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ORIGINAL ARTICLE

Mapping of a new stem rust resistance gene *Sr49* in chromosome 5B of wheat

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Abstract

Key message **A new stem rust resistance gene *Sr49* was mapped to chromosome 5BL of wheat. Usefulness of the closely linked markers *sun209* and *sun479* for marker-assisted selection of *Sr49* was demonstrated.**

Abstract Landrace AUS28011 (Mahmoudi), collected from Ghardimaou, Tunisia, produced low stem rust response against Australian pathotypes of *Puccinia graminis* f. sp. *tritici* (Pgt) carrying virulence for several stem rust resistance genes deployed in modern wheat cultivars. Genetic analysis based on a Mahmoudi/Yitpi F3 population indicated the involvement of a single all-stage stem rust resistance gene and it was temporarily named *SrM*. Bulked segregant analysis using multiplex-ready SSR technology located *SrM* on the long arm of chromosome 5B. Since there is no other all-stage stem rust resistance gene located in chromosome 5BL, *SrM* was permanently designated *Sr49*. The Mahmoudi/Yitpi F3 population was enhanced to generate F6 recombinant inbred line (RIL) population for detailed mapping of *Sr49* using publicly available genomic resources. Markers *sun209* and *sun479* flanked *Sr49* at 1.5 and 0.9 cM distally and proximally, respectively. Markers *sun209* and *sun479* amplified PCR products different than the *Sr49*-linked alleles in 146 and 145 common wheat cultivars, respectively. Six and seven cultivars, respectively, carried the resistance-linked marker alleles $sun209_{148bp}$ and $sun479_{200bp}$; however, none of the cultivars carried both resistance-linked alleles. These results demonstrated the usefulness of these markers for marker-assisted selection of *Sr49* in breeding programs.

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Introduction

Stem rust of wheat (*Triticum aestivum* L.), caused by *Puccinia graminis* f. sp. *tritici* (Pgt), is a very destructive disease in warmer wheat growing regions including the north-eastern wheat belt of Australia. Resistance to stem rust in wheat is classified into two categories: all-stage resistance (ASR) and adult plant resistance (APR). High levels of ASR can be conditioned by a single resistance locus of major effect. In contrast, combinations of three to four APR loci are required to express commercially acceptable level of resistance (Bariana and McIntosh 1995; Bariana et al. 2007; Singh et al. 2000). ASR is often short-lived due to the evolution in Pgt populations to acquire virulence for genes soon after their deployment in commercial cultivars, especially when deployed singly. The combination of two types of resistance has been shown to produce lower stem rust responses (Bariana et al. 1996, 2007).

Deployment of stem rust resistance genes *Sr6* and *Sr11* singly in Australian cultivars Eureka and Gabo, respectively, and the subsequent detection of virulence in the Pgt population for these genes stressed the need to deploy combinations of resistance genes (Watson and Singh 1952). Subsequently, cultivars carrying combinations of stem rust resistance genes were released in Australia (Bariana et al. 2007). Identical infection types produced by several stem rust resistance genes and the expression of high levels of

resistance pose problems for identifying genotypes carrying more than one gene. Although the presence of more than one APR gene can be observed based on additive interactions, it is difficult to ascertain the number of genes present. Molecular markers linked with rust resistance genes offer a solution to this problem. Fifty-eight stem rust resistance (*Sr*) genes have been identified and named (McIntosh et al. 2014). Most of these genes are qualitatively inherited; except *Sr2*, *Sr55*, *Sr56*, *Sr57* and *Sr58* which confer APR (McIntosh et al. 1995, 2014; Bansal et al. 2014). The identification and deployment of genetically diverse sources of resistance is essential to ensure the durability of resistance. A diverse collection of wheat landraces collected by the English botanist Arthur Watkins in the 1920s (Miller et al. 2000) from 32 different geographical regions was used to identify new sources of resistance. Genotype AUS28011 produced low stem rust responses against Australian Pgt pathotypes used in this study. Here, we describe the inheritance of resistance, chromosomal location and development of closely linked markers with the stem rust resistance gene in AUS28011.

