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Evaluation of marker-assisted selection for the stripe rust resistance gene *Yr15*, introgressed from wild emmer wheat

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Abstract Stripe rust disease is caused by the fungus *Puccinia striiformis* f. sp. *tritici* and severely threatens wheat worldwide, repeatedly breaking resistance conferred by resistance genes and evolving more aggressive strains. Wild emmer wheat, *Triticum dicoccoides*, is an important source for novel stripe rust resistance (*Yr*) genes. *Yr15*, a major gene located on chromosome 1BS of *T. dicoccoides*, was previously reported to confer resistance to a broad spectrum of stripe rust isolates, at both seedling and adult plant

stages. Introgressions of *Yr15* into cultivated *T. aestivum* bread wheat and *T. durum* pasta wheat that began in the 1980s are widely used. In the present study, we aimed to validate SSR markers from the *Yr15* region as efficient tools for marker-assisted selection (MAS) for introgression of *Yr15* into wheat and to compare the outcome of gene introgression by MAS and by conventional phenotypic selection. Our findings establish the validity of MAS for introgression of *Yr15* into wheat. We show that the size of the introgressed segment, defined by flanking markers, varies for both phenotypic selection and MAS. The genetic distance of the MAS marker from *Yr15* and the

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number of backcross steps were the main factors affecting the length of the introgressed donor segments. Markers *Xbarc8* and *Xgwm493*, which are the nearest flanking markers studied, were consistent and polymorphic in all 34 introgressions reported here and are therefore the most recommended markers for the introgression of *Yr15* into wheat cultivars. Introgression directed by markers, rather than by phenotype, will facilitate simultaneous selection for multiple stripe rust resistant genes and will help to avoid escapees during the selection process.

Keywords *Triticum dicoccoides* · *Triticum durum* · *Triticum aestivum* · Marker-assisted selection (MAS) · Simple sequence repeats (SSRs) · *Puccinia striiformis* f. sp. *tritici* · Linkage drag

Introduction

Stripe rust caused by the fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*) is one of the most destructive diseases of wheat, resulting in yield losses of 10–70 % in susceptible varieties (Chen 2005; Chen et al. 2010). Total losses of harvestable grain have been reported for severe epidemics (McIntosh et al. 1995; Chen 2005). Introgression of resistance (*R*) genes from wild germplasm has been recognized as an efficient and environmentally safe approach to minimize yield losses due to diseases. For stripe rust, introgression of resistance genes into wheat accessions commenced in the 1980s (Chen 2005), thereby saving hundreds of millions of dollars (Brennan and Murray 1998). Wild emmer wheat, *Triticum dicoccoides*, discovered in 1906 in Rosh Pina, Israel (Aaronsohn 1910), is an important source for novel stripe rust resistance (*Yr*) genes (Grama and Gerechter-Amitai 1974; Fahima et al. 1998; Nevo et al. 2002).

New resistance genes for stripe rust are continuously needed due to breakdown of resistance conferred by currently deployed genes as *P. striiformis* races evolve and migrate (van Silfhout and Grönweggen 1984; Stubbs 1985; Sun et al. 1997; McDonald and Linden 2002). *Yr15* is a stripe rust resistance gene discovered in the 1980s in *T. dicoccoides* accession G25 (Gerechter-Amitai et al. 1989); it was later mapped to chromosome 1BS (Sun et al. 1997; Peng et al. 2000). The resistance conferred by *Yr15* was shown to be effective against 24 *Pst* races from 18

countries around the globe (Gerechter-Amitai et al. 1989) and to 26 international isolates and Chinese races of *Pst* (58893, 59791, 60105, 61009, 68009, 72107, 74187, 75078, 76088, 76093, 78028, 78080, 80551, 82061, 82517, 85019, 86036, 86094, 86106, 86107, PE92, CYR26, CYR27, CYR29, CYR32, CYR-S) (Li et al. 2006; Niu et al. 2000). More recent studies show that *Yr15* is effective against all *Pst* races identified so far in the USA (Murphy et al. 2009; Chen et al. 2010), Australia (Bariana et al. 2007; Randhawa et al. 2014), and India (H.S. Bariana unpublished results). These findings confirm that *Yr15* is a valuable source of resistance to stripe rust. In order to make this resistance gene available to wheat breeders, *Yr15* was introgressed into both durum and bread wheat lines by conventional breeding through phenotypic selection at the Agricultural Research Organization, Volcani Center, Bet Dagan, Israel (Grama and Gerechter-Amitai 1974; Grama et al. 1982; van Silfhout and Grönweggen 1984). Following the initial introgression of *Yr15* in Israel, breeding programs worldwide have used the resulting lines as a source for introgression of *Yr15* into bread and durum wheat cultivars.

Marker-assisted selection (MAS; reviewed in Koebner and Summers 2003; Collard and Mackill 2008; Panigrahi et al. 2013) refers to the use of DNA markers that are tightly linked to target loci as a substitute or assistance for phenotypic selection. By determining the allele of a DNA marker, plants that possess traits linked to that marker may be identified by the marker genotype rather than by their phenotype. MAS is particularly useful for traits with low heritability such as drought tolerance or yield (e.g., Hill et al. 2013), recessive inheritance such as disease resistance (e.g., Tyrka et al. 2008), difficult and costly phenotyping such as baking quality (e.g., Cavanagh et al. 2010), and for pyramiding multiple disease resistance genes (e.g., Tester and Langridge 2010). MAS also provides a rapid means of tracking introgressions (Tester and Langridge 2010). Moreover, it is useful for reducing the length of introgressed chromosome segments in order to minimize the risk of linked genes that can have a negative impact (Holland 2004; Collard and Mackill 2008). MAS has been a successful tool for introgression of genes of interest including disease resistance genes into cultivars in wheat and many other species (Singh et al. 2001; Spielmeier et al. 2003; Yang et al. 2003; Simko et al. 2009).

