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Development of PCR markers linked to resistance to wheat streak mosaic virus in wheat

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Abstract Wheat streak mosaic virus (WSMV), vectored by the wheat curl mite (*Acer tulipae*), is an important disease of wheat (*Triticum aestivum* L.) in the North American Great Plains. Resistant varieties have not been developed for two primary reasons. First, useful sources of resistance have not been available, and second, field screening for virus resistance is laborious and beyond the scope of most breeding programs. The first problem may have been overcome by the development of resistance to both the mite and the virus by the introgression of resistance genes from wild relatives of wheat. To help address the second problem, we have developed polymerase chain reaction (PCR) markers linked to the WSMV resistance gene *Wsm1*. *Wsm1* is contained on a translocated segment from *Agropyron intermedium*. One sequence-tagged-site (STS) primer set (WG232) and one RAPD marker were found to be linked to the translocation containing *Wsm1*. The diagnostic RAPD band was cloned and sequenced to allow the design of specific PCR primers. The PCR primers should be useful for transferring *Wsm1* into locally adapted cultivars.

Key words Wheat · Wheat streak mosaic virus · Markers

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Introduction

Wheat streak mosaic virus (WSMV) causes sporadic, but severe, losses in many areas of the world, and is especially common in the Great Plains of North America (Weiss 1987). WSMV is vectored by the wheat curl mite, which moves from green plant to green plant to spread the disease. WSMV has been of long-standing concern in winter wheat, and changing cultural practices have caused increased severity of the disease in both spring and winter wheat. Important factors have included the movement of winter wheat into traditional spring wheat areas providing a natural "green bridge" for the vector, increases in weedy hosts for the vector due to conservation tillage, and the presence of secondary hosts in non-cropped areas. Thus, the long-standing problem of WSMV for wheat growers in the Great Plains is becoming more severe.

Cultural methods for the control of WSMV rely upon elimination of the "green bridge" for vector movement. Due to the reasons cited above and vagaries of the weather (such as hail storms that provide volunteer wheat to serve as a green bridge), cultural methods do not provide satisfactory control of WSMV. Host plant resistance is an attractive control strategy. However, resistant cultivars have not been developed for two primary reasons. The first of these is that no resistance has been discovered in cultivated wheat. Resistance does exist in relatives of wheat (Friebe et al. 1991), but until recently the laborious process of introgressing the resistance genes into acceptable backgrounds had not been accomplished. Thus, breeders have not had easily usable sources of resistance to introgress into locally adapted cultivars. A second important factor has been the relatively laborious nature of resistance screening. Screening for mite resistance (e.g., Slykhuis 1967; Harvey and Martin 1988) and WSMV resistance (Martin 1978; Stoddard et al. 1987) generally requires resource investments beyond that possible for most varietal breeding programs.

Recent developments have led to renewed optimism that cultivars with resistance to WSMV may be bred. First, several improved sources of resistance are available, generally developed by backcrossing virus resistance or mite resistance from relatives of wheat into acceptable wheat backgrounds. Thus, potentially useful sources of resistance are available. The second development is the advent and refinement of molecular marker technology, including the use of "sequence-tagged-site" (STS) PCR (Chen et al. 1994; Talbert et al. 1994) and random amplification of polymorphic DNA (RAPD) (Williams et al. 1990; Devos and Gale 1992). Identification of markers associated with resistance would facilitate selection in applied breeding, especially given the inherent difficulties in field-based screening for this pathogen.

An excellent source of resistance to WSMV has been developed by the Kansas State University Wheat Genetics Resource Center. Friebe et al. (1991) evaluated eight WSMV-resistant stocks derived from wheat-*Agropyron intermedium* crosses. The most promising line, CI 17884, contained a translocation whereby the short arm of chromosome 4D was replaced by the short arm of chromosome 4 from *Agropyron*. The *Agropyron* segment contains the resistance gene *Wsm1*. This stock has been improved by backcrossing the segment into the hard red winter wheat cultivar "Karl" to produce germ plasm line KS93WGRC27 (Gill et al. 1995). For this report, our objectives were to identify STS-PCR and/or RAPD markers linked to *Wsm1* to facilitate development of locally adapted resistant cultivars.

