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An accurate DNA marker assay for stem rust resistance gene *Sr2* in wheat

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Abstract The stem rust resistance gene *Sr2* has provided broad-spectrum protection against stem rust (*Puccinia graminis* Pers. f. sp. *tritici*) since its wide spread deployment in wheat from the 1940s. Because *Sr2* confers partial resistance which is difficult to select under field conditions, a DNA marker is desirable that accurately predicts *Sr2* in diverse wheat germplasm. Using DNA sequence derived from the vicinity of the *Sr2* locus, we developed a cleaved amplified polymorphic sequence (CAPS) marker that is associated with the presence or absence of the gene in 115 of 122 (95%) diverse wheat lines. The marker genotype predicted the absence of the gene in 100% of lines which were considered to lack *Sr2*. Discrepancies were observed in lines that were predicted to carry *Sr2* but failed to show the CAPS marker. Given the high level of accuracy observed, the marker provides breeders with a selection tool for one of the most important disease resistance genes of wheat.

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Introduction

The resistance gene *Sr2* has been effective against wheat stem rust (*Puccinia graminis* Pers. f. sp. *tritici*) since its transfer from tetraploid emmer wheat (*Triticum dicoccum* Schronk) into the susceptible bread wheat (*T. aestivum* L.) 'Marquis' in the 1920s (McFadden 1930). From the interspecific cross, a hexaploid line with good agronomic type and good levels of field stem rust resistance was released in 1925 as the variety 'Hope' that was used widely as a donor for stem rust resistance in North American and CIMMYT wheat breeding programs (Borlaug 1968). Since then, wheat cultivars with *Sr2* have been grown for many decades and in many regions of the world. The gene is effective only in the adult plant stage against all known pathotypes of stem rust including the recently described Ug99 race which has virulence to many important resistance genes (Pretorius et al. 2000). However, under heavy disease pressure *Sr2* provides insufficient protection when deployed on its own due to its partial resistance and a compatible infection type. The moderate resistance response and recessive gene action makes field selection of *Sr2* by breeders more difficult. Pseudo black chaff, an associated trait which causes a variable and genotype dependant pigmentation of stems and/or glumes, can assist in the selection process (McFadden 1939; Hare and McIntosh 1979; Kota et al. 2006).

An accurate and robust marker for *Sr2* would benefit the wheat breeding community worldwide, because *Sr2* is an important resistance gene which is difficult to select. At least three previous publications reported markers linked to

Sr2: Spielmeyer et al. (2003) described tight linkage of a SSR marker *gwm533* with *Sr2* (approx. 2 cM) and showed that a 120 bp product was associated with the presence of the gene in most lines tested. However, in some susceptible Australian wheats that were predicted not to carry the gene, the marker amplified a 120 bp product. Hayden et al. (2004) improved the marker by utilizing sequence variation within the amplicon of *gwm533* to develop additional markers which predicted the presence of *Sr2* more accurately. McNeil et al. (2008) developed several markers from a BAC contig of chromosome 3B of variety 'Chinese Spring' that were closer to the *Sr2* region than *gwm533* but these markers amplified multiple bands and were difficult to implement in breeding programs. Consequently there is still a need for a reliable marker for *Sr2* that is tightly linked, easy to interpret and informative across a wide range of genotypes.

As part of a larger project aimed at isolating *Sr2*, we compared physical maps in the *Sr2* region from Chinese Spring and resistant variety Hope and exploited sequence variation for the development of a cleaved amplified polymorphic sequence (CAPS) marker. This marker was tested in diverse wheat backgrounds to develop an accurate assay for *Sr2*.

Materials and methods

Plant material

A previously described high-resolution mapping family (Kota et al. 2006) was derived from a cross between the susceptible Chinese Spring (CS) and the resistant chromosome substitution line CS (Hope 3B). This population was used for mapping of *Sr2* and the newly developed marker *csSr2*.

For validating the *csSr2* marker, Australian and international wheat lines were obtained from Australian Winter Cereals Collection (AWCC), Tamworth. The classification of wheat lines with respect to *Sr2* was based on the Wheat Rust Atlas (McIntosh et al. 1995) and discussions with breeders and rust pathologists who previously assessed the level of field resistance and the incidence of pseudo black chaff on lines tested in this study. Two seed sources of Yarolav emmer were obtained from AWCC (AUS3744) and the United States Department of Agriculture-Agriculture Research Service (USDA-ARS), National Small Grains Collection (PI2789). Additional 24 emmer wheats were obtained from AWCC. Seed of North American wheat lines were obtained from the USDA-ARS National Small Grains Collection. In addition, 36 North American wheat lines were evaluated that are parents of mapping populations developed as part of the Wheat Applied Genomics Coordinated Agricultural Project (Wheat CAP) and represent all the wheat market classes in the USA.