Previously, the assignment of *Yr15* to chromosome arm 1BS by McIntosh et al. (1996) facilitated the mapping of the gene, which was begun by Sun et al. (1997) and enabled the finer mapping of the *Yr15* region with the markers reported here. In the present study, we aimed to validate simple sequence repeat (SSR) markers from the *Yr15* region as efficient tools for MAS of *Yr15* into wheat and to compare the outcome of gene introgression by MAS and by conventional phenotypic selection. A set of polymorphic SSR markers was used to genotype 34 *Yr15* introgression lines (ILs) from different breeding programs obtained both by conventional and by MAS backcrossing, as well as on their susceptible recurrent parents (RPs).

Materials and methods

Plant material

*F*₂ mapping population

An *F*₂ population of 151 individuals used for the genetic mapping of the SSR markers was developed by crossing the susceptible *T. durum* accession D447 (LD393/2*Langdon ND58-322) with the resistant BC₃F₉ and BC₃F₁₀ (B9 and B10) introgression lines, which carried the *Yr15* gene within a 1BS chromosome segment introgressed from *T. dicoccoides* accession G25 (Sun et al. 1997; Peng et al. 2000). The *F*₁ plants were tested to verify heterozygosity and then self-crossed to produce a segregating population for linkage analysis.

Yr15 introgression lines

Since its discovery (Gerechter-Amitai et al. 1989), *Yr15* has been introgressed into a range of durum and bread wheat genetic backgrounds in a variety of breeding programs around the globe. The 34 *Yr15* ILs and the 23 susceptible RPs lacking *Yr15*, and the *Yr15* donor line *T. dicoccoides* G25 used in the current study, along with their pedigrees, are described in Table 1. The tetraploid (*T. turgidum* ssp. *durum*) and hexaploid (*T. aestivum*) accessions include cultivars and advanced breeding lines from the Agricultural Research Organization, Volcani Center, Bet Dagan, Israel, the University of Sydney, Cobbitty, Australia, and the University of California, Davis, CA, USA.

Stripe rust resistance tests conducted at the seedling stage

Stripe rust resistance tests were conducted at the seedling stage, as described in Cheng et al. (2010). The highly virulent *Pst* isolate 5006 (that belongs to race 38E134, virulent on *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr22*, *Yr23*, *YrSD*, and *YrHVII*; Cheng et al. 2010), kindly provided by Dr. Jacob Manisterski (Tel Aviv University, Tel-Aviv, Israel), was used to inoculate the plants. Seedlings in the second-leaf stage were infected with stripe rust by spraying with a mixture of a light mineral oil (Soltrol 170, Chevron-Phillips Chemical Co., Houston) and *Pst* spores (2 mg ml⁻¹). The sprayed plants were left under non-humid conditions for 20 min in order to allow the mineral oil to dry. The inoculated plants were then transferred to a dew chamber and kept for 24 h at 100 % humidity, first for 12 h at 9 °C in the dark followed by 12 h under light at 15 °C. The plants were then grown at 70 % humidity under the following day/night regime: 12 h at 15 °C with a light intensity of 150 μmol m⁻² s⁻¹ followed by 12 h at 9 °C in darkness. Infection types (ITs) were scored 2.5 weeks after inoculation. In case of resistant accessions, the test was repeated once again. The IT produced by plant–pathogen interactions was assessed on a scale of 0–9 (Line and Qayoum 1992). The ITs were summarized by combining them into three classes: 0–3 was considered resistant (R), 4–6 moderately resistant (M), and 7–9 susceptible (S) according to Qayoum and Line (1985). Data regarding susceptibility or resistance to stripe rust were recorded for each accession.

DNA extraction

Leaves of month-old plants were collected, frozen in liquid nitrogen, and stored at –80 °C. Genomic DNA was extracted using a large-scale protocol (Kidwell and Osborn 1992) with the modifications described by Xie et al. (2012).

SSR marker analysis

The SSR marker screening of plant DNA samples was performed by PCR in a 15 μl final volume containing 0.2 mM of each dNTP, 250 nM of each primer, 0.6 U of Taq polymerase (DreamTaq, Thermo Fischer Scientific, San Jose, CA), and 100 ng of template DNA.