Materials and methods

Plant materials

Initial screening of PCR primer sets was accomplished using DNA from KS93WGRC27, its wheat recurrent parent Karl, and germ plasm ancestral to KS93WGRC27. This germ plasm included *A. intermedium* accession 7010, CI 17884 (a wheat line containing a 4D/4Ai chromosome substitution), and CI 15092 (an addition line containing 21 pairs of wheat chromosomes and a pair of 4Ai chromosomes). To verify potential linkages of PCR products with *Wsm1*, a set of 91 recombinant F_3-derived F_4 lines were evaluated. The pedigree for these lines was KS93WGRC27/KS84063-9-12-1, where the latter parent is a suceptible winter wheat line from Kansas. The recombinant lines were scored as either homogeneous resistance or susceptible to WSMV after viral inoculation in the field at the Fort Hays Research Center in Kansas (Martin 1978). A subset of lines were subsequently re-screened for WSMV in the greenhouse in Bozeman following the procedures of Carroll et al. (1982) with the exception that 'Michigan Amber' was used as the reservoir host for WSMV. Eight plants of each line, parent, and check cultivar were inoculated. Temperature was maintained at 20–25 °C and each plant was scored based on disease symptoms 4 weeks after inoculation. Classification of selected lines, parents, and check cultivars for WSMV reaction was confirmed by ELISA (Stoddard et al. 1987). Heterogeneity was assumed if all eight individuals did not give the same reaction.

Primer evaluation

Genomic DNA was prepared by the procedure of Dellaporta et al. (1983) and Lassner et al. (1989). Approximately 50 ng of DNA was used per PCR reaction. Two groups of primers were evaluated in order to identify linkages with *Wsm1*. First, we developed and screened ten STS-PCR primer sets derived from RFLP clones that mapped to chromosome 4 in wheat or barley (Gill et al. 1991; Anderson et al. 1992; Tragoonrung et al. 1992; Nelson et al. 1995). Procedures for sequencing RFLP clones and designing primer sets were as previously described (Talbert et al. 1994). The chromosomal origins of amplified products were determined using the nullisomic-tetrasomic stocks developed by Sears (1954). PCR reaction conditions were as described by Talbert et al. (1994).

In addition to STS-PCR primer sets, we screened 100 random 10-bp RAPD primers. These were sets A, B, C, J, and AF from Operon (Alameda, Calif.). Conditions for RAPD analysis were as recommended by Williams et al. (1990), except that 200 M of each dNTP was used instead of 100 μM. The protocol for thermocycling was 4 min at 94°C, followed by 45 cycles of 1 min at 94 °C, 1 min at 34 °C, and 2 min at 72°C. At the end of 45 cycles, a 7-min 72°C incubation was accomplished prior to holding the reactions at 4°C.

To increase the fidelity of a diagnostic band identified by RAPD analysis, the band was cloned and sequenced following the protocol of Talbert et al. (1995). Primers were designed as described by Talbert et al. (1994).

Results and discussion

Ten STS-PCR primer sets developed from chromosome-4 RFLP clones were screened using DNA from susceptible Karl and resistant KS93WGRC27. The RFLP probes were selected as either mapping to chromosome 4S in wheat or barley (WG622, E6a, F8, M149, BCD327, BCD402, CDO662, CDO1338) or to chromosome 4L (WG232, G10). Primer set WG232, used at an annealing temperature of 45 °C, showed a clear polymorphism between susceptible Karl and resistant KS93WGRC27 when PCR products were digested with *Hha*I. Thus, we used this primer set to further screen a set of 91 recombinant inbred progeny that had been scored as either homogeneous resistant or homogeneous susceptible to WSMV (Fig. 1). In sum, 38 of 46 lines classified as resistant were missing the band indicated by the arrow, while 44 of 45 lines classified as susceptible contained the band. Subsequent re-screening of the apparent recombinants indicated that some of the lines were segregating for resistance. This was not unexpected in that the lines were derived from F_3 plants. When these were discarded from the analysis, 37 of 40 resistant lines were missing the diagnostic band, and 41 of 41 susceptibles contained the band. Thus, these data suggest that

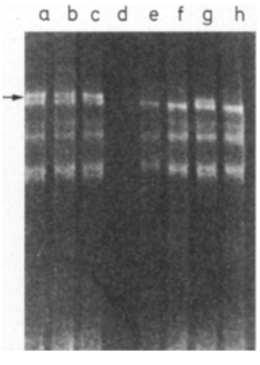

Fig. 1 *Hha*I-digested amplification products from progeny lines derived from the cross KS93WGRC27/3*KS84063-9-12-1. The primer set used for amplification was WG232. *Lanes a, b, c, and g* are susceptible progeny lines, while *lanes e, f, and h* are resistant. *Lane d* is an unsuccessful amplification of a resistant progeny line. The *arrow* points to a band of approximately 1800 bp that is closely linked to *Wsm1*

the polymorphic band is closely linked to the 4D/4Ai translocation breakpoint.

