New Sources of Adult Plant and Seedling Resistance to *Puccinia coronata* f. sp. *avenae* Identified among *Avena sativa* Accessions From the National Small Grains Collection

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Abstract

Accessions of cultivated oat (*Avena sativa* L.) from the United States Department of Agriculture–Agricultural Research Service Small Grains Collection in Aberdeen, ID were characterized for adult plant resistance (APR) and seedling resistance to crown rust, caused by *Puccinia coronata* f. sp. *avenae*. Initially, 607 oat accessions with diverse geographic origins were evaluated in field tests in Baton Rouge, LA. Of those, 97 accessions were not fully susceptible and were tested in the field in St. Paul, MN against a diverse *P. coronata* f. sp. *avenae* population. Thirty-six accessions that had some level of resistance in both field tests and mean coefficients of infection of ≤ 20 were further evaluated for APR and seedling resistance. Among these, four accessions (PI 193040, PI 194201, PI 237090, and PI 247930) were resistant to eight *P. coronata*

f. sp. *avenae* races as seedlings. Twenty-nine accessions had resistance to at least one of the *P. coronata* f. sp. *avenae* races. Three accessions (CIav 2272, CIav 3390, and PI 285583) were fully susceptible to all eight *P. coronata* f. sp. *avenae* races as seedlings. Further evaluation of the three seedling-susceptible accessions at the flag leaf stage in a growth chamber resulted in moderately susceptible to moderately resistant responses. The resistance sources presented here may contain genes not deployed in elite oat varieties, and may be useful for future crown rust resistance breeding. The adult and seedling resistance found in accessions of the cultivated oat species is especially valuable because it avoids problems associated with the transfer of genes from wild species to cultivated oat.

Cultivated oat (*Avena sativa* L.) is an important cereal grown as a dual-purpose crop (forage and grain), mainly in Australia, North America, and Europe. In many environments where oat is cultivated, crown rust disease, caused by *Puccinia coronata* f. sp. *avenae* P. Syd. & Syd., is the main limiting factor for production (Carson 2011). Estimates of significant yield reduction include \$16 million over a 5-year period (2001 to 2005) in Canada (McCallum et al. 2007) and between 1.7 and 20% of oat production in the United States (Carson 2011). Host resistance is the most effective and economically viable method of crown rust control. Over 100 race-specific genes for crown rust resistance have been identified in oat. Unfortunately, the emergence of new races in populations of *P. coronata* f. sp. *avenae* is continuous and rapid as the pathogen undergoes sexual recombination on the alternate host, buckthorn (*Rhamnus carthartica* L.) (Carson 2011). As a result, the effectiveness of newly deployed seedling resistance genes is often short-lived in North America (i.e., 5 years or less) (Carson 2011; Chong and Kolmer 1993; Leonard et al. 2004). Many seedling resistance genes such as *Pc38*, *Pc39*, *Pc48*, and *Pc68* have lost their effectiveness over time (McCallum et al.

2007). Adult plant resistance (APR), where the plant is susceptible at the seedling stage but resistant at the reproductive stage, is an alternative to seedling resistance and may improve the durability of resistance in cereals to rust diseases (Basnet et al. 2013). The effectiveness and durability of APR has been demonstrated in various cereal–rust pathosystems (Basnet et al. 2013; McIntosh et al. 1995; Milus et al. 2015). Combining APR with seedling resistance would provide protection throughout the growing season, and pyramiding multiple APR and seedling resistance types has potential to provide greater durability of resistance than deployment of either resistance type alone (Bariana et al. 2007; Sandhu et al. 2014).

Only a few quantitative trait loci that confer APR to crown rust in oat have been described (Acevedo et al. 2010; Babiker et al. 2015; Cabral et al. 2011; Klos et al. 2017; Lin et al. 2014; Portyanko et al. 2005). Hence, there is a need to identify new sources of resistance to counter the ever-evolving virulence of the oat crown rust pathogen.

The United States Department of Agriculture (USDA) National Small Grains Collection (NSGC) in Aberdeen, ID currently holds over 21,000 accessions of *Avena* spp. that were collected worldwide since the 1920s (Germplasm Resources Information Network [GRIN]; <https://www.ars-grin.gov/npgs/>). Systematic testing of the collection for crown rust resistance was carried out in the early 1980s in Ames, IA and in the 1990s in St. Paul, MN. Since then, only sporadic attempts have been made to test NSGC accessions for crown rust resistance, with the main focus on wild relatives of oat (Cabral and Park 2014; Carson 2009; Tan and Carson 2013). There are nearly 4,000 *Avena* spp. accessions in the NSGC that have not yet been tested for resistance to crown rust, some of which may contain novel genes for adult plant and seedling resistance. The objective of this study was to assess field resistance to crown rust in a diverse set of *A. sativa* accessions and to characterize the level and pattern of seedling resistance and APR in selected accessions.

