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Resistance to *Wheat streak mosaic virus* – a survey of resources and development of molecular markers

M. Fahim[abc], A. Mechanicos[a], L. Ayala-Navarrete[a], S. Haber[d] and P. J. Larkin[a]*

[a]*CSIRO Plant Industry, P.O. Box 1600 Canberra, ACT 2601, Australia;* [b]*Department of Microbiology, Hazara University, Mansehra, KPK, Pakistan;* [c]*Division of Plant Sciences, Research School of Biology, Australian National University, Canberra, ACT, Australia; and* [d]*Cereal Research Centre, Agriculture & Agri-Food Canada, Winnipeg, MB R3T 2M9, Canada*

Wheat streak mosaic virus (WSMV) has been newly documented in Australia. The vulnerability of contemporary Australian elite wheat germplasm prompted a survey for effective resistance against an Australian isolate, WSMV-ACT. This study confirms the effectiveness of previously reported sources of resistance and shows that new sources of resistance also confer protection. The resistance derived from *Thinopyrum intermedium* (*Wsm1*) as a 4D translocation and a new 4A translocation, and two bread wheat resistances, *Wsm2* and the new source c2652, were effective against WSMV-ACT in glasshouse experiments. *Wsm1* was effective at lower temperatures but ineffective above 20°C, a temperature sensitivity shared with many of the derivatives of *Wsm2* except for one new selection which was effective at 26°C. True wheats c2652 and *Wsm2* selection CA745, and amphiploids Zhong1, Zhong2, Zhong4, Zhong5, TAF46, Summer1, Ot38 and OK7211542 were uniformly resistant at 20, 25 and 28°C. New sources of resistance were identified in a *Th. scirpeum*-wheat amphiploid, B84-994, and in chromosome addition lines Z2, Z6 and TAi27, derived from wheat-*Th. intermedium* partial amphiploids. Several new, tightly linked SSR, RAPD and EST-ILP PCR markers were developed for tracking the various *Th. intermedium* translocations associated with *Wsm1*, including the smaller translocations on wheat chromosome 4AS and 4DS. Three markers for the 4A-*Wsm1* translocation were validated on a segregating breeding population.

Keywords: disease resistance, molecular markers, temperature sensitive resistance, *Tritimovirus*, wheat diseases, *Wheat streak mosaic virus*

Introduction

Wheat streak mosaic virus (WSMV: *Tritimovirus, Potyviridae*) is transmitted by the wheat curl mite (WCM) *Aceria tosichella* (Slykhuis, 1955). The presence of the virus was first confirmed in Australia in 2002. Evidence from molecular characterization and demonstration of seed transmission suggest WSMV was accidentally introduced to Australia in the 1980s (Ellis *et al.*, 2003; Dwyer *et al.*, 2007). The virus causes sporadic epidemics in the Great Plains of North America and is of great economic importance, causing severe losses in some years. In Australia, the virus has to-date remained a moderate threat to wheat production, although it has spread rapidly to all the wheat producing states. Both the virus and the leaf curl mite vector are present throughout the Australian wheatbelt. Where environmental conditions have favoured survival over summer and early infestation of crops by viruliferous mites, substantial losses have been observed. Should the combination of management practices and environmental conditions that favour the vector's transmission of the virus become widespread, major industry losses are possible.

Since wheat streak mosaic (WSM) disease was first reported (Mckinney, 1937), efforts have been made to find sources of resistance in cultivated wheat. Sources of resistance or tolerance in true wheat were slow to emerge, prompting efforts to explore resistance in perennial *Triticeae* relatives such as *Thinopyrum intermedium* ($2n = 6x = 42$, JJsS) and *Th. ponticum* ($2n = 10x = 70$, JJJJsJs) (Friebe *et al.*, 1993; Chen *et al.*, 2003). These two species have been valuable sources of resistance and tolerance to various biotic (both against WSMV and its vector WCM) and abiotic stresses and are relatively easy to cross with common and durum wheat (Larson & Atkinson, 1970).

The resistance called *Wsm1* was transferred to wheat initially as Robertsonian translocations 4Ai#2S.4AL and 4Ai#2S.4DL (Chen *et al.*, 1998a,b) and attempts were made to breed with it and deploy it as WSMV resistant varieties (Friebe *et al.*, 1996a; Baley *et al.*, 2001; Sharp *et al.*, 2002; Divis *et al.*, 2006). However, the initial linkage drag associated with *Wsm1* in the absence of WSMV infection affected the deployment of this resistance into the field (Friebe *et al.*, 1996b; Sharp *et al.*, 2002). To assist

*E-mail: philip.larkin@csiro.au

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the development of useful recombinants and crossing of *Wsm1* into elite wheat germplasm, Talbert *et al.* (1996) developed a sequence-tagged site (STS) marker (STS-J15). Backcrossing and selection eventually succeeded in producing lines with *Wsm1* that did not appear to suffer yield penalties. This success, possibly due to recombination between 4DS and the alien chromatin (Divis *et al.*, 2006; Friebe *et al.*, 2009), has led to the release of the cultivar Mace (Graybosch *et al.*, 2009). The development of recombinant shortened *Wsm1* translocations and *Wsm1* translocations on 4A (Haber *et al.*, 2007; Qi *et al.*, 2007) has required new molecular markers to be developed.

More recently, there have been a small number of reports of resistance in conventional wheat lines, not involving alien translocations, such as Hume derived from the cultivar McKenzie (Haber *et al.*, 2011), c2652 (our unpublished data) and CO960293-2 (Haley *et al.*, 2002; Seifers *et al.*, 2006; Lu *et al.*, 2011b). The resistance in CO960293-2 (now called *Wsm2*) has been introgressed into two cultivars, RonL (Seifers *et al.*, 2007) and Snowmass (Haley *et al.*, 2011). With their deployment in cultivars, the few available resistances may be compromised or broken by the selective pressure on the virus, hence the need to find new resistances and deploy them in stacks.

This paper reports the effectiveness of known and new genetic resistances against the Australian isolate of WSMV and the stability of the resistances to increased growth temperatures. It also reports the discovery of new sources of genetic resistance in amphiploids and addition lines and the development of new, more closely-linked, molecular markers for *Wsm1* that should be useful in further refinement of *Wsm1* translocations, marker-assisted breeding, and resistance gene stacking.

Materials and methods

Plant material

Wheat and other cereals

Screening for resistance to WSMV-ACT isolate was carried out on the following: a collection of 53 wheats without reported resistance to WSMV; a collection of wheats with reported resistance including a number of accessions of these sources in different backgrounds from Canada, Nebraska and Kansas (Table 1, Fig. 2); and 42 wheat land races collected in the 1920s (Table 1, Fig. S1), kindly made available by Dr Harbans Bariana, University of Sydney. Bob White selection 26 (BW26) in particular was used as a susceptible control throughout this study.

Amphiploids and chromosome addition and substitution lines

The tertiary gene pool for wheat was also sampled for resistance by including a range of wheat-alien amphiploid hybrids, substitution and chromosome addition lines derived from some of them (Table 1).

Screening for resistance

Virus inoculum was prepared by grinding WSMV infected tissue (stored at −80°C) in 1:10 w/v ratio in 0·02 M potassium phosphate buffer (pH 7) in a Sorvall Omni Mixer (at 10 000 rpm). The virus inoculum used in all experiments was prepared from a WSMV infected wheat collected in Canberra (the Australian Capital Territory) and hereafter called the WSMV-ACT isolate. The homogenate was filtered through four layers of Miracloth® (Calbiochem), abrasive diatomaceous earth (celite; Johns-Manville) was added at 2% w/v to the final volume of inoculum, and the mixture was left on ice for 1 h. Germplasm was inoculated at the 2–3 leaf stage, with the prepared sap extracts from WSMV-infected leaf material. The sap plus celite abrasive was applied with an air-powered spray gun. A side mount gravity type Mini Spray Gun (Star S2F; Rich Star Precision Industrial) with adjustable 0·5–0·8 mm nozzle was used with a portable tank of compressed air (Fig. 1). Following spraying at 270 kPa, the leaves were also gently rubbed with gloved fingers.