Seven primer sets designed from chromosome-4S RFLP clones showed no polymorphic bands between Karl and KS93WGRC27. To determine the chromosomal origin of the amplified products, DNA of the nullisomic-tetrasomic Chinese Spring series was amplified (Sears 1954). The primers tested, their sequences, and the chromosomal origin of the amplification products are shown in Table 1. For four of the primer sets developed from wheat sequences (WG232, WG622) and *T. tauschii* sequences (F8, G10), products were amplified from group-4 chromosomes as predicted from RFLP maps. Additional sequences were also amplified from other homoeologous groups. Primer set F8 amplified a sequence from chromosome 4DL in Chinese Spring based on analysis with ditelosomic Chinese stocks (Sears 1954), yet no polymorphisms were observed between Karl and KS93WGRC27. The use of STS primer sets to target specific regions would be augmented if primer sets were transferrable to identical map positions across related species. However, the primer sets developed from oats (CDO662, CDO1338) and barley (BCD327, BCD402) did not amplify DNA from group-4 homoeologues as predicted from RFLP maps (Table 1). Previous work has shown that many barley-derived primer sets are specific for the predicted homoeologous group in wheat (Talbert et al. 1994). Given the limited number of primer sets tried in this experiment, the general transferability of STS and RFLP map locations across cereal species will require further investigation.

Due to the lack of complete linkage of the WG232 locus to *Wsm1*, and the fact that the polymorphism is not as clear as desired, we extend our study to include the screening of RAPD primers. Of 100 such primers screened using DNA from Karl and KS93WGRC27, 86 revealed clear banding patterns on acrylamide gels. Of these 86, 18 were polymorphic between the two genotypes. These polymorphic RAPD primers were used to amplify DNA from the 91 recombinant inbred lines. Seventeen of eighteen primers either were clearly not linked to *Wsm1*, or else produced inconsistent amplifications. However, RAPD primer J15 produced a clear diagnostic band, not requiring restriction digestion, closely linked to *Wsm1* (Fig. 2). A RAPD of approximately 500 bp was present in germ plasm known to carry *Wsm1* and missing in germ plasm that does not have *Wsm1*. In sum, 42 of 46 lines classified as resistant had the diagnostic band, while 41 of 94 susceptible lines were missing it. We re-screened the apparent recombinants (three of which were the same apparent recombinants as identified by WG232) for virus resistance.

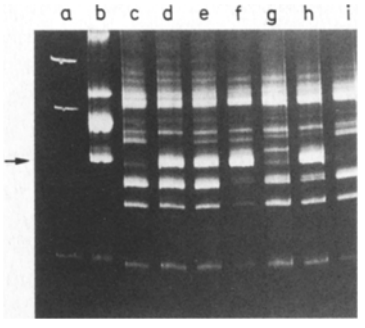

Fig. 2 Undigested DNA amplification products amplified using RAPD primer J15. *Lane a*: *Rsa*I-digested pUC18; *b*: *A. intermedium* accession 7010; *c*: Karl winter wheat; *d*: CI 15092, a susbstitution line containing chromosome 4Ai; *e*: CI 17884, an addition line containing chromosome 4Ai; *f*: KS93WGRC27; *g*: a susceptible progeny line from the cross KS93WGRC27/KS84063-9-12-1; *h*: a resistant progeny line from the same cross; *i*: Pondera spring wheat. The *arrow* points to a band of approximately 500 bp that differentiates resistant and susceptible germ plasm

Table 1 Chromosomal location of products amplified by STS-PCR primer sets based on mapping with nullisomic-tetrasomic Chinese Spring wheat (Sears 1954)