Development of the csSr2 marker

As part of the positional cloning of *Sr2*, we have developed a partial BAC contig of the *Sr2* locus from resistant line Hope (data not shown). This contig was sequenced and primers were designed to amplify a chromosome 3B specific product from CS (Hope 3B). The 3B specific marker was also amplified from the resistant donor Yaroslav emmer and susceptible Marquis. Products from emmer, CS (Hope 3B) and Marquis were sequenced and a 337 bp amplicon in Yaroslav emmer and CS (Hope 3B) contained a single nucleotide polymorphism (SNP) relative to the Marquis sequence (Fig. 1). The SNP was associated with the loss of a *Bsp*HI restriction site in Marquis and was used to develop a CAPS marker named *csSr2*. Amplification and subsequent digestion of the 337 bp product resulted in three fragments (172, 112 and 53 bp) from CS (Hope 3B) and Yaroslav emmer and two fragments (225 and 112 bp) from Marquis (Fig. 2).

DNA extraction, PCR and CAPS analysis

DNA was extracted directly from seeds or leaf tissue using the NucleoSpin Plant II purification kit (Macherey–Nagel, Germany) or the plate extraction protocol described in Mago et al. (2005). PCR reactions were performed in 20 µl volumes with 2–4 µl (100 ng) DNA template, 0.2 mM dNTP, 10 pmol of each primer, 1.5 mM $MgCl_2$, 1× GoTaq Flexi buffer and 0.5 U GoTaq Flexi Taq polymerase (Promega). The primer sequences and PCR conditions are shown in Table 1. For CAPS analysis an additional 5 µl of mix consisting of 2.5 ml of 10× NEB buffer 4 and 0.5 µl of *Bsp*HI (10 U/µl; NEB) was added once the PCR was completed and the tubes were incubated at 37°C for 1 h.

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                                                *
Marquis           GCACAAGCTCTAATTTCTTTGGAATCGTGAGTGTTGGATGTCTCGCACACCTAGCTTAAT
Fultz(landrace)   GCACAAGCTCTAATTTCTTTGGAATCGTGAGTGTTGGATGTCTCGCACACCTAGCTTAAT
Hope              GCACAAGCTCTAATTTCTTTGGAATCATGAGTGTTGGATGTCTCGCACACCTAGCTTAAT
Yarolav Emmer     GCACAAGCTCTAATTTCTTTGGAATCATGAGTGTTGGATGTCTCGCACACCTAGCTTAAT
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Fig. 1 Multiple sequence alignment showing the SNP between *Sr2* resistant lines Hope and Yaroslav emmer and susceptible lines Marquis and Fultz. The position of SNP is shown by *asterisk* and *underlined* sequence shows the *Bsp*HI restriction site

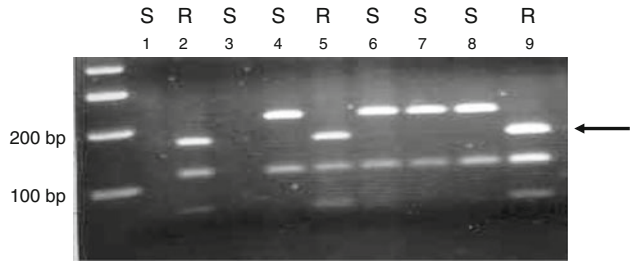

Fig. 2 CAPS marker *csSr2* tested on diverse wheats and run on a agarose gel. Lanes (*1*) Chinese Spring; (*2*) Hope; (*3*) Chinese Spring 3BS deletion line (FLM 0.86); (*4*) Marquis; (*5*) Redman; (*6*) Kenya Plume; (*7*) Thatcher; (*8*) Purplestraw; (*9*) Yaroslav emmer. The *arrow* shows the fragment which is associated with *Sr2*. The rust phenotype is represented as *R* (resistant) and *S* (susceptible)

The CAPS product was separated on a 2.5% (w/v) agarose gel. DNA was extracted from two seeds of each wheat line separately and used for PCR analysis. For wheat varieties that were previously classified, *Sr2* resistant but which lacked the Hope-type marker allele, an additional 8–10 seeds were extracted separately for subsequent marker analysis. These lines included 'Arthur71', 'Derrimut', 'Ellison', 'Kenya Plume', 'Kukri', 'Leichardt', and 'Siete-Cerros'.

