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ORIGINAL PAPER

Characterisation and mapping of gene *Lr73* conferring seedling resistance to *Puccinia triticina* in common wheat

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Abstract

Key message **A gene conferring seedling resistance to *Puccinia triticina* was mapped to chromosome 2BS in the wheat Morocco. The gene was shown to be distinct and was therefore designated *Lr73*.**

Abstract The wheat genotype Morocco, widely susceptible to isolates of *Puccinia triticina*, was resistant to an Australian isolate of this pathogen collected in 2004. Genetic studies established that the resistance in Morocco was also present the Australian wheat genotypes Avocet, Halberd, Harrier, Tincurrin and a selection of cultivar Warigal lacking the resistance gene *Lr20*. Genetic studies based on a cross with Halberd showed that the gene is dominant and located on chromosome 2BS (*XwPt8760*—4 cM—*Lr73*—1.4 cM—*XwPt8235*). The gene was genetically independent of the *Lr13*, *Lr16* and *Lr23* loci, also located on chromosome 2BS, indicating that it is distinct. The locus designation *Lr73* was therefore assigned. On the basis of multi-pathotype tests, it is likely *Lr73* is also present in the Australian wheat cultivars Clearfield STL, Federation (with *Lr10*), Gatcher (with *Lr10* and *Lr27+Lr31*), Marombi (with *Lr1* and *Lr37*), Pugsley (with *Lr1* and *Lr37*), Spear (with *Lr1*), Stiletto and Tarsa (with *Lr1*). Gene *Lr73* is unlikely to be of value in resistance breeding. However, recognising *Lr73* is important to avoid its inadvertent selection in breeding programmes. Furthermore, the apparent rarity of avirulence for genes like *Lr73*, sometimes referred to as "fossil" resistance genes, makes them of interest in terms of the evolution of disease resistance in host plants and of virulence in the respective rust pathogens.

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Introduction

Many genes that confer resistance to rust pathogens in wheat have been identified and utilised by breeders to develop rust resistant cultivars. Genes at 71 (Singh et al. 2013), 53 (Xu et al. 2013) and 54 (Ghazvini et al. 2013) leaf rust, stripe rust and stem rust resistance loci, respectively, have been identified and catalogued in wheat; most of them are expressed at seedling growth stages and known as either seedling or major resistance genes. While many major rust resistance genes have been overcome by pathogen isolates with matching virulence, they continue to play an important role in protecting wheat crops from rust diseases.

In the absence of a perfect marker, a wheat rust resistance gene can only be detected using a pathogen isolate with the matching avirulence allele. Cereal genotypes that are susceptible to all known isolates of a given rust pathogen are often used by rust workers as susceptible checks and to increase inoculum. Such genotypes may carry one or more resistance genes that have not been detected because isolates carrying the matching avirulence allele (s) are unknown. The wheat genotype Morocco, for example, is used widely as a susceptible check because of its

extreme susceptibility to leaf rust, stem rust and stripe rust. Despite this, Ali et al. (1994) reported two isolates of the leaf rust pathogen, *Puccinia triticina*, which were avirulent on Morocco. The isolates were also avirulent on the wheat genotype Little Club, which like Morocco, was used for many years as a susceptible check for *P. triticina* (Ali et al. 1994). A genetic analysis of the resistances identified in Morocco and Little Club indicated a single recessive gene for resistance in the former (*LrMo*), effective against an isolate of *P. triticina* collected from bread wheat in Bulgaria, and a dominant gene for resistance in the latter (*LrLC*), which based on pathogen specificity, differed from *LrMo*. Clearly, the widespread virulence in *P. triticina* for these genes indicates that they have very little or no value in breeding new wheat cultivars with resistance to this pathogen. However, where avirulence for these genes occurs, recognising them can be important to avoid their inadvertent selection in breeding programmes. Furthermore, the apparent rarity of avirulence for these genes, sometimes referred to as "fossil" genes, makes them of interest in terms of the evolution of disease resistance in host plants and of virulence in the respective rust pathogens.

Isolate 040120 of *P. triticina* was collected from wheat cultivar (cv.) Mackellar at Bairnsdale (Victoria, Australia) in late 2004, and was shown to be avirulent on Morocco (R.F. Park unpublished). Greenhouse seedling assessments of the pathogenicity of this isolate on most of the catalogued "*Lr*" genes indicated that it was virulent to many of these genes (Table 1), consistent with it being a typical common wheat attacking form of the leaf rust pathogen *P. triticina*. It was given the Australasian pathotype designation 10-1,3,(7),9,10,11,12 (R.F. Park unpublished). While pt. 10-1,3,(7),9,10,11,12 was virulent on the leaf rust susceptible genotypes Little Club, Sonora and Thatcher, it was avirulent on Morocco and several other wheat genotypes that were either previously thought to lack resistance to leaf rust (e.g. Halberd, Tincurrin) or that showed resistant (low) phenotypes on some wheat cultivars that had not been seen previously with other *P. triticina* isolates (e.g. Avocet, Gatcher, Harrier). The present study was undertaken to characterise the seedling resistance in Morocco to this isolate, and to investigate the genetic basis of the atypical low phenotypes it generated on genotypes Avocet, Halberd, Harrier, Tincurrin and a selection of cultivar Warigal lacking the resistance gene *Lr20* (Warigal −*Lr20*).

