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Seedling resistance to *Puccinia coronata* f. sp. *avenae* in *Avena strigosa*, *A. barbata* and *A. sativa*

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Abstract A study was carried out in which seedling resistance to the crown rust pathogen, *Puccinia coronata* f. sp. *avenae* (*Pca*), was characterised among 385 oat accessions of *Avena strigosa*, *A. barbata* and *A. sativa* from the USDA-ARS National Small Grains Collection. Accessions were tested with eight Australian pathotypes of *Pca* of diverse pathogenicity on a series of differential genotypes carrying known seedling resistance genes. Three diploid accessions, CIav2524, PI78821 and PI83721 of *A. strigosa*, were highly resistant to all eight *Pca* pathotypes, and were postulated to carry *Pc50* and *Pc68*, *Pc91*, or one or more new resistance genes. A total of 58 unidentified resistance specificities were detected among the tetraploid *A. barbata* accessions. All 58 resistances were ineffective against at least one of the *Pca* pathotypes and could therefore be novel. Additionally, evidence was obtained for the presence of genes *Pc39* and *Pc52* (in *A. barbata* and *A. sativa* accessions), *Pc45* (in *A. barbata* accessions), *Pc94* (in *A. strigosa* and *A. barbata* accessions) and the "Saia" resistance (in *A. strigosa* accessions).

Keywords *Avena* spp. · Crown rust · Gene postulation · Resistance

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Introduction

Crown rust of oats (*Avena* spp.), caused by the pathogen *Puccinia coronata* f. sp. *avenae* (*Pca*), has caused significant crop losses world-wide. Previous research has identified seedling (all stage) resistance genes that protect wild and cultivated oats against *Pca*, and where these genes have been catalogued, they have been given *Pc* designations. There are currently 96 known *Pc* genes (CDL 2010), a majority of which have been identified from the hexaploid species *A. sterilis* and the diploid species *A. strigosa*.

Avena spp. exist as hexaploid (2n = 6x = 42; AACCDD), tetraploid (2n = 4x = 28; AABB or AACC) and diploid (2n = 2x = 14; AA or CC) species. Cultivated oat *(A. sativa L.)* is an allohexaploid reported to be a derivative of *A. sterilis* L. (Coffman 1946; Wahl et al. 1960). Both *A. sterilis* L. and *A. barbat*a Brot. are wild species that are indigenous to the Mediterranean region (Dinoor and Wahl 1963).

Diploid oat *A. strigosa* Schreb. (A_sA_s karyotype) is a cultivated species reported to be a good potential source of novel rust resistances (Steinberg et al. 2005).

Cereal rust pathogens, including *Pca*, are known to change with time as new isolates evolve through processes thought to include recombination and rare mutational events, to overcome host-resistance genes. In Australia, the absence of *Rhamnus* spp., the alternate host of *Pca* precludes sexual recombination (Brake et al. 2001). Under these circumstances, pathogenic variability in the rust pathogens is thought to be driven by single-step mutations in clonal lineages that originated from introduced, distinct isolates (e.g. in *Puccinia striiformis* f. sp. *tritici*; Wellings and McIntosh 1990), and/or processes involved in somatic hybridization (e.g. in *Puccinia triticina*; Park et al. 1999).

Virulence matching many of the known *Pc* genes has been recorded. For example, in Australia, the only cultivar resistant to crown rust is Drover, believed to carry *Pc91* (Park and Kavanagh 2009). Given this situation, there is an urgent need to find, introgress and deploy new crown rust resistances in order to prevent or minimise yield losses due to the disease. The objectives of this study were to characterise seedling resistance to *Pca* in accessions of diploid, tetraploid and hexaploid oats, with the aim of identifying potentially new resistance sources for further genetic characterisation and, ultimately, introgression into agronomically suitable hexaploid oat germplasm.

Materials and methods

Plant material

A total of 385 (97 diploid *A. strigosa*, 265 tetraploid *A. barbata* and 23 hexaploid *A. sativa*) oat accessions from the USDA National Small Grains Collection were tested for response to *Pca* under greenhouse conditions at the Plant Breeding Institute (PBI), New South Wales Australia. The accessions were compiled from those available in the USDA collection so as to provide a broad geographic spread and range of species and ploidy levels.

Sowing and raising of seedlings

The 385 wild oat accessions and differential sets were sown in nine centimetre plastic pots, in disease free conditions in the greenhouse. Pots were filled with a medium made up of four parts of pine-bark compost and one part of coarse sand. Prior to seeding, the potting mix was compacted and drenched with a soluble complex fertilizer, Aquasol® N23:P4:K18 (Yates Commercial) at the rate of 3 g/l of water. A total of five accessions were planted in a single pot, in evenly spaced groups of 8–10 seeds of each accession. After sowing, pots were placed in growth rooms maintained at 18–21 °C with natural lighting and a week later seedlings were fertilized with urea as a supplemental nitrogen treatment.