Stem rust testing of wheat CAP lines

The 36 US wheat CAP lines that are parents of mapping populations were grown as rows in the field in Njoro, Kenya in 2008. To ensure heavy inoculum build up of stem rust, spreader rows were planted and artificially inoculated with stem rust collected from experimental plots the previous year. Stem rust severity was assessed following the modified Cobb scale (Peterson et al. 1948). Lines were also tested at seedling stage in the greenhouse in St. Paul, Minnesota using Ug99 race collected in Kenya (see Njau et al. 2010 for detailed description of screening protocol).

Results

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. When the marker was tested in a wide range of germplasm, it amplified a 120 bp product from most lines carrying *Sr2* (Spielmeyer et al. 2003). There were, however, notable exceptions, particularly cultivars from South and West Australian breeding programs carried the 120 bp allele that were susceptible to stem rust. In this study, we expanded the marker survey to a larger set of international lines and found that the 120 bp allele also occurred in many North American and CIMMYT lines which were considered not to have *Sr2* and/or lack field stem rust resistance (Table 2). These findings raised doubts about the utility of *gwm533* as a breeder-friendly marker. We therefore developed a new marker (*csSr2*) based on a SNP which was tightly linked and could distinguish *Sr2* carrying lines with a Hope allele from non-*Sr2* germplasm. The *csSr2* marker detected three alleles: (1) 'null' allele (lack of amplification), (2) Marquis type allele which lacked the *Bsp*HI restriction site and (3) Hope type allele with the *Bsp*HI restriction site.

To evaluate the usefulness of the marker for breeding, it was tested in diverse wheat germplasm from around the world (Table 2). In Australian cultivars, all non-*Sr2* lines carried the 'null' allele except for 'Bindawarra' which had the Marquis type allele. The marker confirmed the presence of the gene in most Australian cultivars that were considered to carry *Sr2* with a few exceptions such as Derrimut, Ellison, Kukri and Leichardt (Bariana pers comm.). Amongst 13 CIMMYT lines, the marker produced two unexpected results. The variety Siete Cerros released in 1966 and the recent variety 'Pastor' were expected to carry *Sr2* but lacked the characteristic SNP from Hope. We also tested a diverse set of Canadian and American germplasm. The marker failed to predict *Sr2* in Arthur 71, although this old American variety is reported to carry *Sr2* in the Wheat Rust Atlas (McIntosh et al. 1995). The *Sr2* marker was present in the parental line Arthur, from which Arthur 71 was derived. Three American landraces ('Fultz', 'Purple-straw' and 'Trumbull') which were used in early wheat breeding before the release of Hope carried alleles associated with the lack of *Sr2*. Among other international lines tested, Kenya Plume unexpectedly failed to carry the allele associated with *Sr2*, but the sib line Kenya Page contained the Hope-type allele. Both Kenya Plume and Kenya Page

Table 1 PCR primers and conditions for the amplification of the *csSr2* marker

Primers	PCR conditions
F-5′ CAAGGGTTGCTAGGATTGGAAAAC	95°C 2 min 1 cycle
R-5′ AGATAACTCTTATGATCTTACATTTTTCTG	95°C 30 s 30 cycles
	60°C 40 s
	72°C 50 s
	72°C 5 min 1 cycle
	15°C 1 min

Table 2 Allele survey of diverse wheat germplasm with known (or presumed) *Sr2* status using SSR marker *gwm533* and CAPS marker *csSr2*