Materials and methods

Pathogen material

Pathotype 10-1,3,(7),9,10,11,12 (isolate 040120) is maintained in the Plant Breeding Institute Cobbitty (PBIC) rust culture collection. The nomenclatural system used to name pathotypes of *P. triticina* in Australia was outlined by McIntosh et al. (1995). Isolates of three other pathotypes of *P. triticina*, all maintained in the PBIC rust culture collection, were also used (Table 1).

Host materials

The wheat genotype Morocco (Sydney University accession W1103) and Australian wheat genotypes Halberd, Harrier, Tincurrin, and Warigal −*Lr20*, were all crossed to cultivar Kelalac, which is susceptible to pt. 10-1,3,(7),9,10,11,12. Morocco was also intercrossed with the genotypes Halberd, Warigal −*Lr20* and Harrier, and cv. Tincurrin was crossed with cvs. Halberd and Harrier, in tests of allelism of the seedling resistance identified in each. In all cases, populations were advanced to the F_3 generation for genetic analyses. All genotypes are held in germplasm collections at the PBIC.

A doubled haploid (DH) population of 82 individuals derived from a cross between the selection Avocet 'S' (a selection of cv. Avocet lacking the stripe rust resistance *YrA*) and cv. Cappelle Desprez was used to map the seedling resistance of Avocet to pt. 10-1,3,(7),9,10,11,12. A DArT map for this population was presented in Nazari et al. (2005). A second DH population derived from a cross between cvs. Cranbrook and Halberd was used to

Table 1 Pathotypes of *Puccinia triticina* used in the present study

Pathotype[a]	Accession no.[b]	Avirulence/virulence formula[c]
10-1,3,(7),9,10,11,12	040120	*Lr3a*, *3ka*, *9*, *15*, *19*, *23*, *24*, *27+31*, *28*, *37/Lr1*, *2a*, *3bg*, *10*, *13*, *14a*, *16*, (*17a*), *17b*, *20*, *26*
26-12	640157	Avirulent to all genes except *Lr10* and *Lr17b*
53-1,4,(6),(7),10,11,12	010047	*Lr1, 2a, 3a, 3bg, 3ka, 9, 14a, 19, 23, 24, 26, 28, 37/Lr10, 13, 15, 16, (17a), 17b, 20, (27+31)*
76-1,3,5,10,12	990423	*Lr1*, *2a*, *9*, *15*, *16*, *17a*, *19*, *23*, *24*, *26*, *27+31*, *28*, *37/Lr3a*, *3bg*, *3ka*, *10*, *13*, *14a*, *17b*, *20*
104-1,2,3,(6),(7),11	890088	*Lr2a*, *3ka*, *9*, *13*, *15*, *17b*, *19*, *24*, *26*, *28*, *37/Lr1*, *3a*, *3bg*, *10*, *14a*, *16*, (*17a*), *20*, *23*, (*27+31*)

[a] Pathotype designations as outlined in McIntosh et al. (1995)

[b] PBIC accession number

[c] Parentheses indicate partial virulence

map the seedling resistance in Halberd. A detailed genetic map for this population was published by Akbari et al. (2006). In both cases, the populations were sown in duplicate and both replicates were inoculated with *P. triticina* pt. 10-1,3,(7),9,10,11,12. Seed of the parental lines used to generate the Cranbrook/Halberd population were not available for testing.

F_3 populations were developed from the crosses Halberd/Egret, Halberd/Selkirk, and Halberd/Thatcher +*Lr23*, and linkage analyses were conducted between the seedling resistances effective against pt. 10-1,3,(7),9,10,11,12 in each parent. Each F_3 population was developed from two F_1 seeds. The two families in each population were analysed separately and once homogeneity was established, the data were pooled.

Inoculation and disease assessment

For greenhouse tests, seedlings of all populations and the parental lines were raised in 9 cm diameter pots containing a mixture of pine bark fines and coarse sand. The pots were watered prior to sowing with a soluble fertiliser (Aquasol®, Hortico Pty. Ltd., Revesby, NSW, Australia) at the rate of 35 g per 3 L for 100 pots. Approximately, 25–30 seeds from each F_2 plant or each F_3 line were sown per pot. Lines of DH populations and parents were sown in clumps (two per pot) of 5–7 seeds each. Seedlings with a fully expanded first leaf (about 8- to 10-day-old) were inoculated with selected isolates of *P. triticina*. Inoculations were conducted by suspending urediniospores in a light mineral oil (Shellsol®, Mobil Oil, Sydney, Australia), at the rate of approximately 10 mg of spores per 10 mL oil per 200 pots, and atomizing the resulting suspension over seedlings in an enclosed chamber using a hydrocarbon propellant pressure pack. Inoculated seedlings were incubated overnight at ambient temperatures in a dark room in which mist was generated by an ultrasonic humidifier. Following the dew treatment, infected seedlings were transferred to a greenhouse growth room in which temperature was maintained within the range 20–25 °C.