Materials and methods

Plant materials and population development

A landrace AUS28011 (local name = Mahmoudi) was collected from Ghardimaou, Tunisia at latitude of 36.45°N and longitude of 8.43°E. This genotype belongs to a set of germplasm assembled by A. E. Watkins during 1920–1930 (Miller et al. 2000). This collection is housed at the Australian Winter Cereal Collection, Tamworth. Mahmoudi was crossed with stem rust susceptible Australian cultivar Yitpi. F1 plants were grown and selfed to produce F2 population. One hundred and ten F2 seeds were sown 10 cM apart in field and 101 plants were successfully harvested and threshed individually to produce F3 population. Single seed from each F3 family was sown and single head from each plant was harvested to generate F4 population. The same process was followed to produce F5 and F6 populations. A set of Australian and Nordic common wheat cultivars was used to check polymorphism of stem rust resistance-linked markers (Table 4).

Pathogen materials and rust response assessment

Details of the Pgt pathotypes used are given in Table 1. The parent 'Mahmoudi' and known gene differential genotypes were sown in the greenhouse in 9-cm pots filled with potting mix comprised of composted pine bark and sand (2:1 ratio) and evaluated against five Australian Pgt pathotypes at the University of Sydney Plant Breeding Institute (PBI), Cobbitty. The potting mix filled pots were fertilised with a water soluble fertilizer Aquasol® (30 g/10 L for 100 pots). For the inheritance study, 30 seeds of each Mahmoudi/Yitpi F3 family were sown as one family per pot, whereas four lines per pot with eight to ten seed clumps of each line were sown for the RIL population. Parents and the susceptible control Morocco were sown with each experiment. Pots were transferred to a rust-free microclimate room maintained at 20 °C after sowing. A second application of the nitrogenous fertilizer urea was applied at the same rate as Aquasol to the 1-week-old seedlings. Ten- to twelve-day-old seedlings were inoculated with the Australian Pgt pathotype 34-1,2,3,6,7,8,9 using the procedure described in Bariana and McIntosh (1993). The inoculated seedlings

Table 1 Infection types produced by parental lines and avirulence/virulence formulae of Pgt pathotypes used

Pathotypes	Culture no.	Infection type		Avirulence/virulence
		Mahmoudi	Yitpi	
34-1,2,3,4,5,6,7	103	2−	3	*Sr8b*, *Sr9e*, *Sr13*, *Sr24*, *Sr27*, *Sr30*, *Sr31,* *Sr32*, *Sr33*, *Sr35*, *Sr37*, *Sr38*, *Sr39*, *Sr40*, *Sr45*, *Sr46*, *SrAgi*, *SrEm*, *Srsatu/Sr6*, *Sr8a*, *Sr9b*, *Sr9g*, *Sr11*, *Sr12*, *Sr15*, *Sr17*, *Sr36*
34-1,2,3,6,7,8,9	205	2−	3+	*Sr8b*, *Sr9e*, *Sr13*, *Sr17*, *Sr24*, *Sr27*, *Sr31,* *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr38*, *Sr39*, *Sr40*, *Sr45*, *Sr46*, *SrEm*, *Srsatu/Sr6*, *Sr8a*, *Sr9b*, *Sr9 g*, *Sr11*, *Sr12*, *Sr15*, *Sr30*, *SrAgi*
98-1,2,(3),(5),6	279	2−	3	*Sr8b*, *Sr9e*, *Sr13*, *Sr15*, *Sr24*, *Sr27*, *Sr30*, *Sr31*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr38*, *Sr39*, *Sr40*, *Sr45*, *Sr46*, *SrAgi*, *SrEm*, *Srsatu/Sr6*, *Sr8a*, *(Sr9b)*, *Sr9 g*, *Sr11*, *Sr12*, *(Sr17)*
40-1,2,3,4,5,6,7	383	2=	3	*Sr8b*, *Sr13*, *Sr24*, *Sr27*, *Sr30*, *Sr31*, *Sr32*, *Sr33*, *Sr35*, *Sr37*, *Sr38*, *Sr39*, *Sr40*, *Sr45*, *Sr46*, *SrAgi*, *SrEm*, *Srsatu/Sr6*, *Sr8a*, *Sr9b*, *Sr9e*, *Sr9g*, *Sr11*, *Sr12*, *Sr15*, *Sr17*
343-1,2,3,5,6,(8),9	465	2−	33+	*Sr8b*, *Sr9e*, *Sr9 g*, *Sr13*, *Sr15*, *Sr24*, *Sr27*, *Sr31,* *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr38*, *Sr39*, *Sr40*, *Sr45*, *Sr46*, *SrEm*, *Srsatu/Sr6*, *Sr8a*, *Sr9b*,*Sr11*, *Sr12*, *Sr17*, *(Sr30)*, *SrAgi*