Pca pathotypes, inoculation and disease scoring

Eight Australian pathotypes of *Pca* were used in this experiment, each being identified by a PBI rust isolate accession number. The pathotype accession numbers, with culture numbers in parentheses, were: 691074 (=135), 853023 (=440), 933015 (=504), 982774 (=529), 982604 (=530), 203594 (=550), 013535 (=567), and 073518 (=604). A differential set comprising 39 oat genotypes carrying known or uncharacterized seedling resistances to *Pca* was used to characterise virulence and avirulence among these eight isolates (Table 1). The isolates were chosen based on diverse pathogenicities, plus virulence and avirulence for several known *Pc* genes present in diploid *Avena* genotypes (*Pc15*, *Pc16* and *Pc17* (the "Saia" resistance), *Pc92*, and *Pc94*). The pathogenicity of each of the eight isolates to the differential genotypes is given in Table 2.

Inoculations were conducted using 2 week-old seedlings, which were placed in an inoculation chamber, and sprayed evenly with urediniospores suspended in a light mineral oil [Pegasol® 3440 Special (Mobil Oil)] at the rate of 10 mg of spores/10 ml of oil. The suspension was allowed to settle for 2–3 min and seedlings were then allowed to air-dry prior to incubation for 24 h, at room temperature (18–21 °C) in a saturated misting chamber. The following day, the seedlings were transferred to a greenhouse micro-climate in which temperature was maintained within the range 21–23 °C. Rust reactions on the inoculated primary leaves were recorded 14 days later on a "0"–"4" scale, as described by Murphy (1935), with minor modifications (Table 3).

Table 1 Infection types (ITs) of 39 seedling differentials to eight *Puccinia coronata* f. sp. *avenae* isolates, scored on a "0–4" scale described by Murphy (1935)

Differential	*Pc* genes	Rust isolate							
		691074	982774	073518	853023	933015	203594	982604	013535
PI296251	*Pc-H458*	;cn	0;	1cn	0;	2cn	12cn	12n	3+
WIX4361-9	*Pc-Wix 1, 2*	;cn	;1cn	;cn	;1−cn	; cn	; c	3+	0;
Amagalon	*Pc91*	0, 2c	0;	12	0	; ws	0ws	;1n	0;
Culgoa	*Pc-Cul*	;cn	0;	;nws	;−	23cn	12cn	23	3+
Cleanleaf	*Pc38, 39, Cl*	;cn	0;	0;	;	0;	;cn	3+	0;
CAV4904	*Pc68*	0, 23c[a]	0;	0	0;−	0	0	0	0;
TAM 0.301	*Pc58*	0;	0	;n[b]	;c	3+	3+	12	3+
TAM 0.312	*Pc59*	0;− ws[c]	0;	;n	;c	3+	3+	12	3+
Coker 234	*Pc61*	0	0;	;ws−	;c	3+	3+	;1n	3+
PC38	*Pc38*	3+[d]	12	;n	3	;ws	;cn	3+	0;
PC39	*Pc39*	0;	12cn	23	;c	3	3c	3+	3+
Swan	*Pc1*	3+	3	3+	3+	3+	3+	3+	3+
PI267989-1	*Pc36*	0;	12cn	;1	;12	;cn	12cn	12	;1
CAV5115	*Pc46*	23cn	3+	;ws	3+	;cn	;cn	;1n+	0;
PC50	*Pc50*	0	0	0	0;−	0;−	0	;1	0
PC51	*Pc51*	0	0	;1−	0;	12cn	23cn	3	3+
CI8001	*Pc52*	0;−	;	;1	;c	12cn	0	3+	;1
Enterprise	*Pc55*	0;	1	;1	;1−c	12cn	12cn	3+	3+
PC56	*Pc56*	0;	;1	;n	;c, 3	2cn	3c	12	3
CAV4540	*Pc63*	12cn	12cn	;n	12cn	0;	;cn	;1	0;
CAV4248	*Pc64*	0;−, 2c	;1	;n	;c	0;	0;	;n+	0;
CI8044	*Pc71*	0;−	12cn	23	;−c	23	23	3+	3+
X716	Unknown	0;	1c	;n	;−c	0;	12cn	3	;1
Warrego	Unknown	0;	0;	;n	;1	;1cn	;cn	3	0;
Bettong	Unknown	0	0;	;nws	0;	0;ws	12−ws	0;	3+
Barcoo	Unknown	0;−	0;	;1	0;−	3n	3n	12	3+
Landhafer	*Pc5*	0;	;	1n	;c	23c	3c	3+	3
Santa Fe	*Pc6, 7, 8, 21*	;1cn	;	12	;c	23cn	3c	3+	3
Ukraine	*Pc3c,4c,6c,9*	12cn	;	0;	;c	3+	3+	3+	33+
Trispernia	*Pc6d*	12cn	;1	2	1+cn	3+	3	3+	0;
Bondvic	–	12cn	23n	12	;1cn	3	23cn	3	3
PC45	*Pc45*	0;	2cn	3	;c	3c	3c	3	3
PC48	*Pc48*	0;	0;	3	;−c	3	23cn	;1	3
Nugene	Unknown	0;	0;	;n	;c	0;	0;−	;1	0;
Gwydir	Unknown	0;	0;	;n	0;	0;	0;	;1	0;
Volta	*Pc50+*	0	0;	0	0;−	0;ws	;1	;1	0;
PC92	*Pc92*	0;−	0;	3+	0;−	23cn	2cn	0;	3
PC94	*Pc94*	0;	0;	;n	;c	0;	3+	;c	0;
Saia	*Pc15, 16, 17*	3+	;	;ws−	3+	0;ws	0;	3	0