Disease assessments were made at 12 days after inoculation, and followed the '0'–'4' infection type (IT) scoring system, in which IT '0' indicated no visible symptoms, IT ';' indicated hypersensitive flecks, IT '1' indicated small uredinia with necrosis, IT '2' indicated small to medium sized uredinia with green islands and surrounded by necrosis or chlorosis, IT '3' indicated medium to large sized uredinia with chlorosis, IT '4' indicated large uredinia without chlorosis, and IT 'X' indicated heterogeneous ITs, similarly distributed over a given leaf. Plus and minus signs were used to indicate variation in ITs, and the letters 'C' and 'N' were used to indicate more than normal chlorosis or necrosis, respectively. Infection types of '3+' or higher were regarded as compatible (high infection type; high IT), whereas ITs of '3' or lower were regarded as incompatible (low infection type; low IT).

Statistical and molecular analyses

The data obtained from the rust tested F_2, F_3 and DH populations were subjected to Chi squared (χ^2) analysis to confirm the goodness-of-fit of observed ratios to theoretical expectations.

Segregation data for molecular markers and phenotypes of each population were combined and partial linkage maps were constructed with the computer programme JOINMAP® 3.0 (Van Ooijen and Voorrips 2001). The Kosambi mapping function was applied to convert recombination fractions into map distances (Kosambi 1944). Charts of genetic linkage maps were drawn with MAPCHART 2.1 software (Voorrips 2002).

Allelism tests and linkage analyses

For linkage studies, F_2 individuals and F_3 families using approximately 20 seedlings per family were tested. Chi squared analyses were performed to check the goodness-of-fit to a 7 Non-segregating Resistant:8 Segregating:1 Non-segregating Susceptible genetic ratio, expected for two independent genes. The maximum recombination frequency (r) at $P = 0.05$ and $P = 0.01$ was calculated for each cross (assuming resistance based on different genes) using the method of Hanson (1959).

Results

Responses to selected *P. triticina* pathotypes

The resistant phenotype of Morocco when challenged with pt. 10-1,3,(7),9,10,11,12 was ';1=' (Table 2; Fig. 1), and to all other pathotypes, '3+' (Table 2). When tested with pt. 10-1,3,(7),10,11,12, Halberd, Harrier, Tincurrin, and Warigal −*Lr20* all displayed low phenotypes similar to that on Morocco, whereas Kelalac was susceptible (Table 2; Fig. 1). Prior to the detection of pt. 10-1,3,(7),9,10,11,12, all known Australian isolates of *P. triticina* were virulent on Morocco, Halberd, and Tincurrin. With the exception of Warigal −*Lr20*, all genotypes were susceptible to pt. 53-1,4,(6),(7),10,11,12 (Table 2; Fig. 2). The resistance of Warigal −*Lr20* to this pathotype is likely due to the presence of *Lr1*, for which it is avirulent. The genotypes Little Club, Line E and Thatcher were susceptible to all five pathotypes (Table 2).

Table 2 Comparative infection types of a range of wheat genotypes when infected with selected Australian isolates of *Puccinia triticina*

Genotype	Pathotype				
	10-1,3,(7),9,10,11,12	53-1,4,(6),(7),10,11,12	26-12	76-1,3,5,10,12	104-1,2,3,(6),(7),11
Morocco	;1=	3+	3+	3+	3+
Harrier	;	3+	3+	3+	X+
Warigal −Lr20	;	0;	0;	0;	3+
Halberd	;1=	3+	3+	3+	3+
Cranbrook	;12−	;12	;1−	;1−	3+
Tincurrin	;1=	3+	3+	3+	3+
Kelalac	3+	3+	3+	3+	3+
Little Club	3+	3+	3+	3+	3+
Line E	3+	3+	3+	3+	3+
Thatcher	3+	3+	3+	3+	3+
Thatcher +Lr23	;1+	;1+	;1+	;1+	3+
Avocet 'S'	0;	3+	X+	3+	X++
Cappelle Desprez	22+	3+	?[a]	?	?
Selkirk	3CN	33+CN	12CN	12CN	3CN
Egret	3+	3+	X+	3+	X++

[a] not tested

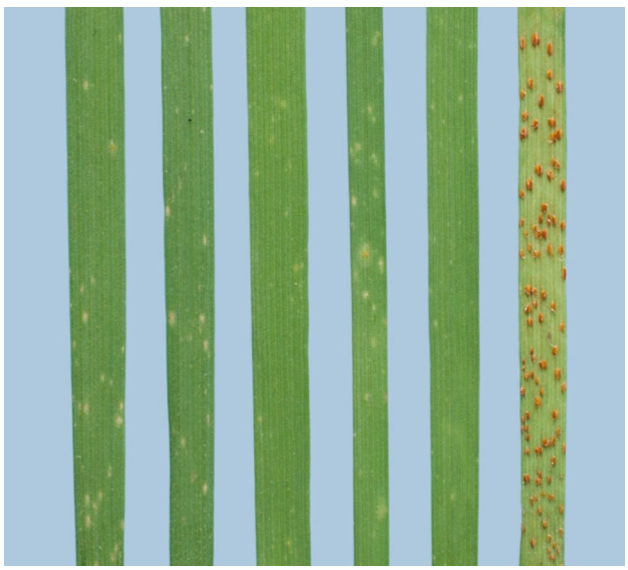

Fig. 1 Seedling responses of (from *left*) Morocco, Halberd, Harrier, Tincurrin, Warigal −*Lr20*, and Kelalac, when tested in the greenhouse against *Puccinia triticina* pathotype 10-1,3,(7),9,10,11,12