were transferred to a microclimate room maintained at 25 °C. Stem rust seedling responses were noted 14 days after inoculation using the scale described in Luig (1983). In addition to the numeric value of the disease score, symbols '+' (plus), '−' (minus) and '=' (double minus) were used to note deviations from standard responses. The F3 population was classified into non-segregating resistant (NSR), segregating (SEG) and non-segregating susceptible (NSS) categories. The RIL population was categorised as homozygous resistant (HR) and homozygous susceptible (HS).

Molecular mapping

DNA was extracted from the Mahmoudi/Yitpi F3 population, RIL population, parents and 152 wheat cultivars following the method described in Bansal et al. (2010). Bulk segregant analysis was performed using multiplex-ready SSR technology (Hayden et al. 2008) to determine chromosomal location of the underlying resistance gene. The resistant and susceptible bulks comprised of equal amounts of pooled DNA from 20 homozygous resistant and 20 homozygous susceptible F3 families, respectively. Nine hundred seventy-six microsatellite (SSR) markers were tested for polymorphism and linkage between the bulks. SSR markers linked between the bulks were genotyped on the entire Mahmoudi/Yitpi F3 population. To confirm chromosomal location of the linked marker *wmc471*, genotyping of Chinese Spring (CS) and nulli-tetrasomic and di-telosomic stocks in the CS background (Table 2) was performed.

To saturate the chromosome 5BL region carrying the resistance gene, two sources of published DNA markers were used. The first corresponded to STS and SSR markers linked with *Sr56* in chromosome 5BL (Bansal et al. 2014) and these were derived from DArT clones (http://www.triticarte.com.au) and assembled flow-sorted chromosome survey sequence of cultivar Chinese Spring (IWGSC 2014), respectively. The second source included SNPs from the iSelect 90K wheat SNP assay (Wang et al. 2014) that were genetically mapped to the distal region of chromosome 5BL. These SNPs were genotyped using the KASPar assay (http://www.lgcgenomics.com/genotyping/kasp-genotyping-chemistry/). Markers that were polymorphic between the parents were genotyped on the entire RIL population. SSR markers designed in this study were named as '*sun*' (Sydney University) and KASP markers from 90K Infinium Arrays as '*IWB*'.

Statistical analysis and genetic mapping

Chi-squared analysis was performed to test the goodness-of-fit of the observed segregation to the expected genetic ratios. Linkage analysis was performed using MapManager version QTXb20 (Manly et al. 2001). Map distances were computed using the Kosambi mapping function (Kosambi 1943).