[a] Chlorosis

[b] Necrosis

[c] Water-soaked

[d] "+" or "−" signs following an IT score denotes the level of intensity of infection or an intermediate IT response

Table 2 Pathogenicity of the eight Australian *Puccinia coronata* f. sp. *avenae* isolates used to assess *Avena* accessions

Isolate number	Virulence[a]
691074	*Pc38*, Swan[b], Saia
853023	Swan, *Pc46*, Saia
933015	*Pc58*, *Pc59*, *Pc61*, Swan
982774	*Pc46*
982604	Wix43619, Clean leaf, *Pc38*, *Pc39*, Swan, *Pc52*, *Pc55*, *Pc71*
203594	*Pc58*, *Pc59*, *Pc61*, Swan, *Pc94*
013535	H458, Culgoa, *Pc58*, *Pc59*, *Pc61*, *Pc39*, Swan, *Pc51*, *Pc55*, *Pc71*, Bettong, Barcoo
073518	Swan, *Pc92*

[a] For *Pc* genes/differentials-*Pc1*, *Pc38*, *Pc39*, *Pc46*, *Pc51*, *Pc52*, *Pc55*, *Pc58*, *Pc59*, *Pc61*, *Pc71*, *Pc92*, *Pc94*, Saia (*Pc15*, *Pc16*, *Pc17*), Bettong, Barcoo, Cleanleaf, Culgoa, H458, Wix43619

[b] Swan likely carries *Pc1* (Park, Unpublished)

Table 3 Seedling infection types (ITs) in response to *Puccinia coronata* f. sp. *avenae*, that are denoted by a "0–4" scale, described by Murphy (1935)

IT	Denoted as	Description
0	Immune	No visible signs of infection
;	Highly resistant	Chlorotic and necrotic flecking
1	Resistant	Minute uredinia, usually surrounded by a distinct necrotic area
2	Moderately resistant	Small to medium-size uredinia, sometimes in green islands, surrounded by chlorosis or necrosis
3	Moderately susceptible	Medium to large uredinia, no necrosis, but chlorosis often present
4	Completely susceptible	Large uredinia, no necrosis, but little or no chlorosis

Results

Infection type (IT) responses to the eight *Pca* pathotypes on the control seedling differentials were used as a basis to postulate *Pc* genotypes. These were matched with ITs of the 385 diploid, tetraploid and hexaploid accessions. Of the total 385 accessions, 169 produced compatible ITs to all eight pathotypes, and hence lacked seedling resistance effective against the pathotypes used (data not presented; see Supplemental information, Table S1).

Among the remaining 216 accessions, 45 were postulated to carry four known genes (*Pc39*, *Pc45*, *Pc52* and *Pc94*), the "Saia" resistance, and unknown resistance, and 171 accessions carried unidentified resistance effective against one to seven of the eight *Pca* pathotypes used. Of these 171 accessions, 128 could be placed into 15 "Unidentified Resistance" groups (URGs), each comprising accessions with similar IT responses to the eight pathotypes. All of the remaining 43 accessions (of 171) displayed unique IT responses to the eight pathotypes.

Accessions with unknown resistance(s) effective against all pathotypes

Three diploid accessions, CIav2524, PI78821 and PI83721, produced immune to highly resistant ITs of "0" and "0;" to all eight pathotypes (Table 4). These were identical to those produced on differentials PC50 and PC68 and the cultivar Volta. The accessions were tested with two additional pathotypes with virulence for *Pc50* (761063 = 221) and *Pc68* (033521 = 582), and were resistant to both. Based on these results, the accessions cannot carry either gene singly, but could carry both *Pc50* and *Pc68* in combination, *Pc91*, or an unknown resistance gene(s).

