PCR-Based High-resolution Melt Markers for Pc genes. Part 2: SNPs linked to *Pc38*, *Pc48*, *Pc68*, and *Pc71*.

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This article is the second part of our informal release of molecular marker information for crown rust resistance genes. In the first part, we reported High-resolution Melt (HRM) markers for the seedling resistance genes *Pc58a* and *Pc91* and for the QTLs *QCr.cdl11-13A* and *QPc.crc-14D* (Yimer and Esvelt Klos 2016). Here, we report HRM markers and assays for the genes *Pc38*, *Pc48*, *Pc68,* and *Pc71*. Previously published markers for these *Pc* genes include RFLP markers for *Pc38, Pc48,* and *Pc71* (Wight *et al.* 2004; Bush and Wise 1998); and RFLP and AFLP markers for *Pc68* (Bush and Wise 1996; Kulcheski *et al.* 2010). In order to increase the options available for marker-assisted selection, we have created PCR-based HRM markers from SNPs in the target genomic regions. First, we used the placement of RFLP and AFLP markers on the consensus map of Chaffin *et al.* (2016) to identify probable linked SNPs. The criteria used to select SNPs for assay development included proximity to the marker (within 5cM; Table 1), availability of sufficient nucleotide sequence before and after the SNP to accommodate primers, and presence of information on minor allele frequency from the CORE genotyping panel. The sequence around these SNPs is available on T3/Oat at http://triticeaetoolbox.org/oat/, should you need to design primers for a different assay type.

Table 1. Selected SNPs near published RFLP and AFLP markers linked to *Pc* genes.

Pc gene	Marker	Position (cM)[a]	Distance (cM)[b]	SNP	Position (cM)[a]
Pc38	CDO341	Mrg02 85.2	2	GMI_ES17_c4223_141	Mrg02 85.2
				GMI_DS_LB_459	Mrg02 85.2
Pc48	UMN401	Mrg20 127.4	6	GMI_ES01_c10310_366	Mrg20 135.8
	CDO1471	Mrg20 119.7	6	GMI_ES_LB_9185	Mrg20 115.6
Pc68	avnA	Mrg19 64	12	GMI_DS_LB_8147	Mrg19 59.2
				GMI_ES02_c11450_462	Mrg19 64.7
	CDO309A	Mrg19 73.1	10.1	GMI_DS_A3_340_378	Mrg19 71.3
Pc71	BCD1270	Mrg05 126.1	0.4	GMI_ES01_c3435_183	Mrg05 125.1
				GMI_ES15_c14779_89	Mrg05 125.1

a. Position on the consensus map of Chaffin *et al.* (2016).
b. Distance (cM) between marker and *Pc* gene in the original linkage map.

Primers are listed in Table 2. The basic assay protocol, reagents, and PCR conditions for the High-resolution Melt Assay were described in a previous article (Yimer and Esvelt-Klos, 2016).

Table 2. HRM marker primers for SNPs linked to crown rust resistance *Pc* genes.

***Pc* gene**	**SNP**	**HRM primer**
Pc38	GMI_ES17_c4223_141	F-GATGCCCAAGCACTTCTCC R-GAAGAAGGTACAATGATAGGAGCTG
	GMI_DS_LB_459	F-GTCTGAGCAGCTAGGGATCG R-TGCCCAAGTTCAGTTCAGTG
Pc48	GMI_ES01_c10310_366	F- CTGGTACGACCGTTCTGTCA R- CCTTCTTCGATCATCGGCTA
	GMI_ES_LB_9185	F-CATCTCTGGATTTGAGGGTGA R-CTTGGGCTCATTGATCTGGT
Pc68	GMI_DS_LB_8147	F- GGCACCATACGAGGTAGTTTATG R- TGTGGGTAGATTTTTGTGACCA
	GMI_ES02_c11450_462	F-GGGCGATTTCTTGTAGTGGA R-TCTCAGATGGTGAGGTTAGTATCG
	GMI_DS_A3_340_378	F-AAAGTGGCAACAAAGGGATGAC R-GCGGTATTTTGATCTGATTTGC
Pc71	GMI_ES01_c3435_183	F- CAAGCTCTGTGAAGATGTTGC R- AAAGCGACGAATTTCAAGCA
	GMI_ES15_c14779_89	F-GATACTACAGACGAGGCATCCA R-CAAGATCACAACTGGCCTCA