Table 2 Genotyping of Chinese spring (CS) nulli (N)–tetra (T) and CS di-telosomic (DT) 5BL stocks with *wmc471*

Genotype	PCR product (bp)[a]	Chromosome
CSN3B-T3A	254	3B
CSN3B-T3D	254	3B
CSN4A-T4B	196	4A
CSN4A-T4D	196	4A
CSN5B-T5A	214	5B
CSN5B-T5D	214	5B
CSN7B-T7A	210	7B
CSN7B-T7D	210	7B
Mahmoudi	214	5B
Yitpi	Null	5B
CSDT 5BL	214	5BL

[a] CS amplified all four products

Fig. 1 Seedling (**a**) and adult plant (**b**) responses produced by Mahmoudi (*left*) and Yitpi (*right*)

Results

Inheritance studies

Infection types (ITs) produced by Mahmoudi varied from 2= to 2− and Yitpi exhibited higher ITs ranging from 3 to 3+ against Australian Pgt pathotypes (Table 1; Fig. 1a). Pathotype 34-1,2,3,6,7,8,9 produced a relatively higher infection type on Yitpi and, therefore, was used for

screening Mahmoudi/Yitpi F3 and RIL populations. Stem rust response variation among Mahmoudi/Yitpi F3 families conformed to monogenic segregation with 21 families scored as NSR (IT 2−), 51 SEG (ITs 2−, 3+) and 29 NSS (IT 3+). Chi-squared analysis of the segregation data was good fit for a 1 NSR:2 SEG:1 NSS ratio ($\chi^2_{1:2:1} = 1.28$, non-significant at $P = 0.05$ and 2 *df*). The Mahmoudi/Yitpi RIL population also showed segregation at single locus (43 HR: 49 HS; $\chi^2_{1:1} = 0.39$, non-significant at $P = 0.05$ and 1 *df*). The resistance locus was temporarily named *SrM*.

Molecular mapping

Bulk segregant analysis using multiplex-ready PCR showed association of SSR markers *gwm160*, *barc190*, *barc282* and *wmc471* with *SrM*. Markers *gwm160*, *barc190* and *barc282* inherited independently of *SrM* when genotyped on the entire F3 population. In contrast, *wmc471* was mapped 7.2 cM away from *SrM*. Marker *wmc471* was previously mapped on chromosomes 4A, 3B and 7B (http://wheat.pw.usda.gov/cgi-bin/graingenes/report) and it was also observed to map on chromosome 5B (M. H. Hayden unpublished data). To confirm the chromosome location of *wmc471*, we tested DNA from the Chinese Spring nullitetrasomic stocks (Table 2). In Chinese Spring, *wmc471* produced four amplicons: 196, 210, 214 and 254 bp, which could be assigned to chromosomes 4A, 7B, 5B and 3B, respectively. In this study, *wmc471* amplified a chromosome 5B-specific 214 bp product in Mahmoudi and failed to amplify any product in Yitpi. These results indicated the location of *wmc471* in chromosome 5B. To confirm the chromosomal arm, di-telosomic 5BL stock was genotyped with *wmc471*. The amplification of a 214 bp product indicated the location of *wmc471* in chromosome 5BL. By virtue of its linkage with *wmc471*, *SrM* was concluded to be located in chromosome 5BL. Since there is no other all-stage stem rust resistance gene located in chromosome 5BL, *SrM* was permanently designated *Sr49*.

Marker *wmc471* mapped on the Mahmoudi/Yitpi F6 RIL population. To detect close marker–trait associations, allele specific primers for 42 SNPs from the distal region of chromosome 5BL (Wang et al. 2014) were designed. Six SNPs revealed polymorphism between the parental lines and were genotyped on the entire Mahmoudi/Yitpi RIL population. Four SNPs (*IWB61646*, *IWB24987*, *IWB35866* and *IWB28598*) showed linkage with *SrM*. Of the 45 SSR markers derived from assembled flow-sorted chromosome 5BL survey sequence contigs of cultivar Chinese Spring that were orthologous to *Brachypodium* genes *Bradi1g00760* to *Bradi1g01470* (Bansal et al. 2014), six were polymorphic between Mahmoudi and Yitpi. These markers (denoted by the prefix 'sun') were genotyped on the entire RIL population. In total, 12 loci (including *Sr49*) spanned

