) and *T. monococcum* accession RL5244 (Kerber and Dyck 1973)). It was then transferred to tetraploid and hexaploid wheat through interspecific hybridizations. But so far no one has found it in durum. There may be occasional out-crossing between tetraploid wheat and *T. monococcum* and therefore we may find the gene from *T. monococcum* in tetraploid wheat (Ravi Singh personal comm.). But the use of this gene in wheat breeding is limited due to a yield penalty and a delay in heading date associated with the *T. monococcum* chromosome segment carrying this gene (Olson et al. 2010). But recently hexaploid lines with *Sr22* which have reduced *T. monococcum* genome have been produced due to the effectiveness of this gene against Ug99 (Olson et al. 2010). Therefore, the varieties and landraces that showed haplotype loci for the diagnostic markers of this gene will be utilized in further breeding program to combat Ug99 and related races of *Pgt*.

Haplotype analysis of markers associated with *Sr22* indicated the presence of the *Sr22* gene in varieties and landraces which showed susceptibility response in the field testing. But only variety 'Sebatel' and 'Boohai' showed a MR and MS, respectively response to *Pgt* race Ug99 during the field testing and showed the haplotype for this gene. The presence of *Sr22* in 'Sebatel' was indicated by a minor QTL in the respective genomic region in the 'Kristal' × 'Sebatel' mapping population (Haile et al. 2012a). Therefore, based on the current study, the markers used to haplotype *Sr22* are not completely diagnostic and thus may produce false positive result as reported in UCDavis website (http://maswheat.ucdavis.edu/protocols/Sr22/Disease_rust_Sr22.htm) or *Sr22* may be only partially effective for resistance to Ug99.

Sr35 originated from *T. monococcum* and is effective against *Pgt* races of TTKSK (Ug99) and its variants TTKST and TTKSK. There is no clear report where *Sr35* was transferred to durum wheat. But since the source of resistance in some of the Ethiopian tetraploid wheat varieties is not clearly known, we have employed markers that are associated with *Sr35* (*CFA2193*, *CFA2170*, *GWM480*, *BE423242*, *BF485004* and *BE405552*) to check the presence of this gene. Based on the reference line, most of the tetraploid wheat varieties of Ethiopia including the susceptible ones showed the haplotype for this gene which is unlikely since *Sr35* is considered as one of the most highly effective genes against the new African race Ug99 (Jin et al. 2007). We have also observed that all markers used to haplotype *Sr35* produced the same fragment size for 'KINGBIRD#1' and for the line carrying *Sr35* (W3763-SR35). Therefore, KINGBIRD might also carry *Sr35* in addition to *Sr2*.

Admassu (2010) reported, based on testing for stem rust resistance genes in Ethiopian wheat varieties, that it was difficult to postulate the resistance gene(s) responsible for their resistance. The author indicated in his study that varieties 'Cocorit-71', 'Ld-357', 'Kilinto', 'Bichena', 'Tob-66', 'Quamy', 'Robe', 'Ude', 'Yerer', 'Oda', 'Bakelcha' and 'Leliso' displayed low ITs against all the *Pgt* races they have used, which made it difficult to postulate the type of genes present in these genotypes. Thus, they concluded that either a single gene or a combination of genes may be responsible for the resistance displayed by these varieties. Therefore, the subject requires further analysis with more molecular markers accompanied by gene postulation based on wider virulence spectra races.

Conclusion

The tetraploid wheat has been a source of resistance genes *Sr2*, *9d*, *9e*, *12*, *13*, *14* (Roelfs et al. 1992). According to Bechere et al. (2000) Ethiopian tetraploid wheat accessions were noted for their good source of resistance to stem rust. The presence of some genes in the landraces, in this study, also strengths this fact and showed that Ethiopian cultivated tetraploid wheat accessions are still good sources of stem rust

resistance. Beteselassie et al. (2007) reported the same scenario by postulating the *Sr* genes in Ethiopian tetraploid wheat accessions through multipathotype testing.

Most of the genes that are catalogued were transferred to bread wheat from alien sources. *Sr2* and *Sr13* were transferred to bread wheat from tetraploid emmers and *Sr35* was transferred from *T. monococcum*. It is reported on UCDavis website (http://maswheat.ucdavis.edu/protocols/stemrust/), that most of the molecular markers linked to *Sr* resistance genes are not diagnostic. This might be one of the reasons why we did not identify these genes in most of the tested durum wheat varieties. Dominance for the undesirable allele, lack of amplification, amplification of the wrong locus, recombination between the marker and the gene, and lack of polymorphism between the source and recurrent parents are also some of the reasons because of which markers can fail to predict the presence of a gene (Yu et al. 2010).