In all experiments eight plants per genotype were used; six were inoculated and two were left uninoculated. The plants were scored for symptoms at 14 and 28 days post-inoculation (dpi) as described previously (Fahim *et al.*, 2010). Newly emerged leaf samples were collected at either 14 or 28 dpi for WSMV-specific ELISA using Agdia® reagents following manufacturer's instructions and extracted at a standard weight to extract ratio as detailed in Fahim *et al.* (2010). Plates were read at $A_{405\ nm}$ in ELISA Reader Spectra Max 340 PC (Molecular Devices) 60 min after addition of substrate. Every sample, inoculated and uninoculated healthy controls, had duplicate wells on the ELISA plate, and means were used in calculating the ELISA value ratio between inoculated and healthy controls.

Temperature sensitivity of resistance to the Australian isolate of WSMV

Lines that expressed resistance to WSMV in glasshouse experiments were evaluated for resistance against WSMV in controlled temperature growth cabinets. When screening for temperature sensitivity of the resistance, two sets of eight plants per genotype were used; one set in a cabinet at 18°C night, 20°C day temperature and the other set in a cabinet with 18°C night, 25 or 28°C day temperature. Each set of eight plants per genotype was grown in 10 cm pots and six of these were inoculated with WSMV at the three leaf stage; the remaining two plants were kept as uninoculated controls. Light conditions varied from 190 to 375 μE m^{-2} s^{-1} depending upon experiment (Table 2) and the day length was set at 16 h, with 8 h dark. After inoculation plants were rated for symptoms at 7, 14, 21 and 28 dpi, and the youngest fully expanded leaf was harvested from each plant for ELISA analysis as described above.

Table 1 Germplasm screened for resistance against WSMV-ACT. Eight wheat plants per genotype were grown in a glasshouse, six inoculated at the three-leaf stage with *Wheat streak mosaic virus* (WSMV) and two left as healthy controls. Each experiment included susceptible BW26 controls. Germplasm with resistance was subsequently assessed at controlled temperatures. Resistance (R) is recorded only if resistance was confirmed in subsequent testing at 20°C

Germplasm	Source	Identifiers/accession	Description	WSMV	References/source
Wheat	Wheat cultivars and breeding lines		Annuello, Barian, Bolac, Brennan, Bob White26, Camm, Chara, Corella, CSP44, Currowong, Declic, Diamond Bird, Drysdale, EGA Gregory, Frame, GBA Ruby, Giles, H45, HD2009, WC03.87, WC03.008, WC03.1010.3, Janz, Jinan 177, Karl, Kellalac, Kennedy, Lalbahadur, Mackellar, Marombi, Myna, Pugsley, Romany, Rosella, Rudd, Silver Star, Sunvale, Sunbri, Sunstate, Super Seri, Tennant, Ventura, Vilmorin, Vl404, Wedgetail, WL711, Wylah, Xiaoyan 54, Xiaoyan 6, Yitpi, Young, Z1-sib	S	
	Wheat land races	Figure S1	42 wheat land races collected during 1920s	S	Harbans Bariana (Sydney University)
	c2652	CA716, CPI146018	Spring wheat selected from breeding line C2652	R	Steve Haber (AAFC, Winnipeg)
		CA742, Haber9379, CPI146896	Advanced resistant derivative of CA716	R	
Wheat (contd)	CO960293-2 (*Wsm2*) various	CA744 Original	PI222668/TAM 107//(NOVI SAD 14/NOVI SAD 603//NEWTON/3/PROBRAND 835_ CO850034). PI222668 is ex- Azerbaijan	R	Haley *et al.* (2002) Seifers *et al.* (2006, 2007) Lu *et al.* (2011b)
		CA717, AUS34288, PI615160	As above but sourced ex- AWCC Tamworth, Australia	R	
		CA743, Haber7760, CPI146635	Winter wheat '4th cycle' WSMV selection seed, CO960293-1-1-3gen	R	
		CA745, Haber9104, CPI146894	Spring wheat, Superb2*/CO960293-2 (BC1F9)	R	
		CA833, RonL, CPI147321	Hard white winter wheat (awned) KS03HW158, selected from the cross Trego/CO960293-2	R	
	Wsm1 various	CA739, KS93WGRC27	Pedigree CI17884/4* KARL, *Wsm1* translocation (4Ai#-2S.4DL) from intermedium 4Ai-2, CI17884 also contained *Ae. speltoides* 7S substituting for 7A	R	Gill *et al.* (1995)
		CA740, KS95H10-3	*Wsm1* translocation (4Ai#2S.4DL); an improved selection of KS93WGRC27	R	Dallas Seifers, Kansas State University
		CA741, Haber9376, CPI146895	Pai Toborochi/CI15091 F_{12} derivative, where CI15092 is a disomic substitution line 4Ai#2 (4A). Selection for resistance and against an J15 marker. Likely *Wsm1* translocation on 4AS (T4Ai#2S-4AS.4AL)	R	Haber *et al.* (2007)
		CA 837, Rec213, KS09WRGC51	Shorter *Wsm1* recombinant translocation, derived from KS93WGRC27. 4Ai#2S-4DS.4DL (where only 18% of S arm is alien)	R	Friebe *et al.* (2009)
		CA832, Mace, CPI147322	Winter wheat with *Wsm1*, bred from CI17884	R	Graybosch *et al.* (2009)

Table 1 (Continued)

Germplasm	Source	Identifiers/accession	Description	WSMV	References/source
Wheat-alien amphiploids and partial amphiploids	*Th. intermedium* derived	Zhong1	2*n* = 56 wheat/*Th. intermedium*	R	Qi *et al.* (1979) Sun (1981) Chen *et al.* (2003)
		Zhong2	2*n* = 56 wheat/*Th. intermedium*	R	
		Zhong4	2*n* = 56 wheat/*Th. intermedium*	R	
		Zhong5	2*n* = 56 wheat/*Th. intermedium*	R	
		TAF46	2*n* = 56 wheat/*Th. intermedium*	R	Cauderon (1966)
		Summer 1, CPI119107, CA830	2*n* = 56 wheat/*Th. intermedium*	R	NE Normal University, Changchun, China
		Otrastajuscaja 38, Ot38, CPI114085, CA596,	2*n* = 56, wheat/*Th. intermedium*. Produced in 1920–30s. Released in Russia in 1978 as a grain and fodder plant	R	Berezhnoi (1987)
	Th. ponticum derived	OK7211542, CPI114084, CA595	2*n* = 56 wheat/*Th. ponticum*	R	
	Th. scirpeum derived	B84-994, CPI109887, CA574	2*n* = 56 *T. turgidum*/*Th. scirpeum* (J1J1J2J2)	R	Mujeeb-Kazi & Miranda (1984)
	L. elongatum derived	CS-LE, CA731	2*n* = 56 Wheat/*Lophopyrum elongatum* amphiploid	R	Rana Munns, CSIRO
	Haynaldia villosa derived	CA657, CPI115741	2*n* = 42 T. durum/*Haynaldia villosa* amphiploid, from Nanjing Agricultural University	S	
Addition lines (2*n* = 44)	TAi Series	TAi11	TAI11: 2*n* = 44, ex Zhong2	S	He *et al.* (1989)
		TAi12	2*n* = 44, ex Zhong2	S	
		TAi14	2*n* = 42 + 2t, ex Zhong2	S	
		TAi15	2*n* = 44, ex Zhong2	S	
		TAi21	2*n* = 44, ex Zhong4. Pedigree Ken149 *2/Zhong4	S	
		TAi22	2*n* = 44, ex Zhong4. Pedigree Ken149 *2/Zhong4	S	
		TAi23	2*n* = 44, ex Zhong5. Pedigree Zhuocheng1 *2/Zhong5	S	
		TAi24	2*n* = 44, ex Zhong5. Pedigree Zhuocheng1 *2/Zhong5	S	
		TAi26	2*n* = 44, ex Zhong3. Pedigree 3B2 *2/Zhong3	S	
		TAi27	2*n* = 44, ex Zhong3. Pedigree 3B2 *2/Zhong3 (see Discussion)	R	Dong *et al.* (2004); Jiang *et al.* (2005); Gao *et al.* (1994)
	Z-Series	Z2	2*n* = 44, ex Zhong5	R	Larkin *et al.* (1995)
		Z3	2*n* = 44, ex Zhong5	S	
		Z4	2*n* = 44, ex Zhong5	S	
		Z5	2*n* = 44, ex Zhong5	S	
		Z6	2*n* = 44, ex Zhong5	R	
	L-Series	L1	2*n* = 44 ex-TAF46 group 7 addition	S	Cauderon (1966)
		L2	2*n* = 44 ex-TAF46 group 3 addition	S	
		L3	2*n* = 44 ex-TAF46 group 1 addition	S	
		L4	2*n* = 44 ex-TAF46 group 4 addition	S	
		L5	2*n* = 44 ex-TAF46 group 5 addition	R	
		L7	2*n* = 44 ex-TAF46 group 6 addition	S	
Substitution lines (2*n* = 42)		L1 (7A)	L1 substituting for 7A	S	Bob McIntosh, University of Sydney

