Title
Genotyping of U.S. Wheat Germplasm for Presence of Stem Rust Resistance Genes Sr24, Sr36 and Sr1RSAmigo

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Haplotype diversity of stem rust resistance loci in uncharacterized wheat lines

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Abstract Stem rust is one of the most destructive diseases of wheat worldwide. The recent emergence of wheat stem rust race Ug99 (TTKS based on the North American stem rust race nomenclature system) and related strains threaten global wheat production because they overcome widely used genes that had been effective for many years. 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 genotypes of widely used wheat germplasm. In this study, a diverse collection of wheat germplasm was haplotyped for stem rust resistance genes *Sr2*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr36*, *Sr40*, and *1A.1R* using linked microsatellite or simple sequence repeat (SSR) and sequence tagged site (STS) markers. Haplotype analysis indicated that 83 out of 115 current wheat breeding lines from the International Maize and Wheat Improvement Center (CIMMYT) likely carry *Sr2*. Among those, five out of 94 CIMMYT spring lines tested had both *Sr2* and *Sr25* haplotypes. Five out of 22 Agriculture Research Service (ARS) lines likely have *Sr2* and a few have *Sr24*, *Sr36*, and *1A.1R*. Two out of 43 Chinese accessions have *Sr2*. No line was found to have the *Sr26* and *Sr40* haplotypes in this panel of accessions. DArT genotyping was used to identify new markers associated with the major stem resistance genes. Four DArT markers were significantly associated with *Sr2* and one with *Sr25*. Principal component analysis grouped

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wheat lines from similar origins. Almost all CIMMYT spring wheats were clustered together as a large group and separated from the winter wheats. The results provide useful information for stem rust resistance breeding and pyramiding.

Keywords Stem rust · *Sr* gene · Haplotype · Pyramiding · Genetic relationship · Marker-assisted selection

Introduction

Stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) is one of the most serious diseases of wheat worldwide. In the mid-twentieth century, yield losses caused by stem rust reached 20–50% in many regions/countries including Europe, Asia, Australia, and United States (Zadoks 1963; Rees 1972; Joshi and Palmer 1973; Leonard et al. 2001a, b). Stem rust resistance genes were successfully deployed in wheat cultivars worldwide from the mid-1950s, effectively controlling the disease. However, in 1998, a new race of stem rust pathogen, Ug99 (TTKS; Pretorius et al. 2000), was first identified in Uganda and has now spread throughout East Africa, the Middle East, and West Asia (Singh et al. 2008). Ug99 and related strains threaten global wheat production because they are virulent on widely used major genes that had been effective for many years.

To date, about 50 stem rust resistance (*Sr*) genes have been identified and most mapped to specific chromosome positions (McIntosh et al. 2008; see also summarized table for stem rust resistance genes and markers at http://rustopedia.get-traction.com/traction). However, only a few are effective against Ug99 (Singh et al. 2006, 2008) and many of those are associated with undesirable effects on agronomic traits (McIntosh et al. 1995). Among the stem rust resistance genes that are still effective, *Sr2* is one of the most widely used (McIntosh et al. 1995). *Sr2* was transferred from *T. turgidum* into hexaploid wheat in the 1920s (McFadden 1930). Since then, the *Sr2* gene has been deployed in wheat breeding programs worldwide and has provided durable adult plant rust resistance for more than 50 years. However, it only provides partial adult plant resistance and is associated with the pseudo-black chaff trait (Hare and McIntosh 1979).

The *Sr22* gene was originally identified in the diploid wheat species *Triticum monococcum* ssp. *boeoticum* accession G-21 (Gerechter-Amitai et al. 1971) and *T. monococcum* L. accession RL5244 (Kerber and Dyck 1973). It was then transferred to tetraploid and hexaploid wheat through interspecific hybridizations. *Sr22* is effective against all pathotypes of the stem rust pathogen in Australia. However, a yield penalty associated with the *T. monococcum* ssp. *boeoticum* chromosome segment carrying *Sr22* has limited its use (The et al. 1988). Lines with *Sr22* and reduced *T. monococcum* segments have been recently produced (Olson et al. 2010). *Sr25* and *Sr26* had been transferred into wheat from *Thinopyrum ponticum* Barkworth and Dewey and are effective against new stem rust races such as Ug99 and related strains. *Sr25* and the linked leaf rust resistance gene *Lr19* were translocated onto the long arm of wheat chromosome 7D (Friebe et al. 1994) and 7A (Zhang et al. 2005). The use of germplasm containing *Sr25/Lrl9* was initially limited because of linkage with another *Th. ponticum* derived gene causing undesirably yellow flour. Knott (1980) developed a mutant line, Agatha-28, containing *Sr25/Lr19* with reduced yellow color due to a mutation in the *PSY-E1* gene (Zhang and Dubcovsky 2008). It was then backcrossed into the Australian wheat backgrounds and has been used in the CIMMYT breeding program where it is present in the variety Wheatear (Bariana et al. 2007). *Sr26* was used as a source of resistance in Australia in the 1960s and the cultivar Eagle was released in 1971 (Martin 1971). Since then various other popular cultivars such as Kite and Avocet were released that carry *Sr26*. A report that yield penalty is associated with the *Th. ponticum* segment (The et al. 1988) reduced its use in breeding programs. New lines with shortened alien segments have been developed to overcome the yield penalty of the original *Sr26*-containing lines (Dundas et al. 2007). *Sr40* is another stem rust resistance gene from *Triticum timopheevii*. Although it is not widely deployed in current cultivars, it has been transferred into common wheat to determine if there is any negative effect on agronomic traits.

Several other effective genes, including, *Sr13*, *Sr32*, *Sr35*, *Sr39*, *Sr44*, *Sr45*, *Sr46*, and a few unnamed genes, have also been introduced into wheat but have not been deployed in commercial cultivars. These genes are currently effective against

Ug99 (Jin et al. 2007) and are potentially useful for pyramiding with other stem rust resistance genes to develop durable rust resistant cultivars. *Sr24* and *Sr36* were originally transferred from alien species to bread wheat (Smith et al. 1968; McIntosh and Gyarfas 1971) and were widely used by wheat breeders. Initially, wheat lines with *Sr24* and *Sr36* were resistant to Ug99; however, recent field stem rust screening in Kenya identified susceptible infection types indicating that *Sr24 and Sr36* are no longer effective against new races of Ug99 relatives, such as TTKST (Jin et al. 2008) and TTTSK (Jin et al. 2009). However, since *Sr24* and *Sr36* were introduced in a wide range of commercial varieties and are still effective against most races of stem rust, they may still be useful for pyramiding with other genes to obtain rust resistant wheat varieties.

The purpose of this study was to assess the prevalence of several known stem rust resistance genes in a large collection of elite wheat breeding lines representing the current breeding gene pools from an international breeding program that could be exposed to the Ug99 stem rust races. In addition, genome-wide DArT markers were used to identify new markers for rust resistance genes, assess genetic variation for rust resistance in the germplasm collection and to identify potentially new sources of stem rust resistance. Breeders can use this information to design crosses that assemble new, potentially durable combinations of stem rust resistance genes.

Materials and methods

Genetic resources

The wheat accessions for this study were selected by the wheat breeders from the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT) and the International Center for Agricultural Research in the Dry Areas (ICARDA) to be representative of their spring and winter wheat breeding programs in Africa, Turkey, and Mexico. Leading cultivars from the major Chinese wheat growing areas were also included. In the present study, we analyzed 228 wheat lines of diverse origins including 94 lines from the CIMMYT elite spring wheat program, 21 lines from the CIMMYT winter and facultative program, selected for stem rust resistance in Kenya, 43 cultivars from China, and 70 lines of miscellaneous origins. Their names, types, origins, and pedigrees are presented in Table S1. For most accessions, the rust resistance or major gene status was included in Table S1. The information on marker primers used in the present study is presented in Table 1 (see also Yu et al. 2009 for the complete marker list).