Materials and Methods

Plant and pathogen materials. In total, 607 *A. sativa* accessions originating from 44 countries (Table 1) were obtained from the

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NSGC and evaluated for field and seedling resistance to crown rust. The accessions were randomly chosen from among all the accessions, with a bias toward those that had no crown rust resistance evaluations on record. Accessions that previously had not been evaluated

comprised 63% of the sample. The accessions were classified as cultivars (48%) or landraces (30%), or had uncertain improvement status (22%).

Eight *P. coronata* f. sp. *avenae* races—three from the USDA Agricultural Research Service (ARS) Small Grains and Potato Germplasm Research Unit, Aberdeen, ID and five from the USDA-ARS Cereal Disease Laboratory, St. Paul, MN—were sourced for seedling tests.

Evaluation of accessions for field resistance. The 607 oat accessions, along with the susceptible check cultivar Otana and the resistant check cultivar TAM-O-301 carrying the *Pc58* seedling resistance gene complex, were planted at the Louisiana State University experiment station in Baton Rouge, LA in November 2015. Each accession was planted in a nonreplicated head row. The susceptible and resistant checks were planted every 40 rows. Natural *P. coronata* f. sp. *avenae* infection occurred and crown rust was rated twice in March and April 2016, first at the booting stage and then when most plants were at the flag leaf stage. Disease severity was measured from 0 to 100% based on the modified Cobb scale (Peterson et al. 1948). Infection response was recorded as resistant, with necrotic flecks (R); moderately resistant, with small uredinia surrounded by necrosis or chlorosis (MR); moderately susceptible, with medium to large uredinia surrounded by chlorosis (MS); and susceptible, with large

Table 1. Geographic origin of *Avena sativa* accessions and their infection response under field conditions in Baton Rouge in 2016

Origin	Tested (n) ^y	Infection response ^x				Some resistance (%) ^z
		S	MS	MR	R	
Europe	418	387	15	10	6	7.4
North America	86	67	4	12	3	22.1
Asia	23	20	1	2	0	13
South America	44	10	14	10	10	77.3
Africa	19	17	0	1	1	10.5
Australia	17	9	3	3	2	47.1
Total	607	510	37	38	22	16

^x Abbreviations: S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant.

^y Number of accessions tested.

^z Percentage of accessions with some level of disease resistance, with infection responses including MS, MR, or R.

Table 2. Reaction of 36 oat accessions to crown rust under natural infection in the field as adult plants in Baton Rouge (BR) LA and St. Paul (SP) MN in 2016 and 2017