Table 1 Graphical genotypes of wheat breeding lines that harbor the stripe rust resistance gene, *Yr15*, introgressed from *T. dicoccoides*

Wheat line	Xgwm911	Xwmc406	Xbarc8	Response to stripe rust	Xgwm413	Xgwm273	Xgwm498	Xgwm762	Pedigree	Ploidy
<i>T. dicoccoides</i> G25	338	213	221	R	93	164	154	153	<i>T. dicoccoides</i> donor of <i>Yr15</i> (G25 hereafter)	4X
<i>T. durum</i> D447	334	211	260	S	95	156	158	158	LD393/2 Langdon ND58-322 (D447 hereafter)	4X
B1	369	213	221	R	93	164	154	153	<i>T. durum</i> D447/3/G25	4X
B2	369	213	221	R	93	164	154	153	<i>T. durum</i> D447/3/G25	4X
B9	369	213	221	R	93	164	154	153	<i>T. durum</i> D447/3/G25	4X
B10	369	213	221	R	93	164	154	153	<i>T. durum</i> D447/3/G25	4X
B70	338	224	238	R	93	164	154	153	<i>T. durum</i> D447/3/G25	4X
280-1	334	218	230	R	91	156	160	158	<i>T. durum</i> *13/G25	4X
280-2	334	218	230	R	91	156	158	111	<i>T. durum</i> *13/G25	4X
Nursit 163	336	210	263	S	97	158	160	165	Land race, Israel. (Eyal and Levy, 1987)	4X
Merav	281	210	251	S	89	166	158	118	GABO/KENYA-324/GENERAL-URQUIZA	6X
Sel07-97	279	213	221	R	93	164	154	153	G25/N163/2/Merav ^a	6X
Sel46	281	213	221	R	93	164	154	153	G25/N163/2/Merav ^a	6X
Sel7	279	213	221	R	93	164	154	153	G25/N163/2/Merav ^a	6X
V763	283	213	221	R	93	164	154	153	BTL/3/NURIST163/G25/MERAV ^a	6X
Mexico 708	281	210	251	S	89	166	158	118	AQUILERA/KENYA-324/MARROQUII/SUPREMO/3/GABO	6X
Sel04-34H	279	213	221	R	93	164	154	153	G25/N163/2/M708	6X
Sel20	281	213	221	R	93	164	154	153	G25/N163/2/M708	6X
Sel32	281	213	221	R	93	164	154	153	G25/N163/2/M708	6X
V879-C1-1	312	213	248	R	93	164	154	153	N163/G25/M708/3/VC293	6X
AvocetS	283	217	257	S	101	168	158	140	WW-119/WW-15/EGRET	6X
Avocet-Yr15	283	213	221	R	93	164	154	153	V763/6/Avocet	6X
Corrijin	283	210	264	S	103	176	158	120	TINCURRIN/2/GAMENYA/IASUL	6X
Corrijin-Yr15	283	213	221	R	93	164	154	153	V763/5/Corrijin	6X
Excalibur	281	210	251	S	93	166	158	118	RAC-177/S(26)UNICULUM-492/RAC-311-S	6X
Excalibur-Yr15	281	210	251	R	93	166	158	118	V763/5/Excalibur	6X
Kulin	281	210	267	S	88	168	158	120	BODALLIN/GAMENYA/INIA-66	6X
Kulin-Yr15	281	213	221	R	93	164	154	153	V763/5/Kulin	6X
Stillete	281	210	255	S	105	168	158	161	Veranopolis/3/RAC177/2/3/Spear/3/Dagger	6X
Stillete-Yr15	281	213	221	R	93	164	155	153	V763/Yr15/5/Stillete	6X
Suncea	283	211	251	S	89	166	158	118	Ciano/2/Spica/Amber Mutant Sonora 64	6X
Suncea-Yr15	283	213	221	R	93	164	154	118	Avocet-Yr15/6/Suncea	6X
Baxter	281	210	267	S	87	168	158	120	Inia66/Garut/Cook/4/Jupateco/3/Lerma Rojo 64/Sonora 84A/Timgalen sib	6X
HSB-3177	283	213	221	R	87	168	158	168	Avocet-Yr24Yr15//2/Baxter	6X
Combat	283	217	260	S	95	168	158	140	GBA005/Banks	6X
HSB-2408	283	217	221	R	93	164	158	140	Avocet-Yr24Yr15//2/Combat	6X
Sapphire	283	217	257	S	101	168	158	140	GBA008/Janz	6X
HSB-2527	283	217	257	S	101	168	158	168	Avocet-Yr24Yr15//2/Sapphire	6X
Shenton	281	212	254	S	89	164	158	118	ALTAR-84/AE/SQI/SERI/3/SERI	6X
HSB-2801	281	212	254	R	93	164	158	118	Avocet-Yr24Yr15//2/Shenton	6X
Ruby	281	211	254	S	89	168	158	120	Ineia/Weever	6X
HSB-2944	281	213	254	R	93	167	158	120	Avocet-Yr24Yr15//2/Ruby	6X
HSB-2949	281	213	221	R	93	168	158	168	Avocet-Yr24Yr15//2/Ruby	6X
HSB-2955	281	213	221	R	93	164	158	120	Avocet-Yr24Yr15//2/Ruby	6X
UC1037	281	210	267	S	87	168	158	120	CLEO/66/ANZA/3/SOLAR	6X
UC1037-Yr15	281	213	221	R	93	164	154	153	UC1037/3/Sunfield-Yr15	6X
UC1041	281	210	266	S	87	170	158	122	Tadnia/Yecora Rojo	6X
UC1041-Yr15	281	213	221	R	93	164	154	153	UC1041/3/Sunfield-Yr15	6X
UC1128	281	211	254	S	89	168	158	120	OPATA/KILL/S	6X
UC1128-Yr15	281	213	221	R	93	164	154	153	UC1128/3/Sunfield-Yr15	6X
UC1107	281	210	248	S	103	168	158	122	TADORNAINIA66/PRLS	6X
UC1107-Yr15	281	213	222	R	93	164	154	153	UC1107/3/UC1128-Yr15	6X
UC1110	281	210	258	S	109	156	158	150	BB/CHA/YD/S/3/CKR/S	6X
UC1110-Yr15	281	213	221	R	93	164	158	118	UC1110/3/UC1128-Yr15	6X
UC1358	281	211	257	S	89	168	158	118	RSIS/Express	6X
UC1358-Yr15	281	213	221	R	93	164	154	153	UC1158/3/Sunfield-Yr15	6X
UCKern	281	210	257	S	89	168	158	118	Tadonia/Inia 66/Yecora Rojo/3/Klasic	6X
UCKern-Yr15	281	213	221	R	93	164	154	153	UCKern/3/Sunfield-Yr15	6X