Country	Wheat line	*Sr2* resistance	*gwm533*	*csSr2*	*Bsp*HI SNP
Australia					
	Angas	–	120	Null	
	Aroona	–	120	Null	
	Arrino	–	155	Null	
	Babbler	–	Null	Null	
	Banks	–	Null	Null	
	Bindawarra	–	120	+	–
	Brookton	–	155	Null	
	Braewood	–	120	Null	
	Cadoux	–	Null	Null	
	Camm	–	Null	Null	
	Chara	–	Null	Null	
	Cocomba	–	Null	Null	
	Condor	–	Null	Null	
	Cook	–	Null	Null	
	Currawong	–	Null	Null	
	Dagger	–	155	Null	
	Datatine	–	No data	Null	
	Ega-hume	–	Null	Null	
	Ega-Jaegar	–	Null	Null	
	Federation	–	155	Null	
	Frame	–	155	Null	
	Gabo	–	120	Null	
	Gladius	–	120	Null	
	Grebe	–	Null	Null	
	Gutha	–	Null	Null	
	H45	–	Null	Null	
	Halberd	–	155	Null	
	Janz	–	Null	Null	
	Katunga	–	155	Null	
	Kelalac	–	Null	Null	
	Kite	–	Null	Null	
	Krichauff	–	120	Null	
	Meering	–	Null	Null	
	Mitre	–	Null	Null	
	Molineux	–	120	Null	
	Oxley	–	Null	Null	
	Perouse	–	Null	Null	
	Qual2000	–	No data	Null	
	Quarrion	–	Null	Null	
	Rosella	–	Null	Null	
	Spear	–	155	Null	
	Stiletto	–	155	Null	
	Sunland	–	Null	Null	
	Sunvale	–	Null	Null	
	Swift	–	Null	Null	

Table 2 continued

Country	Wheat line	*Sr2* resistance	*gwm533*	*csSr2*	*Bsp*HI SNP
	Tasman	−	120	Null	
	Tincurrin	−	No data	Null	
	Trident	−	155	Null	
	Westonia	−	120	Null	
	Wyalkatchem	−	120	Null	
	Yaralinka	−	120	Null	
	Yitpi	−	155	Null	
	Baxter	+	120	+	+
	Brennan	+	120	+	+
	Carnamah	+	120	+	+
	Derrimut	+	120	+	−
	Diamondbird	+	120	+	+
	Dollarbird	+	120	+	+
	Drysdale	+	120	+	+
	Ega-Wiley	+	120	+	+
	Ellison	+	120	+	−
	Eradu	+	120	+	+
	Hartog	+	120	+	+
	Houtman	+	120	+	+
	Kukri	+	120 + 155	+	−
	Leichardt	+	120	Null	
	Crusader	+	120	+	+
	Lowan	+	120	+	+
	Machete	+	120	+	+
	Pelsart	+	120	+	+
	Rowan	+	120	+	+
	Sunbrook	+	120	+	+
	Suneca	+	120	+	+
	Sunstate	+	120	+	+
	Sunzell	+	120	+	+
	Timgalen	+	120	+	+
	Cranbrook	+	120	+	+
	Ventura	+	120	+	+
North America					
Canada					
	Glenlea	−	120	Null	
	Marquis	−	120	+	−
	RL-6058	−	120	+	−
	RL-6071	−	120	+	−
	Thatcher	−	120	+	−
	Lancer	+	120	+	+
	Pembina	+	120	+	+
	Redman	+	120	+	+
	Renown	+	120	+	+
	Selkirk	+	120	+	+

Table 2 continued

Country	Wheat line	*Sr2* resistance	*gwm533*	*csSr2*	*Bsp*HI SNP
USA					
	Langdon	−	Null	Null	
	Abe	−	Null	+	−
	Arthur	+	No data	+	+
	Arthur71	+	120	+	−
	Yaroslav emmer	+	120	+	+
	Hope	+	120	+	+
	Oasis	+	120	+	+
	Ottawa	+	120	+	+
	Scout	+	120	+	+
	Sullivan	+	120	+	+
Land Races					
	Fultz	−	120	+	−
	Purplestraw	−	120	+	−
	Trumbull	−	Null	+	−
CIMMYT					
	Anza	−	Null	Null	
	Avocet	−	155	Null	
	Bluebird	+	No data	+	+
	Ciano67	+	120	+	+
	Kingbird	+	No data	+	+
	Kiritati	+	No data	+	+
	Lerma Rojo	+	120	+	+
	Nuri70	+	120	+	+
	Opata	−	120	+	−
	Parula	+	120	+	+
	Pastor	+	No data	+	−
	Pavon76	+	120	+	+
	Siete-Cerros	+	120	+	−
Others					
Brazil	Frontana	+	No data	+	+
Chile	Forno	−	120	+	−
China	Chinese Spring	−	155	Null	
India	**Sonalika**	+	120	+	+
Kenya	Kenya Page	+	120	+	+
	Kenya Plume	+	120	+	−
UK	**Excalibur**	−	155	Null	