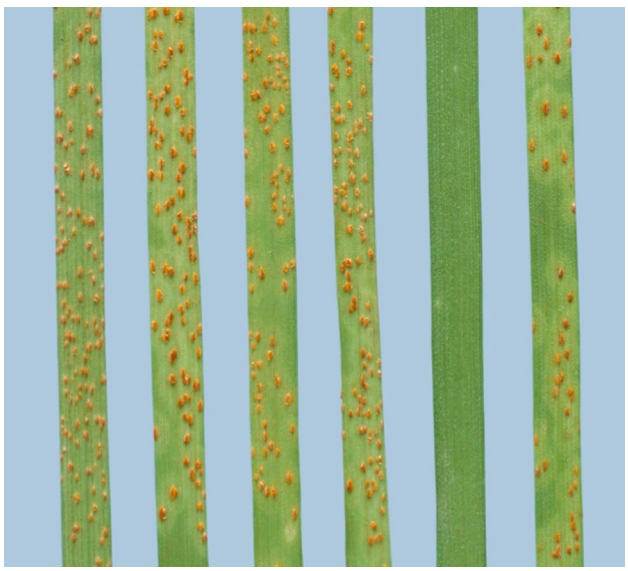

Fig. 2 Seedling responses of (from *left*) Morocco, Halberd, Harrier, Tincurrin, Warigal −*Lr20*, and Kelalac, when tested in the greenhouse against *Puccinia triticina* pathotype 53-1,4,(6),(7),10,11,12. The low reaction of Warigal −*Lr20* is due to *Lr1*

Inheritance studies

F_3 populations derived from crossing cv. Kelalac with cvs Morocco, Halberd, Harrier, Tincurrin and Warigal −*Lr20* segregated in patterns expected for a single locus when inoculated with pt. 10-1,3,(7),9,10,11,12 (Table 3). The pattern of segregation within lines in each of the five populations indicated a dominant mode of inheritance conforming to an expected segregation of three resistant: one susceptible (Table 3).

Linkage analysis

No susceptible plant was identified among the progeny of the F_3 populations derived from various intercrosses

Table 3 Seedling responses and segregation ratio of F_3 families derived from five crosses inoculated with pathotype 10-1,3,(7),9,10,11,12

Cross	Number of F_3 families					Pooled segregating F_3 individuals			
	NSR	Seg	NSS	$\chi^2_{1:2:1}$	*P*	Res	Susc	$\chi^2_{3:1}$	*P*
Harrier/Kelalac	14	29	13	0.11	0.9	293	111	1.32	0.2
Tincurrin/Kelalac	23	43	24	0.20	0.9	539	187	0.22	0.6
Warigal−Lr20/Kelalac	18	26	14	1.17	0.6	371	117	0.27	0.6
Morocco/Kelalac	16	30	18	0.37	0.8	348	120	0.10	0.7
Halberd/Kelalac	19	27	13	1.64	0.4	288	109	1.27	0.2
Pooled[a]	90	155	82	1.27	0.5	1839	644	1.16	0.3

NSR non-segregating resistant, *Seg* segregating, *NSS* non-segregating susceptible, *R* resistant, *S* susceptible

[a] Heterogeneity $\chi^2_{1:2:1} = 2.13$ (8 d.f.), $P > 0.9$ and Heterogeneity $\chi^2_{3:1} = 1.99$, $P > 0.7$ (4 d.f.)

Table 4 Seedling responses and genetic ratios of F_2 individuals and F_3 progeny derived from five crosses inoculated with pathotype 10-1,3,(7),9,10,11,12 and maximum recombination frequency (*r*) for each cross assuming resistance based on different genes

Cross	F_2 phenotypic response			F_3 phenotypic response					*r* (cM)[a]	
	Res	Sus	$\chi^2_{15:1}$	NSR	Seg	NSS	No data	$\chi^2_{7:8:1}$	$P = 0.05$	$P = 0.01$
Morocco/Halberd	99	0	6.6	87	0	0	12	111.85**	1.5	2.3
Morocco/War−Lr20	81	0	5.4	77	0	0	4	99.00**	1.8	2.8
Halberd/Tincurrin	82	0	5.4	80	0	0	2	102.85**	1.8	2.7
Harrier/Tincurrin	92	0	6.1	92	0	0	0	118.29**	1.6	2.4
Morocco/Harrier	123	0	8.2	116	0	0	7	149.14**	1.2	1.8
Pooled	477	0	1642.3	452	0	0	25	1,356**	0.3	0.5

Res resistant, *Sus* susceptible, *NSR* non-segregating resistant, *Seg* segregating, *NSS* non-segregating susceptible

[a] Maximum recombination frequency, estimated from Hanson (1959)

** $P < 0.001$

among the genotypes Morocco, Warigal −*Lr20*, Halberd, Tincurrin and Harrier, when tested with pt. 10-1,3,(7),9,10,11,12, indicating that the seedling resistance gene present in each was most likely the same (Table 4). The maximum recombination frequency, estimated according to Hanson (1959) at $P = 0.05$, ranged from 1.2 cM in the population Morocco/Harrier to 1.8 cM in the Morocco/Warigal −*Lr20* and Halberd/Tincurrin populations (Table 4). The gene shared by these five genotypes was given the tentative designation *LrMorocco*. Across 452 F_3 lines, this value was estimated at 0.3 cM ($P = 0.05$) and 0.5 cM ($P = 0.01$).