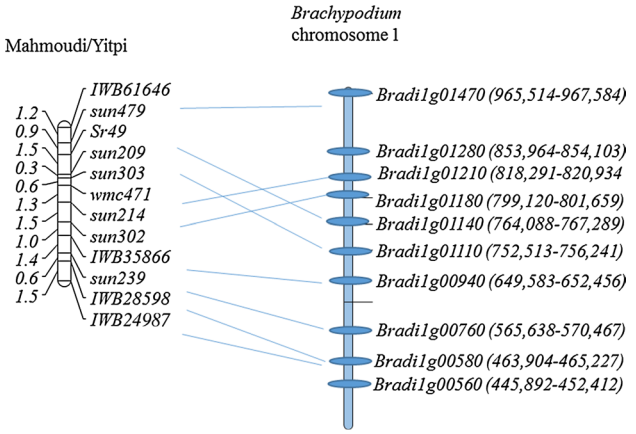

Fig. 2 Genetic linkage map showing location of *Sr49* in Mahmoudi/Yitpi RIL population and diagrammatic presentation of orthologous region in *Brachypodium* chromosome 1

11.8 cM interval. *SrM* was flanked by *sun479* (proximal) and *sun209* (distal) at a distance of 0.9 and 1.5 cM, respectively (Fig. 2). Comparison of the wheat marker loci order and *Brachypodium* gene order in the region of interest indicated an inversion. Primer sequences of markers used in this study are listed in Table 3.

Polymorphism of flanking markers among wheat cultivars

Flanking markers *sun209* and *sun479* were tested on a set of 69 Australian and 83 Nordic (Finland, Norway, Sweden) wheat cultivars lacking the stem rust resistance gene *Sr49* (Bariana and co-workers unpublished results; Table 4). Marker *sun209* amplified a 148 bp product in Mahmoudi and a 158 bp fragment in Yitpi. Of 152 test cultivars, 145 produced amplicons (140–158 bp) different than the *Sr49*-linked $sun209_{148bp}$ allele. Nordic genotypes Blanka, Nora and Norrøna and Australian genotypes Axe, Correll, EGA Bonnie Rock and Wedin amplified the resistance-linked 148 bp product. All Nordic and 63 Australian genotypes carried the $sun479_{185bp}$ allele, whereas six Australian genotypes possessed the *Sr49*-linked $sun479_{200bp}$ allele. None of the 152 genotypes amplified *Sr49*-linked alleles for both markers. These results demonstrated the usefulness of both markers in marker-assisted selection of *Sr49* in breeding programs. These markers can be used singly following assessment of polymorphism between parents to be crossed.

Discussion

Landraces are known to be a rich source of genetic diversity for resistance to biotic stresses (Bansal et al. 2013; Daetwyler et al. 2014). A genome-wide association study on the

Table 3 Primer sequences of markers used to refine the *Sr49* map location

Marker	Primer	Sequence
sun479	F	CAAATGAAATGTGATCCTGTT[a]
	R	TCATCTAACCAGCAATGGTAT
sun209	F	AGACTATGAGCTTCGCTATTG
	R	GTGATTGGTTCGGATTACTTA
sun214	F	TCTGTTGTATTGTGGATACGA
	R	GCTAAACACACTATCCTGCAC
sun239	F	AAGGTTCTCAGGTTCTCAAAC
	R	GAGGCATTCAAACATAACAAA
sun302	F	CTCGCTGAATTATTTTGTCAT
	R	CAATAAATAAATTGCGCTCAG
sun303	F	GCACGAGTAATAAATCATCCA
	R	ACACCAAACATAGCACAACTC
IWB61646	A1	AGCTGACCCATGCAACG[b]
	A2	CAGCTGACCCATGCAACA[c]
	C	AACAAAGCTGAGGGATAGTTCG
IWB24987	A1	GACTTGCGGCCATGAGAG
	A2	GACTTGCGGCCATGAGAA
	C	CCGATGATGAAAGTGATGACC
IWB35866	A1	CATCGACGGCAAGAAGAAG
	A2	CATCGACGGCAAGAAGAAA
	C	CGTAGGAACTCCTGCTCCAT
IWB28598	A1	CCATGGTTTAGCAAGCACG
	A2	CCATGGTTTAGCAAGCACA
	C	TGAACTGATCCATCATACTCACG