The alleles obtained for seven simple sequence repeat (SSR) markers spanning the *Yr15* gene (Fig. 1) are shown for G25, the *Yr15* donor, *Yr15* ILs, and RPs. Alleles in yellow values are identical to those obtained for G25. Alleles in green values are different from those of G25. Resistance or susceptibility to *Pst* is specified and highlighted, respectively, in yellow or green values. For each wheat line, the pedigree and ploidy are specified if applicable. (Color table online)

^a Gerechter-Amitai et al. (1992) reported that the pedigree of these *T. durum* lines as LD393/2 Langdon ND58-322 (D447)

^b Lines with pedigree: BTL/3/NURIST163/G25//MERAV

^c Line referred here as Avocet-Yr24Yr15 was obtained by the following crosses: The donor line for the *Yr24* stripe rust resistance gene was crossed with Avocet S, and after six backcrosses, this line was crossed with Avocet-Yr15 to obtain a new Avocet line that carried two stripe rust resistance genes, designated as Avocet-Yr24Yr15

Table 2 Simple sequence repeat (SSR) marker information

Marker	Primer sequence	CS ^a (bp)	D447 (bp)	G25 (bp)	Allele number in 58 ILs and RPs
<i>Xwmc406-F</i> ^b	TATGAGGGTCGGATCAATACAA	217	211	213	7
<i>Xwmc406-R</i>	CGAGTTTACTGCAAAACAAATGG				
<i>Xbarc8-F</i> ^c	GCGGGAATCATGCATAGGAAAACAGAA	250	260	221	15
<i>Xbarc8-R</i>	GCGGGGGCGAAACATACACATAAAAACA				
<i>Xgwm413-F</i> ^d	TGCTTGCTAGATTGCTTGGG	93	95	93	11
<i>Xgwm413-R</i>	GATCGTCTCGTCCTTGGCA				
<i>Xgwm273-F</i> ^d	ATTGGACGGACAGATGCTTT	171	156	162	8
<i>Xgwm273-R</i>	AGCAGTGAGGAAGGGGATC				
<i>Xgwm498-F</i> ^d	GGTGGTATGGACTATGGACACT	159	158	155	4
<i>Xgwm498-R</i>	TTTGCATGGAGGCACATACT				
<i>Xgwm762-F</i> ^e	Unpublished	147	158	153	12
<i>Xgwm762-R</i>	Unpublished				
<i>Xgwm911-F</i> ^e	Unpublished	272	334	338	8
<i>Xgwm911-R</i>	Unpublished				

For the seven SSR markers used in the current study, this information includes primer name, sequence (if publicly available), and fragment length in *Triticum aestivum* Chinese Spring, *Triticum durum* D447, and *Triticum dicoccoides* G25

^a The model wheat Chinese spring was included in each run as a positive control and a reference line for allele size

^b Somers et al. (2004)

^c Song et al. (2002)

^d Röder et al. (1998)

^e Ganal and Röder (2007)

PCR was performed on the Veriti[®] 96-Well Fast Thermal Cycler (Applied Biosystems Inc. (ABI), Foster City, CA, USA). The PCR program comprised a 5-min initial denaturation at 94 °C followed by 35 cycles of: 94 °C for 30 s; 55–60 °C (depending of annealing temperature of the primer set used) for 30 s; and 72 °C for 30–60 s (depending on the size of the amplicon). Fragment analysis was carried out on a Model 3130xl DNA sequencer (ABI). Allele fragment sizes and genotypes were determined using GeneMapper V3.7 software (ABI). The bread wheat accession Chinese Spring (CS) served as a size reference in each electrophoresis run. The SSR markers that were used in the current study for genetic mapping of *Yr15*, as well as for screening the IL and RP lines, are listed in Table 2. These markers were selected from a large set of SSR markers mapped to chromosome 1B of bread wheat (Röder et al. 1998; Peng et al. 2000; Song et al. 2002; Somers et al. 2004; Ganal and Röder 2007), based on screening for polymorphism between the parental lines of the *Yr15* mapping population, as well as between the IL and RP lines.

Genetic mapping

For each polymorphic SSR marker, a χ^2 analysis was performed to test for deviation from the 1:2:1 expected segregation ratio in the F₂ mapping population. Linkage analysis and map construction were performed based on the evolutionary strategy algorithm included in the MultiPoint package (Mester et al. 2003), as described in Peleg et al. (2008).