DNA extraction and DNA fragment analysis

DNA was extracted from young leaves of seedlings according to Heun et al. (1991). PCR amplifications were carried out in 10 µl reactions containing 200 µM dNTPs, 1.5 mM Mg^{++}, 100 nM of each primer, 0.5 U of *Taq*-polymerase, and 25 ng of DNA. Most PCR markers were amplified as follows: 94°C for 4 min, 30 cycles of 94°C for 45 s, 60°C for 45 s and 72°C for 1 min, followed by the final extension of 72°C for 7 min. A touch-down protocol was used for marker cfa2123 consisting of decreasing annealing temperature from 65–50°C with a 5°C decrease every 5 cycles, followed by 25 cycles at 50°C. PCR products were analyzed using both polyacrylamide gel electrophoresis (PAGE) and ABI 3730 DNA Analyzer. For PAGE, 4% (w/v) polyacrylamide denaturing gel was used and PCR bands were detected by silver staining. A 25-bp DNA ladder was used for size standard. For the ABI 3730 genotyping, the direct labeling method was used with fluorescent dyes (6-FAM, VIC, NED and PET) and PCR bands were analyzed using the Genographer Version 2.1.4 (http://sourceforge.net/projects/genographer).

Data analysis

For SSR and STS markers, the sizes of PCR amplicons were recorded. For DArT markers, genotyping data were analyzed using TASSEL (http://www.maizegenetics.net/) for principal component analysis and NTSYSpc Version 2.2 (Exeter Software, Setauket, NY) for cluster analysis. The Jauovach module was used for calculating similarity coefficients between wheat lines, and the unweighted pair group method with arithmetic mean (UPGMA) was used for clustering (Sneath and Sokal 1973). To identify DArT markers associated with the haplotypes of stem rust resistance genes, we conducted an association analysis using the haplotyping results of

Table 1 Markers used for haplotyping major stem rust resistance genes and their primer sequences

Sr gene	Chr. interval	Marker	Forward primer	Reverse primer
1A.1R	1A.1R	BARC28	5′-ctccccggctagtgaccaca	5′-gcggcatctttcattaacgagctagt
		SCM9	5′-tgacaacccccttttccctcgt	5′-tcatcgacgctaaggaggaccc
Sr2	3BS	GWM533	5′-aaggcgaatcaaacggaata	5′-gttgctttaggggaaaagcc
		BARC133	5′-agcgctcgaaaagtcag	5′-ggcaggtccaactccag
Sr22	7AL	CFA2123	5′-cggtctttgtttgctctaaacc	5′-accggccatctatgatgaag
		CFA2019	5′-gacgagctaactgcagaccc	5′-ctcaatcctgatgcggagat
		BARC121	5′-actgatcagcaatgtcaactgaa	5′-ccggtgtctttcctaacgctatg
Sr24	3DL	BARC71	5′-gcgcttgttcctcacctgctcata	5′-gcgtatattctctcgtcttcttgttggtt
		Sr24#12	5′-cacccgtgacatgctcgta	5′-aacaggaaatgagcaacgatgt
Sr25	7DL	Gb	5′-catccttggggacctc	5′-ccagctcgcatacatcca
		BF145935	5′-cttcacctccaaggagttccac	5′-gcgtacctgatcaccaccttgaagg
Sr26	6AL	Sr26#43	5′-aatcgtccacattggcttct	5′-cgcaacaaaatcatgcacta
		BF518379	5′-agccgcgaaatctactttga	5′-ttaaacggacagagccacacg
Sr36	2BS	WMC477	5′-cgtcgaaaaccgtacactctcc	5′-gcgaaacagaatagccctgatg
		GWM271	5′-caagatcgtggagccagc	5′-agctgctagcttttgggaca
		GWM 319	5′-ggttgctgtacaagtgttcacg	5′-cgggtgctgtgtgtaatgac
		STM773	5′-aaacgccccaaccacctctctc	5′-atggtttgttgtgttgtgtgtagg
Sr40	2BS	GWM 344	5′-caaggaaataggcggtaact	5′-atttgagtctgaagtttgca
		WMC661	5′-ccaccatggtgctaatagtgtc	5′-agctcgtaacgtaatgcaactg
		WMC474	5′-atgctattaaactagcatgtgtcg	5′-agtggaaacatcattcctggta

Sr2 and *Sr25* and DArT genotyping data for the CIMMYT wheat lines. The haplotype results were converted to digital scores, "1" present and "0" absent for the respective haplotypes, and designated as traits. The linkage disequilibrium (LD) analysis used the genotyping scores of 828 DArT markers in the mixed linear model with population structure controlled by the kinship matrix using TASSEL.

Results

Haplotyping stem rust resistance loci using SSR makers

Major stem rust resistance genes were characterized for source, available markers, current research activities, and prioritized for this project (http://rustopedia.get-traction.com/traction). To evaluate the functionality and polymorphism for the available markers, we first screened 58 markers associated with 21 stem rust resistance genes on a panel of 24 accessions. Forty-six (80%) of the markers amplified clear fragments and, of those, 35 (75%) showed polymorphism. Twenty were chosen for haplotyping eight major stem rust resistance genes in the present study (Table 1). 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).

Fig. 1 **a** Haplotype diversity at stem rust resistance genes *Sr2*, *Sr22*, *Sr24*, and *Sr25* using microsatellite markers in a diverse collection of wheat lines. Multiple markers used are listed below the respective gene. *Numeric values* are the sizes (bp) of PCR amplicons for the respective marker and wheat line. 'NA' means null allele. Amplicons with same size were sorted together as a haplotype group and color-coded as follow: *red and pink highlight* the common haplotypes similar to the known gene resources; *other colors highlight* other haplotypes differed from the known gene resources. **b** Haplotype diversity at stem rust resistance genes *Sr26*, *Sr36*, *Sr40*, and *1A.1R* using microsatellite markers in a diverse collection of wheat lines. Multiple markers used are listed below the respective gene. *Numeric values* are the sizes (bp) of PCR amplicons for the respective marker and wheat line. 'NA' means null allele. Amplicons with same size were sorted together as a haplotype group and color-coded as follow: *red and pink highlight* the common haplotypes similar to the known gene resources; *other colors highlight* other haplotypes differed from the known gene resources