Table 1 (Continued)

Germplasm	Source	Identifiers/accession	Description	WSMV	References/source
L1 (7B)	L1 substituting for 7B	S			
L1 (7D)	L1 substituting for 7D	S			
6E (6A)	6E (ex-*L. elongatum*) substituting for 6A	S			
6E (6B)	6E (ex-*L. elongatum*) substituting for 6B	S			
6E (6D)	6E (ex-*L. elongatum*) substituting for 6D	S			

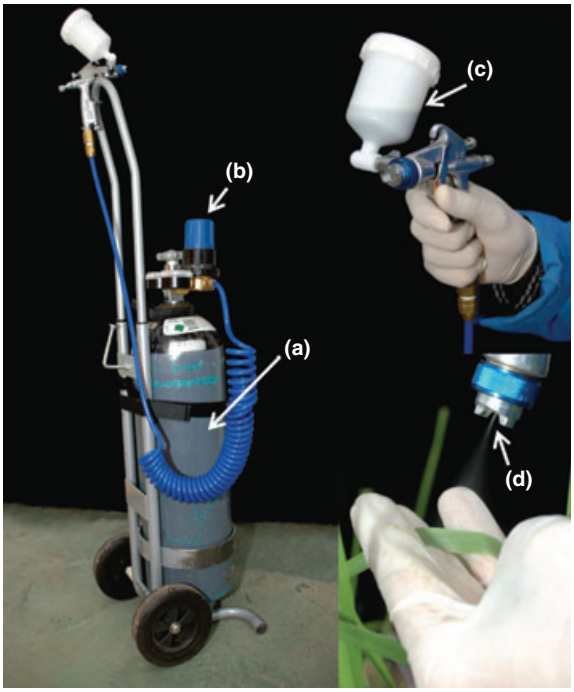

Figure 1 Mechanical inoculation system: spray gun with 0·8 mm nozzle. (a) air cylinder, (b) air pressure regulator at 40 psi, (c) container filled with infected sap and suspended celite shaken regularly to keep them suspended, (d) the nozzle and sap–celite spray is directed at leaf lamina. Celite acts as abrasive to assist in the infection process.

Molecular markers

New molecular markers were developed for the *Wsm1*-carrying translocations. Initial screening of potential markers was carried out on DNA obtained from *Th. intermedium* and Chinese Spring wheat. The polymorphic markers between wheat and *Th. intermedium* were tested on the following material: *Wsm1*-CA739 (4Ai#2S·4DL); *Wsm1*-CA740 (4Ai#2S·4DL); *Wsm1*-CA837 (Rec213, the shortest translocation on 4D; 4Ai#2S-4DS·4DL; Friebe *et al.*, 2009); *Wsm1*-Mace (Original 4Ai#2S·4DL with no yield penalty); and *Wsm1*-CA741 (4Ai#2S-4AS·4AL; Haber *et al.*, 2007). The controls included: *Th. intermedium*; wheats (Chinese Spring and Karl); Yi4212, a substitution of a group 2 *Th. intermedium* chromosome for 2D (Xin *et al.*, 2001); and Mackellar, a wheat carrying a group 7 *Th. intermedium* translocation on 7DL (Larkin *et al.*, 2002). A total of 120 primer pairs were designed and tested using the following approaches; all primers were ordered either from Sigma or Invitrogen.

Wild grasses derived ESTs and SSRs

The NCBI database was searched for expressed sequence tags (ESTs) and simple sequence repeats (SSRs) from the following *Triticeae* species: *Th. intermedium*, *Th. ponticum*, *Th. bessarabicum*, *Th. junceiforme*, *Th. caespitosum*, *Th. junceum*, *Agropyron cristatum*, *Lophopyrum elongatum*, *Pseudoroegneria*

Table 2 Temperature sensitivity of resistance in germplasm against *Wheat streak mosaic virus* (WSMV)

Resistance source/ germplasm	Accessions	Resistance at the following growth temperatures (°C)		
		20°C day, 18°C night 190 μE m^{-2} s^{-1}	25°C day, 18°C night 250 μE m^{-2} s^{-1}	28°C day, 18°C night 242 μE m^{-2} s^{-1}
c2652, *n* = 42	CA716, CA742	R	R	R
CO960293-2 (*Wsm2*), *n* = 42	CA717, CA744, CA743, RonL	R	S	S
	CA745	R	R	R
Translocation-*Wsm1*, *n* = 42	CA739, CA740, CA741, Rec213, Mace	R	S	S
Wheat/*Th. intermedium* partial amphiploids, *n* = 56	Zhong (1,2, 4, 5), TAF46, Summer 1, OT38	R	R	R
Wheat/*Th. ponticum* partial amphiploid, *n* = 56	OK7211542	R	R	R
T. turgidum/*Th. scirpeum* partial amphiploid, *n* = 56	B84-994	R	S	S
Wheat/*L. elongatum* partial amphiploid, *n* = 56	CS-LE	R	S	S
Th. intermedium addition lines to wheat	Z2, Z6, L5[a], TAi27	R	S	S

[a]L5 showed necrosis at 21 dpi, and at 28 dpi all plants were dead.

stipifolia, *Ps. spicata*, *Ps. strigosa*, *Elymus hispidus*, *Secale cereale* and *Avena vaviloviana*.

Wheat ESTs

As the original source of the *Th. intermedium* derived gene *Wsm1* is a compensating translocation 4Ai#2S·4DL, and *Wsm1* has since been associated with the telomeric region (Friebe *et al.*, 2009), wheat ESTs associated with the deletion bin 4DS2-0.82-1.00 were sought (Grain Gene 2·0; http://wheat.pw.usda.gov). A total of 49 primer pairs were designed based on ESTs mapped to deletion bin 4DS2-0.82-1.00. The ESTs were CoGe Blasted against rice and *Brachypodium* genomic sequences. The sequences were exported as FASTA files and aligned using AlignX (Vector NTI 10). Where alignment revealed the likely position of an intron, primers were designed to interspecific conserved exonic regions spanning at least one intron. This approach has previously been used for isolating polymorphism in genes and is known as intron-length polymorphism (ILP) or exon-primed intron-crossing PCR (EPIC-PCR) (Wang *et al.*, 2006).