Results

Identification of highly polymorphic SSR markers for genetic mapping of *Yr15*

A total of seven SSR markers, among those previously mapped to chromosome 1B, were screened in 58 *Yr15* IL and RP lines (Table 2). Among these markers, six polymorphic SSRs showed 7–15 different alleles within these lines, while one marker, *Xgwm498*, showed a lower polymorphism level with only four

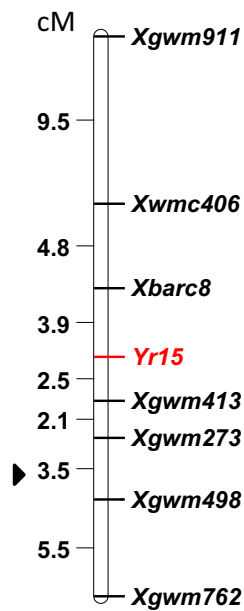


Fig. 1 Genetic map of the stripe rust resistance gene *Yr15*. The map is of chromosome 1BS of wild emmer wheat, developed by genotyping of 155 F₂ plants derived from the cross of susceptible D447 line with B9 or B10 ILs that contain *Yr15* and genotyped with seven SSR markers. The genetic distance in cM is shown on the left. The approximate position of the centromere, according to deletion bin mapping of markers *Xgwm273* to 1BS and *Xgwm498* to 1BL (Sourdille et al. 2004), is indicated by an arrowhead

alleles (Tables 1, 2). These seven markers were chosen to construct a genetic linkage map of chromosome 1B spanning the *Yr15* segment introgressed from the *T. dicoccoides* donor line G25 (Fig. 1) using an F₂ mapping population of 151 plants derived from a cross of the susceptible line D447 with B9 or B10 ILs containing *Yr15*. The resulting genetic map showed that the seven SSR markers span a region of 31.8 cM on chromosome 1B, with *Xgwm911* being the most distal marker (18.2 cM distal to *Yr15*) and *Xgwm762* the most proximal (13.6 cM proximal to *Yr15*). Sourdille et al. (2004) have used deletion bin mapping to assign marker *Xgwm273* to 1BS, whereas *Xgwm498* was assigned to 1BL. On this basis, the centromere location was marked in the current study on the *Yr15* genetic map between markers *Xgwm273* and *Xgwm498* (Fig. 1). Therefore, *Xgwm273* is the most proximal marker to the *Yr15* gene on the short arm of chromosome 1B. The *Yr15* gene was mapped to a 6.4 cM interval flanked by marker *Xbarc8*, located 3.9 cM to the distal side, and by *Xgwm413* located

2.5 cM to the proximal side. The obtained genetic map was used as a reference to determine the size of the introgressed chromosome segments harboring *Yr15* in the different ILs.

Evaluation of *Yr15* donor fragment sizes carried by introgression lines obtained by conventional and MAS backcrossing

The graphical genotypes concept, introduced by Young and Tanksley (1989), was implemented in our study for comparison of the size of *Yr15* donor fragments carried by ILs developed by conventional and MAS backcrossing. This method allowed the donor and RP chromosomal segments to be distinguished easily. The seven highly polymorphic SSR markers used for developing the genetic map described above (Fig. 1) were also used to genotype 58 wheat lines (34 ILs and the *Yr15* donor line resistant to *Pst* and 23 susceptible RPs) obtained from three different breeding programs scattered around the globe (Israel, Australia and California). These *Yr15* ILs were proved to be resistant at the adult plant stage to a wide range of *Pst* isolates, prevalent in the respective continents. The spectrum of marker alleles obtained for the seven SSR markers by screening of the 58 wheat lines is presented in Table 1 in a graphical genotype format. The order of the markers was defined by the *Yr15* genetic map described above (Fig. 1), and the genetic distances in cM were used to calculate the length of the introgressed donor segment.

In the Israeli breeding program, *Yr15* was introgressed from the donor *T. dicoccoides* line G25 through recurrent backcrossing directed by phenotypic selection. Lines B1, B2, B9, and B10 are ILs derived from the cross between G25 and the susceptible tetraploid *T. durum* line D447. In these ILs, the *Yr15* donor segments expand from the region between *Xgwm911* (not present) and *Xwmc406* in the short arm to a region beyond *Xgwm762* in the long arm. B70 is an additional IL derived from the same cross, which contains a shorter *T. dicoccoides* donor segment that spans from *Yr15* in the short arm to *Xgwm762* in the long arm. The B70 alleles for markers *Xwmc406* and *Xbarc8* are not present in either the donor G25 or the susceptible line D447. ILs 280-1 and 280-2 were found to be resistant to *Pst* in our survey (Table 1), yet exhibit alleles distinct from those of the *Yr15* donor line for all tested SSR markers.

Yr15 was also introgressed into two hexaploid (bread) wheat cultivars, Merav and Mexico 708, resulting in the selection lines designated here as Sel07-97, Sel46, and Sel7. Based on the available markers, these ILs contained the same *Yr15* donor segment as the tetraploid lines B1, B2, B9, and B10 described above. The same segment was detected in the hexaploid lines designated as V763 (Table 1). These lines were obtained in the Israeli breeding program by crossing tetraploid durum wheat containing *Yr15* (described above) with adapted bread wheat cultivars and then further advancing the progeny with high economic potential (Grama and Gerechter-Ami-tai 1974). Some of the V763 lines produced in the Israeli program were later used by the Australian Cereal Rust Control Program (ACRCP) at the University of Sydney for introgression of *Yr15* into elite Australian cultivars.