Mapping studies

When tested with pt. 10-1,3,(7),9,10,11,12, Avocet 'S' was highly resistant (IT '0;'), and Cappelle Desprez resistant (IT '22+') (Table 2; Figs. 3, 4). The Avocet 'S'/Cappelle

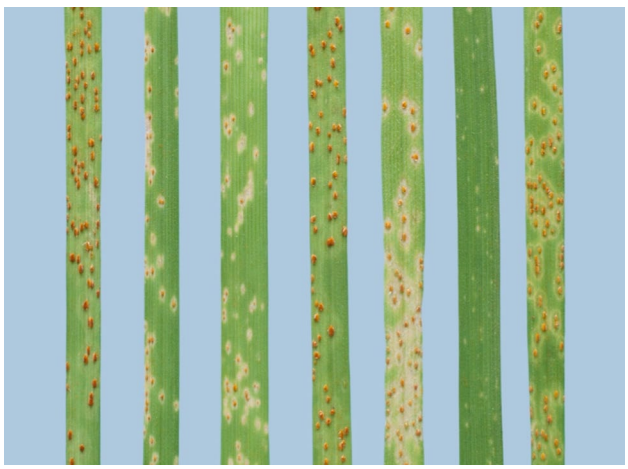

Fig. 3 Seedling responses of (from *left*) Thatcher, Thatcher +Lr23 (*Lr23*), Cranbrook (*Lr23*), Egret (*Lr13*), Selkirk (*Lr16*), Avocet 'S' (*Lr73*), and Cappelle Desprez, when tested in the greenhouse against *Puccinia triticina* pathotype 10-1,3,(7),9,10,11,12

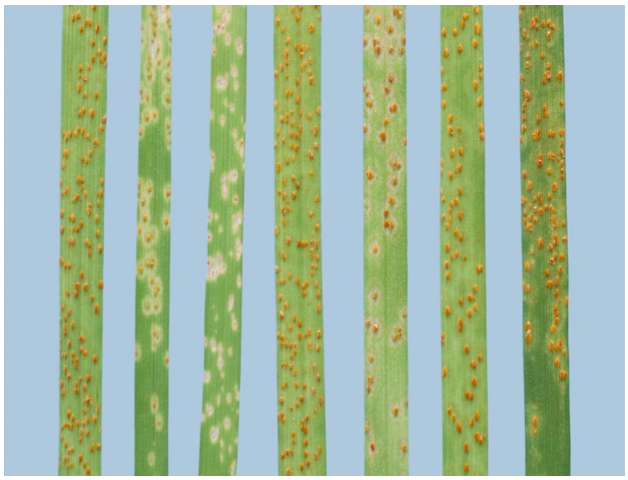

Fig. 4 Seedling responses of (from *left*) Thatcher, Thatcher +Lr23, Cranbrook, Egret, Selkirk, Avocet 'S', and Cappelle Desprez, when tested in the greenhouse against *Puccinia triticina* pathotype 53-1,4,(6),(7),10,11,12

Desprez DH population segregated for the resistance contributed by Avocet 'S', as expected, for a single gene (42 IT '0;':40 IT '22+' or '3+', $\chi^2_{1:1} = 0.02$, $P = 0.89$). This gene was temporarily designated *LrAvS* and was mapped to the short arm of chromosome 2B (Fig. 5).

Cultivar Cranbrook was postulated to carry *Lr23* by McIntosh et al. (1995). When tested with pt. 10-1,3,(7),9,10,11,12, both Halberd and Cranbrook were resistant (Table 2; Figs. 1, 3). These responses, along with those to the other pathotypes used (Table 2), were consistent with the presence of *LrMorocco* in the former, and *Lr23* in the latter. When tested with pt. 53-1,4,(6),(7),10,11,12, Halberd was susceptible, and the resistant response of Cranbrook was again consistent with the presence of *Lr23* in this cultivar (Table 2; Figs. 2, 4). On the basis of these results, it was expected that the Cranbrook/Halberd DH population would display a two gene segregation when tested with pt. 10-1,3,(7),9,10,11,12 (*Lr23* and *LrMorocco*). However, a segregation ratio conforming to that expected for a single gene was observed in tests using this pathotype (66 resistant:61 susceptible, $\chi^2_{1:1} = 0.12$, $P = 0.73$). This indicated that unlike the Cranbrook accession used in the multipathotype tests (Table 2; Figs. 3, 4), the Cranbrook plant (s) used to generate the DH population must have lacked *Lr23*. A subset of 64 individuals for which molecular marker data was available was used for