HRM genotypes are recorded as cluster number (1, 2, or 3) and presented in Table 3 for a variety of germplasm, including susceptible lines and several *Pc* differential lines. Assays were robust, with fewer than 10% missing calls. In all cases, the differential line associated with the linked *Pc* gene was categorized as genotype group 1.

Table 3. Genotype calls (as a cluster number) for 5 SNPs linked to *Pc* genes.

Line	Notes	*Pc38*		*Pc48*		*Pc 68*			*Pc71*	
		GMI_ES17_c4223_141	GMI_DS_LB_459	GMI_ES01_c10310_366	GMI_ES_LB_9185	GMI_ES02_c11450_462	GMI_DS_A3_340_378	GMI_DS_LB_8147	GMI_ES01_c3435_183	GMI_ES15_c14779_89
94197A1-9-2-2-2-5	*QCr.cdl11-13A* donor	1	3	3	3	1	1	1	1	1
Ajay	Susceptible	1	3	2	1	1	3	2	3	3
Amagalon	*Pc91* donor	2	2	1	2	1	3	2	2	2
Ascencao	Seedling resistance from *Pc14*	1	3	1	1	2	1	3	1	1
Assiniboia/S42	Seedling resistance from *Pc38* and *Pc68*	1	3	1	1	1	3	2	3	1
Bia		1	3	3	1	1	1	2	1	3
Boyer CDC	APR resistance	3	3	1	1	2	NC	3	3	1
Calibre		1	3	3	1	2	3	3	3	1
CAV 5041	Seedling resistance from *Pc48*	1	3	3	3	1	1	2	2	2
CAV 4904	Seedling resistance from *Pc68*	1	1	1	1	1	3	2	2	2
CAV 2648	Seedling resistance from *Pc38*	1	1	3	1	1	1	2	1	2
CAV 1832	Seedling resistance from *Pc54*	1	3	3	1	1	3	2	2	2
CAV 1830	Seedling resistance from *Pc54*	1	3	3	1	1	3	2	2	2
CDC Dancer	Susceptible	3	3	1	3	2	3	3	1	3
Clav 6661	Susceptible	1	3	1	1	1	3	1	3	1
CN 33027	Seedling resistance from *Pc54*	1	3	1	1	2	3	3	1	1
CN 1967	Seedling resistance from *Pc38*	1	1	1	1	2	1	3	1	1
CN 1962	Seedling resistance from *Pc48*	1	3	3	1	1	3	2	1	1
CN 1956	Seedling resistance from *Pc38*	1	1	1	1	2	3	3	1	1
CW 491	Seedling resistance from *Pc38*	1	1	3	1	1	1	2	1	2
Dumont	Seedling resistance from *Pc38*	1	1	1	1	2	2	1	3	1
D526	Seedling resistance from *Pc71*	1	1	3	1	1	3	1	1	1
Exeter	Susceptible	2	3	1	1	2	3	2	1	3
Fidler	Susceptible	3	3	1	1	2	1	3	1	3
FLO772-R3		2	3	3	1	1	3	2	1	NC
Florida 501	Some seedling resistance	2	1	NC	1	1	3	NC	1	1
Gem	Susceptible	1	1	NC	NC	1	1	NC	1	1
Gerard		1	3	1	1	1	3	2	NC	NC
Goslin	Seedling resistance from *Pc48*	1	3	1	1	1	2	NC	1	3