Markers from literature

Markers reported in the literature to amplify sequences from the alien genomes were also tested as potential markers. These included: 3P3/3P4 (Wang & Wei, 1995); STSJ-15 (Talbert *et al.*, 1996); BG263898 (without restriction digestion in this study; Qi *et al.*, 2007); wheat resistance gene analogue (RGA)-ILPs (Shang *et al.*, 2010); and ILP markers to *Thinopyrum* TiERF1 (Liang *et al.*, 2008).

DNA extraction and marker assessment

DNA was extracted using a QIAGEN kit, according to manufacturer's instructions. Four microlitres of 10 ng μL^{-1} genomic DNA was amplified using the HotStartTaq® DNA polymerase and Master Mix buffer from QIAGEN following the manufacturer's guidelines. Amplification was performed in a ThermoHybaid PX2 or Corbette thermacycler as follows: one cycle of 15 min at 95°C; 35 cycles of 30 s at 94°C, 30 s at 52–65°C (depending on the individual pair of primers' temperature melting point, T_m) and 30 s at 72°C; and a final 5 min extension step at 72°C. Amplification products were separated on 1%, 2% or 3% agarose gels and visualized with ethidium bromide under UV light; where higher resolution was required products were separated by electrophoretic microchip (MultiNA; Shimadzu). Primers amplifying polymorphic sequences from *Thinopyrum* and wheat were then tested on lines derived from *Wsm1* carrying translocations. When a pair of primers amplified a *Thinopyrum* band from the translocation lines but not from the corresponding wheat line, the band was considered a dominant marker for the translocation. Likewise, if a pair of primers amplified a band in wheat but not in *Thinopyrum* it was

considered dominant for wheat. Co-dominant markers were those that gave a different size product on wheat and *Thinopyrum*.

Results

Resistance in common wheat

The inoculation method adopted achieved almost 100% of susceptible controls being infected and showing symptoms by 14 dpi. Almost all the common wheat accessions, including Australian and international cultivars and land races, were susceptible and developed characteristic wheat streak symptoms with attendant accumulation of virus as detected by ELISA. The results of many experiments are summarized in Table 1 (land race results in Fig. S1). The differences in early virus accumulation between susceptible cultivars and land races in one experiment were not reproducible and all were considered susceptible. The assessment is largely confined to substantial and reproducible resistance shown by greatly reduced symptoms and ELISA readings that do not differ significantly from background. All the true wheats examined, other than those with previously reported resistance (see below), were susceptible to the WSMV-ACT isolate and accumulated virus to a significant level by 14 dpi.

All lines previously reported to resist infection with North American isolates of the virus also succeeded in resisting the Australian WSMV-ACT isolate (Table 1, Fig. 2). Wheats with c2652 resistance and all five wheats carrying the CO960293-2 (*Wsm2*) resistance prevented systemic infection, as shown by the absence of symptoms and failure to accumulate virus in the youngest fully expanded leaf at 14 and 28 dpi (Table 1).

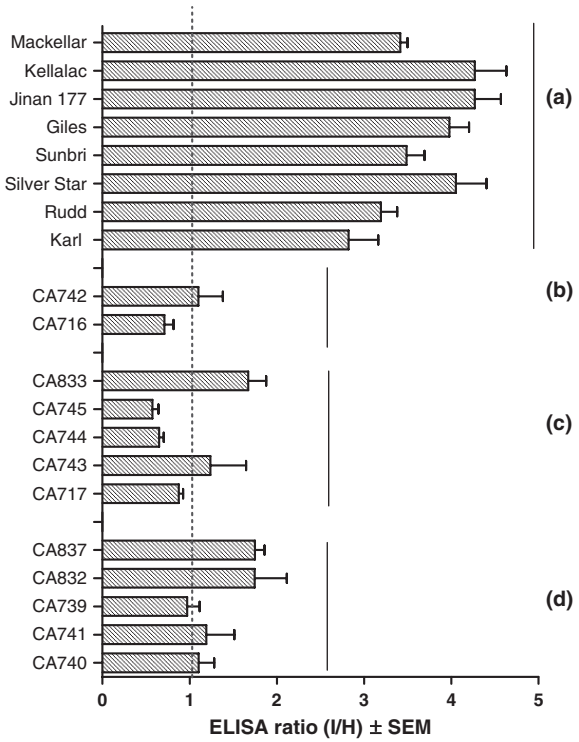

Figure 2 Resistance in wheat against WSMV-ACT. Eight plants per genotype were grown in the glasshouse. At the three leaf stage, six plants per genotype were inoculated and two plants were left as healthy controls. At 14 dpi, a newly emerged, fully expanded leaf was sampled for ELISA. $OD_{450\ nm}$ values for infected plants were divided by $OD_{450\ nm}$ values from healthy controls and presented as ELISA ratio ± SEM. (a) Selection of susceptible Australian wheat lines, (b) two accessions of c2652, (c) five accessions derived from CO960293-2, (d) five accessions derived from *Wsm1* translocation lines. The raw ELISA values show the group of varieties in (a) are significantly different to all the other genotypes at $P \leq 0{\cdot}05$.

Resistance in wheat carrying the *Wsm1*-alien translocation

The *Wsm1* gene is derived originally as a Robertsonian translocation from *Th. intermedium* to wheat, 4Ai#2S.4DL. Subsequently some derivatives have had the alien portion shortened by recombination and can be symbolized as 4Ai#2S-4DS.4DL, where *Wsm1* appears to reside in the terminal 18% of the short arm (Friebe *et al.*, 2009). All lines carrying the *Wsm1* were also resistant to WSMV-ACT (Table 1, Fig. 2). This includes the shortened translocation rec213 (CA837) and Mace, an advanced *Wsm1* line recently released as a cultivar.

CA741-*Wsm1* is derived from the same *Th. intermedium* group 4 chromosome (4Ai#2) contributing *Wsm1*, but is an independently derived translocation to 4AS developed from the 4Ai#2(4A) substitution, CI15092 (Seifers *et al.*, 1995; Haber *et al.*, 2007). Evidence that it is the same group-4 chromosome from the hexaploid *Th. intermedium* comes from the amplification of the same 341-bp STS marker sequence from the 4A substitution line CI15092 and the 4D translocation (Talbert *et al.*, 1996). In accession CA741 this translocation appears to be smaller than the 4DS translocations and is no longer linked to the STS marker (Haber *et al.*, 2007 and later under molecular markers), but appears also to contain *Wsm1* as it protects against WSMV-ACT (Fig. 2). Being on 4A makes it a suitable source of resistance for introgression into durum wheat (AABB) (Haber *et al.*, 2007).

Resistance in amphiploids and partial amphiploids

The responses of various alien-wheat hybrids to inoculation with WSMV are given in Table 1. Wheat hybrids derived from *Th. intermedium* were highly resistant to WSMV-ACT, including $2n = 56$ partial amphiploids Zhong1, Zhong2, Zhong4, Zhong5, Summer1, TAF46 and Otrastajuscaja 38. The wheat/*Th. ponticum* partial amphiploid OK7211542, the wheat/*Agropyron scirpeum* (B84-994) and wheat/*Lophopyrum elongatum* amphiploids were also resistant. While some of these had been shown previously to be resistant to North American isolates (Chen *et al.*, 2003; Li *et al.*, 2004), this is the first report of the resistance of Summer1, the *scirpeum* and

elongatum amphiploids. The wheat/*Haynaldia villosa* amphiploid was not resistant when challenged with WSMV.