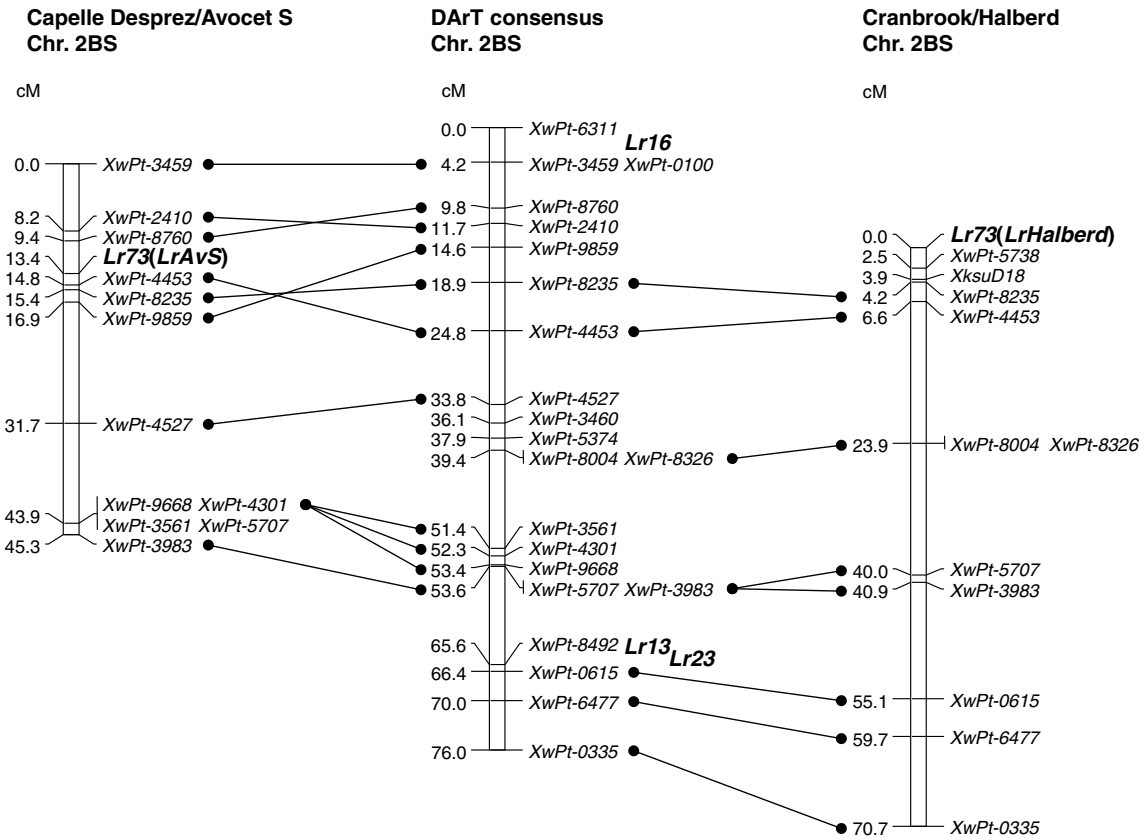

Fig. 5 Mapping of the seedling resistance genes present in Halberd and Avocet S based on tests with Australian *Puccinia triticina* pathotype 10-1,3,(7),9,10,11,12 and 26-12 respectively. The genes are located distal to marker loci *XwPt-4453* and *XwPt-8235* in each population. A DArT consensus map for the region is also shown, with marker loci common to both maps connected by *solid lines*

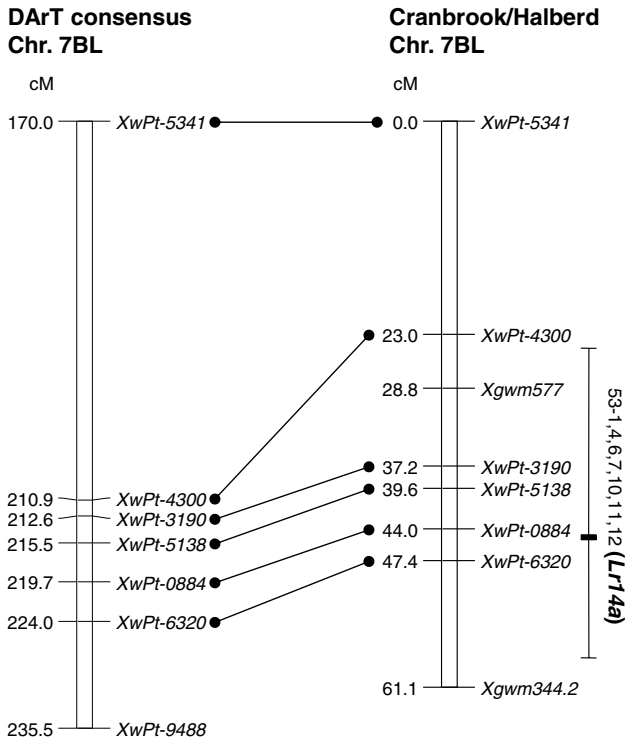

Fig. 6 Mapping of the seedling resistance gene, likely *Lr14a*, present in Cranbrook based on phenotypic data from tests with Australian *Puccinia triticina* pathotype 53-1,4,(6),(7),10,11,12. A DArT consensus map for the region is also shown, with marker loci common to both maps connected by *solid line*

genetic mapping. Gene *LrHalberd* (syn. *LrMorocco*), was mapped to the terminal region on the short arm of wheat chromosome 2B, closely linked to DArT markers *XwPt-4453* and *XwPt-8235* (Fig. 5).