a

		Sr2				*Sr22*					*Sr24*				*Sr25*	
Wheat line	SR	GWM533	BARC133	Wheat line	SR	CFA2019	BARC121	CFA2123	Wheat line	SR	BARC71	Sr24#12	Wheat line	SR	BF145935	GB
CIMMYT79	R-MR	120	117	Sr22TB	Sr22	235	215	246	LcSr24Ag	Sr24	108, 104	500	LcSr25Ars	Sr25	198	130
CIMMYT83	R-MR	120	117	ARS05-0456	NA	235	215	246	Agent	Sr24	108, 104	500	Agatha	Sr25	198	130
CIMMYT87	R	120	117	CHINA11	NA	235	215	246	McCormick	Sr24	108, 104	500	CIMMYT69	R	198	130
CIMMYT2	MR	120	117	CIMMYT5	R-MR	235	215	246	ARS05-0046	Sr24	108, 104	500	CIMMYT70	R	198	130
CIMMYT3	MS	120	117	CIMMYT6	R-MR	235	215	246	ARS05-0469	NA	108, 104	500	CIMMYT11	R-MR	198	130
CIMMYT6	R-MR	120	117	CIMMYT35	R-MR	235	215	246	ARS05-0005	NA	108, 104	500	CIMMYT12	R-MR	198	130
CIMMYT35	R-MR	120	117	CIMMYT 111	NA	235	215	246	CIMMYT87	R	126	500	CIMMYT42	MR-MS	198	130
CIMMYT39	MS	120	117	CIMMYT19	S	215	215	246	CIMMYT1	MS	126	200	CIMMYT1	MS	202	NA
CIMMYT43	MR-MS	120	117	CIMMYT22	MS	215	215	246	CIMMYT2	MR	126	200	CIMMYT2	MR	202	NA
CIMMYT45	MR-MS	120	117	CIMMYT25	MR	215	215	246	CIMMYT3	MS	126	200	CIMMYT3	MS	202	NA
CIMMYT46	MR-MS	120	117	CIMMYT32	S	215	215	246	CIMMYT4	Sr-ND643	126	200	CIMMYT4	Sr-ND643	202	NA
CIMMYT50	MR-MS	120	117	CIMMYT33	MR	215	215	246	CIMMYT6	R-MR	126	200	CIMMYT5	R-MR	202	NA
CIMMYT52	MR	120	117	CIMMYT59	MR-MS	215	215	246	CIMMYT7	Sr-HUW234	126	200	CIMMYT6	R-MR	202	NA
CIMMYT56	MR-MS	120	117	CIMMYT66	MS	215	215	246	CIMMYT8	R-MR	126	200	CIMMYT7	Sr-HUW234	202	NA
CIMMYT58	MR-MS	120	117	CIMMYT76	NA	215	215	246	CIMMYT10	MS	126	200	CIMMYT8	R-MR	202	NA
CIMMYT59	MR-MS	120	117	CIMMYT107	NA	215	215	246	CIMMYT11	Sr25	126	200	CIMMYT9	S	202	NA
CIMMYT60	MR	120	117	CIMMYT109	NA	215	215	246	CIMMYT12	Sr25	126	200	CIMMYT10	MS	202	NA
CIMMYT62	MR-MS	120	117	ARS03-6180	Amigo	215	215	246	CIMMYT13	MR	126	200	CIMMYT13	MR	202	NA
CIMMYT64	S	120	117	CHINA2	NA	215	215	246	CIMMYT14	MR	126	200	CIMMYT14	MR	202	NA
CIMMYT66	MS	120	117	CHINA28	NA	215	215	246	CIMMYT15	Sr33?	126	200	CIMMYT15	MS	202	NA
CIMMYT67	SrHUW234	120	117	LineA sel	Sr14	215	215	246	CIMMYT16	R-MR	126	200	CIMMYT16	R-MR	202	NA
CIMMYT73	S	120	117	LcSr24Ag	Sr24	215	215	246	CIMMYT17	MR	126	200	CIMMYT17	MR	202	NA
CIMMYT76	NA	120	117	Agent	Sr24	215	215	246	CIMMYT19	S	126	200	CIMMYT18	S	202	NA
CIMMYT77	MR	120	117	CnsSr32	Sr32	215	215	246	CIMMYT20	MS	126	200	CIMMYT19	S	202	NA
CIMMYT80	MR	120	117	CIMMYT102	NA	215	230	246	CIMMYT22	MS	126	200	CIMMYT20	MS	202	NA
CIMMYT81	MR	120	117	CIMMYT106	NA	215	230	246	CIMMYT23	SrTmp	126	200	CIMMYT21	MS	202	NA
CIMMYT82	R-MR	120	117	CIMMYT112	NA	215	230	246	CIMMYT24	R-MR	126	200	CIMMYT22	MS	202	NA
CIMMYT90	R-MR	120	117	CIMMYT2	MR	235	230	NA	CIMMYT25	MR	126	200	CIMMYT23	SrTmp	202	NA
CIMMYT55	MR-MS	120	117	CIMMYT98	NA	235	230	NA	CIMMYT26	MR	126	200	CIMMYT24	R-MR	202	NA
CIMMYT61	MS	120	117	GENEVA	NA	235	230	NA	CIMMYT29	MS	126	200	CIMMYT25	MR	202	NA
CIMMYT63	R-MR	120	117	CIMMYT1	MS	235	215	NA	CIMMYT31	MR	126	200	CIMMYT26	MR	202	NA
CIMMYT70	Sr25	120	117	CIMMYT18	S	235	215	NA	CIMMYT32	S	126	200	CIMMYT27	MS	202	NA
ARS05-0037	NA	120	117	CIMMYT29	MS	235	215	NA	CIMMYT34	MR	126	200	CIMMYT28	Sr-Sharp	202	NA
ARS05-1266	NA	120	117	CIMMYT57	MR-MS	235	215	NA	CIMMYT35	R-MR	126	200	CIMMYT29	MS	202	NA
ARS03-3806	NA	120	117	MT0495	Tmp?	235	215	NA	CIMMYT36	MS	126	200	CIMMYT30	NA	202	NA
China7	NA	120	117	CHINA5	NA	235	215	NA	CIMMYT37	MS	126	200	CIMMYT31	MR	202	NA
CIMMYT15	Sr33?	120	114	CIMMYT108	NA	215	NA	246	CIMMYT38	MR	126	200	CIMMYT32	S	202	NA
CIMMYT7	Sr-HUW234	120	114	CHINA27	NA	215	NA	246	CIMMYT42	Sr25	126	200	CIMMYT33	MR	202	NA
CIMMYT54	MS	120	114	Khapstein	Sr13	215	NA	246	CIMMYT43	MR-MS	126	200	CIMMYT34	MR	202	NA
CIMMYT1	MS	120	114	CIMMYT10	MS	235	217	NA	CIMMYT44	MR-MS	126	200	CIMMYT35	R-MR	202	NA
CIMMYT11	Sr25	120	114	CIMMYT 103	NA	235	NA	NA	CIMMYT46	MR-MS	126	200	CIMMYT36	MS	202	NA
CIMMYT12	Sr25	120	114	CHINA24	NA	235	NA	NA	CIMMYT48	MS	126	200	CIMMYT37	MS	202	NA
CIMMYT13	MR	120	114	CHINA38	NA	235	NA	NA	CIMMYT49	MR-MS	126	200	CIMMYT38	MR	202	NA
CIMMYT14	MR	120	114	Combination	Sr13(+17)	223	215	246	CIMMYT50	MR-MS	126	200	CIMMYT39	MS	202	NA
CIMMYT16	R-MR	120	114	CIMMYT24	R-MR	NA	215	246	CIMMYT52	MR	126	200	CIMMYT40	MR	202	NA
CIMMYT20	MS	120	114	CIMMYT36	MS	NA	215	246	CIMMYT53	R-MR	126	200	CIMMYT41	MR-MS	202	NA
CIMMYT21	MS	120	114	CIMMYT37	MS	NA	215	246	CIMMYT54	MS	126	200	CIMMYT43	MR-MS	202	NA
CIMMYT24	R-MR	120	114	CIMMYT38	MR	NA	215	246	CIMMYT55	MR-MS	126	200	CIMMYT44	MR-MS	202	NA
CIMMYT26	MR	120	114	CIMMYT39	MS	NA	215	246	CIMMYT59	MR-MS	126	200	CIMMYT45	MR-MS	202	NA
CIMMYT28	Sr-Sharp	120	114	CIMMYT43	MR-MS	NA	215	246	CIMMYT60	MR	126	200	CIMMYT46	MR-MS	202	NA
CIMMYT30	NA	120	114	CIMMYT44	MR-MS	NA	215	246	CIMMYT61	MS	126	200	CIMMYT47	MR	202	NA
CIMMYT31	MR	120	114	CIMMYT46	MR-MS	NA	215	246	CIMMYT62	MR-MS	126	200	CIMMYT48	MS	202	NA
CIMMYT34	MR	120	114	CIMMYT48	MS	NA	215	246	CIMMYT63	R-MR	126	200	CIMMYT49	MR-MS	202	NA
CIMMYT36	MS	120	114	CIMMYT49	MR-MS	NA	215	246	CIMMYT64	S	126	200	CIMMYT50	MR-MS	202	NA
CIMMYT37	MS	120	114	CIMMYT58	MR-MS	NA	215	246	CIMMYT65	MR	126	200	CIMMYT51	MR-MS	202	NA
CIMMYT38	MR	120	114	CIMMYT60	MR	NA	215	246	CIMMYT67	SrHUW234	126	200	CIMMYT52	MR	202	NA
CIMMYT42	Sr25	120	114	CIMMYT61	MS	NA	215	246	CIMMYT69	Sr25	126	200	CIMMYT53	R-MR	202	NA
CIMMYT44	MR-MS	120	114	CIMMYT63	R-MR	NA	215	246	CIMMYT70	Sr25	126	200	CIMMYT54	MS	202	NA
CIMMYT47	MR	120	114	CIMMYT64	S	NA	215	246	CIMMYT71	SrSha7	126	200	CIMMYT55	MR-MS	202	NA
CIMMYT53	R-MR	120	114	CIMMYT68	MR	NA	215	246	CIMMYT74	MS	126	200	CIMMYT56	MR-MS	202	NA
CIMMYT69	Sr25	120	114	CIMMYT70	Sr25	NA	215	246	CIMMYT77	MR	126	200	CIMMYT57	MR-MS	202	NA
CIMMYT78	MR	120	114	CIMMYT71	SrSha7	NA	215	246	CIMMYT78	MR	126	200	CIMMYT58	MR-MS	202	NA
CIMMYT84	MR	120	114	CIMMYT72	SrSha7	NA	215	246	CIMMYT79	R-MR	126	200	CIMMYT59	MR-MS	202	NA
CIMMYT85	MR	120	114	CIMMYT77	MR	NA	215	246	CIMMYT81	MR	126	200	CIMMYT60	MR	202	NA
CIMMYT88	SrSynthetic	120	114	CIMMYT79	R-MR	NA	215	246	CIMMYT82	R-MR	126	200	CIMMYT61	MS	202	NA
CIMMYT92	R-MR	120	114	CIMMYT86	MR	NA	215	246	CIMMYT83	R-MR	126	200	CIMMYT62	MR-MS	202	NA