Resistance in chromosome addition and substitution lines

A total of 28 single chromosome alien addition lines ($2n = 44$) and substitution lines ($2n = 42$) were assessed for resistance. Several addition lines derived from *Th. intermedium* partial amphiploids ($2n = 56$) were resistant to the WSMV-ACT (Table 1). Addition line L5 (ex-TAF46) was shown previously to resist North American isolates of WSMV (Stoddard *et al.*, 1987) and it likewise resisted the Australian isolate, at least at low growth temperatures. However, the other addition lines in the L-series were uniformly susceptible to WSMV-ACT, including L2 which was previously reported to be resistant (Table 1, Fig. 3). Other addition lines Z2, Z6 (ex-Zhong5) and TAi27 (ex-Zhong3) are newly reported sources of resistance to WSMV. Z2 and Z6 have a group 2 *Th. intermedium* chromosome (Larkin *et al.*, 1995). TAi27 is a novel and complex $2n = 44$ addition line which appears to involve introgressions from a number of *Th. intermedium* chromosomes including from groups 2, 4, 7 and possibly others (Liu *et al.*, 2001; Dong *et al.*, 2004; our unpublished data), perhaps as recombinant chromosomes, perhaps as both an addition and a substitution (Han *et al.*, 1998). Line L5 has a 5J chromosome (Forster *et al.*, 1987; Chen *et al.*, 1999b). Therefore, it can be concluded that *Th. intermedium* has various resistances to WSMV at least on chromosomes of groups 2, 4 and 5.

Temperature stability of resistance to WSMV

The germplasm which showed WSMV resistance in glasshouse experiments in cool but not strictly controlled conditions was retested in growth chambers with different controlled temperature regimes. In all experiments where the day temperature was 18–20°C, only susceptible controls developed symptoms when inoculated with the virus. All the genotypes found to be resistant in the earlier glasshouse experiments were also resistant in the controlled temperature chambers at this low growth temperature (Table 2).

ELISA readings were taken at 4-weekly time points. As previously reported, all accessions of *Wsm1* and most accessions of CO960293-2 (*Wsm2*) lost resistance at the higher growth temperatures (Table 2, Fig. 4). The plants showed characteristic virus symptoms and accumulated virus, as shown by ELISA, at least by 14 dpi. In susceptible control germplasm, virus symptoms appeared as early as 5 dpi at the elevated temperatures, reflecting a faster rate of virus replication.

Similar to the *Wsm1* lines, the wheat/*L. elongatum* amphiploid CS-LE and durum wheat/*Th. scirpeum* amphiploid B84-994 were both resistant at 20°C, had delayed symptoms at 25°C (Fig. 4d), but were totally susceptible at 28°C (Table 2).

The resistance in addition lines Z2, Z6, L5 and TAi27 behaved in a similar fashion as *Wsm1* translocation lines, ceasing to be effective at 25 and 28°C (Table 2, Fig. 4d). The resistances in Z2, Z6 and L5 were already overcome at 25°C at 14 dpi, whereas TAi-27 was only overcome after 21 dpi. Despite the breakdown of resistance in the addition lines, they did delay symptoms and virus accu-

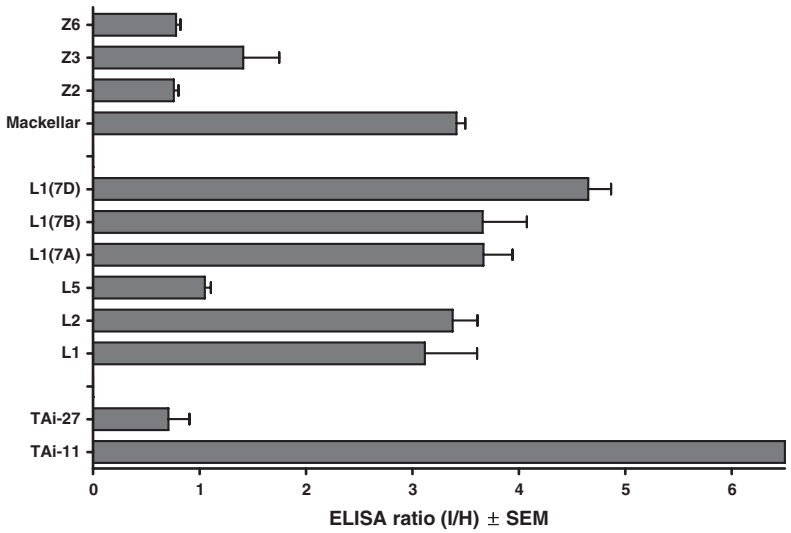

Figure 3 Resistance in Addition lines. Eight plants per genotype were grown in the glasshouse. At the three leaf stage, six plants per genotype were inoculated with WSMV and two plants left as healthy controls. At 14 dpi, a newly emerged, fully expanded leaf was sampled and ELISA performed. The apparent resistance by ELISA ratio of the lines Z6, Z2, L1 and TAi27 was confirmed by the total absence of symptoms in these lines and the development of symptoms in all the other lines.

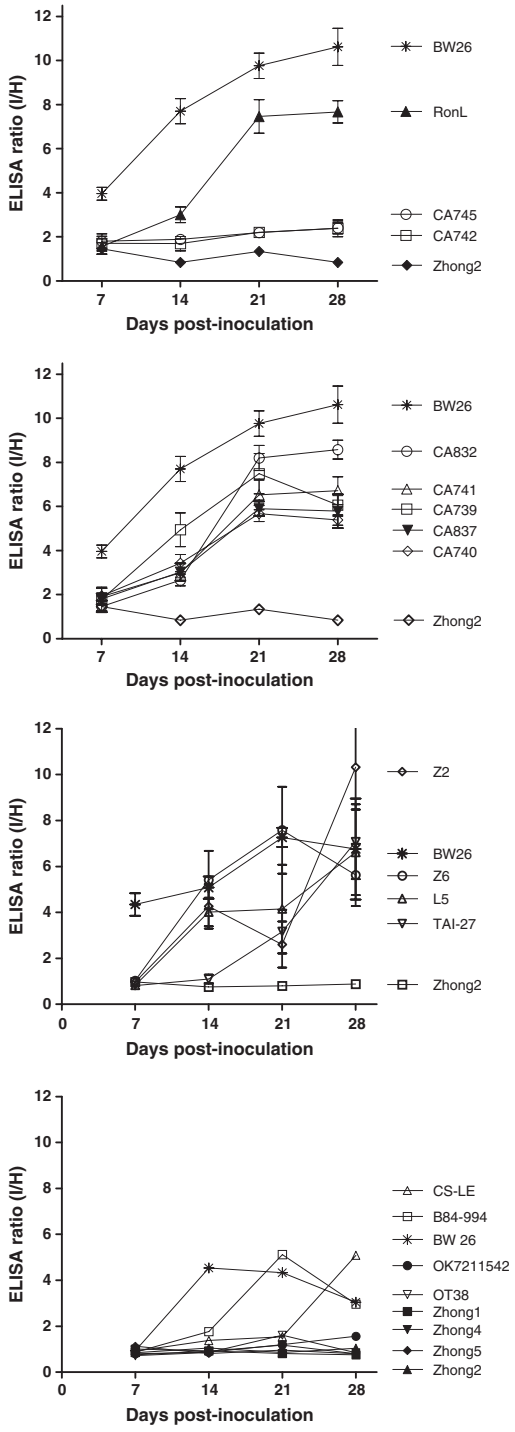

Figure 4 Temperature sensitive resistance in wheat. Germplasm with resistance against WMSV in the glasshouse was grown at controlled elevated temperature in a growth cabinet at 18°C night, 25°C day with 250 μE m^{-2} s^{-1} of light. Plants (n = 6) were inoculated at the three leaf stage. New expanded leaf samples were collected at 7, 14, 21 and 28 dpi and stored at –80°C until assayed together. Presented are the ELISA ratios (inoculated/healthy) at each time point. The first two plots are data from genotypes in one experiment; the third and fourth plots are from two further independent experiments.

mulation at the higher temperatures compared to the fully susceptible wheat control BW26.