Accession	Origin	Status ^z	Adult plant rating ^x				Mean (±SD) ^y	
			BR-2016	BR-2017	SP-2016	SP-2017	Severity	CI
Otana	...	Cultivar	40S	50S	60S	60S	52.5 ± 6.6 a	52.5 ± 6.6 a
PI 197790	Australia	Uncertain	30MR	40MS	25MR	20MS	28.8 ± 5.5 ab	17.5 ± 6.9 b
PI 287298	Italy	Landrace	30MR	30MS	30MR	30MS	30 ± 0 ab	18 ± 6.9 b
Clav 4318	United States	Cultivar	30MR	20MR	30MS	40MR	30 ± 5.2 ab	15 ± 6.8 b
TAM-O-301	...	Cultivar	10MR	15MR	25MR	15MR	16.3 ± 3.3 b	6.5 ± 2.5 b
PI 137599	Argentina	Landrace	5R	1R	30R	10R	11.5 ± 9.8 b	2.3 ± 0.8 b
PI 140902	Australia	Cultivar	5R	20MS	30MR	20R	18.8 ± 7.3 b	8.3 ± 3.9 b
PI 140903	Australia	Uncertain	10MR	20MR	20MR	20R	17.5 ± 2 b	6 ± 2.3 b
PI 163404	Argentina	Unknown	10MR	10R	30MR	10MS	15 ± 7 b	6.5 ± 3.4 b
PI 168091	Turkey	Landrace	20R	10R	30MR	10R	17.5 ± 6.6 b	5 ± 2.8 b
PI 168092	Turkey	Landrace	10R	10R	25MR	10R	13.8 ± 4.5 b	4 ± 3 b
PI 175023	India	Landrace	10MR	10MR	25MR	20MS	16.3 ± 4.5 b	8.5 ± 4.7 b
PI 177842	Turkey	Landrace	10R	10R	30MS	20MR	17.5 ± 6.6 b	9 ± 7.3 b
PI 186268	Argentina	Cultivar	1R	10R	20MR	1R	8 ± 6.1 b	2.6 ± 1.7 b
PI 186270	Argentina	Uncertain	10MR	20R	30MR	5MS	16.3 ± 8.1 b	6 ± 3 b
PI 186619	Brazil	Cultivar	10MR	10R	30MR	20MR	17.5 ± 6.6 b	6.5 ± 3.4 b
PI 189628	Brazil	Uncertain	10MR	10R	25MR	5MR	12.5 ± 5.7 b	4.5 ± 2.8 b
PI 193040	Argentina	Unknown	10R	10R	20R	Missing	13.3 ± 2.8 b	2.7 ± 0.96 b
PI 194201	Uruguay	Uncertain	5R	10R	30MR	10MR	13.8 ± 8.1 b	4.8 ± 2.9 b
PI 198224	Argentina	Cultivar	5R	10R	30MR	1MR	11.5 ± 8.9 b	3.9 ± 2.4 b
PI 219765	Greece	Uncertain	20MR	30MS	30MR	30MS	27.5 ± 2 b	17 ± 6.2 b
PI 237090	Brazil	Uncertain	10MR	10MR	25MR	Missing	15 ± 4.1 b	6 ± 2.8 b
PI 244465	Unknown	Unknown	5R	missing	20MR	Missing	12.5 ± 3.4 b	4.5 ± 2.3 b
PI 247930	Brazil	Uncertain	1R	5R	30MR	10MR	11.5 ± 8.9 b	4.3 ± 2.3 b
PI 247932	Brazil	Cultivar	5R	5R	30MR	10MR	12.5 ± 7.9 b	4.5 ± 2.3 b
PI 285583	Poland	Cultivar	20MR	30MS	20MR	10MR	20 ± 5.2 b	11 ± 6.8 b
PI 287288	Greece	Unknown	10R	10MR	20MR	Missing	13.3 ± 2.8 b	5.3 ± 2.6 b
PI 287295	Italy	Landrace	20R	20MS	30MR	Missing	23.3 ± 2 b	15 ± 5.1 b
PI 287296	Italy	Landrace	20MR	20MS	30MR	20MS	22.5 ± 5 b	13 ± 3.8 b
PI 287300	Greece	Landrace	20R	20MR	25MR	20MR	21.3 ± 1.5 b	7.5 ± 2.5 b
PI 287302	Greece	Landrace	10R	10MR	35MR	20MR	18.8 ± 8.8 b	7 ± 3.3 b
PI 287306	Albania	Landrace	30MR	30MR	30MR	20MS	27.5 ± 2 b	13 ± 2 b
PI 287307	Italy	Landrace	30MR	30MR	30MR	20MS	27.5 ± 2 b	13 ± 2 b
Clav 2272	United States	Uncertain	30MR	10R	25MR	10R	18.8 ± 7.3 b	6.5 ± 3.3 b
Clav 3390	United States	Cultivar	20MR	30MS	30MR	20MR	25 ± 6.6 b	15 ± 9.5 b
Clav 3412	United States	Cultivar	30MR	20MS	35MR	20MS	26.3 ± 4.5 b	14.5 ± 1.9 b
Clav 4042	United States	Cultivar	10R	20MS	40MS	30MS	25 ± 9.9 b	18.5 ± 9.7 b
Clav 4097	United States	Cultivar	40MR	20MS	30MS	20MS	27.5 ± 6.6 b	18 ± 4 b

^x Abbreviations: S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant.

^y SD = standard deviation and CI = coefficient of infection. Means followed by the same letter in a column are not significantly different ($\alpha = 0.05$).