Accessions carrying the "Saia" resistance, Pc39, Pc45, Pc52 and Pc94

Resistance genes *Pc39* and *Pc52* were postulated in tetraploid *A. barbata* and hexaploid *A. sativa* accessions, while *Pc45* was postulated only in tetraploid accessions of *A. barbata*. Further, *Pc94* was postulated in tetraploid *A. barbata* and diploid *A. strigosa* accessions, whereas the "Saia" resistance (*Pc15*, *Pc16*, *Pc17*) was detected only in diploid accessions of *A. strigosa*. The ITs observed on select accessions and differentials, along with their respective *Pc* genes, are given in Table 5.

"Saia" resistance

The "Saia" resistance was postulated in 18 diploid accessions (CIav4639, Clav5082, Clav6858, Clav6956, Clav7010, Clav7280, Clav8089, Clav9020, Clav9021, PI291991, PI292226, PI436082, PI436103, PI436104, PI436105, PI436109, PI436113 and PI436117). These 18 accessions produced near

Table 4 Seedling infection types produced by *Avena* accessions that were resistant to all isolates of *Puccinia coronata* f. sp. a*venae* used, along with differential testers for resistance genes *Pc50*, *Pc68* and *Pc91* (bold typeface)

Accession	Species	*Pc* gene	Rust isolate									
			691074	982774	073518	853023	933015	203594	982604	013535	761063	033521
Clav2524	*A. strigosa*	*?*	0;	0	0;	0	0;	0;	0	0	0	0
PI78821	*A. strigosa*	*?*	0	0	0	0	0	0	0	0	0	0
PI83721	*A. strigosa*	*?*	0;	0;−	0	0	0	0	0;−	0	0	0
PC50	***A. sativa***	***Pc50***	**0**	**0**	**0**	**0;−**	**0;−**	**0**	**;1**	**0**	**3+**	**1cn**
PC68	***A. sativa***	***Pc68***	**0;−**[a]	**0**	**0**	**0;**	**0**	**0**	**0**	**0;**	**12**	**3+**
Amagalon	***A. sativa***	***Pc91***	**0;**	**0;**	**12**	**0**	**; ws**	**0; ws**	**;1n**	**0;**	**0;**	**0;**

[a] "+" or "−" signs following an IT score denotes the level of intensity of infection or an intermediate IT response

Table 5 Seedling infection types produced by *Avena* accessions that displayed response patterns similar to differential testers, along with the relevant resistance genes *Pc15*, *Pc16*, *Pc17*, *Pc39*, *Pc45*, *Pc52*, *Pc94* (bold typeface), when tested against eight isolates of *Puccinia coronata* f. sp. a*venae*

Accession	*Pc* gene (s)	Species	Rust isolate							
			691074	982774	073518	853023	933015	203594	982604	013535
CIav4639		*A. strigosa*	3c	;1n	;ws	3+	0;	0;	33+	0;
Saia	***Pc15*, *16*, *17***	***A. strigosa***	**3**	;	**;ws−**	**3+**	**0; ws**	**0;**	**3**	**0**
PI337809		*A. barbata*	23c	12+	23n	12+	33+	3	3+	3+
CIav4571		*A. sativa*	0; ws	0;n	2	;n ws	3+	3+	3+	3+
PC39	***Pc39***	***A. sativa***	**0;**	**12c**	**23**	**;c**	**3**	**3c**	**3+**	**3+**
PI337905		*A. barbata*	23cn	12	3+	;12	3	3	33+	33+
PC45	***Pc45***	***A. sativa***	**0;**	**2cn**	**33+**	**;c**	**3c**	**3c**	**3**	**3+**
PI282710		*A. barbata*	12c	;1+n	2c	;1−	2cn	12cn	3+	;1cn
CIav4568		*A. sativa*	0;	;1n	12	;n ws	;1c ws	12cn ws	33+	0;
CI 8001	***Pc52***	***A. sativa***	**0;**−[a]	;	**;1**	**;c**	**12cn**	**0**	**3+**	**;1**
PI337896		*A. barbata*	12cn	;1cn	12cn	;cn	12cn	3cn	;1n	;1
PI131641		*A. strigosa*	;c ws	;1	;1	;1n	;cn	3cn	;1n	12
PC94	***Pc94***	***A. sativa***	**0;**	**0;**	**;n**	**;c**	**0;**	**3+**	**;c**	**0;**