Primer		Sequence	Chromosome[a]
BCD327	L	GGCACGAGGCACAAACTTGA	Not determined[b]
	R	ACGAGCAGAAGCCAAGCCAA	
BCD402	L	CACCTGATCCACCTTCATGG	2D, 5B(*Hha*I)
	R	TGACCGCATGCACCGTTGCC	
CDO662	L	CTTTTTACAGCAGGAGGCC	5B(*Hha*I)
	R	AGCTCCAGAAGGCAAAGAAG	
CDO1338	L	GCTTTCCTTTTGCAGCAACA	5A(*Hha*I)
	R	GCACTAAGGCATACACCAGT	
WG232	L	CCTCAGTGTTTCAGGGTAAA	7A(*Dde*I), 4A, 4D (*Hin*fI)
	R	TGGACTCGTGTTCAATAATG	
WG622	L	CTCCTGTCAAACTGTTCCCTGA	4A, 5D (*Hin*fI)
	R	AGGTGGGACTGGAACCTGCATC	
E6a	L	GTATCTCTTCATGCACGTTC	5B (*Hin*fI)
	R	CCAACGAGTACTCCCTCGGC	
F8	L	GCATTATCATCAGCTGAAAG	4A, 4B, 4D (*Rsa*I)
	R	GTTCAAGGCAGACCTTGACT	
G10	L	GTGTTGATGTCCTTGAGGCC	4A, 4B, 4D (*Hin*fI)
	R	TGTCCAGCTTCAGCGAGTAC	
M149	L	GCTCATCGTTCACTTGTACC	Not determined[b]
	R	ACAACTCCCCTTCACTGTTC	

[a] Enzyme that resulted in a clear missing fragment given parenthetically

[b] No missing bands were observed in Chinese Spring nullisomic-tetrasomic lines

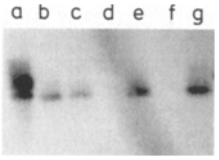

Fig. 3 Southern blot containing DNA from wheat lines amplified with J15 and probed with the 500-bp fragment amplified by J15 from *A. intermedium*. *Lane a*: *A. intermedium* accession 7010; *b*: CI 15092; *c*: CI 17884; *d*: Karl; *e*: KS93WGRC27; *f*: susceptible progeny line; *g*: resistant progeny line

When the segregating lines were discarded from the analysis, 40 of 40 resistant lines had the diagnostic band, and 41 of 42 susceptible lines were missing the band.

Data with J15 suggested that the diagnostic RAPD was being amplified from the *Agropyron* segment containing *Wsm1*. This was confirmed by Southern-blot analysis by using the 500-bp RAPD from *A. intermedium* as a probe on J15-amplified DNA from resistant and susceptible germ plasm (Fig. 3). Hydridization to the diagnostic band occurred in all resistant germ plasms. An additional RAPD also hybridized in *A. intermedium* (lane a). The origin of this RAPD is unknown, although it may reflect the hexaploid nature of *A. intermedium*.

Southern-blot results suggested that the diagnostic J15 RAPD was amplified from the *Agropyron* segment containing *Wsm1*. A correlation of 100% between the presence of the RAPD and resistance might be anticipated given the lack of recombination expected between wheat and *Agropyron* chromosome segments (Friebe et al. 1991). However, one recombinant inbred line was scored as having the J15 RAPD, yet also being susceptible to WSMV. To verify this result, we screened seven individuals from this family for both WSMV reaction and the presence of the J15 RAPD. All were scored as susceptible, based both on visual symptoms and on ELISA results. Additionally, all seven individuals contained the diagnostic J15 RAPD. Thus, there is clearly not a 100% correlation between the J15 RAPD and resistance. Whether this is due to recombination between the *Agropyron* segment and a wheat chromosome or due to some other genetic mechanism is not known.

In the primer development work thus far reported, we used the procedure of Dellaporta et al. (1983) to prepare DNA. Our interest in developing markers linked to *Wsm1* is due to our need for such markers in backcrossing *Wsm1* into wheat varieties adapted to Montana. We prefer a method that requires small amounts of DNA. We generally use the procedure of Lassner et al. (1989) to prepare DNA; however, poor success was obtained using RAPD primer J15 with DNA prepared in this manner. For example, Fig. 4 A contains three DNA samples prepared according to Dellaporta et al. (1983) and amplified using J15 (lanes a–c). Lanes d–k are segregating progeny DNAs prepared according to Lassner et al. (1989) in which amplification was unsuccessful.