b

Sr26					*Sr36*						*Sr40*				*1A.1R*		
Wheat line	SR	Sr26#43/ BE518379	Wheat line	SR	WMC477	STM773	GWM319	GWM271	Wheat line	SR	WMC474	WMC661	GWM344	Wheat line	SR	SCM9	BARC28
Eagle	Sr26	207	W2691SrTt-1	Sr36	187	153	168	170	RL 6088	Sr40	150	188	264	TAM107	1A.1R	224	260
PW327	Sr26	207	CI12632	Sr36	187	153	168	170	CIMMYT21	MS	135	225	132	Amigo	1A.1R	224	260
CIMMYT1	MS	303	ARS05-0886	NA	187	153	168	170	CIMMYT34	MR	135	225	132	ARS04-1267	NA	224	260
CIMMYT2	MR	303	ARS05-0456	NA	187	153	168	170	CIMMYT36	MS	135	225	132	ARS03-6180	NA	224	260
CIMMYT3	MS	303	ARS05-0005	NA	187	153	168	170	CIMMYT37	MS	135	225	132	ARS05-0897	NA	224	260
CIMMYT4	Sr-ND643	303	ARS05-0146	NA	187	153	168	170	CIMMYT46	MR-MS	135	225	127	ARS05-1041	NA	224	260
CIMMYT5	R-MR	303	CIMMYT37	MS	187	185	190	155	CIMMYT47	MR	135	225	127	ARS05-0005	NA	224	260
CIMMYT6	R-MR	303	CIMMYT67	SrHUW23	187	185	190	155	CIMMYT35	R-MR	135	230	125	CIMMYT87	R	205	260
CIMMYT7	Sr-HUW234	303	CIMMYT87	R	187	185	190	155	CIMMYT40	MR	135	230	125	CIMMYT56	MR-MS	205	260
CIMMYT8	R-MR	303	CIMMYT92	R-MR	187	185	190	152	CIMMYT50	MR-MS	135	230	125	CIMMYT101	NA	205	260
CIMMYT9	S	303	CIMMYT58	MR-MS	187	185	190	150	CIMMYT67	NA	135	230	125	CIMMYT86	MR	205	250
CIMMYT10	MS	303	Kofa	R	187	185	190	150	CIMMYT69	Sr25	135	230	125	CIMMYT40	MR	205	250
CIMMYT11	R	303	CIMMYT36	MS	187	185	190	155	CIMMYT70	Sr25	135	230	125	CIMMYT64	S	205	250
CIMMYT12	R-MR	303	CIMMYT49	MR-MS	187	185	190	155	CIMMYT61	MS	135	230	127	CIMMYT71	SrSha7	205	250
CIMMYT13	MR	303	CIMMYT11	Sr25	187	185	190	152	CIMMYT74	MS	135	230	127	CIMMYT89	MS	205	250
CIMMYT14	MR	303	CIMMYT61	MS	187	185	190	152	CIMMYT77	MR	135	230	127	CIMMYT94	SrSha7	205	250
CIMMYT15	MS	303	Millenium		187	185	190	NA	CIMMYT82	R-MR	135	230	127	NY7388	NA	205	250
CIMMYT16	R-MR	303	CIMMYT77	MR	183	185	190	155	CIMMYT84	MR	135	230	127	ClarksCream	NA	205	270
CIMMYT17	MR	303	CIMMYT88	SrSynthe	183	185	190	155	CIMMYT92	R-MR	135	230	127	China30	NA	205	285
CIMMYT18	S	303	CIMMYT90	R-MR	183	185	190	155	CIMMYT71	SrSha7	135	230	132	CIMMYT55	MR-MS	205	NA
CIMMYT19	S	303	CIMMYT35	R-MR	183	185	190	150	CIMMYT7	NA	135	230	NA	CIMMYT103	NA	205	NA
CIMMYT20	MS	303	CIMMYT59	MR-MS	183	185	190	150	CIMMYT63	R-MR	135	230	NA	CIMMYT109	NA	205	NA
CIMMYT21	MS	303	CIMMYT84	MR	183	185	188	155	CIMMYT88	NA	135	182	127	CIMMYT111	NA	205	NA
CIMMYT22	MS	303	CIMMYT7	Sr-HUW23	183	185	188	155	CIMMYT15	NA	135	182	NA	GA96693-4E16(	R	205	NA
CIMMYT23	SrTmp	303	CIMMYT50	MR-MS	183	185	188	152	CIMMYT90	R-MR	135	225	125	ChineseSpring	S	205	NA
CIMMYT24	R-MR	303	CIMMYT60	MR	183	185	188	152	CIMMYT3	MS	135	240	132	ARS05-1266	NA	224	270
CIMMYT25	MR	303	CIMMYT76	NA	183	185	188	152	Kofa	R	135	250	127	CIMMYT76	NA	172	250
CIMMYT26	MR	303	CIMMYT73	S	183	185	188	152	CIMMYT16	R-MR	135	NA	127	CIMMYT66	MS	175	250
CIMMYT27	MS	303	CIMMYT19	S	183	185	188	155	CIMMYT18	S	135	NA	127	CIMMYT69	Sr25	195	250
CIMMYT28	Sr-Sharp	303	CIMMYT95	NA	187	185	NA	150	CIMMYT24	R-MR	135	NA	127	CIMMYT1	MS	NA	250
CIMMYT29	MS	303	PW327	26	187	185	NA	150	CIMMYT30	NA	135	NA	127	CIMMYT2	MR	NA	250
CIMMYT30	NA	303	NY7388		187	185	NA	150	CIMMYT44	MR-MS	135	NA	127	CIMMYT3	MS	NA	250
CIMMYT31	MR	303	China43	NA	187	185	NA	152	CIMMYT45	MR-MS	135	NA	127	CIMMYT4	Sr-ND643	NA	250
CIMMYT32	S	303	CIMMYT40	MR	187	185	NA	155	CIMMYT56	MR-MS	135	NA	127	CIMMYT7	Sr-HUW234	NA	250
CIMMYT33	MR	303	CIMMYT39	MS	187	185	NA	155	CIMMYT59	MR-MS	135	NA	127	CIMMYT11	R	NA	250
CIMMYT34	MR	303	CIMMYT46	MR-MS	187	185	NA	155	CIMMYT75	S	135	NA	127	CIMMYT12	R-MR	NA	250
CIMMYT35	R-MR	303	CIMMYT 103		187	185	NA	155	CIMMYT76	NA	135	NA	127	CIMMYT13	MR	NA	250
CIMMYT36	MS	303	CIMMYT17	MR	187	185	NA	155	CIMMYT78	MR	135	NA	127	CIMMYT14	MR	NA	250
CIMMYT37	MS	303	CIMMYT91	R-MR	187	185	NA	NA	CIMMYT83	R-MR	135	NA	127	CIMMYT15	NA	NA	250
CIMMYT38	MR	303	ARS05-0469	NA	187	188	190	155	China25	NA	135	NA	127	CIMMYT16	R-MR	NA	250