The wheat lines c2652, CO960293-CA745 and amphiploids Zhong1, Zhong2, Zhong4, Zhong5, TAF46, Summer1, Ot38 and OK7211542 were uniformly resistant at all time points in all three temperature regimes: 20, 25 and 28°C (Table 2). One accession derived from CO960293-2 (*Wsm2*), namely CA745, was stably resistant at higher growth temperatures in a number of experiments; this interesting trait, distinguishing it from the other *Wsm2* derivatives, may reflect the *de novo* alteration of a trait's expression that can be identified and subsequently fixed with the iterative exposure to cycles of virus inoculation and selection over several generations (Seifers *et al.*, 2006; Haber *et al.*, 2011).

Development of molecular markers for *Wsm1* translocation

A total of 130 pairs of primers from various origins were initially tested using DNA from *Th. intermedium*, wheat and *Wsm1*–CA740. Included in this were 28 resistance gene analogue (RGA)-ILP markers developed by Shang *et al.* (2010), selected based on their location on 4A or 4D; none of these RGA primers detected any useful polymorphisms for *Wsm1* translocations. Nine markers in total were useful in differentiating the presence of some or all of the *Wsm1* carrying lines, the published STS-J15 (Talbert *et al.*, 1996), and BG263898-STS (Qi *et al.*, 2007) markers, and six new ones (Fig. 5, Table 3).

WSR2 amplified only from *Th. intermedium* and the 4A translocation CA741, but not from any of the wheats or 4D translocations, CA739, CA740, CA837 (rec213) or CA832 (Mace). WSR9 amplified *Th. intermedium* but not the wheats. The product from the 4A translocation (CA741) was of similar size to the *Th. intermedium* band. It also amplified from all the 4D translocations except the reduced CA837 (rec213), but the product size was much smaller (Fig. 5). Surprisingly it also amplified from the group 2 substitution line Yi4212. WSR11 amplified no bands in wheat, but amplified a band of the same size from all alien carrying lines including Yi4212 (group 2 substitution), and Mackellar (group 7 translocation); the exception was the 4A translocation, CA741. WSR17 amplified from *Th. intermedium*, the 4D *Wsm1* translocations, (except CA837), the 4A *Wsm1* translocation and from Yi4212 (group 2 substitution), but not from Mackellar (group 7 translocation). WSR65 is an EST-ILP co-dominant marker, amplifying a wheat band and a *Th. intermedium* band in all the 4D translocations including CA837 (rec213), but not from the 4A *Wsm1* translocation CA741. SCM4 amplified from all the *Wsm1* lines except CA837 (rec213). It also amplified from Yi4212 (group 2 substitution), but none of the wheats and not Mackellar. CL167 amplified only from the large *Wsm1* translocations and Yi4212 (group 2 substitution).

The previously reported marker STS-J15 (Talbert *et al.*, 1996) identified *Wsm1* translocations in various backgrounds with the exception of the 4A translocation

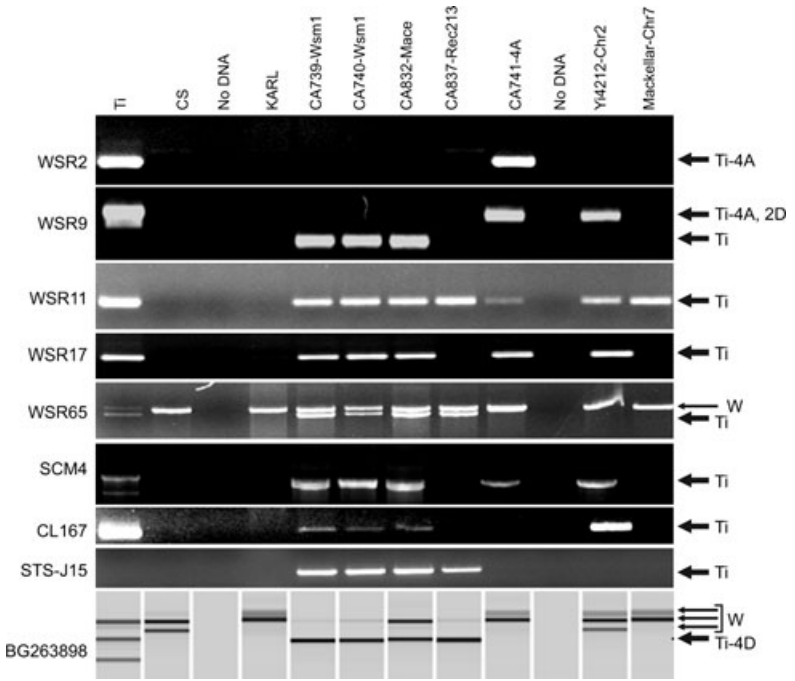

Figure 5 Markers for *Wsm1* translocations on 4D and/or 4A. Markers from various sources were used to detect polymorphisms associated with *Wsm1* translocations in wheat. *Th. intermedium* (Ti) and wheats Chinese Spring, Karl and Mackellar were used as controls. Karl was the background for CA739. Yi4212 is wheat line with Ti group 2 chromosome substituting for 2D. Mackellar is carrying a group 7 Ti fragment on wheat chromosome arm 7DL. *Wsm1* translocations on wheat 4D are represented by CA739, CA740, CA832, CA837, while the *Wsm1* translocation on 4A is represented by CA741. PCR products associated with some or all the tested Ti fragments are shown with an arrow and Ti; products associated specifically with the Ti 4A translocation or the 2D substitution, are notated as Ti-T4A or Ti-sub2D, respectively. Wheat specific products are shown with a light arrow and W. PCR product for marker BG263898 (last row) was resolved with MultiNA® for better resolution.

in CA741, as this line had been deliberately selected as expressing the resistance phenotype but lacking the marker in populations descended from a cross between Pai Toborichi (susceptible to WSMV, no *intermedium* translocation, marker-negative) and 4A translocation-bearing CI15092 (Haber *et al.*, 2007). BG263898-STS was used without post-PCR digestion with restriction enzyme (Qi *et al.*, 2007) and was able to detect a polymorphic band for *Wsm1* on 4D, but as with STS-J15, it did not detect the 4A translocation in CA741, the group 2 substitution, or the group 7 translocation.

The new markers may be useful in further efforts to produce recombinant translocations and for marker-assisted selections, especially to enable pyramiding multiple resistances into a single background. Most immediately pertinent is the fact that there are now markers that can follow the 4A translocation in CA741, which STS-J15 is unable to do.

Validation of new molecular markers for *Wsm1* translocation on wheat arm 4A in a breeding population

Some of the promising markers were tested on a population of 65 BC_1F_1 individuals from the breeding pedigree CA741/2* CW3.87, where CW3.87 is an elite breeder's genotype. A total of 65 plants were assessed for WSMV resistance and with markers WSR2, WSR9 and SCM4. Figure 6 shows that 24 segregants were positive for the 4A *Wsm1* molecular markers and all 24 were also resistant or moderately resistant to WSMV as indicated by lack of symptoms and low ELISA values. Of the 41 segregants without the markers, 40 were fully susceptible and one moderately susceptible. All tested individuals of parent CA741 were resistant, while the individuals of CW3.87 were susceptible (Fig. 6).