Sr22 and *Sr35* are rarely used genes (Yu et al. 2010) that have been confirmed to be resistant to Ug99 (Jin et al. 2007). But some susceptible varieties and landraces showed a haplotype for these genes. For example, LR1 and Mamouri (50S) showed a haplotype for *Sr22*. Based on the reference line, W3763-SR35, most durum wheat varieties of Ethiopia showed similar fragment size for the tested diagnostic markers. Even some susceptible (40S) varieties, 'Denbi', 'JennahKhetifa' and 'Hitosa', showed a haplotype for *Sr35*. As a result, these haplotypes may not be diagnostic for *Sr22* and *Sr35* and further evaluation is needed. Using more molecular markers closely linked to the gene of interest could be useful for distinguishing the false positives.

Based on the result of this study, the resistance against race Ug99 (TTKSK) of *Pgt* in 'Sebatel' might be due to combinations of *Sr* resistance genes *Sr2* and *Sr22*. The other resistant Ethiopian durum wheat varieties, 'Yerer', 'Boohai', 'Ude' and 'Gerardo', which also showed a MS reaction to *Pgt* race of Ug99 (TTKSK) might be due to *Sr35*. It was not possible to accompany the findings with pedigree tracking since the source of resistance genes in these varieties is not clearly known. Moreover, it is likely that these varieties also had resistant genes not detected because of a limited number of *Sr* genes with diagnostic markers available for durum wheat. Therefore, it will be helpful to accompany this approach with association analysis combined with pedigree and rust race reaction for better gene identification and postulation. But, as this study is the first report on the presence of *Sr* genes in Ethiopian durum wheat varieties and tetraploid wheat landraces based on linked or associated molecular markers, it gives some preliminary information for further research.

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References

Admassu B (2010) Genetic and virulence diversity of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia and stem rust resistance genes in wheat. Ph.D. thesis, Giessen University, Cuvillier Verlag Göttingen, Germany

Admassu B, Embet F, Zerihun K (2004) Physiological races and virulence diversity of *Puccinia graminis* f. sp. *tritici* on wheat in Ethiopia. In: 12th regional wheat workshop for Eastern, Central, and Southern Africa, Nakuru, Kenya, 22–26 November 2004, pp 145–150

Admassu B, Lind V, Friedt W, Ordon F (2009) Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia with special consideration of Ug99. Plant Pathol 58:362–369

Admassu B, Perovic D, Friedt W, Ordon F (2011) Genetic mapping of the stem rust (*Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn) resistance gene *Sr13* in wheat (*Triticum aestivum* L.). Theor Appl Genet 122:643–648

Babiker E, Ibrahim A, Yen Y, Stein J (2009) Identification of a microsatellite marker associated with stem rust resistance gene *Sr35* in wheat. Aust J Crop Sci 3:195–200

Badebo A (2002) Breeding bread wheat with multiple disease resistance and high yield for the Ethiopian highlands: broadening the genetic basis of yellow rust and tan spot resistance. Ph.D. thesis, Georg-August University, Gottingen, Germany

Badebo A, Stubbs RW, Van Ginkel M, Getinet G (1990) Identification of resistance genes to *Puccinia striiformis* in seedlings of Ethiopian and CIMMYT bread wheat varieties and lines. Neth J Plant Pathol 96:199–210

Bechere E, Kebede H, Belay G (2000) Durum wheat in Ethiopia: an old crop in an ancient land. Institute of Biodiversity