CIMMYT39	MS	303	CIMMYT94	SrSha7	187	188	190	NA	China29	NA	135	NA	127	CIMMYT17	MR	NA	250
CIMMYT40	MR	303	China19	NA	187	180	190	155	CIMMYT49	MR-MS	135	NA	125	McCormick	NA	195	260
CIMMYT41	MR-MS	303	CIMMYT22	MS	187	NA	188	155	CIMMYT85	MR	135	NA	129	CIMMYT10	MS	NA	260
CIMMYT42	Sr25	303	China2	NA	187	NA	190	152	CIMMYT20	MS	135	NA	NA	CIMMYT80	MR	NA	260
CIMMYT43	MR-MS	303	CIMMYT52	MR	187	NA	190	155	CIMMYT48	MS	135	NA	NA	CIMMYT88	NA	NA	260
CIMMYT44	MR-MS	303	CIMMYT 106	NA	187	NA	190	155	CIMMYT 104	NA	135	NA	NA	CIMMYT97	NA	NA	260
CIMMYT45	MR-MS	303	ARS05-0605	nd	187	NA	190	155	CIMMYT 107	NA	135	NA	NA	CIMMYT 102	NA	NA	260
CIMMYT46	MR-MS	303	73.214.3-1	27	187	NA	190	155	CIMMYT 114	NA	135	NA	NA	CIMMYT 105	NA	NA	260
CIMMYT47	MR	303	Agatha	Sr25	187	NA	NA	150	ARS05-0037	NA	135	NA	NA	CIMMYT 106	NA	NA	260
CIMMYT48	MS	303	GA98401-5E45	R	183	180	190	150	China33	NA	135	NA	NA	CIMMYT 114	NA	NA	260
CIMMYT49	MR-MS	303	GA991209-6E33		183	180	190	NA	China36	NA	135	NA	NA	ARS05-0146	NA	NA	260
CIMMYT50	MR-MS	303	CIMMYT54	MS	183	185	NA	152	China37	NA	135	NA	NA	China1	NA	NA	260
CIMMYT51	MR-MS	303	CIMMYT64	S	183	185	NA	155	China38	NA	135	NA	NA	China19	NA	NA	260
CIMMYT52	MR	303	CIMMYT 114	NA	183	185	NA	155	Geneva2	NA	135	NA	NA	NY8080	NA	NA	260
CIMMYT53	R-MR	303	CIMMYT31	MR	183	185	NA	155	K253	Sr9e	135	NA	NA	CS_T_mono	Sr21	NA	195
CIMMYT54	MS	303	CIMMYT53	R-MR	183	185	NA	155	ARS04-1267	Amigo	116	NA	127	CnsSr32	Sr32	NA	195
CIMMYT55	MR-MS	303	CIMMYT28	Sr-Sharp	183	185	NA	NA	ARS05-0005	NA	116	NA	127	CIMMYT95	NA	NA	200
CIMMYT56	MR-MS	303	CIMMYT 109	NA	183	NA	190	155	Mq(2)5*G2919	Sr35	120	250	NA	CIMMYT96	NA	NA	200
CIMMYT57	MR-MS	303	CIMMYT 111	NA	183	NA	190	155	Millenium	NA	122	NA	NA	CIMMYT79	R-MR	NA	245
CIMMYT58	MR-MS	303	GA001435-6E23	NA	183	NA	190	155	China12	NA	124	NA	NA	CIMMYT85	MR	NA	245
CIMMYT59	MR-MS	303	GA991371-6E12	NA	183	NA	190	155	Eagle	Sr26	127	NA	NA	ARS03-3806	NA	NA	245
CIMMYT60	MR	303	CIMMYT29	MS	183	NA	NA	150	Agatha	Sr25	128	NA	NA	Geneva	NA	NA	260
CIMMYT61	MS	303	CIMMYT 107	NA	183	NA	NA	150	China10	NA	129	NA	127	Caledonia	S	NA	245

Fig. 1 continued

Sr2

Sr2 is located on the short arm of wheat chromosome 3B (Hare and McIntosh 1979). Two closely linked microsatellite markers gwm533 and BARC133 and one unpublished STS marker (W. Spielmeyer, personal communication) were used for haplotyping *Sr2*. Among them, marker gwm533 amplified a 120 bp PCR fragment which is diagnostic for *Sr2* (Spielmeyer et al. 2003), while the STS marker amplified a 400 bp fragment. Using these markers, we found that 83 out of 115 CIMMYT's current wheat breeding lines showed the *Sr2* haplotype (Fig. 1). Of those, three stem rust resistant lines 'Kingbird', 'Pavon F76' and 'Kiritati,' with CIMMYT entry numbers 87, 79, and 83, respectively, are known (based on phenotype) to have the *Sr2* complex consisting of *Sr2* and other unknown genes (Singh et al. 2009). We used them as *Sr2* sources for comparison with other haplotypes. In addition to the CIMMYT lines, five ARS lines and two Chinese lines appeared to have the *Sr2* haplotype.

Marker BARC133 amplified various sizes of PCR fragments in our germplasm collection. Among *Sr2* positives, about half of them had a 117-bp and half had a 114-bp fragment. Because of the similarity in size, the same primer set and PCR conditions were used to produce amplicons that were run on PAGE and the ABI 3730. Identical results confirmed the size difference, indicating that both 117 and 114 bp fragments are associated with *Sr2*. Thus, the two haplotypes for *Sr2* positive lines were "120-117" (Fig. 1, *Sr2* red highlight) and "120-114" (Fig. 1, pink highlight) for gwm533 and BARC133, respectively. This result was consistent with the unpublished STS marker.