^z Improvement status.

uredinia without chlorosis or necrosis (S) (McCartney et al. 2011). Ninety-seven accessions that were not susceptible (with infection response R, MR, and MS) in Baton Rouge were further evaluated at the Matt Moore Buckthorn Nursery of the Cereal Disease Laboratory (CDL) in St. Paul, MN in summer 2016. The test at the CDL was conducted following experimental procedures similar to those in Baton Rouge, except that two resistant (TAM-O-301 and MN841801) and two susceptible (Otana and Protégé) oat genotypes were planted every 20 rows. The average coefficient of infection (CI) was calculated from the two field scores (severity on modified Cobb scale and the infection response). The scores were converted to CI by multiplying severity by an assigned constant value for the field response (R = 0.2, MR = 0.4, MS = 0.8, and S = 1.0) (Stubbs et al. 1986). Thirty-six accessions that were not susceptible in either location and with CI ≤ 20 were further evaluated in Baton Rouge and St. Paul in 2017. The same accessions were also used in the seedling resistance tests.

Evaluation of landraces for seedling resistance. The 36 accessions that were not susceptible at either field location were tested for seedling resistance against eight *P. coronata* f. sp. *avenae* races. In addition, *Pc* differential lines were included in the seedling evaluation. A single pustule from each of the eight races was propagated on Otana plants. Three or four seeds of each test material were sown

in 3.8-cm-diameter cone-tainers containing a 3:2:2 (vol/vol/vol) mix of sand, peat moss, and vermiculite in a greenhouse. The experiment was conducted in two replications. Two weeks after planting, seedlings were inoculated with uredinia of each race suspended in Sol-trol170 isoparaffin (Chevron Phillips, The Woodlands, TX) using a motorized sprayer (GAST Manufacturing, Inc., Benton Harbor, MI) and a small atomizer (G-R Manufacturing Manhattan, KS). Plants were left in the open for 1 h to dry, then transferred to a dew chamber set at 20°C with no light. After 18 h in the dew chamber, plants were placed in a growth chamber adjusted to 20 to 22°C with an 18-h photoperiod. Genotype reactions to each *P. coronata* f. sp. *avenae* race were recorded as infection type (IT) on the first and second leaves 14 days after inoculation. IT were based on a 0-to-4 scale (Chong et al. 2000), with + and – indicating intermediate types. Infection types 0 to 2+ were considered resistant and 3– to 4+ susceptible. The tests were repeated and, when there was discrepancy in disease rating between the first and second test, a third test was conducted.

Growth chamber evaluation of accessions for APR. Three accessions (Clav 2272, Clav 3390, and PI 285583) that were susceptible to the eight *P. coronata* f. sp. *avenae* races at the seedling stage but resistant in the field were evaluated in the growth chamber to confirm their APR status. Seed of the three accessions and checks (Otana and TAM-O-301) were planted in containers and placed in a

Table 3. Mean disease severity, coefficient of infection, and standard error (SE) on Otana and TAM-O-301 in Baton Rouge (BR-16 and BR-17) and St. Paul (SP-16 and SP-2017)

Cultivar	Disease severity (±SE)				Coefficient of infection (±SE)			
	BR-16	BR-17	SP-16	SP-17	BR-16	BR-17	SP-16	SP-17
Otana	40 ± 2.2	50 ± 1.7	60 ± 2.1	60 ± 1.9	40 ± 2.2	50 ± 1.7	60 ± 2.1	60 ± 1.9
TAM-O-301	10 ± 1.1	15 ± 1.1	25 ± 0.9	15 ± 1.0	3.7 ± 0.38	4.9 ± 0.58	10 ± 0.34	5.3 ± 0.31

Table 4. Infection types produced by eight *Puccinia coronata* f. sp. *avenae* races on 31 *Pc* gene differentials^z