Conservation and Research (IBCR), Addis Ababa, Ethiopia, p 68
Beteselassie N, Fininsa C, Badebo A (2007) Sources of resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in Ethiopian tetraploid wheat accessions. Genet Resour Crop Evol 54:337–343
CIMMYT (2000)1998/1999 World wheat facts and trends. Global wheat research in a changing world, challenges and achievements. CIMMYT, Mexico
CSA (2009) Central Statistical Agency, 2009/2010, agricultural sample survey, report on area and production of crops. Statistical bulletin 446, vol IV. Addis Ababa, Ethiopia, p 14
Das BK, Saini A, Bhagwat SG, Jawali N (2006) Development of SCAR markers for identification of stem rust resistance gene *Sr31* in the homozygous or heterozygous condition in bread wheat. Plant Breed 125:544–549
Dawit W (2008) Analysis of pathogen virulence and cultivar resistance to yellow rust, *Puccinia striiformis* f. sp. *tritici*, in Ethiopia. Ph.D. thesis, Martin-Luther-Universität Halle-Wittenberg, Germany
Doyle JJ, Doyle JL (1990) A rapid total DNA preparation procedure for fresh plant tissue. Focus 12:13–15
FAOSTAT (2003) Crop protection compendium CD. CAB International, UK
Forster BP, Ellis RP, Thomas WTB, Newton AC, Tuberosa RTD, El-Enein RA, Bahri MH, Ben Salem M (2000) The development and application of molecular markers for abiotic stress tolerance in barley. J Exp Biol 51:19–27
Gerechter-Amitai ZK, Wahl I, Vardi A, Zohary D (1971) Transfer of stem rust seedling resistance from wild diploid einkorn to tetraploid durum wheat by means of a triploid hybrid bridge. Euphytica 2:281–285
Haile JK, Nachit MM, Hammer K, Badebo A, Röder MS (2012a) QTL mapping of resistance to race Ug99 of *Puccinia graminis* f. sp. *tritici* in durum wheat (*Triticum durum* Desf.). Mol Breed. doi:10.1007/s11032-012-9734-7
Haile JK, Hammer K, Badebo A, Nachit MM, Röder MS (2012b) Genetic diversity assessment of Ethiopian tetraploid wheat landraces and improved durum wheat varieties using microsatellites and markers linked with stem rust resistance. Genet Resour Crop Evol. doi:10.1007/s10722-012-9855-1
Hare RA, McIntosh RA (1979) Genetics and cytogenetics studies of durable adult-plant resistance in 'Hope' and related cultivars to wheat rusts. Z Pflanzenzüchtung 83:350–367
Hayden MJ, Kuchel H, Chalmers KJ (2004) Sequence tagged microsatellites for the Xgwm533 locus provide new diagnostic markers to select for the presence of stem rust resistance genes *Sr2* in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1641–1647
Hiebert CW, Fetch TG Jr, Zegeye T (2010) Genetics and mapping of stem rust resistance to Ug99 in the wheat cultivar Webster. Theor Appl Genet 121:65–69
Huang XQ, Hsam SLK, Zeller FJ (1997) Chromosomal locations of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em. Thell.). 4. Gene pm24 in Chinese landrace Chiyacao. Theor Appl Genet 95:950–953
Huerta-Espino J (1992) Analysis of wheat leaf and stem rust virulence on a worldwide basis. Ph.D. thesis, University of Minnesota, USA
Jin Y, Singh RP, Ward RW, Wanyera R, Kinyua MG, Njau P, Fetch T Jr, Pretorius ZA, Yahyaoui A (2007) Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis 91:1096–1099
Kerber ER, Dyck PL (1973) Inheritance of stem rust resistance transferred from diploid wheat (*Triticum monococcum*) to tetraploid and hexaploid wheat and chromosome location of the gene involved. Can J Genet Cytol 15:397–409
Khan R, Bariana H, Dholakia B, Naik S, Lagu M, Rathjen A, Bhavani S, Gupta V (2005) Molecular mapping of stem and leaf rust resistance in wheat. Theor Appl Genet 111: 846–850
Klindworth D, Miller J, Jin Y, Xu SS (2007) Chromosomal locations of genes for stem rust resistance in monogenic lines derived from tetraploid wheat accession STS464. Crop Sci 47:1441–1450
Lakew B, Semeane Y, Alemayehu F, Genre H, Grando S, van Leur JAG, Ceccarelli S (1997) Exploiting the diversity of barley landraces in Ethiopia. Genet Resour Crop Evol 44:109–116
Leppik EE (1970) Gene centers of plants as a source of disease resistance. Annu Rev Phytopathol 8:323–344
Liu S, Yu L-X, Singh RP, Jin Y, Sorrells ME, Anderson JA (2010) Diagnostic and co-dominant PCR markers for wheat stem rust resistance genes *Sr25* and *Sr26*. Theor Appl Genet 120:691–697
Mago R, Bariana HS, Dundas IS, Spielmeyer W, Lawrence GJ, Pryor AJ, Ellis JG (2005) Development of broadly useful PCR-markers for the wheat stem rust resistance genes *Sr24* and *Sr26*. Theor Appl Genet 111:496–504
Mago R, Brown-Guedira G, Dreisigacker S, Breen J, Jin Y, Singh R, Appels R, Lagudah ES, Ellis J, Spielmeyer W (2011) An accurate DNA marker assay for stem rust resistance gene *Sr2* in wheat. Theor Appl Genet 122:735–744
Masresha A (1996) Wheat rust races identified in virulence surveys in Ethiopia. In: Tanner DG, Payne TS, Abdalla OS (eds) The ninth wheat regional workshop for Eastern Central and Southern Africa. CIMMYT, Addis Ababa, Ethiopia
McIntosh RA (1988) The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico
McIntosh RA, Dyck PL, The TT, Cusick J, Milne DL (1984) Cytogentical studies in wheat XIII: *Sr35*—a third gene from *Triticum monococcum* for resistance to *Puccinia graminis tritici*. Z Pflanzenzüchtung 92:1–14
McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO, Canberra
McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat. In: Pogna NE, Romano M, Pogna A, Galterio G (eds) Proceedings of the 10th International Wheat Genetics Symposium, Paestum, Italy
Mujeeb-Kati A, Rajarm S (2000) Transferring alien genes from related species and genera for wheat improvement. In: Citrus BC, Rajarm S, MacPherson Gomez H (eds) Bread wheat improvement and production. FAO, Rome
Olivera PD, Rouse M, Badebo A, Abeyo B, Woldeab G, Wanyera W, Jin Y (2011) Races of *Puccinia graminis* f. sp.