[a] "+" or "−" signs following an IT score denotes the level of intensity of infection or an intermediate IT response

identical responses to those produced by the differential "Saia" in tests with the eight *Pca* pathotypes. All produced immune to resistant ITs ranging from "0" to "12" in response to isolates 982774, 933015, 073518, 203594 and 013535. In response to pathotype 691074, all accessions with the exception of two (CIav4639 and PI291991) produced compatible ITs, while the differential Saia produced a moderately compatible IT of "3". The two accessions CIav4639 and PI291991 produced moderately compatible ITs of "3c". A similar response to isolate 982604 was observed, with compatible ITs produced on 17 of the 18 accessions, in addition to a moderately compatible IT of "3" produced on a single accession PI436082, and on the differential Saia. Three accessions CIav8089, PI292226 and PI436082 produced higher ITs ("12c" to "2c") against isolate 933015 compared to other accessions ("0" to ";1"), indicating that the three accessions lacked one of the genes present in the remaining accessions and the differential tester, that was responsible for conditioning an immune to highly resistant response to isolate 933015.

Pc39

Seven tetraploid accessions (PI337809, PI337863, PI337865, PI337900, CIav9121, PI320657, PI411366) and one hexaploid accession (CIav4571) were all postulated to carry *Pc39*, *Pc55* and/ or *Pc71*. While the pathotypes used could not discriminate between these three resistance genes, the intermediate ITs produced by isolate 073518 on all lines and on the differential line carrying *Pc39* suggested that this gene was present in each accession. All of these accessions produced incompatible ITs in response to isolates 691074, 982774, 073518, 853023, and compatible ITs to isolates 982604 and 013535. In contrast, both compatible and moderately compatible ITs were produced in response to isolates 933015 and 203594. In response to isolate 691074, differential PC39 produced an IT of "0;" while ITs of the accessions ranged between "0" and "23". Additionally, the hexaploid accession CIav4571 produced an immune IT of "0;n", while a resistant IT of "12c" was produced on the differential genotype PC39.

Pc45

Seven tetraploid accessions (PI337905, PI337910, CIav9122, PI282709, PI287199, PI287200 and PI1317921) were postulated to carry *Pc45*. All produced resistant to moderately incompatible ITs in response to isolates 691074, 982774 and 853023, moderately compatible to compatible ITs in response to isolates 073518, 933015 and 203594, and compatible ITs in response to isolates 982604 and 013535. The low ITs observed with isolate 691074, while incompatible, were significantly higher than that observed on the differential PC45. While this could indicate that the gene present in the seven accessions is not *Pc45*, it is also possible that this isolate is avirulent for a second gene in the differential genotype, which conferred the low IT "0;". In response to isolate 982604, a moderately compatible IT of "3" was observed on the differential genotype PC45, in contrast to compatible ITs on all of the seven accessions, also possibly indicating the presence of a second gene in the differential.

Pc52

The gene *Pc52* was postulated in three hexaploid accessions (Clav4568, Clav4701 and Clav4713) and a single tetraploid accession (PI282710). In response to isolate 982604, accessions Clav4701 and Clav4713 produced moderately compatible ITs, while accessions Clav4568, PI282710 and the differential tester CI8001 produced compatible ITs. In contrast, incompatible ITs were produced in response to the remaining seven pathotypes.

Pc94

Four tetraploid accessions (PI337896, PI337904, PI295885 and PI337795) and a single diploid accession (PI131641) were postulated to carry the gene *Pc94*. All accessions produced incompatible ITs of "0;" to "23" to the seven isolates avirulent on *Pc94*, and moderately compatible ITs of "3", in response to isolate 203594, the only isolate virulent on *Pc94*. Further, the low ITs observed on the differential carrying *Pc94* were lower than those generated on the five accessions, suggesting the possible presence of a second gene in the differential genotype.

Unidentified resistance groups (URGs)

A total of 171 accessions were resistant to one to seven of the eight *Pca* pathotypes, displaying reaction ITs that did not match any of those produced on the differential testers, hence indicating the presence of unidentified resistance. Of these 171 accessions, 128 were placed into 15 URGs, each comprising more than one accession with similar ITs to the eight pathotypes. For a comprehensive classification of these 128 accessions, IT scores of "3" and greater were considered compatible. Each of the remaining 43 accessions differed in response to the eight pathotypes, producing unique ITs that differed from all differential testers. Therefore, a total of 58 unidentified resistances (i.e. 15 URGs + 43 accessions with unique response ITs) were identified. Overall, accessions of *A. strigosa* carried 17 unidentified resistances (7 URGs + 10 accessions with unique response ITs), accessions of *A. barbata* were postulated to carry 47 unidentified resistances (15 URGs + 32 accessions with unique response ITs) and accessions of *A. sativa* carried three unidentified resistances (2 URGs + 1 accession with a unique response IT).