The hexaploid line Avocet S, susceptible to stripe rust, was crossed with V763 and subsequently backcrossed to obtain the *Yr15*-containing line Avocet-*Yr15* (Zakari et al. 2003). In a similar way, V763 derivatives (including Avocet-*Yr15*) were used to introgress *Yr15* into five additional Australian bread wheat cultivars (Corrigin, Excalibur, Kulin, Stilleto, and Suncea), which were used as RPs in conventional introgressions by backcrossing. The six ILs derived from these introgressions were screened with the seven SSR markers (Table 1). Among the six ILs, four lines (Avocet-*Yr15*, Corrigin-*Yr15*, Kulin-*Yr15*, and Stilleto-*Yr15*) contained the long segment including *Xwmc406* in the short arm and *Xgwm762* in the long arm (likely more than 30 cM) detected in V763. In the other two ILs, shorter donor intervals were found. For the IL Excalibur-*Yr15*, a donor fragment of 2.5–8.5 cM was identified by markers *Xbarc8* distal and *Xgwm273* proximal to *Yr15*. In Excalibur-*Yr15*, only *Yr15* and *Xgwm413* were retained from the original G25 donor chromosome. For IL Suncea-*Yr15*, a donor fragment of less than 31.8 cM was identified by markers *Xgwm911* distal and *Xgwm762* proximal to *Yr15*.

In a different set of crosses produced by the ACRCP, the susceptible Avocet S was crossed with the donor of another stripe rust resistance gene, *Yr24*, and backcrossed six times to produce the Avocet-*Yr24* NIL. This line was then crossed with Avocet-*Yr15* to obtain a new Avocet line carrying the two stripe rust resistance genes, designated as Avocet-*Yr15Yr24*

(Zakari et al. 2003). In these crosses, both MAS and phenotypic selection were applied. The construction of an Avocet IL combining both *Yr15* and *Yr24* was accomplished by double selection, first for the phenotype of *Yr15* and then for the genotype of the marker *Xgwm11* [located in 1BS bin 1BS9-0.84-1.06 (Sourdille et al. 2004)] linked to *Yr24* (Zakari et al. 2003). We screened five bread wheat RPs (i.e., Baxter, Combat, Sapphire, Shenton, and Ruby) and seven ILs derived from crosses with Avocet-*Yr15Yr24*. For all IL lines derived from these crosses, the G25 donor fragment did not include the 1B long-arm markers *Xgwm498* and *Xgwm762*. This likely reflects the recombination event between the *Yr15* and *Yr24* genes, since *Yr24* was mapped proximal to *Yr15* (Li et al. 2006). The shortest G25 donor fragment in the *Yr15Yr24* lines was found in lines HSB-2801 (4.6–12 cM; retained G25 alleles *Yr15* and *Xgwm273*) and HSB-3177 (8.7–20.7 cM; retained G25 alleles *Xwmc406* and *Yr15*). Among the *Yr15Yr24* lines, the longest G25 segment was detected in HSB-2955 (<24.2 cM; Table 1).

In the breeding program at the University of California at Davis, accession Sunfield-*Yr15* (pedigree WW31/4/W3566/Topo/3/Waite Line/IRN70.511//SUN15B), which harbors the *Yr15* donor fragment introgressed from line V763, was used to transfer *Yr15* into breeding lines and commercial varieties. In this breeding program, MAS was carried out using markers *Xbarc8* and *Xgwm273* (<http://www.plantsciences.ucdavis.edu/MASWheat/protocols/Yr15/index.htm>), which span a 8.5 cM interval (Fig. 1). From this program, we screened seven ILs and their corresponding susceptible RPs with the seven SSR markers. Analysis of ILs UC1037-*Yr15*, UC1041-*Yr15*, UC1128-*Yr15*, UC1107-*Yr15*, UC1358-*Yr15*, and UCKern-*Yr15*, derived from Sunfield-*Yr15* crosses, revealed that these lines contain *Yr15* donor segments including the short-arm marker *Xwmc406* and the long-arm marker *Xgwm762*. A shorter segment was detected only in IL UC1110-*Yr15*, where the two long-arm markers include non-G25 alleles. These lines, together with the Avocet-*Yr15*, have been tested in California for the last 13 years with no virulence detected in any of the lines. This result indicates that *Yr15* is still effective for the dozen of *Pst* races that have been detected during recent years in California (Chen 2005). This is particularly relevant because wheat varieties carrying *Yr15* now cover more than 88,000 acres in California

(http://smallgrains.ucdavis.edu/CWC_Wheat_Var_Survey_2013.pdf).

Taking these data together, marker analysis with seven SSRs of the 34 *Yr15* ILs obtained from three breeding programs revealed that the G25 donor fragment was delimited by SSR marker *Xgwm911* on the distal side of the gene; the most distal G25 allele was detected by the *Xwmc406* marker. In the majority of ILs (23/34), we found no additional recombination events between *Xwmc406* on the short arm and *Xgwm762* on the long arm, indicating that the complete centromeric region of G25 1B is present in these lines. Four lines showed shorter G25 1BS segments in the distal region (B70, Excalibur-15, HSB-2408, and HSB2801) and eight have lost the G-25 alleles for the two markers on the 1B long arm (Excalibur-*Yr15*, HSB-3177, HSB-2408, HSB2801, HSB-2944, HSB-2949, HSB2955, and UC1110-*Yr15*).