Sr22

Sr22 was previously mapped on the long arm of chromosome 7A (Khan et al. 2005). Three linked markers, cfa2019, cfa2123, and BARC121 (Miranda et al. 2007), were used for haplotyping this locus. PCR amplification by cfa2019 and cfa2123 showed 235- and 245-bp fragments, respectively, in the *Sr22* source germplasm, *Sr22* TB (Fig. 1, *Sr22*). BARC121 amplified two polymorphic bands of 215 and 230 bp in most germplasm. However, only the 215-bp fragment was amplified in *Sr22* TB, indicating that only the 215-bp fragment was specific to *Sr22*, while the 230-bp fragment was not associated with *Sr22*. Therefore, the haplotype for *Sr22* was "235-215-245" for markers cfa2019, BARC121, and cfa2123, respectively. Among our germplasm accessions, besides *Sr22* TB, only two lines, ARS05-0456 and China 11 showed this haplotype. However, pedigree tracking indicated that none of the parentages is known to have *Sr22*. Consequently, this haplotype may not be diagnostic for *Sr22* and further evaluation is needed.

Sr24

Two markers, BARC71 and *Sr24*#12, were used for haplotying *Sr24* which is on chromosome 3DL (Mago et al. 2005) in AGENT- or 1BS in Amigo-derived lines. BARC71 amplified two fragments of 108 bp and 104 bp, and marker *Sr24*#12 amplified a 500-bp PCR fragment in *Sr24*-carrying lines such as AGENT and Lc*Sr24*Ag (Fig. 1, *Sr24*). McCormick and three ARS lines, ARS05-0046, ARS05-0469, and ARS05-0005, had the same haplotype as the check lines. McCormick is known to carry *Sr24*, while the three ARS lines were unknown. In addition to the 3DL translocation, *Sr24* was introgressed into wheat lines with US origin via the 1BS-*Lophopyrum* translocation present in Amigo (Jiang et al. 1994; The et al. 1992). Mago et al. (2005) reported that BARC71 amplified 85- and 103-bp fragments in *Sr24* containing lines and a 107-bp fragment in most but not all susceptible lines. Olson et al. (2010) reported that BARC71 amplified 83-, 88-, and 101-bp fragments from the translocated segment containing *Sr24* locus; however BARC71 also amplified a wheat fragment 107 bp in length when the *Lophopyrum* translocation was on 1BS. Of the six lines identified to carry *Sr24* in our study, all of them amplified the 101- and 107-bp (104- and 108-bp in our study, respectively) wheat fragments, indicating the presence of the 1BS translocation and the *Sr24*-containing segment. The pedigrees of ARS05-0046 and ARS05-0469 have the same parentage (TX98D1170*2/TTCC365), whereas ARS05-0005 was selected from the cross of TX85-264*2/TTCC512 (Table S1). Stem rust race tests indicated that ARS05-0046 and ARS05-0469 were resistant to TTKSK (avirulent on *Sr24*) and more susceptible to TTKST (virulent on *Sr24*) indicating that they have *Sr24*, whereas ARS05-0005 showed a considerable level of resistance to both races (but a higher reaction to QCCL virulent on *Sr24*), therefore the gene could not be postulated (David Marshall, Yue Jin, personal communication). This might be due to multiple resistance genes that were introduced in ARS05-0005, and the contradictory race reactions. A CIMMYT line, CIMMYT#87 (Kingbird) amplified the positive 500-bp fragment using marker Sr24#12 while the non-*Sr24* 126-bp fragment was amplified using BARC71. Similarly, the haplotype for 1A.1R was ambiguous in this line; however, it does have *Sr2* based on both haplotype and phenotype. Kingbird has TAM-200 parentage that had both Amigo and 1A.1R; however, seedling tests indicated that Kingbird does not have *Sr24* (Ravi Singh, personal communication). Consequently, a detailed mapping study will be required to elucidate the stem rust resistance genotype of Kingbird.

Sr25

The co-dominant marker, BF145935, derived from wheat EST (Ayala-Navarrete et al. 2007) and the dominant marker, Gb, were used for haplotyping *Sr25*. Liu et al. (2010) validated marker BF145935 using 42 wheat lines and indicated that two DNA fragments amplified in most lines. The upper band of Wheatear was smaller than that of non-*Sr25* lines. This DNA fragment is located on the 7Ae#1 segment that was translocated onto wheat chromosome 7DL (Ayala-Navarrete et al. 2007; Liu et al. 2010). In the present study, we used marker BF145935 for haplotyping *Sr25* and found that the fragment sizes were 198 and 180 bp in *Sr25* lines and 202 and 180 bp in wheat lines without *Sr25* (Fig. 2). CIMMYT lines 11, 12, 42, 69 (heterozygous), and 70 showed the *Sr25*-haplotype. The dominant marker Gb amplified a 130-bp fragment only in the *Sr25*-positive lines and no PCR product was obtained in wheat lines that lack *Sr25*. Both markers were in agreement that these CIMMYT lines likely carry *Sr25*. This was consistent with the rust race test (Jin et al. unpublished). Pedigree tracking showed that all the CIMMYT *Sr25*-carrying lines contain Wheatear in their parentages (Table S1). These lines had relatively high resistance to stem rust as tested in Kenya in 2007 and 2008 (Singh et al. unpublished), and both *Sr25* and *Sr2* were identified in these lines.

Sr26

Sr26 is located on the distal portion of chromosome 6AL/6Ae#1 translocation (Dundas et al. 2007). A dominant marker, *Sr26*#43, derived from an AFLP fragment (Mago et al. 2005) amplified a 207-bp PCR product in wheat line carrying *Sr26* while no amplification product occurred in wheat lines without *Sr26*. Liu et al. (2010) developed an STS marker, BE518379, that was specific to chromosome 6AL and also developed a complex co-dominant marker by combining this marker and marker *Sr26*#43. Using this combination, 2 PCR fragments of 207 and 303 bp were amplified in *Sr26*-carrying lines, Eagle and Agent/9*LMPG, while only a 303-bp fragment was amplified in wheat lines lacking *Sr26*. As expected with the exception of the *Sr26*-containing checks, no other wheat line was found to contain this gene (Fig. 1, *Sr26*).

Sr36

Sr36 is located on the short arm of chromosome 2B (Gyarfas 1978). Four linked markers, wmc477, stm733-2, gwm319, and gwm271 (Tsilo et al. 2008), were used for haplotyping *Sr36*. Polymorphic fragments of 187 bp and 153 bp were amplified by markers wmc477 and stm773-2, respectively, in the

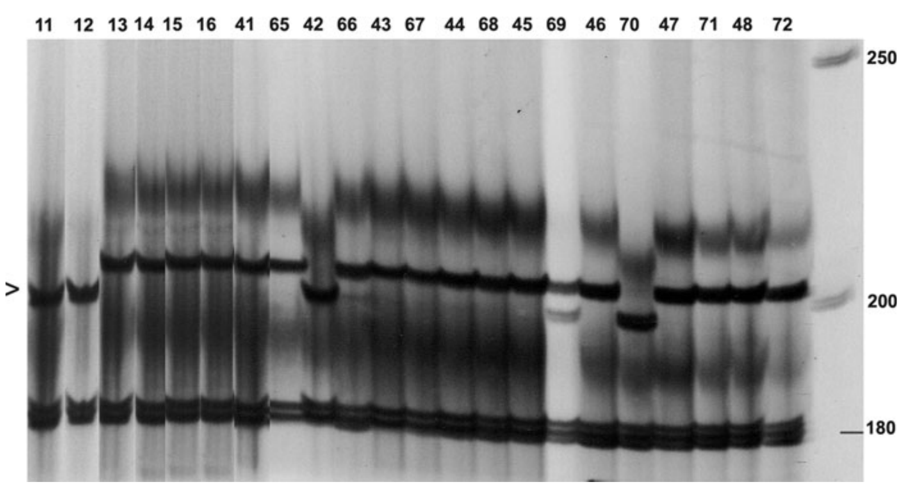

Fig. 2 PAGE image of PCR products amplified by marker BF145935 in parts of germplasm with CIMMYT accessions. The *number* indicated on each *lane* corresponds to the CIMMYT entry #. Among them, CIMMYT #11, 12, 42, 69 (heterozygous), and 70 show *Sr25* loci (*arrow*). Size standards are shown on the *right*