Wheat lines in bold were tested previously for *gwm533* (Spielmeyer et al. 2003; Hayden et al. 2004)

were considered to carry *Sr2*. We also evaluated the marker on 36 parents of mapping populations developed by the Wheat Coordinated Agricultural Project which represent germplasm adapted to most climatic zones of the United States (Table 3). These mapping parents also included a few lines developed by CIMMYT. Because the *Sr2* status of the lines was uncertain, only those that lacked effective race-specific stem rust resistance were included in this study. Stem rust reaction of the lines ranged from moderately susceptible (30MS) to fully susceptible (90S) in the field in Kenya. The marker predicted the presence of *Sr2* in moderately susceptible lines 'Louise' and 'Panawawa', both of which have Hope in their pedigree. The remaining lines carried a null allele or amplified the Marquis type allele, including those with moderate levels of stem rust resistance such as 'Eltan' and 'Foster'. Lack of the Hope allele in these moderately resistant cultivars suggests that they may possess other genes conferring APR to stem rust.

Table 3 Allele survey of North American wheat lines of unknown *Sr2* status using SSR marker *gwm533* and CAPS marker *csSr2*

Wheat line	Stem rust reaction[a]	*gwm533*	*csSr2*	*Bsp*HI SNP
UC1110	90S	143	Null	
CIMMYT-2 PI 610750	60MSS	120	Null	
IDO444	40S	Null	+	−
IDO556	70S	120	+	−
Zak	40S	120	+	−
Mc Neal	60S	120	Null	
Thatcher	40MS	120	+	−
Stephens	60SMS	155	+	−
OR9900553	60SMS	Null	+	−
Louise	50MSS	120	+	+
Panawawa	60MSS	120	+	+
Finch	80S	155	+	−
Eltan	20S	155	+	−
GRN*5/ND614-A	30MRMS	120	Null	
NY18/CC 40-1	30MSMR	120	+	−
Reeder/Bw-277 "R"	40S	120	Null	
Reeder/Bw-277 "S"	40MSS	146	Null	
CO940610	60MSMR	144	Null	
TAM 105	50MRMS	120	+	−
Heyne	80S	Null	Null	
Wesley	30MRMS	Null	Null	
Jagger	70S	Null	Null	
2174	5S	120	+	−
Weebill	70MSS	120	+	−
JupetecoS	90S	120	+	−
26R46	50S	120	+	−
P92201	70SMS	146	Null	
Cayuga	0	120	Null	
Caledonia	80S	120	Null	
25R26	60S	Null	+	−
Foster	30S	Null	+	−
RSI5 Source 97039	90S	No data	+	−
Express	60S	120	+	−
26R61	80S	120	+	−
Kanqueen	60MSS	Null	+	−
Clark's Cream	60MS	Null	+	−

[a] Level of stem rust resistance was assessed in the field in Kenya in 2008

The *csSr2* marker was also tested on a diverse set of CIMMYT lines that were grown in a stem rust resistance screening nursery in Njoro, Kenya in 2007. Lines and screening protocol were described in detail by Njau et al. (2010). According to Njau et al. (2010) a subset of 22 lines lacked effective race-specific resistance and was scored from moderately susceptible (40MSS) to highly resistant (5MS) in the field. The marker predicted the presence of *Sr2* in 13 lines that expressed some level of adult plant resistance (Table 4). In three moderately susceptible lines (40MSS–30MSS) and six resistant/moderately resistant lines (5S–30M) the marker predicted the absence of *Sr2*. It is possible that lines with APR but lacking the Hope marker allele possess other useful sources of stem rust resistance. In summary, the new CAPS marker *csSr2* is associated with the presence of *Sr2* in most genetic backgrounds that were tested in this study. In a small number of lines, the marker did not coincide with the presumed *Sr2* genotype.

Analysis of emmer wheats

McFadden (1930) reported the transfer of *Sr2* from Yaroslav emmer into hexaploid wheat Marquis. We tested two genebank accessions of Yaroslav emmer from the USDA and Australian collections with the *csSr2* marker and confirmed that both accessions carried the Hope type allele. The marker was null or Marquis type in an additional 23 emmer wheats that were tested in this study with the exception of the Italian emmer cultivar 'Villoso Americano' which amplified a Hope type allele (Table 5). The *Sr2* status of this emmer wheat remains uncertain.