[a] M13 seq: CACGACGTTGTAAAACGAC attached at the 5' end of *sun* primers

[b] Fam seq: GAAGGTGACCAAGTTCATGCT attached at the 5' end of A1 primers

[c] Hex seq: GAAGGTCGGAGTCAACGGATT attached at the 5' end of A2 primers

Watkins collection of landraces using DArT markers identified marker–stem rust resistance associations in chromosomes 1BL, 3A, 3B, 4BL, 5BL, 6BS and 7BS (Bansal et al. 2013). To validate these predictions, mapping populations based on several resistant parents were developed. Genetic analysis of stem rust resistance in landrace Mahmoudi from the Watkins collection revealed the involvement of a single gene and the locus was temporarily named *SrM*. Association of marker *wmc471* with *SrM* led us to plan critical experiments to confirm the genomic location of *wmc471* and consequently *SrM*. The location of *wmc471*in chromosome 5BL was confirmed and *SrM* was permanently named *Sr49*. These results validated the prediction of Bansal et al. (2013) for involvement of chromosome 5BL in controlling stem rust resistance.

To refine position of the *Sr49* resistance locus in chromosome 5BL, published genomic and genetic marker resources were used. We tested SSR markers developed by Bansal et al. (2014) for the 5BL region and SNPs located in the most distal region of chromosome 5BL (Wang et al. 2014). SSR markers *sun479* and *sun209* flanked *Sr49* at 0.9 cM (proximal) and 1.5 cM (distal) genetic distances, respectively (Fig. 2). A comparison of synteny between the consensus SNP map (Wang et al. 2014) and *Brachypodium* genes suggested the telomeric location of *Sr49*. An adult plant stem rust resistance gene *Sr56* has been previously mapped 2.6 cM distal to *sun209* in chromosome 5BL (Bansal et al. 2014), whereas *Sr49* is 1.5 cM proximal to *sun209*. The comparison of *Brachypodium* genes indicated an inversion in the region flanking *Sr49* distally.

Sr49 was observed to be effective against all commercially important Australian Pgt pathotypes and produced a resistant to moderately resistant (R-MR) response under field conditions (Fig. 1b). It could, therefore, be used effectively in wheat breeding in combination with other stem rust resistance genes. The amplification of alleles other than those linked with *Sr49* for both markers in more than 95 % wheat cultivars (145 and 146/152) indicated the usefulness of *sun209* and *sun479* for marker-assisted selection of this gene in these backgrounds. Markers for stem rust resistance genes *Sr2*, *Sr13*, *Sr15*, *Sr22*, *Sr25*, *Sr26*, *Sr28*, *Sr33*, *Sr35*, *Sr39*, *Sr44*, *Sr45*, *Sr47*, *Sr52*, *Sr55/Yr46/Lr67*, *Sr56* and *Sr57/Yr18/Lr34* (http://maswheat.ucdavis.edu/protocols/index.htm; ES Lagudah pers comm; Bansal et al. 2014; Jayatilake et al. 2013) have been reported. *Sr2*, *Sr55/Yr46/Lr67*, *Sr56* and *Sr57/Yr18/Lr34* belong to the APR category. Although these genes are considered to provide durable rust control, they do not provide an acceptable level of resistance individually. *Sr49* can be pyramided with these marker-tagged genes to provide enhanced level of protection. Unlike many wild relatives-derived sources of stem rust resistance, the common wheat origin of *Sr49* should not involve linkage drag of deleterious effects.