HiFi	*Pc91* mapping parent	3	3	1	1	1	1	2	2	2
Horizon	Susceptible	2	1	3	1	2	3	NC	1	NC
Hudson	Susceptible	1	3	3	1	1	3	NC	1	3
Kangaroo	Susceptible	1	3	3	1	1	2	2	2	2
Kanota		1	3	NC	1	2	3	2	2	NC
Lang	Susceptible	1	3	3	1	1	2	2	1	1
La 07065SBSBSB-32	Seedling resistance	1	3	1	1	1	NC	3	1	2
Leggett	Seedling resistance from *Pc94*	3	3	3	1	2	2	2	3	3
Makuru	Susceptible	3	NC	1	1	NC	3	3	3	3
MAM 17-5	APR resistance	1	1	3	1	1	1	1	1	3
Marion		2	3	3	3	2	1	3	1	3
Milford		1	3	1	1	1	3	3	3	3
MN841801	*QPc.crc-14D* donor	1	3	3	1	1	3	1	1	2
Morgan AC	Susceptible	3	3	3	1	1	3	2	1	3
Morton	Seedling resistance	3	3	3	1	2	1	3	1	3
Noble 2	Susceptible mapping parent	3	1	3	1	1	3	2	1	3
Ogle	Susceptible mapping parent	2	3	3	NC	3	NC	3	1	NC
Otana	Susceptible mapping parent	3	3	3	3	NC	1	3	3	3
OT62	Resistant Ogle/TAMO-301 RIL	2	NC	3	NC	NC	NC	1	2	NC
Pc14	*Pc* differential line	1	3	3	3	2	3	2	3	1
Pc35	*Pc* differential line	1	3	1	1	2	3	2	1	1
Pc36	*Pc* differential line	1	1	3	1	1	3	1	1	NC
Pc38	*Pc* differential line	1	1	1	1	2	3	2	1	1
Pc39	*Pc* differential line	NC	3	1	1	2	3	1	1	1
Pc40	*Pc* differential line	1	3	1	1	2	3	2	1	1
Pc45	*Pc* differential line	1	3	1	1	2	3	2	1	1
Pc46	*Pc* differential line	1	3	1	3	2	1	3	1	1
Pc48	*Pc* differential line	1	3	1	1	3	3	3	1	1
Pc50	*Pc* differential line	1	3	1	1	2	NC	2	1	1
Pc51	*Pc* differential line	1	1	3	1	1	1	2	3	1
Pc52	*Pc* differential line	1	3	1	1	2	NC	3	1	1
Pc53	*Pc* differential line	1	3	1	1	1	1	2	1	1
Pc54	*Pc* differential line	1	3	3	1	1	3	1	1	1
Pc55	*Pc* differential line	1	3	1	1	2	3	2	1	1
Pc56	*Pc* differential line	1	3	1	1	2	3	2	1	1
Pc57	*Pc* differential line	1	1	3	1	1	1	1	3	1
Pc58	*Pc* differential line	1	3	1	1	1	1	2	2	2
Pc59	*Pc* differential line	1	1	3	1	1	1	2	1	1
Pc60	*Pc* differential line	1	3	1	1	2	NC	2	1	1
Pc61	*Pc* differential line	1	3	3	1	1	2	1	1	1