Discussion

Although the *Sr2* gene has played an important role in conferring durable resistance to stem rust worldwide, the gene is difficult to select in breeding programs. The partial resistance phenotype can be difficult to score in the field and is often masked by other effective resistance genes in the background. Breeders often rely on the expression of pseudo-black chaff to transfer the gene, but this trait is also an unreliable indicator due to variable levels of pigmentation observed in different *Sr2* carrying wheats. The durable *Sr2* gene is, therefore, an ideal candidate for deploying a breeder-friendly marker that will remove some of the contingencies and permit more accurate selection in breeding.

A useful marker for *Sr2* must fulfil at least three criteria: (1) The marker must be tightly linked to the resistance gene which minimises the risk of recombination separating the marker from the gene. (2) The marker allele that is associated with the donor line Hope must be rare or absent in non-*Sr2* germplasm. (3) The marker must be easy to assay using PCR.

Our newly developed *csSr2* marker offers significant advantages over the SSR marker *gwm533*. Firstly, no recombinant between *csSr2* and the gene was identified in 3,000 gametes derived from the cross between Chinese

Table 4 Allele survey of CIMMYT lines from rust screening nursery Njoro (Kenya) in 2007 with the *csSr2* marker

No.	Cross	Sr Njoro 2007	Level of APR	*csSr2*	*Bsp*HI SNP
6032	BABAX/3/OASIS/SKAUZ//4*BCN/4/PASTOR	10MSS	APR	+	–
6033	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ/6/FRET2	30MSS	APR	+	+
6035	DOLLARBIRD	20M	APR	+	+
6036	FRET2*2/KUKUNA	30M	APR	+	–
6038	HE1/3*CNO79//2*SERI/3/ATTILA/4/WH 542	40MSS	Mod Sus	+	–
6039	HPO/TAN//VEE/3/2*PGO/4/MILAN/5/SSERI1	10MSS	APR	+	+
6041	KAMB1*2/KHVAKI	10MSS	APR	+	+
6042	KIRITATI	10MS	APR	+	+
6044	PASTOR/MILAN	5S	APR	Null	
6045	PAVON F 76	5M	APR	+	+
6046	PFAU/WEAVER*2//KIRITATI	5MS	APR	+	+
6050	PFAU/WEAVER*2//KIRITATI	10MSS	APR	+	+
6051	PFAU/WEAVER//KIRITATI	5MSS	APR	+	+
6054	PFAU/WEAVER//KIRITATI	40MSS	Mod Sus	+	–
6055	PGO//CROC_1/AE.SQUARROSA (224)/3/2*BORL95/4/CIRCUS	20MSS	APR	+	+
6057	PGO/SERI//BAV92	10MSS	APR	+	+
6058	PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1	10MS	APR	Null	
6059	KINGBIRD (=TAM200/TUI/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1)	5MSS	APR	+	+
6062	TEMPORALERA M 87*2/4/HD2281/TRAP#1/3/KAUZ*2/TRAP//KAUZ	30MSS	APR	+	–
6063	WL6736/5/2*BR12*3/4/IAS55*4/CI14123/3/IAS55*4/EG,AUS//IAS55*4/ALD/6/OASIS/5*BORL95/7/BORL95	10MSS	APR	+	+
6064	CHIL/CHUM18/4/BUC/BJY/3/CNDR/ANA//CNDR/MUS	30M	Mod Sus	+	–
6104	JUCHI F2000	30M	APR	+	–

Spring and the Chinese Spring 3B substitution line placing the marker much closer to the *Sr2* gene than *gwm533* (Kota et al. 2006; data not shown). Secondly, the new marker was more accurate in lines known (or presumed) to carry the resistance gene. The *csSr2* marker was able to predict the presence or absence of the gene in 95% of lines with presumed *Sr2* status, whereas the accuracy of *gwm533* was only 84%. Amongst presumed non-*Sr2* wheats, *csSr2* marker was null or amplified the Marquis type allele, whereas *gwm533* detected the Hope like allele in several susceptible, non-*Sr2* wheats (Table 2). No comparison was made with the sequence-tagged microsatellite marker developed by Hayden et al. (2004) as this marker amplified several alleles which require PCR products to be separated by capillary electrophoresis.