tritici in Ethiopia and Kenya. In: McIntosh RA (ed) Proceedings of the Borlaug Global Rust Initiative 2011 technical workshop, June 13–16, St. Paul, MN, USA, p 157

Olson EL, Brown-Guedira G, Marshall D, Stack E, Bowden RL, Jin Y, Rouse M, Pumphrey MO (2010) Development of wheat lines having a small introgressed segment carrying stem rust resistance gene *Sr22*. Crop Sci 50:1823–1830

Peterson RF, Champbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can J Res (C) 26:496–500

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Phytopathology 84:203

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of diseases management. CIMMYT, Mexico City

Saal B, Wricke G (1999) Development of simple sequence repeat markers in rye (*Seale cereale* L.). Genome 42:964–972

Simons K, Abate Z, Chao S, Zhang W, Rouse M, Jin Y, Elias E, Dubcovsky J (2011) Genetic mapping of stem rust resistance gene *Sr13* in tetraploid wheat (*Triticum turgidum* ssp. *durum* L.). Theor Appl Genet 122:649–658

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, Ward RW (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. Perspect Agric Vet Sci Nutr Nat Resour 1:1–13

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Ward WR (2008) Will stem rust destroy the world's wheat crop? Adv Agron 98:271–309

Singh RP, Huerta-Espino J, Bhavani S, Singh D, Singh PK, Herrera-Foessel SA, Njau P, Wanyera R, Jin Y (2009) Breeding for minor gene-based resistance to stem rust of wheat. In: Proceedings of the Borlaug Global Rust Initiative, C.D. Obregon, Mexico

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad-spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum* L.). Crop Sci 43:333–336

Sunderlund SD, Roelfs AP (1980) Greenhouse evaluation of the adult plant resistance of *Sr2* to wheat stem rust. Phytopathology 70:634–637

Teklu Y, Hammer K (2009) Diversity of Ethiopian wheat germplasm: breeding opportunities for improving grain yield potentials and quality traits. Plant Genet Resour 7:1–8

Temam H (1984) Distribution and prevalence of physiologic races of *Puccinia graminis* f. sp. *tritici* in some parts of Ethiopia and reaction of wheat cultivars to these races. M.Sc. thesis, Addis Ababa University, Ethiopia

Tsilo TJ, Jin Y, James A, Anderson JA (2008) Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Sci 48:253–261

Vanzetti LS, Campos P, Demichelis M, Lombardo LA, Aurelia PR, Vaschetto LM, Bainotti CT, Helguera M (2011) Identification of leaf rust resistance genes in selected Argentinean bread wheat cultivars by gene postulation and molecular markers. Electron J Biotechnol 14(3). http://dx.doi.org/10.2225/vol14-issue3-fulltext-14

Vavilov NI (1929) Wheats of Ethiopia. Bull Appl Bot Genet Plant Breed 20:324–356

Wu S, Pumphrey M, Bai G (2009) Molecular mapping of stem rust-resistance gene *Sr40* in wheat. Crop Sci 49:1682–1686

Yu LX, Liu S, Anderson JA, Singh RP, Jin Y, Dubcovsky J, Guidera GB, Bhavani S, Morgounov A, He Z, Huerta-Espino J, Sorrells ME (2010) Haplotype diversity of stem rust resistance loci in uncharacterized wheat lines. Mol Breed 26:667–680

Yu LX, Lorenz A, Rutkoski J, Singh RP, Bhavani S, Huerta-Espino J, Sorrells ME (2011) Association mapping and gene–gene interaction for stem rust resistance in CIMMYT spring wheat germplasm. Theor Appl Genet 123:1257–1268

Zhang W, Olson E, Saintenac C, Rouse M, Abate Z, Jin Y, Akhunov E, Pumphrey M, Dubcovsky J (2010) Genetic maps of stem rust resistance gene *Sr35* in diploid and hexaploid wheat. Crop Sci 50:2464–2474