Unidentified resistance group 1 (URG1)

Five tetraploid accessions (PI317953, PI320588, PI367334, PI367357, PI367360) were susceptible to isolate 982774 and resistant to the seven remaining isolates. Given the susceptibilities of these accessions to isolate 982774, it is possible that they may carry *Pc46* plus an additional resistance gene that is effective against isolate 853023.

Unidentified resistance group 2 (URG2)

URG2 comprised five tetraploid accessions (Clav9060, PI287194, PI317933 PI317937, PI320641), each susceptible to pathotypes 982774, 982604 and 013535, and resistant to the five remaining pathotypes.

Unidentified resistance group 3 (URG3)

Four tetraploid accessions (PI337943, Clav9037, PI367297, PI411364) and seven diploid accessions (PI131695, Clav7121, Clav9110, Clav9116, PI304557, PI436107, PI436115) comprised URG3. All produced resistant ITs in response to pathotypes 073518, 933015 and 203594, and compatible ITs in response to the five remaining pathotypes.

Unidentified resistance group 4 (URG4)

URG4 comprised four tetraploid accessions (PI287189, PI317948, PI367322, PI367323) that were resistant to pathotypes 691074, 073518, 933015 and 203594, and susceptible to the four remaining pathotypes.

Unidentified resistance group 5 (URG5)

URG5 comprised three tetraploid accessions (PI337986, PI295891, PI320612), each with incompatible ITs to pathotypes 691074, 853023 and 933015, and compatible ITs in response to the five remaining pathotypes.

Unidentified resistance group 6 (URG6)

URG6 comprised five tetraploid accessions (PI337935, PI365624, Clav9093, PI320595, PI320600) and five diploid accessions (Clav7122, Clav9012, Clav9030, PI287315, PI436126). All 10 produced incompatible ITs to pathotypes 073518 and 933015, and compatible ITs in response to the six remaining pathotypes.

Unidentified resistance group 7 (URG7)

A total of 17 tetraploid accessions (PI337856, PI337933, PI282729, PI337738, PI337777, PI337779, PI337825, PI337894, PI337927, PI337959, PI362375, PI282772, PI287198, PI320572, PI320575, PI337750, PI411374) and two diploid accessions (PI436118, PI436124) comprised URG7. Each was resistant to pathotypes 933015 and 203594, and susceptible to the six remaining pathotypes. It is possible that these accessions carry both *Pc39* and *Pc61*.

Unidentified resistance group 8 (URG8)

This group comprised tetraploid accession PI320598, hexaploid accession PI487306, and the two diploid accessions Clav9011 and Clav9019. All four produced incompatible ITs to pathotypes 073518 and 203594, and compatible ITs to the six remaining pathotypes.

Unidentified resistance group 9 (URG9)

Nine tetraploid accessions (PI295895, PI320620, PI320625, PI320631, PI320652, PI320659, PI320675, PI320703, PI320704) comprised URG9. All produced incompatible ITs to pathotypes 691074 and 853023, and compatible ITs to the six remaining pathotypes.

Unidentified resistance group 10 (URG10)

URG10 comprised three tetraploid accessions (PI337873, PI337881, PI320637), all resistant to pathotypes 691074 and 982774, and susceptible to the six remaining pathotypes.

Unidentified resistance group 11 (URG11)

In URG11, two tetraploid accessions PI287202 and PI287204 produced incompatible ITs to pathotypes 691074 and 073518, and compatible ITs to the six remaining pathotypes.

Unidentified resistance group 12 (URG12)

URG12 comprised 10 tetraploid accessions (PI337822, PI337899, PI337911, PI282725, PI295894, PI295896,

PI320586, PI320619, PI320629, PI320684) and one diploid accession (PI436131). All 11 were resistant to pathotype 853023, and susceptible to the seven remaining pathotypes.

Unidentified resistance group 13 (URG13)

URG13 comprised five tetraploid accessions (PI337923, PI293342, PI320556, PI320685, PI367303), two hexaploid accessions (Clav4704, PI487309) and 10 diploid accessions (Clav2520, Clav2523, Clav2525, Clav3214, PI274610, PI306419, PI361910, PI436080, PI436111, PI436127). All 17 were resistant to pathotype 073518, and susceptible to the seven remaining pathotypes.

Unidentified resistance group 14 (URG14)

This group comprised 10 tetraploid accessions (PI337839, PI337930, PI337934, PI282724, PI282726, PI295887, PI295888, PI295889, PI296232, PI337752) and four diploid accessions (PI83720, Clav2920, PI111261, Clav9007). All 14 produced incompatible ITs to pathotype 203594, and compatible ITs in response to the seven remaining pathotypes.