The frequency of the null allele in Australian, non-*Sr2* germplasm was much higher than in North American germplasm. The *csSr2* marker can distinguish the heterozygote susceptible from homozygote resistant genotypes when used in backgrounds that carry the Marquis type allele, but is dominant in germplasm that is null at the marker locus. We conclude that the *csSr2* marker can be used by breeders to transfer the gene from donor lines to adapted, non-*Sr2* wheats.

But is the marker also useful to accurately predict the gene in unknown germplasm? The answer depends on how the few cases are interpreted where the marker genotype fail to match the presumed *Sr2* phenotype. It is possible that some lines presumed to carry *Sr2*, were wrongly classified and lack the gene. These lines were then correctly identified as such by the marker. For example, it is unknown how Siete Cerros was classified as carrying *Sr2*, according to R. Singh (unpublished data) this line does not express pseudo black chaff and a report linking Siete Cerros with *Sr2* was speculative and failed to present strong evidence for its presence (Mishra 1992). It is also not uncommon that wheat varieties released by breeders consist of mixtures and are heterogenous for important traits such as disease resistance or seed storage proteins (Lawrence 1986). In this study different *gwm533* marker alleles were amplified from Kukri, it is possible that Kukri is also mixed for *Sr2* and that another seed source may carry the *csSr2* Hope allele. Alternatively, the SNP associated with resistance may have mutated or recombined

Table 5 Allele survey of diverse emmer wheats with CAPS marker *csSr2*

AUS	Name	Origin	Stem rust resistance[a]	*csSr2*	*Bsp*HI
3511	Villoso Americano no. 380	ITA	MR	+	+
3728	Vernal Emmer	?	R	Null	
3741	Emmer	?	R	Null	
3743	Emmer	?	R	Null	
3745	Emmer	?	R	Null	
3746	Emmer 1	?	S	Null	
3748	Emmer 6	?	HS	Null	
3749	Emmer	?	R	Null	
6360	ECH 969	AUS	R	Null	
10731	Emmer W11	?	R	Null	
11436	Emmer	?	R	Null	
15520	Valki 74	CZE	R	Null	
17349	Kajanus Landskrona	SWE	HS	Null	
17384	Black Winter	USA	HS	Null	
18175	Bari 7360	ETH	R	+	−
19385	T. dicoccum-Asiaminor	IRN	R	Null	
21048	T. dicoccon 8	FRA	R	Null	
21758	T. dicoccon var Rufum	DEU	MR	Null	
27537	KU 1058	ESP	S	Null	
28781	Ukraine W94614	UKR	R	Null	
28791	Bulgaria W94659	BGR	R	Null	
28792	Morocco W74633	MAR	MR	Null	
95052	Emmer	?	MR	Null	
2657	Iumillo	?	R[b]	Null	
3744	Yaroslav Emmer	RUS	R	+	+
PI2789	Yaroslav Emmer	RUS	R	+	+

R resistant, *S* susceptible, *MR* moderately resistant, *HS* highly susceptible

[a] Level of stem rust resistance assessed in the field at Plant Breeding Institute, Cobbitty, Australia in 2009

[b] Field stem rust resistance previously evaluated

with the gene and is, therefore, in some lines not diagnostic for *Sr2*. For example, the marker failed to predict the presence of *Sr2* in Pastor, although this line expressed PBC under field conditions (R. Singh unpublished data) suggesting that in this line PBC was either separated from *Sr2* or that the marker was not diagnostic. Future genetic studies of populations derived from Pastor and other lines where the marker failed to predict the presence of the gene, could clarify if the marker lacked accuracy or if wheat lines were wrongly classified.

One reason for isolating the *Sr2* gene is to develop a gene-derived marker that will further improve the selection accuracy. Recently, gene-derived markers were developed from the durable leaf and stripe rust resistance gene *Lr34/Yr18* (Lagudah et al. 2009). Although these markers were more accurate than previously published linked markers, there were still a few examples where the marker predicted the presence of *Lr34/Yr18* in susceptible wheats (Lagudah et al. 2009). Determining the DNA sequence of the *Sr2* gene may, therefore, not necessarily deliver a 'perfect' marker but is expected to enhance the accuracy even further by which *Sr2* can be transferred into future wheat cultivars.

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