This confirmed the usefulness of the markers in following the apparently shortened 4A translocation in CA741 and that *Wsm1* expresses dominant resistance, effective in the heterozygotes. However, this experiment also indicated that the 4A translocation segregates in a manner distorted from the expected 1:1 ratio. In three other populations (two BC_1 populations in different backgrounds, and an F_2 population) assayed in the glasshouse at a different time of year, the presence of the alien marker corresponded almost perfectly (after virus inoculation at around the three leaf stage) with plant death at about the tillering or early elongation stage. The leaves from plants carrying molecular markers for CA741-4A translocation did not develop normal virus symptoms; instead the leaf

Table 3 Potential markers for some or all *Wsm1* translocations (see Fig. 5)

Marker	Source	Description	Forward primer	Reverse primer	Size (bp)	T_m
WSR2	EF174397.1	*R. fastigiata* isolate LMCB1 mRNA sequence	TAGCTCATACACGACAGCGG	GAGAGTGTGCAACTACCGCA	220 Ti	60
WSR9	U58022	*Ps. stipifolia* RAPD fragment Primer OPB08	GTTTCATGCAGATTGGCCTT	TGTTAGGTCGTCCGATAGGG	~320 Ti4A ~250 Ti4D	60
WSR11	U43516	*Th. bessarabicum* RAPD marker DNA	TCCCGGTACTTATCGAGGTG	CCGCAAGTCTTACTGCAACA	200 Ti	60
WSR17	AY618664	*Th. intermedium* repeat sequence	TACCAATGTCTTCAGCTGCG	ACTGCTCCTCCGTCTCAAAA	220 Ti	60
WSR65	BE443500_RC	Deletion Bin 4DS2-0.821.00	TGTTGTGACCAGTAGTGCTGC	CCTCAAAAGCTGCTACGACA	1300 Ti	60
SCM4	EF566899	*Pseudoroegneria spicata* RAPD sequence (Zhang *et al.* 2002)	GCCCTGCCATTGATCCCAAGCTG	TGGGCCAGGTCTTTCAGGTGACG	1300 Ti	60
CL167	BQ172287	2DL9-0.76-1.00; 2AL1-0.85-1.00; 2BL6-0.89-1.00	CGGAAGGACTTCATCATCATTTGT	CCTCTGCTGCTTCTCCTTCTCAG	300 Ti	66
STS-J15	RFLP Probe WG232/4L	*Wsm1* STS marker Talbert *et al.* (1996)	GTAGCAGGGGAAGCTGAAGA	CCGAGCTCACACGCTAATTT	341 Ti	50
BG263898-STS	BG263898	Ex- Qi *et al.* (2007) (without RE digestion)	TGCTCAATAAGAACTGGCAGAACG	GGAATCACAACTCAGGGGAAACAG	310 Ti	56

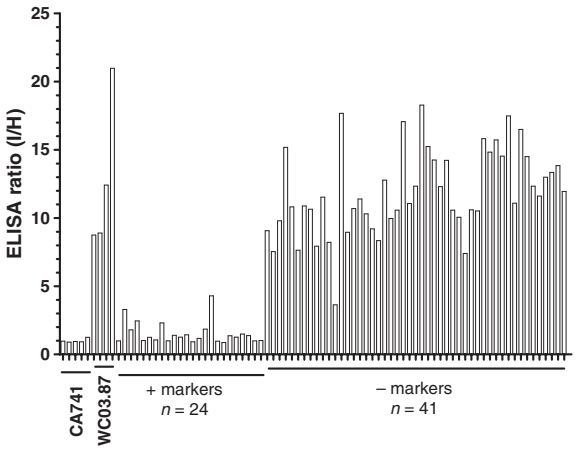

Figure 6 Segregation of resistance with molecular markers in *Wsm1*-4A population. Wheat lines segregating for *Wsm1* translocation on 4A (in accession CA741) were tested for resistance and the resistant phenotype was correlated with markers for the *Wsm1* translocation (WSR2, WSR9, SCM4) (see Fig. 5). The segregating population derived from CA741 was grown in the glasshouse along with resistant parent CA741 and susceptible parent WC03.087. At the three leaf stage plants were inoculated with WSMV-ACT isolate. At 14 dpi, a newly emerged, fully expanded leaf was sampled for ELISA. ELISA values for infected plants were divided by ELISA values from healthy controls (CA741 parental line in this case) and presented as an ELISA ratio.

lamina narrowed, followed by rapid and spreading leaf senescence, and plant death at 21–28 dpi. The phenomenon is suggestive of virus-induced plant hypersensitivity or programmed cell death, rather than the usual symptoms of WSMV. Circumstantial evidence suggests periods of elevated temperature can trigger the phenotype. In all these experiments the homozygous CA741 parent, with and without inoculation, was not dying.

Similar observations were made in Canada in F_6–F_9 generations during development of CA741; the hypersensitivity was selected out of homozygotes at F_{10} and seed made available for the Canberra experiments at F_{12}. During that development, resistance was selected by virus assay and the absence of the STS-J15 marker, and many of those individuals in each generation displayed necrotic leaves that led to plant death (Haber *et al.*, 2007; S. Haber, Agriculture and Agrifood Canada, unpublished data). The preliminary observations of this phenomenon can be summarized as follows: it is a senescence phenotype associated with heterozygosity of the 4A alien translocation, is triggered by the presence of virus, and may be accentuated by short periods of high temperatures.

Discussion

In susceptible common wheat and land race genotypes WSMV symptoms generally appeared at 4–7 dpi in summer and 8–10 dpi in winter in glasshouse experiments. Symptoms usually appear as light green and yellow streaking of the leaves, and as the disease progresses, the streaks coalesce, leading to general chlorosis of the leaf lamina and reduced photosynthetic efficiency. Susceptible plants accumulated moderate to high level of virus titre, displayed foliar symptoms and, as is characteristic

of severe WSMV infections, were stunted with shortened tillers lying prostrate. In the most severe cases, heads were shrivelled or failed to emerge and produced little or no seed.

Although all were susceptible to virus inoculation, some Australian wheats and international land races appeared to have a degree of tolerance as shown by plants growing to near-normal height. There has been no attempt to define this further in this present work. These lines would need to be studied in controlled yield trials involving WSMV inoculation to determine what contribution, if any, tolerance and/or partial resistance made in protecting yield under disease pressure.

This survey has confirmed that the genetic sources previously shown to resist North American isolates also resist the Australian WSMV-ACT isolate. Amongst the true wheats the most stable resistance was the c2652 source, which proved effective at higher growth temperatures. This resistance (Haber *et al.*, 2006) now appears to be an example of a *de novo*-evolved trait that became genetically fixed with repeated selection (Haber *et al.*, 2011; our unpublished data). It has been possible to introgress c2652 into both hexaploid and tetraploid germplasm by simple backcrossing and it was inherited in a manner consistent with that of a single dominant gene. Because c2652 resistance is easily introgressed into durum wheat, this gene must be in the A or B genome and therefore should also be amenable to combining with one of the *Wsm1*-4D translocations in bread wheat.

The resistance in CO960293-2 (*Wsm2*) was identified under natural field infections in a population being bred for Russian wheat aphid resistance (Haley *et al.*, 2002). The resistance has been characterized as temperature sensitive (Seifers *et al.*, 2006). Recently the resistance gene has been mapped to chromosome arm 3BS and named *Wsm2* (Lu *et al.*, 2011b) with flanking SSR markers identified. CO960293-2 was readily introgressed into wheat with no apparent yield penalty and released as cultivars RonL (Seifers *et al.*, 2007) and Snowmass (Haley *et al.*, 2011). Most of the *Wsm2* lines available, including RonL, were resistant in the low temperature cabinet but failed at higher temperatures. However, the CA745 derivative of CO960293-2, which had been iteratively selected in a regime that included exposure to elevated temperatures, continued to be effective even at 28°C. Since both CA745-*Wsm2* and c2652 were effective at elevated temperature, combining them could confer protection that should be particularly valuable for wheat in warmer zones. The location of *Wsm2* on 3BS ensures that it can be combined with *Wsm1* (either on 4A or 4D), but it remains to be determined whether it can be combined with c2652.

This study has also confirmed the effectiveness of the *Wsm1* translocations against the Australian isolate of WSMV, and confirmed that this resistance is temperature sensitive. The temperature sensitivity of *Wsm1* to North American isolates has been well documented (Friebe *et al.*, 1996a, 2009; Sharp *et al.*, 2002; Divis *et al.*, 2006). Friebe *et al.* (2009) have recently substantially reduced the size of the alien translocation in a recombinant called rec213. The original *Wsm1* translocation has been released in cultivar Mace in Nebraska (Graybosch *et al.*, 2009).