two *Sr36*-carrying lines, CI12632 and W2691*SrTt-1*, whereas markers gwm319 and 271 amplified 169- and 170-bp fragments, respectively. With the four-marker combination, a haplotype of "189-160-170-170" was identified in *Sr36*-carrying lines, including the two checks, and four ARS lines, ARS05-0886, ARS05-0456, ARS05-0005, and ARS05-0146. The pedigree indicated that ARS05-0456 contains Neuse in its parentage, which is known to carry *Sr36*. Neuse was not found as a parent in the other lines. The haplotype result was consistent with that of stem rust race tests (Singh et al. unpublished), suggesting that haplotyping with the four marker combination is predictive of the presence of *Sr36*.

Sr40

Sr40 was mapped on chromosome 2BS (Dyck 1992) and is closely linked to *Sr36* (Wu et al. 2009). Microsatellite markers wmc474, wmc661, and gwm344 were used for haplotyping *Sr40* and they produced polymorphic bands of 150, 188, and 264 bp, respectively, in the *Sr40*-carrying line, RL6088. Although a few haplotypes were identified in other wheat lines, none were the same as RL6088. These lines are unlikely to carry *Sr40*. Among them, four CIMMYT lines, CIMMYT 21, 34, 36, and 37, shared the 135-225-132 haplotype and all have Waxwing in their pedigrees (Fig. 1, *Sr40*, pink highlight). Waxwing has been shown to have moderate resistance to stem rust. Since *Sr23* (linked to *Lr16*) is also located on chromosome 2BS (McIntosh et al. 1995) it might contribute some resistance in Waxwing. Because both *Sr23* and *Sr40* are located in the same chromosome region, the markers used for *Sr40* may coincidentally haplotype *Sr23* and produce the 135-225-132 banding pattern; however this observation requires confirmation.

1A.1R

The resistance of wheat-rye chromosome translocation, *1A.1R*, is due to an unnamed rust resistance gene. A rye SSR marker, SCM9, that was developed to detect the presence of the 1RS rye translocation (Saal and Wricke 1999), was used for haplotyping. This marker amplified a 224-bp band in two *1A.1R*-carrying lines, TAM107 and Amigo, and five ARS accessions including ARS03-6180, ARS04-1267, ARS05-0897, ARS05-1041, and ARS05-0005 (Fig. 1, *1A.1R*, red highlight). Another SSR marker, BARC28, was also used for haplotyping *1A.1R* and amplified a 260-bp fragment in all *1A.1R* positives, as well as a few non-*1A.1R* containing lines, suggesting BARC28 is not diagnostic. Although other haplotypes were identified in other lines with unknown sources of resistance, the banding patterns were different from that of the check lines in the case of the SCM9 marker (Fig.1, *1A.1R*, yellow-colored). A known source of the *1B.1R* translocation, PBW343 (CIMMYT 89)- was included in this haplotype, indicating that a different rye parent contributed the 1R chromosome arm and it was likely the common *1B.1R* translocation.

Genome-wide genotyping using DArT markers

Genome-wide DArT markers can be effectively used for genotyping hexaploid wheat (Akbari et al. 2006). In the present study, the DArT array containing 3,500 DArT markers was used for genotyping the wheat lines and generated 843 polymorphic markers. The genotyping data was used for principal component analysis to assess the genetic relationships among the accessions. As illustrated in Fig. 3, three major groups were found. Group 1 included almost all spring lines from CIMMYT (Fig. 3, left panel). Among them, most contain the *Sr2* haplotype (Fig. 3, CTsp(*Sr2*)) and few contain both *Sr2* and *Sr25* (Fig. 3, CTsp(*Sr2* + *25*)). Group 2 included 21 CIMMYT winter lines, 42 Chinese lines, and most US lines (Fig. 3, right-lower panel). Among 21 CIMMYT winter lines tested, only five were detected to have *Sr2* (Fig. 3, CTwin(*Sr2*)). Few Chinese lines were found to have *Sr2*. All "ARS" lines were clustered into this group and five likely have *Sr2* (Fig. 3, ARS(*Sr2*). A few contain *Sr24*, *Sr36* or *1A.1R*. Several accessions with durum/emmer background were classified into the third group (Fig. 3, right-upper panel).

Identification of DArT markers associated with the *Sr2* and *Sr25* haplotypes

Association analysis was used to identify DArT markers associated with the haplotypes of stem rust resistance genes, *Sr2* and *Sr25*, in the CIMMYT

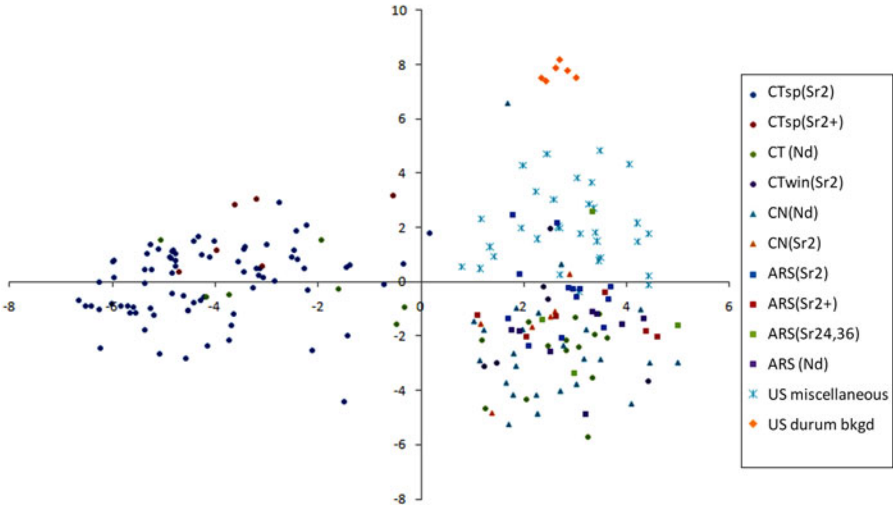

Fig. 3 Principal component analysis in wheat lines using DArT genotyping data. The first two PC columns were used for plot. Each data point represents a genotype. *Sharps and colors* were used, as indicted in the *right panel*, to illustrate the various origins and genetic backgrounds and suggested major resistance genes in wheat lines. CTsp(*Sr2*), (*Sr2* + *25*), CIMMYT spring lines with the *Sr2* and both the *Sr2* and *Sr25* haplotypes, respectively; CT(Nd), CIMMYT lines (spring and winter) *Sr* gene was not detected; CTwin(*Sr2*), CIMMYT winter lines with *Sr2*; CN(Nd), Chinese lines *Sr* gene was not detected; CN(*Sr2*), Chinese lines with *Sr2*; ARS(*Sr24*), (*Sr36*), (1A.1R), ARS accessions with *Sr24*, *36* and *1A.1R*, respectively; US miscellaneous, wheat lines from different origins in USA., US durum bkgd, US wheat lines with durum/emmer background