Pc62	*Pc* differential line	1	3	1	1	1	1	1	3	1
Pc63	*Pc* differential line	1	NC	1	1	1	1	1	NC	2
Pc64	*Pc* differential line	1	3	3	1	1	1	1	1	1
Pc67	*Pc* differential line	1	3	3	1	1	1	1	3	1
Pc68	*Pc* differential line	1	3	3	1	1	1	1	1	1
Pc70	*Pc* differential line	1	3	3	1	1	3	1	1	1
Pc71	*Pc* differential line	1	1	NC	1	1	1	1	1	1
Pc91	*Pc* differential line	1	1	3	1	2	2	1	1	1
Pc94	*Pc* differential line	1	1	3	1	1	1	1	3	1
Pc96	*Pc* differential line	1	3	1	1	2	3	2	3	1
Pinnacle AC	Seedling resistance from *Pc68*	3	3	1	1	2	3	3	3	3
Provena	Susceptible mapping parent	3	3	3	1	2	3	3	1	3
Rigodon		3	1	NC	1	1	3	2	3	3
Ronald	Seedling resistance from *Pc68*	3	3	1	3	2	2	2	3	3
Shadow	Susceptible	1	3	3	1	1	3	2	3	1
Sol-Fi CDC	Susceptible mapping parent	1	1	3	1	1	1	2	1	2
Summit	Seedling resistance from *Pc38*, *Pc48*, and *Pc68*	1	NC	3	1	1	1	1	1	2
Sun II	Susceptible	1	3	3	1	1	3	1	3	1
TAM-O-301	*Pc58a* donor	3	3	1	3	1	1	3	2	2
TAM-O-405	Seedling resistance	1	3	1	1	1	3	3	1	2
Tardis	Susceptible	3	1	1	1	2	3	3	2	2
UFRGS 8	Susceptible	1	1	NC	NC	1	2	NC	1	1
X48411		1	1	3	1	1	1	2	1	3
Y345	Seedling resistance from *Pc71*	1	1	3	1	1	1	1	2	2

NC= no call

Table 4 summarizes the presence of the genotype possessed by the relevant *Pc* differential line in several categories of germplasm. The marker GMI_ES17_c4223_141 uniformly categorized all presumed carriers of the *Pc38* gene into a single genotype class along with the *Pc* differential line. Although most likely physically linked to their respective genes of interest, the other markers misclassified carrier lines with susceptible germplasm to some extent. This may reflect the potential for recombination between markers and *Pc* genes.

The rare alleles of GMI_ES01_c10310_366, GMI_ES02_c11450_462, GMI_DS_A3_340_378, GMI_ES01_c3435_183, and GMI_ES15_c14779_89 may occur so infrequently in the oat population at large that they will seldom be of use for marker-assisted selection in breeding. Markers GMI_ES17_c4223_141, GMI_DS_LB_459, GMI_ES_LB_9185, and GMI_DS_LB_8147 could be used in marker-assisted selection in crosses where they are polymorphic between parents.

For all markers linked to these *Pc* genes, some susceptible lines had the same marker genotype as the relevant differential line. Therefore, none of these markers would be appropriate for diagnosing the presence of a *Pc* gene in germplasm with an uncharacterized genetic background.

Table 4. Frequency of the genotype class carried by the *Pc* differential lines for *Pc38*, *Pc48*, *Pc68,* and *Pc71*.

Pc Gene	SNP	*Pc* gene carriers (#)[a]	Susc.& diff. lines[b]	Other lines[c]	Total[d]
Pc38	GMI_ES17_c4223_141	1.00 (7)	0.78	0.63	0.74
Pc38	GMI_DS_LB_459	0.71 (7)	0.27	0.21	0.29
Pc48	GMI_ES01_c10310_366	0.25 (4)	0.47	0.49	0.47
Pc48	GMI_ES_LB_9185	0.75 (4)	0.91	0.86	0.89
Pc68	GMI_ES02_c11450_462	0.4 (5)	0.55	0.69	0.61
Pc68	GMI_DS_A3_340_378	0.4(5)	0.33	0.42	0.37
Pc68	GMI_DS_LB_8147	0.4 (5)	0.29	0.21	0.28
Pc71	GMI_ES01_c3435_183	0.50 (2)	0.69	0.51	0.62
Pc71	GMI_ES15_c14779_89	0.50 (2)	0.68	0.33	0.53

a. Lines known or conjectured to derive seedling resistance from the *Pc* gene linked to the relevant marker with the number of lines in this class in parentheses.
b. Fifty-one lines susceptible to crown rust and differential lines not carrying the relevant *Pc* gene.
c. All other lines genotyped.
d. The frequency of the marker genotype class over the successfully genotyped 93 lines.

References

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