Previous work in our laboratory has shown that STS-PCR primer sets worked well using crude DNA preparations (unpublished data). Thus, we wished to convert the J15 RAPD marker to a more specific STS primer set. To accomplish this, the 500-bp RAPD band was cloned and sequenced and primers were designed from the sequence data. The new primer set, designated STSJ15, has the 5′ to 3′ sequences of (left primer: GTAGCAGGGGAAGCTGAAGA) and (right primer:

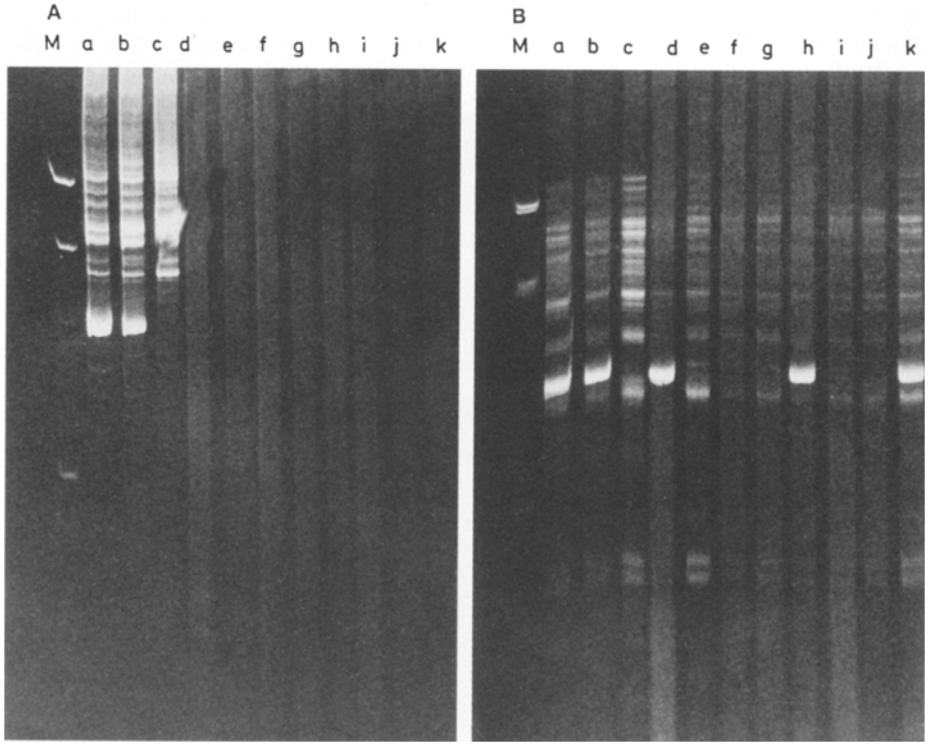

Fig. 4 Panel A Amplification reactions using RAPD primer J15. *Lane M* is pUC 18 digested with *Rsa*I, producing fragments of 1.8, 0.7 and 0.2 kb, respectively. *Lanes a, b, and c* are from DNA prepared according to Dellaporta et al. (1983), while *lanes d–k* were prepared according to Lassner et al. (1989). Specific genotypes are *lanes a*: KS93WGRC27; *b*: CI 17884; *c*: Karl; *d–g*: progeny of Amidon/KS93WGRC27/ /MTWS10; *h–k*: progeny of Amidon/KS93WGRC27/ /MTWS11. Progeny are expected to be segregating 1:1 for the diagnostic band. **Panel B** Same DNA samples amplified with STSJ15

CCGAGCTCACACGCTAATTT). This primer set was used to amplify DNA from the 91 recombinant lines tested with RAPD J15, and gave identical results. Additionally, these primer sets worked using DNA samples that failed to amplify using RAPD J15. An amplification reaction with these primers using the same DNA samples as shown in Fig. 4 A is indicated in Fig. 4 B. In this case, successful amplification of the segregating progeny lines occurred, and allowed identification of progeny containing the STSJ15 marker linked to *Wsm1*.

Devos and Gale (1992) suggested that RAPD primers may find a use for tracking introgressed chromosome segments in wheat. Our results support this contention, yet suggest that at least in this one instance derivation of specific primer sets from RAPD fragments may be desirable. Given the inherent difficulties in field screening for wheat streak mosaic virus resistance, primer set STSJ15 will be useful in backcrossing programs to introgress *Wsm1* into locally adapted materials.

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