Unidentified resistance group 15 (URG15)

URG15 comprised 10 tetraploid accessions (PI287197, PI295879, PI296217, PI296224, PI320624, PI320639, PI320642, PI320671, PI320672, PI320721), all of which were resistant to pathotype 691074, and susceptible to the seven remaining pathotypes.

Discussion

The eight *Pca* pathotypes used in this experiment enabled postulation of four known genes *Pc39*, *Pc45*, *Pc52* and *Pc94*, the "Saia" resistance, unknown resistance(s) effective against all eight pathotypes, and 58 unidentified resistances among the 385 accessions. While *Pc94*, the "Saia" resistance, an unknown resistance(s) and 17 unidentified resistances were postulated in diploid accessions of *A. strigosa* ($2n = 2x = 14$; A_sA_s), genes *Pc39*, *Pc45*, *Pc52*, *Pc94* and 48 unidentified resistances were postulated in tetraploid accessions of *A. barbata* ($2n = 4x = 28$; AABB), and genes *Pc39*, *Pc52* and three unidentified resistances were postulated in hexaploid accessions of *A. sativa* ($2n = 6x = 42$; AACCDD). Additionally, tetraploid *A. barbata* accessions carried a majority of the 58 unidentified resistances. The unknown resistances effective against all eight pathotypes were detected in three diploid accessions (CIav2524, PI78821 and PI83721) and might be conditioned by genes *Pc50* and *Pc68* in combination, *Pc91* or one or more new crown rust resistance genes. In a previous study involving the characterisation and comparison of SSR variability in accessions of diploid *A. strigosa* and tetraploid *A. barbata* (Cabral et al., 2013), two of the above three accessions (PI78821 and PI83721) produced resistant ITs to five of the eight *Pca* pathotypes used in this study. Although both accessions collected in Australia produced near-identical immune responses to all five pathotypes, DNA fingerprints with 11 SSR markers placed them in separate clusters of a UPGMA similarity dendrogram (data not shown).

Evidence was found for the presence of the same *Pc* gene(s) in two different species of *Avena*. The wild hexaploid species *A. sterilis* is reported to be the original source of genes *Pc39*, *Pc45* and *Pc52* (CDL 2010), however in the present study, evidence was obtained for the presence of all three genes in tetraploid accessions of *A. barbata*. Similarly, a single tetraploid accession was postulated to carry the gene *Pc94*, which was first identified in the diploid species *A. strigosa*. The presence of the same resistance gene in species of different ploidy levels could be due to a common genome in each. For example, the wheat leaf rust resistance gene *Lr14a* (Dyck and Samborski 1970), originally identified in emmer wheat, has also been reported in durum wheat (Herrera-Foessel et al. 2008), a subspecies derived from wild emmer; both carry AABB genomes. Additionally, Isidore et al. (2005) reported two highly conserved, stable haplotypes of the *Lr10* locus in the A genome of diploid, tetraploid and hexaploid species.

Based on karyotype and interspecific hybridization studies, there is strong evidence of the involvement of only the A and C genomes in the evolution of hexaploid oat (AACCDD) species (Loskutov 2008). Both A and D genomes of hexaploid oat are similar, and have evolved from the A genome of a diploid progenitor (Linares et al. 1998; Loskutov 2008), which is also the source of origin of other diploid A-genome variants like A_sA_s of *A. strigosa* (Loskutov 2008). Further, the A genome of tetraploid *A. barbata*

(AABB) is morphologically identical to the A_s genome of diploid *A. strigosa* (A_sA_s), the probable source of origin of the B genome in *A. barbata* (Rajhathy and Thomas 1974; Nishiyama et al. 1989). Given the above evolutionary relationships, it might be safe to assume the existence of highly conserved regions of the A genome, across ploidy, that carry identical *Pc* resistances. In the current study, similar gene(s) identified in accessions of *A. strigosa*, *A. barbata* and *A. sativa* might all be associated with the A genome, which is present in all three species.

The "Saia" resistance was postulated in 18 diploid accessions of *A. strigosa*. In response to tests with eight pathotypes, ITs produced on these accessions were almost identical to those observed on the differential Saia, reported to carry genes *Pc15*, *Pc16* and *Pc17* (Murphy et al. 1958). Thus, it could be assumed that these accessions carry one or more of the resistance genes present in Saia. Based on response ITs to pt. 691074 (data not shown), which was virulent for *Pc38* and produced a moderately susceptible IT of "3" on the cultivar Saia, it could be concluded that these accessions might carry one or two of the genes in Saia only, and that the third gene is effective against this pathotype.