It is perhaps worth noting that even the temperature sensitive *Wsm1* and *Wsm2* lines appear to be effective in the field in Canberra (our unpublished data). The reason may be that during the critical times of the growing season, day temperatures only reach permissive levels for certain times of the day and are followed by long periods of non-permissive cool night temperatures (Seifers *et al.*, 1995).

Wild relatives of wheat, the so called tertiary gene pool, have been important sources for traits of agronomic importance, especially resistance to biotic and abiotic stresses (Jauhar & Peterson, 1996; Qi *et al.*, 2007). In order to become deployable in wheat improvement, a series of introgression steps are often required as follows: wheat-alien hybrids, full or partial amphiploids, chromosome additions, chromosome substitutions, and finally compensating translocation lines (Friebe *et al.*, 1991; Banks *et al.*, 1995; Luan *et al.*, 2010; Niu *et al.*, 2011).

Two species among these wild relatives, *Th. intermedium* and *Th. ponticum*, have previously displayed very high levels of resistance to WSMV and WCM (Fedak & Han, 2005). There was some evidence in *Th. ponticum* that the genes controlling resistance to WCM and WSMV are closely linked (Martin *et al.*, 1976) and therefore might be introgressed together, however even the WSMV resistance from *Th. ponticum* has proved to be complex (Hakizimana *et al.*, 2004).

All seven wheat-*Th. intermedium* amphiploids tested in the present study were highly resistant to WSMV-ACT at all tested temperatures. The resistance of this type of amphiploid has been noted previously (Chen *et al.*, 1998a). Zhong1 and Zhong2, produced by Qi *et al.* (1979) and characterized by Han *et al.* (2003), were reported resistant to both WSMV and its vector WCM (Chen *et al.*, 2003) and also highly resistant to *Barley yellow dwarf virus* (BYDV), leaf rust and stem rust (Banks *et al.*, 1993; Zhang *et al.*, 1996; Fedak *et al.*, 2001; Xin *et al.*, 2001). The diversity of resistances in the Zhong series adds to the justification for attempting to generate translocations to wheat.

All of the four Zhong lines tested in the present study showed high levels of resistance to WSMV-ACT (Table 1). The amphiploids or partial amphiploids with *Th. ponticum* (OK7211542), *Th. scirpeum* (B84-994) and *L. elongatum* (CS-LE) were all resistant to WSMV in glasshouse and lower controlled temperatures. B84-994 and CS-LE showed various degrees of instability at controlled elevated temperatures.

Screening chromosome addition lines ($2n = 44$) revealed new sources of WSMV resistance in Z2, Z6 and

TAi27 (this study), as well as confirming the already reported L5 (Stoddard *et al.*, 1987). The resistance in these addition lines was effective at low temperature and in the glasshouse experiments. Unlike the amphiploids from which they were derived, all these addition lines become susceptible at elevated temperature.

The alien chromosome present in Z2 and Z6 belongs to homoeologous group 2 (2Ai#2) and carries *Barley yellow dwarf virus* (BYDV) resistance (Larkin *et al.*, 1995; Tang *et al.*, 2000; Zhang *et al.*, 2000). At least some derivatives of Z2 have become substitution lines where wheat chromosome 2D was replaced by 2Ai#2 and was resistant to *Fusarium graminearum* (Han *et al.*, 2003). At least some accessions of Z6 ($2n = 44$) appear to have one pair of chromosomes derived from *Th. intermedium* plus another pair of translocated chromosomes involving B-genome chromosomes of wheat (Han *et al.*, 2003). In the present study resistance to WSMV-ACT was present in Z2 and Z6.

TAi27 is the third new addition line ($2n = 44$) identified with resistance to WSMV (this study). It is one of 14 in the TAi series involving *Th. intermedium* chromosomes added to wheat (He *et al.*, 1989). The literature suggests uncertainty whether TAi27 was derived from Zhong3 (Dong *et al.*, 2004), Zhong4 (Jiang *et al.*, 2005, 2009), or Zhong5 (Liu *et al.*, 2002). However, TAi27 is complex and appears to involve introgressions from a number of *Th. intermedium* chromosomes including from groups 2, 4, 7 and possibly others (Liu *et al.*, 2001; Dong *et al.*, 2004; our unpublished data), perhaps as recombinant chromosomes, or perhaps as both an addition and a substitution (Han *et al.*, 1998). Isozyme data implicated a *Th. intermedium* group 4 chromosome (Gao *et al.*, 1994). TAi27 has other useful traits such as BYDV resistance (Jiang *et al.*, 2009) and stem rust resistance (our unpublished data) which would help justify the effort required to generate translocations.

Stoddard *et al.* (1987) previously evaluated resistance in the L-series addition lines against WSMV and concluded that L2 carries intermediate resistance while L5 conferred stronger resistance at least at low temperature. In the experiments here L2 became readily infected with WSMV-ACT in the glasshouse, while L5 was resistant. L5 possesses a 5J chromosome (Forster *et al.*, 1987; Chen *et al.*, 1999a); Z2 and Z6 have a group 2 *Th. intermedium* chromosome (Larkin *et al.*, 1995); TAi27 has chromatin from at least groups 2 and 4, possibly others (Liu *et al.*, 2001; Dong *et al.*, 2004); *Wsm1* derives from a group 4 *Th. intermedium* chromosome. Therefore, it can be concluded that *Th. intermedium* has various resistances to WSMV at least on chromosomes of groups 2, 4 and 5. The cumulative effect of multiple genes in *Th. intermedium* and the derived amphiploids would explain their high level of resistance. This would also encourage ongoing efforts to render further wheatgrass WSMV resistance genes deployable and to pyramid multiple genes in cultivars.

The stability and strength of the WSMV resistance in the wheat/*Th. intermedium* partial amphiploids, and the observation that multiple genes on multiple chromosomes are involved, demonstrates the value of gene stacking in breeding for resistance to this serious disease. The general wisdom of resistance gene pyramids to increase the longevity of protection to agricultural diseases is well accepted (Ayliffe *et al.*, 2008).

Even before new alien sources of WSMV resistance become available, there are opportunities to pyramid resistance genes. Using molecular markers makes this much easier to accomplish. *Wsm1* is available on 4D or 4A, and CO960293-2 (*Wsm2*) is on 3B (Lu *et al.*, 2011b), allowing for their combination. This study has developed a number of new markers for the various *Wsm1* translocations; importantly this includes three simple markers for the previously unmarked CA741 version of *Wsm1* on chromosome 4A. Although the flanking markers to *Wsm2* in Lu *et al.* (2011b) are not close enough to be safely used in marker assisted selection, more closely linked markers are now available (Lu *et al.*, 2011a). Furthermore this study has shown that the CA745 derivative of CO960293-2 consistently performed well at elevated temperatures, as did c2652, making them attractive for warmer agroclimatic wheat zones. Even without markers for the heat stable resistances, it might be possible to combine them with *Wsm1*; segregants with both might be distinguished by the presence of a *Wsm1* marker and the presence of heat stable resistance. Despite that theoretical possibility, geneticists and breeders concerned with this disease will welcome the development of tightly linked molecular markers for both c2652 and *Wsm2*.

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Supporting Information

Additional Supporting Information can be found in the online version of this article:

Figure S1. Reaction of land races to infection by WSMV-ACT isolate. Eight plants per genotype were grown in glasshouse. At three leaf stage, six plants per genotype were inoculated and two plants were left as healthy controls. At 14 dpi, newly emerged, fully expanded leaf was sampled for ELISA. ELISA values for infected plants were divided by ELISA values from healthy controls and presented as ELISA ratio ± SEM.

Table S1. Molecular markers amplifying diagnostic bands between *Thinopyrum intermedium* and wheat.

Table S2. Potential markers to detect polymorphism between wheat and *Thinopyrum intermedium*.

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