Table 2 Significant DArT markers associated with *Sr2* and *Sr25*

Haplotype	DArT marker	*P* value	Chromosome	cM	Population	Linked marker
Sr2	wPt-8446	5.23E-10	3BS	3.0	Carnamah/WAWHT2046	gwm533*(12.6) Barc133*(14.5) gwm389(0)
Sr2	wPt-7225	7.67E-07	3BS	2.1	Carnamah/WAWHT2046	gwm533*(12.6) Barc133*(14.5) gwm389(0)
Sr2	wPt-8093	3.40E-04	3BS	2.7	Carnamah/WAWHT2046	gwm533*(12.6) Barc133*(14.5) gwm389(0)
Sr2	wPt-7984	7.95E-04	3BS	8.6	Carnamah/WAWHT2046	gwm533*(12.6) Barc133*(14.5) gwm389(0)
Sr25	wPt-2258	1.48E-06	7DL	60.4	Synthetic/Opata	wg420(64.6) Gb*

* marker used for haplotyping the same loci. The numbers in the parentheses indicate distance in cM

wheat lines (Table 2). Four DArT markers were associated with the *Sr2* haplotype and are in the approximate location of *Sr2* on chromosome 3BS (http://www.triticarte.com.au/). The most significant marker was wpt-8446 ($P = 5.23$E–10), which was within 1 cM of the other two markers, wpt-7225 and wpt-8093. Marker wpt-7984 was approximately 4 cM distal to gwm533 and 6 cM proximal to wpt-8446 but had a higher *P*-value ($P = 7.95$E–4). The chromosome location indicates that these DArT markers are linked to *Sr2* and could be used for haplotyping this gene. The association analysis for the *Sr25* locus identified a significant DArT marker, wpt-2258 (Table 2). This marker was located on chromosome 7DL, about 4 cM distal to the STS marker, wg420, which is linked to *Sr25/Lr19* (Prins et al. 2001). Marker wg420 is approximately 2 cM distal to marker Gb that was used for haplotyping *Sr25* in the present study (Table 1).

Discussion

We examined haplotype diversity of important stem rust resistance genes in a diverse collection of wheat germplasm being used as sources of resistance or important susceptible parents to combat the spread of Ug99. The use of multiple, linked microsatellite and DArT markers for haplotyping stem rust resistance genes provided information on the reliability of the haplotypes and their frequency in elite germplasm of international breeding programs. Similar haplotypes among different genotypes facilitates hypothesis testing for resistance genes. However, haplotypes alone are inadequate to confirm the presence of a specific allele in uncharacterized lines. Combining haplotype 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 marker haplotypes. It is not always possible to obtain the reaction of breeding lines to specific races of rust so haplotyping can be quite useful for strategic crossing and selection. In this study, haplotyping indicated that 83 out of 115 current CIMMYT wheat breeding lines are likely to carry *Sr2*, thus illustrating the importance of *Sr2* in the CIMMYT breeding program. Among those identified, CIMMYT spring lines with entry #11, 12, 42, 69, and 70 are likely to have both *Sr2* and *Sr25*. Some lines are likely to have what is referred to as the *Sr2*-complex that consists of *Sr2* and other unknown genes that provide adult plant resistance (Singh et al. 2009). The result that many CIMMYT wheat lines carry the *Sr2* gene is not surprising because *Sr2* has been widely deployed in CIMMYT wheat breeding programs (McIntosh et al. 1995; Singh et al. 2009). This result is consistent with the observed phenotypes. However, among those *Sr2*-positives, CIMMYT 73 was rated susceptible in rust tests (Table S1). Pedigree checking did not show any parentage known to carry *Sr2*; however, recent investigation at CIMMYT indicated that this line shows pseudo-black chaff (Pbc), a trait linked to *Sr2*, suggesting that this line does carry *Sr2*. Its pedigree is most likely not correct and it appears to be derived from a synthetic wheat. High susceptibility to Ug99 suggests that *Sr2* is suppressed in this line—a phenomenon also observed in some other synthetic wheat derived lines that have *Pbc* and *Sr2* haplotype but show high susceptibility to Ug99. Definitive proof for the presence or absence of *Sr2* in CIMMYT 73 will become clearer with the eventual cloning of *Sr2*.

Three and four ARS lines are likely to have *Sr24* and *Sr36*, respectively. *Sr24* and *Sr36* have been widely used in US stem rust resistance breeding programs (McIntosh et al. 1995). Although *Sr24* and *Sr36* are no longer resistant to new races related to Ug99, they are still useful if combined together or for pyramiding with other Ug99 effective genes. Although *Sr22* provides resistance to Ug99 (Roelfs and McVey 1979), only one cultivar, Schomburgk, containing *Sr22* was released in Australia (McIntosh et al. 1995). Surprisingly, two lines in our germplasm collection were found to have the same haplotype as the check lines. A similar result by a Chinese group (Dr. Y.Y. Cao, personal communication) found that several Chinese wheat lines have the *Sr22* haplotype. These lines are most likely "false positives" since none of them were found to have related parentage in their pedigrees and most of the Chinese wheat materials tested to date were found to be susceptible to Ug99. However, race-specific tests are required to eliminate the possibility that *Sr22* was introduced into these lines. None of the lines had *Sr26* in our panel of accessions except the original source lines, confirming that this gene has not been widely deployed in breeding programs. The same is true for *Sr40*. Only recently, *Sr40* and other rarely used genes including *Sr22*, *Sr35*, *Sr39*, and *Sr44* have been confirmed to be resistant to Ug99 (Jin et al. 2007). Their value has increased dramatically for pyramiding multiple genes into single cultivars to achieve durable rust resistance to Ug99.

Optimization of markers for marker-assisted selection is a major bottleneck regardless of marker type or platform. Markers can fail to predict the presence of a gene for a variety of reasons including 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. The use of multiple markers for haplotyping provided information in relevant germplasm on dominance, frequency of amplification, allelic distribution, and polymorphism. Association analysis combined with pedigree and rust race reaction can provide evidence for recombination; however, follow-up linkage and complementation tests are required for confirmation. Diagnostic markers are critical for molecular breeding strategies such as marker-assisted selection (MAS), but developing such markers is often difficult and time-consuming. "Perfect markers", defined as markers that detect the functional mutation in the desired allele, are the gold standard, but are not yet available for any of the wheat stem rust resistance genes. False positives are a common problem in MAS. The variety Thatcher does not carry *Sr2* but was positive for the unpublished marker in this study. Additional markers linked to the *Sr2* gene were useful for distinguishing the false positives such as Thatcher. Moreover, using multiple markers, two additional *Sr2* haplotypes were identified in the *Sr2* positive lines. This information is valuable for the *Sr2* MAS efforts in the CIMMYT breeding program.

The use of DArT markers for whole-genome genotyping can benefit molecular breeding programs in three areas. First, it assays the whole genome and provides opportunity for identifying new markers linked to known major genes. For instance, in the present study, we identified five DArT markers associated with *Sr2* and *Sr25* by association analysis. These markers could be used for developing primers for new markers or in place of the previous ones. Second, when combined with phenotypic data it facilitates the discovery of new resistance loci. Using the DArT genotyping and phenotyping of a subset of CIMMYT lines, we identified several loci linked to known major stem rust resistance genes as well as putative new genes by association mapping (Yu et al. in preparation). Third, prediction models can be developed for implementing the genomic selection approach (Heffner et al. 2009). This could be a more efficient approach for traits controlled by many loci than a gene-by-gene approach. Genome-wide markers provide a more complete picture of the genetic variation in the breeding program and could be useful in choosing parental lines for stem rust resistance breeding. Further investigation of the putative new stem rust resistance loci is required to determine if they are useful in wheat breeding. As the cost of marker platforms continues to decline, the genome-wide approach to molecular breeding will become the methodology of choice.

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