Evaluation of stripe rust resistance in the *Yr15* ILs and RPs

Phenotypic tests of the 58 wheat lines listed in Table 1 were made by inoculating with *Pst* race 38E134, which is avirulent on *Yr15* but virulent on *Yr2*, *Yr6*, *Yr7*, and *Yr9*, *Yr22*, *Yr23*, *YrSD* and *YrHVII* -containing plants (Cheng et al. 2010). IT types of 0 or 1 (resistant phenotype) were identified in our survey for all accessions that carry the G25 introgressed segment harboring *Yr15*. No moderate resistance ITs (4–6) were observed in our survey. Plants that were susceptible to stripe rust showed ITs of 7–9. Generally, RPs were found to be susceptible to this race of *Pst*, whereas ILs that contain the G25 chromosome segment, as identified by the SSR markers used here, showed resistance to *Pst*, indicating that these lines possess *Yr15*. Two cases were exceptions: lines 280-1 and 280-2 were found to be resistant, but no alleles of G25 could be identified by SSR genotyping. These lines may possess a stripe rust resistance gene other than *Yr15*. Alternatively, these two lines may contain a donor chromosomal segment that is shorter than could be detected by the markers used here, i.e., <6.4 cM, as a result of two recombination events, one between *Xbarc8* and *Yr15* and the other between *Yr15* and *Xgwm413*. In contrast, line HSB-2527 lacked both G25 donor alleles and *Pst* resistance, indicating that the segment introgressed into this line was probably lost during subsequent generations.

Discussion

Although the wild progenitors of cultivated crops harbor great reservoirs of genes and alleles conferring pathogen resistance, they also carry numerous traits that are undesirable in cultivated crops (Brouwer and St. Clair 2004). Introgression of resistance from wild progenitors is complicated by the need for many backcrossing and selection steps in order to eliminate undesirable donor traits (Niks et al. 2011). Decreasing the size of a donor fragment can remove genes with deleterious effects that are linked to the target gene (Hospital 2001). Therefore, the practical use of a target gene may be dependent on reducing linkage drag during IL development (Jacobsen and Schouten 2007). Molecular markers can be used to accelerate the removal of linked undesirable traits by specifically selecting for recombination between markers linked to the desirable and undesirable traits.

Here, we evaluated the introgression of the stripe rust resistance gene *Yr15*, which was derived from wild emmer wheat, *T. dicoccoides*, into commercial wheat cultivars. The ILs investigated here originated from several conventional and MAS breeding programs that differed from each other regarding their experimental design, use of markers, and the number of backcrosses performed. The lines included in our study had not undergone directed selection for limiting the size of the donor fragment surrounding the target gene, except for the lines combining *Yr15* and *Yr24* where the proximal region of *Yr15* was removed to incorporate *Yr24*. Thus, one of our aims was to evaluate donor fragment sizes in ILs developed by backcrosses under conventional phenotypic selection compared with those developed with MAS.

Our results demonstrate that the donor fragment can be dramatically shortened through conventional breeding. Among 19 ILs obtained by conventional breeding, two (10.5 %) possessed donor fragments delimited at both sides by the SSR markers used in the current study. Among the lines containing the *Yr15* gene that were investigated, the smallest donor fragment, less than 8.5 cM, was found in Excalibur-*Yr15*, which was developed by conventional backcross breeding. In comparison, the shortest donor fragment developed by MAS was less than 12 cM (IL HSB-2801). Reduction in the length of the donor fragment by conventional breeding depends not only on the recombination rate in the chromosomal interval

surrounding the target gene, but also on the severity of linked negative traits and on the difficulty of recognizing these traits during phenotyping. Hence, although the shortest donor fragment was indeed obtained by conventional breeding, MAS provided a more consistent reduction in linkage drag even when no selection for recombination was applied.

In the Australian program, Avocet-*Yr24Yr15* was obtained by MAS using marker *Xgwm11* and was then backcrossed conventionally to introgress *Yr15* into additional wheat cultivars (Zakari et al. 2003; Bariana unpublished results). All ILs obtained by these crosses contained relatively short donor fragments, bordered at both sides of *Yr15* with RP alleles as identified in the current study by SSR markers. In contrast, in the American breeding program, where two markers spanning the target gene served in marker-assisted foreground selection of *Yr15* (<http://www.plantsciences.ucdavis.edu/MASWheat/protocols/Yr15/index.htm>), all but one (UC110-*Yr15*) IL contained a long donor fragment, which was not delimited on the proximal side of *Yr15* (Table 1). The shorter segments in the *Yr24Yr15* lines were determined by specific selection for recombination events on the proximal side of *Yr15*. Since *Yr24* is close to *Yr15*, this resulted in the elimination of most of the original G25 alleles on the proximal side.