Seed of the parental lines used to develop the Cranbrook/Halberd population was not available for testing. To confirm the absence of *Lr23* in the population, it was screened with pt. 53-1,4,(6),(7),10,11,12, to which all individuals should have been susceptible. However, a segregation of 37 resistant: 66 susceptible was observed ($\chi^2_{1:1} = 8.16$, $P = 0.004$), and the ratio deviated from expected for a single gene. The Chi squared value showed even higher deviation for a two gene model ($\chi^2_{3:1} = 83.87$, $P < 0.000$) indicating that segregation was skewed and that a single gene was likely involved and the gene responsible was mapped to chromosome 7BL (Fig. 6). Further tests of the population with two additional leaf rust pathotypes virulent for *Lr23* and avirulent for *Lr14a* (data not shown) indicated that the gene was likely *Lr14a*. The presence of *Sr17* in Cranbrook (R. F. Park unpublished), which is linked to *Lr14* (McIntosh et al. 1995), is consistent with this conclusion. While the source of Cranbrook used to generate the DH population must have lacked *Lr23* and carried *Lr14a*, it seems probable from the results presented here, along with previous studies (McIntosh et al. 1995), that other sources of this cultivar, including that shown in Figs. 3 and 4, carry both *Lr14a* and *Lr23* (R. F. Park unpublished).

Allelism tests

Three other genes conferring resistance to leaf rust are known to be located on chromosome 2BS: *Lr13*, *Lr16*, and *Lr23* (McIntosh et al. 1995). Allelism tests were therefore conducted for three genes and *LrMorocco* using three crosses involving these genes (Table 5). The F_3 phenotypic classification conformed to a digenic segregation model (7 Non-segregating Resistant:8 Segregating:1 Non-segregating Susceptible) for all three crosses when tested with pathotypes 10-1,3,(7),9,10,11,12 and 26-12, indicating that *LrMorocco* is genetically independent from *Lr13, Lr16* and *Lr23* (Table 5). Further, F_2 genotypes (AABB, AABb, AAbb, AaBB, AaBb, Aabb, aaBB, aaBb, aabb) were predicted based on joint segregation of F_3 families of each cross using pathotypes 10-1,3,(7),9,10,11,12 and 26-12. For each cross, the predicted genotypic classification fitted a 1AABB:2AABb:1AAbb:2AaBB:4AaBb:2Aabb:1aaBB:2aaBb:1aabb genetic ratio (*LrMorocco/Lr13*, $P > 0.9$; *LrMorocco/Lr16*, $P > 0.8$; *LrMorocco/Lr23*, $P > 0.5$) expected for segregation at two independent loci.

Discussion

Initial greenhouse seedling tests with *P. triticina* isolate 040120 indicated that it was unusual in that it was virulent on the differential genotype Norka (*Lr1*, *Lr20*), but avirulent on Tarsa, which at that time was thought to carry

Table 5 Joint segregation analysis of F_3 families in three test crosses for allelism using *Pt* pathotypes 10-1,3,(7),9,10,11,12 and 26-12

Cross	Genes involved	Number of families			$\chi^2_{7:8:1}$	>*P*
		NSR	Seg	NSS		
Halberd/Egret	*LrMorocco/Lr13*	27	31	3	0.18	0.9
Halberd/Selkirk	*LrMorocco/Lr16*	27	22	2	3.18	0.3
Halberd/Tc +Lr23	*LrMorocco/Lr23*	37	35	6	0.91	0.6

NSR non-segregating resistant, *Seg* segregating, *NSS* non-segregating susceptible

only *Lr1* (R.F. Park unpublished). To study the isolate in greater detail, several attempts were made to subculture it on the susceptible check genotype Morocco, all of which proved unsuccessful. It was eventually realised that the isolate was avirulent on Morocco, and it was subcultured on Mildress (*Lr26*). Subsequent studies established that it was virulent for *Lr1* and avirulent for an unknown resistance gene in Tarsa. In the present study, the resistance gene in Morocco was shown to be present in five Australian wheat genotypes including Halberd, and genetic studies based on Halberd established that it was dominant in action and located on chromosome 2BS. The genetic independence of this locus from *Lr13*, *Lr16* and *Lr23*, also located on chromosome 2BS, indicated that it is distinct, and hence it was given the locus designation *Lr73*. In our studies, *Lr73* is located distal to marker loci *XwPt-4453* and *XwPt-8235*. *Lr16* is also distal to these markers and is reported to be the most terminal gene on 2BS spanning about 9 cM region corresponding to a cluster of closely linked SSRs (*Xgwm 210*—1 cM—*Xwmc661*—1 cM—*Xwmc764*—4 cM—*Xwmc614/Lr16/xwmc635*—3 cM—*Xbarc*) in the microsatellite consensus maps of Somers et al. (2004) and McCartney et al. (2005). Based on a DArT consensus map of 2BS (Fig. 5) and the consensus map of Somers et al. (2004), *Lr73* is mapped 8.9 cM (Fig. 5; Cranbrook/Halberd map) to 10.9 cM (Fig. 5; Capelle Desprez/Avocet S map) proximal to *Lr16*. Despite this, the F_3 tests in this study depicted that *Lr16* and *Lr73* are genetically independent. The low probability level ($P = 0.31$) associated with the Chi squared test could suggest that the two genes are not completely independent. It is possible that the small F_3 population used (51 families) and/or differences in the level of recombination between the different mapping populations used in the present and previous studies may have contributed to this discrepancy. Nevertheless, the closely linked genes *Lr13* and *Lr23* (McIntosh et al. 1995) are proximal to *Lr73* (*Lr73*—35.1 cM—*barc55*—5.1 cm—*Lr13*) based on the consensus map of Somers et al. (2004) and the partial linkage map developed by Bansal et al. (2008).