Wheat breeding is a complex process and sometimes improvement of resistance to one rust pathogen can result in susceptibility to another. The deployment of stripe rust resistance gene *Yr10* in cultivar Angas in Australia resulted in the loss of stem rust resistance gene *Sr22* from the recurrent parent in 1991. The availability of markers linked with *Yr10* and *Sr22* at that stage would have facilitated the selection of combination of these genes. Triple rust resistance is a foremost selection criterion in wheat breeding programs to save production losses. Markers linked with several stripe rust and leaf rust resistance genes have been identified (http://maswheat.ucdavis.edu/protocols/index.htm; Bansal et al. 2010; Bariana et al. 2007; ES Lagudah pers comm; Mandoulakani et al. 2015; Randhawa et al. 2014). These markers can be used to deploy triple rust resistance in future wheat cultivars. Development of triple rust resistant donor sources would add to the training population candidates for wheat programs aiming to perform genomic selection.

Table 4 Polymorphism of *Sr49*-linked markers among Australian and Nordic wheat cultivars

Cultivars/genotypes	Allele size (bp)	
	Sun209	*sun479*
Mahmoudi (*Sr49*)	148	200
EGA Bounty—Australian	138	185
Eroica—Nordic	140	185
Aros—Nordic	145	185
Gazelle, King Rock, Orion—Australian	146	185
Blanka, Nora, Norrøna—Nordic	148	185
Axe, Correll, EGA Bonnie Rock, Wedin—Australian		
Diamant ll, Fram l, Jokikylä ME0505 Apu, Landvårkveite, Lavett, Møystad, MS 273–150, Progress, Trym—Nordic	150	185
Baxter, Bolac, Catalina, Derrimut, EGA Wedgetail, Espada, Gladius, Impose CL Plus, Janz, Kord CL Plus, Mace, Merinda, Merlin, Naparoo, Spitfire, SQP Revenue, Sunco, Sunguard, Sunvale, Sunvex, Waagan, Wallup, Wylah, Young—Australian		
Apu, Atson, Avle, Bastian, Bjarne, Boru, Brons, Canon, Dacke, Drabant, Dragon, Ergo, Extra Kolben, Fram ll, Haarajärvi ME0102 Apu, Horsmanaho ME201 Timantti, Kadett, Kärn ll, Kimmo, Kiuru, Laitiala AP0103, Lantvete-från-Dalarna, Lantvete-från-Halland, Monola ME1301, Naxos, Nemares, Østby, Reno, Rubin, Runar, Ruso, Saffran, Sibirian, Skirne, Snøgg II, Snøgg l, Touko, Vinjett, Vitus, Walter, William, Zebra—Nordic	152	185
Beaufort, Chara, Cobra, Corack, Giles, Impala, Phantom, Preston, Sentinel, Shield—Australian		
Børsum, Dala, Dalarna, Diamant 1, Fagott, Fylgia l, Fylgia ll, Halland, Järvenkylä ME0302 Timantti, Kärn, Manu, Polkka, Pompe, Pondus, Prins, Rang, Ring, Rival, Rollo, Sappo, Sopu, Sport, Svenno, Timantti Paavo, Tjalve, Troll, Ulla—Nordic	154	185
AGT Katana, Calingiri, EGA Gregory, EGA Wylie, Fortune, GBA Sapphire, Kunjin, Mackellar, Strzelecki, Yandanooka—Australian		
Carnamah, Crusader, Diamondbird, EGA Burke, Envoy, Livingston, Suntop, Sunzell, Ventura—Australian	185	156
Estoc, Justica CL Plus, Scout, Yitpi—Australian	158	185
Gauntlet, Lang—Australian	150	200
Dart, Forrest, Lincoln—Australian	152	200
Magenta—Australian	154	200

The development of donor sources is not only limited to rust resistance, careful choice of initial parental material followed by top crosses can also ensure capturing of other marker-tagged traits.

Author contribution statement HB and UB developed segregating population and conducted phenotypic assessments. UB, SM, KF and MH conducted molecular genotyping. All authors contributed towards writing up of this manuscript.

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