Pc39 has been mapped using restriction fragment length polymorphism (RFLP) markers in a hexaploid Kanota/Ogle population (KO_37 linkage group) that is homologous to linkage group 1 in a Pendek-*39*/Pendek-*48* population (Wight et al. 2004). In our study, *Pc39* was postulated in seven tetraploid accessions and a single hexaploid accession. In response to pathotypes 933015 and 203594, mild differences in compatible ("3+") and moderately susceptible ("3" to "3c") IT scores were observed between accessions and the differential control for *Pc39*. In response to pt. 982774, the hexaploid accession Clav4571 produced an immune IT of "0;n", in contrast to "12c" produced on the differential genotype. This could suggest the presence of a gene in addition to *Pc39* in Clav4571.

Gene *Pc45* was postulated in seven tetraploid *A. barbata* accessions. In response to pt. 691074, all produced resistant to moderately resistant ITs of "12cn" to "23", while an immune IT of "0;" was observed on the differential control for *Pc45*. Similarly, compatible responses to pathotype 982604 in the accessions, and a moderately susceptible IT of "3c" were also observed in the differential control for *Pc45*. The higher ITs observed on the accessions compared with the differential could be due to genetic background, including differences in ploidy between the tetraploid accessions and hexaploid differential. Research in oat and wheat has shown that the expression of rust resistance genes can be affected by ploidy levels. The suppression of resistance to *Pca* in interspecific gene transfers from lower to higher ploidies in oat was reported by Rines et al. (2007) and in wheat by Bai and Knott (1992).

Pc52 was postulated in three hexaploid accessions and a single tetraploid accession. The hexaploid accessions produced immune ITs in response to pathotype 013535, and a resistant IT of ";1" on the differential control CI8001 and the tetraploid accession. This could suggest the presence of a resistance gene in addition to *Pc52* in the three hexaploid accessions.

Pc94 was reported to be a good source of crown rust resistance in North America and was initially transferred from the diploid *A. strigosa* genotype RL1697 to the hexaploid genotype SUNII (Aung et al. 1996). In the present study, *Pc94* was postulated in four tetraploid accessions and a single diploid accession. It has been mapped in a hexaploid population of a Calibre/S42 cross (CaS42_1 linkage group) using sequence-characterized amplified region (SCAR) markers that were developed from amplified fragment length polymorphisms (AFLP) between resistant and susceptible F_2 DNA bulks (Chong et al. 2004). The accessions produced resistant to moderately resistant ITs compared with immune responses observed on the differential PC94. These variable IT responses could be due to differences in genetic background (e.g. ploidy level) or could suggest that the gene identified is not *Pc94*. Genetic confirmation of the gene identified in the present study could be established by conducting a test of allelism between the diploid accession PI131641 and the original diploid accession RL1697. However, undertaking similar studies with the tetraploid accessions postulated to carry *Pc94* might not be as straight forward.

An interesting outcome of this study was the detection of crown rust resistance genes *Pc39*, *Pc45*, *Pc52* and *Pc94* in species/ploidy that were different from those reported previously. *Pc39* was first detected in a hexaploid *A. sterilis* L. line F366 (Fleischmann and McKenzie 1968), which however, in our study was postulated in seven tetraploid *A. barbata* accessions and a single hexaploid *A. sativa* accession. Similarly, *Pc45*

which was first identified in the hexaploid *A. sterilis* L. line F169 (Fleischmann et al. 1971), but was postulated in seven tetraploid *A. barbata* accessions of our study. Further, *Pc52* postulated in three hexaploid *A. sativa* accessions and a single tetraploid *A. barbata* accession of our study, was initially identified in hexaploid *A. sterilis* L. and carried in Iowa isoline X421 (Simons et al. 1978). The four tetraploid *A. barbata* accessions postulated to carry *Pc94*, in his study, could act as an additional donor-source (besides *A. strigosa* genotype RL1697) for interspecific-introgression into cultivated oat. A proper deployment of such resistances from different genetic background/ploidy levels could help create and maintain the diversity of a resistance gene(s) which in turn might provide effective protection against the *Pca* pathogen.

The wild hexaploid *A. sterilis* L. is the progenitor of hexaploid oat species including the cultivated *A. sativa* (Loskutov 2008). As such, it would be interesting to characterize and compare resistances in the two species. However, this comparison was not possible in our study due to the absence of *A. sterilis* accessions from the collection. In future, it would be worthwhile to undertake similar studies that involve accessions of the above two species.

One hundred and seventy one accessions were resistant to up to seven of the eight pathotypes of *Pca* used. A total of 58 unidentified resistances (15 URGs + 43 accessions with unique ITs) were detected among the 171 accessions, a majority of which were in the tetraploid species *A. barbata*. The ITs produced on these accessions in response to the eight pathotypes did not match those observed on the differential tester lines. This suggests that accessions of *A. barbata* are a potential source of new crown rust resistance. Further studies are, however, needed to confirm these findings and to identify resistances that might be useful.

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