It is not known if *Lr73* is the same as *LrMo*, reported in Morocco by Ali et al. (1994). These researchers reported that *LrMo* conferred an IT '0', which is different but similar to the resistant phenotype (;1 =) displayed by Morocco to pt. 10-1,3,(7),9,10,11,12. *LrMo* displayed recessive inheritance based on susceptibility of F_1 plants and progeny tests of 95 F_2 plants from a cross involving Morocco (Ali et al. 1994). Although *Lr73* showed dominant inheritance in the present studies, it remains possible that *LrMo* and *Lr73* are the same. Dominance reversal has been described for rust resistance genes in wheat, for example, *Yr6* (El-Bedewy and Röbbelen 1982) and *Sr6*, where in the latter example gene action differed with temperature and different isolates of *P. graminis* f. sp. *tritici* (Knott and Anderson 1956).

On the basis of multipathotype tests, it is likely that in addition to Morocco, Avocet, Halberd, Harrier, Tincurrin and Warigal −*Lr20*, *Lr73* is also present in Australian wheat cultivars Clearfield STL, Federation, Gatcher (with *Lr27* +*Lr31)*, Marombi (with *Lr1* and *Lr37*), Pugsley (with *Lr1* and *Lr37*), Spear (with *Lr1*), Stiletto (R.F. Park unpublished), and Tarsa (with *Lr1*). The origin of this resistance gene in Australian wheat germplasm is unknown, and it seems unlikely that it would have originated from Morocco given that there is no record indicating it was used as a parent of any Australian wheat cultivar. The occurrence of *Lr73* in other international wheat germplasm is currently not known, although all entries tested in two international wheat nurseries (39th International Bread Wheat Screening Nursery, 209 lines, distributed by CIMMYT in 2002 (Dadkhodaie et al. 2011); 14th Facultative and Winter Wheat Observation Nursery, 86 lines, distributed by CIMMYT in 2007 (Loladze 2011)) were susceptible to pt. 10-1,3,(7),9,10,11,12, indicating that they lacked *Lr73*.

The *P. triticina* isolate shown by Ali et al. (1994) to be avirulent on Morocco originated from bread wheat in Bulgaria in 1989, and was similar to isolate 040140 in being virulent for *Lr1*, *Lr2a*, *Lr2c* and *Lr26*, but differed from it in being virulent for *Lr3a*, *Lr3ka* and *Lr11*, and avirulent for *Lr10*. A second isolate that was virulent on Morocco but avirulent on an uncharacterized dominant resistance gene in Little Club originated from durum wheat in Ethiopia, and was widely avirulent on bread wheat cultivars (Ali et al. 1994). The avirulence on Morocco of two leaf rust isolates, presumed to be *P. triticina* based on their virulence for many other genes conferring resistance to this pathogen in bread wheat, is interesting, given the high susceptibility of Morocco to this pathogen. The use of Tarsa (presumed to carry *Lr1* and *Lr73*) and Norka (*Lr1*+*Lr20*) in the Australasian differential set for *P. triticina* allowed the identification of avirulence for *Lr73* in pt. 10-1,3,(7),9,10,11,12. These two differentials have been used since at least 1988 in Australia, and this is the first time avirulence for *Lr73* has been detected since then (R.F. Park unpublished). In regions in which pathogenicity surveys for *P. triticina* are undertaken using differential genotypes such as the Thatcher near isogenic series (e.g. USA, Canada, France, Czech Republic; Huerta-Espino et al. 2011), it would not be possible to assess the frequency of virulence/avirulence for *Lr73*. The origin of pt. 10-1,3,(7),9,10,11,12 is unknown, but given its distinctiveness from all other known Australian pathotypes of *P. triticina*, it seems most likely that it was a foreign incursion into this region. National pathogenicity surveys of wheat-attacking rust pathogens in Australia have documented 11 such exotic incursions since 1925, five of which (including pt. 10-1,3,(7),9,10,11,12) were *P. triticina* (Park et al. 2011).

Gene *Lr73* is unlikely to be of value in resistance breeding. At present in Australia, cultivars Clearfield STL, Marombi

and Pugsley are protected from pt. 10-1,3,(7),9,10,11,12 by *Lr73*, but are moderately susceptible to one or more of the pathotypes prevailing in 2011 (Wellings et al. 2011). This pathotype is nonetheless potentially important because it combines virulences for *Lr1* and *Lr13*, detected only once previously in Australia, and that was in a single isolate of pathotype 104-1,3,5,10,12 recovered from South Australia in 2002 (R.F. Park unpublished). The combination of *Lr1* and *Lr13* is present in several Australian wheat cultivars including Diamondbird, EGA Burke, Hartog, Kukri, Lincoln and Sunbrook (Wellings et al. 2011), and the vulnerability of these cultivars to this pathotype is currently being assessed.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments reported in this manuscript comply with the current laws of Australia.

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