The evidence presented here thus highlights the beneficial role of marker choice in MAS programs and the consequent power to both rapidly shorten the segment around a selected gene and even to combine the gene with other desirable traits (e.g., *Yr15Yr24*). Marker choice in turn depends on the availability of sufficient markers to choose among, which can be limited by lack of polymorphism when transferring markers between populations or affected by variation in genetic positions between different mapping projects (Spielmeyer et al. 2003; Ben-David 2011). The feasibility of using MAS in practical breeding programs is determined by the reproducibility of the marker-gene association across generations and populations (Collard and Mackill 2008). Ideally, markers used for MAS should be diagnostic for traits in a wide range of breeding materials, clearly discriminating between cultivars by their expression of the target trait. SSR markers are widely used in major cereals because these markers are reproducible, codominant, and polymorphic, relatively simple and cheap to use, and

generally highly heritable (Gupta et al. 1999; Gupta and Varshney 2000).

Given a particular recombination rate for the region carrying the introgressed gene of interest, the backcross number will affect the length of the donor fragment. It has been calculated that a minimum of six backcrosses are normally required to restore (on average) 99 % of the genome of the recurrent parent when transferring one dominant gene (Stam and Zeven 1981). Because of the phenotype bias of conventional breeding methods, the donor segment can remain very large even with many BC generations (Young and Tanksley 1989). Young and Tanksley (1989) estimated that the size of donor segments may vary from 4 to more than 51 cM in NILs of tomato even after 11 backcross generations. In the current study, lines produced with the shortest donor segments were obtained by the Australian breeding program through five or six backcrosses for both conventional and MAS breeding. The *Yr15Yr24* lines, however, were specifically selected to reduce the G25 proximal region of *Yr15*. Timonova et al. (2013) showed that the length of the A^t chromosome fragment introgressed into common wheat from *Triticum timopheevii* was significantly reduced after the third backcross generation. Zhou et al. (2005), who developed 33 wheat NILs for 22 powdery mildew resistance genes, reported that some NILs developed by backcrossing for four generations with MAS were as similar to the RPs as those obtained after six or more conventional backcross generations, therefore demonstrating the high efficiency of MAS.

By screening a large number of *Yr15* ILs and RPs, we assessed the utilization of MAS for the practical introgression of *Yr15* into various tetraploid and hexaploid wheat accessions. Our results show that markers *Xbarc8* and *Xgwm413* can be used efficiently to select for *Yr15* and, if desired, the further markers *Xwmc406* and *Xgwm273* can be used in combination with these to select for recombination events in the *Yr15* flanking regions (Table 1). However, in some cases, recombination may occur between the marker and the target gene due to loose linkage (Sharp et al. 2001), and therefore, the use of two markers, flanking the gene on both sides, is preferable.

Yr15 confers remarkably broad spectrum resistance against a large collection of *Pst* isolates from all over the world (Gerechter-Amitai et al. 1989; Niu et al. 2000; Bariana et al. 2007; Murphy et al. 2009;

Chen et al. 2010; Randhawa et al. 2014). The genetic mapping of *Yr15* indicates that *Yr15* behaves as a single dominant gene. Nevertheless, because plant resistance genes tend to cluster (Hammond-Kosack and Jones 1997; McHale et al. 2006; Michelmore and Meyers 1998; Staskawicz et al. 1995), it remains formally possible that *Yr15* resides within a tightly linked cluster of genes that confers resistance in a more complex fashion. Indeed, wheat chromosome arm 1BS contains additional stripe rust resistance genes including *Yr9*, *Yr10*, *Yr24*, and *YrH52* (Li et al. 2006; Lin and Chen 2007; McIntosh et al. 2008). However, the high virulence of isolate 5006 used in mapping (Chen et al. 2010) increases the probability that the resistance conferred by the introgressed segment is due to a monogenic *Yr15*. Final proof of the single-gene hypothesis will need to await positional cloning of *Yr15* and verification of its utility in the absence of flanking regions.

For pyramiding genes for one or more traits of interest, MAS is highly useful, especially if these genes cannot be distinguished by phenotypic selection (Stuber 1991; He et al. 2004). MAS had been earlier used to pyramid three bacterial blight resistance genes (*xa5*, *xa13*, and *Xa21*) into indica rice (Singh et al. 2001). Gene pyramiding has a great advantage in the introgression of disease resistance genes because, while individual genes may not confer durable resistance, the combination of four or five genes can provide durable resistance for many years (Joshi and Nayak 2010). Although *Yr15* has already succeeded in conferring stripe rust resistance for many years in many introgression lines around the world, its application in a pyramid of resistance genes could extend its lifetime. Indeed, *Yr15* has been combined with *Yr24* in all HSB lines reported in Table 1. All the commercial varieties including *Yr15* in California also include *Yr5* specifically for this purpose. These varieties, which currently cover 14 % of the acreage of common wheat in California, have not shown negative traits associated with the introgression of either of these genes.

Conclusion

The molecular markers validated here can serve the wheat breeding community to accelerate the introgression of *Yr15* into lines of interest. The nearest

flanking markers *Xbarc8* and *Xgwm413* were consistent in distinguishing between the allele of the segment originating from the G25 donor line and the alleles originating from the susceptible recurrent parents and hence are the current best choice for MAS of *Yr15*. The introgression of *Yr15* has proved to be very useful in controlling the stripe rust epidemics in California and might be of use in the rest of the western USA (Chen 2005, 2007; Chen et al. 2002, 2010). The practical exploitation of wild emmer wheat for genes of interest beneficial to agriculture serves as a strong argument for conservation of the natural habitats in which wild emmer wheat populations grow.

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