Differential	<i>Pc</i> gene	Race designation							
		MGBH	LLMG	LCBB	LQBG	MGLB	NSGG	LSGG	LBBB
Ascencao	<i>Pc14</i>	3	3+	3	4	34	3	4	3
D-137	<i>Pc35</i>	0	0;	;	3	4	;	2	1
CI 8081	<i>Pc36</i>	33-	;	;	3	34	34	4	;
CW491-4	<i>Pc38</i>	21+	34	;	3	;	3	4	;
F-366	<i>Pc39</i>	4-	;	;	4	3	4	34	1
F-83	<i>Pc40</i>	4	3	3	43	3	34-	43	4
F-169	<i>Pc45</i>	0;	0	;	;	;	;	;	0
F-290	<i>Pc46</i>	1+2	;	;	;	;	3-	2	;
F-158	<i>Pc48</i>	0	0	0;	;	;	4	34-	0
CW-486	<i>Pc50</i>	3	0;	;	;	0;	0	0	;
Wahl number 8	<i>Pc51</i>	;	3	;	;	2	;	;	2
Wahl number 2	<i>Pc52</i>	0	;	;	;	;	33-	3+	0
6-112-1-15	<i>Pc53</i>	34	;	0;	;	0	;	;	0
CAV 1832	<i>Pc54</i>	0;	0;	11+	;	;	;	;	;
CAV 4963	<i>Pc55</i>	4	;	;	43	3	34	34-	;
CAV 1964	<i>Pc56</i>	4	43+	;	3	;	34	34-	2
CI 8295	<i>Pc57</i>	3	3-	;	;	;	3	34	1+2
TAM-O-301	<i>Pc58</i>	0;	0;	0;	;	;	;	;	0;
TAM-O-312	<i>Pc59</i>	1	34-	;	;	;	;	;	1
Coker 227	<i>Pc60</i>	3	;	1+	;	;	2	1-	0
Coker 234	<i>Pc61</i>	3	3	3	2	11+	;	2	2
Fraser Pc62	<i>Pc62</i>	;	;	;	;	;	;	;	;
Fraser Pc63	<i>Pc63</i>	3	0	;	0;	;	11+	22+	0
Makuru//Sun II Pc64	<i>Pc64</i>	4-	0;	;	2	;	;	;	;
Makuru//Sun II Pc67	<i>Pc67</i>	4-	4	1+	;	1+	2	2	1
Makuru//Sun II Pc68	<i>Pc68</i>	0	01+	3+	0	0;	;	;	0
H547	<i>Pc70</i>	;	;	;	43	3	;	;	;
IA Y345	<i>Pc71</i>	2+	;	;	;	4	3	3	;
Amagalon	<i>Pc91</i>	0	;	;	0;	;	;	;	0
	<i>Pc94</i>	0;	;	;	;	;	;	;	;
	<i>Pc96</i>	0	3	34	3	3-	3	3	0

^z Based on the 0-to-4 scale described by Chong et al. (2000).

greenhouse. Later, the flag leaf of each plant was inoculated with uredinia of the eight *P. coronata* f. sp. *avenae* races used in the seedling evaluation and placed in a growth chamber adjusted to 20 to 22°C with an 18-h photoperiod. Flag leaves were evaluated for infection response and disease severity 2 weeks after inoculation. The experiment was repeated following the same procedure as above. Both experiments were conducted in three replications.

Statistical analyses. Standard error of disease severity and CI within field plots in each location–year was estimated using the replicated data of Otana and TAM-O-301. Data for disease severity and CI from Baton Rouge and St. Paul in the 2 years were subjected to analysis of variance. When there was significance difference for disease severity or CI among accessions, post hoc multiple comparison of means was performed using the Tukey's studentized range test (honestly significant difference). All statistical tests were performed using the software SAS Enterprise Guide v. 7.1 (SAS Institute 2014).

Results

Field resistance. Of the 607 oat accessions tested in Baton Rouge in 2016, most (84%) had an infection response of S. The remaining 97 accessions (16%) were MS, MR, or R (Table 1). These 97 accessions were selected for further evaluation at the buckthorn nursery in St. Paul. The raw data for the 607 oat accessions was submitted to the GRIN (<https://www.ars-grin.gov/npgs/>).

In St. Paul, 72 of 97 accessions (74.2%) exhibited MR infection response, followed by MS (22.7%) and R (2.1%). One accession, PI 174543, which exhibited an MR infection response in Baton Rouge, was S in St. Paul. Most accessions rated as R in Baton Rouge exhibited MR or MS reactions in St. Paul.

Of the 97 accessions tested at both field locations in 2016, one was discarded for having an S reaction in St. Paul, and 60 accessions were dropped because their mean CI was above 20. The remaining 36 accessions with infection responses of MS, MR, or R in both locations and a mean CI ≤ 20 were selected for a second round of field evaluations and for seedling tests.

After testing the 36 accessions in two locations for 2 years (four location–years), the mean CI was 7.2 (range: 0.6 to 20) at Baton Rouge and 11.9 (range: 4 to 20) at St. Paul. The average disease severity of the 36 accessions was 16% (range: 3 to 35%) at Baton Rouge and 22.2% (range: 10.5 to 35%) at St. Paul. The mean CI and disease severity of the resistant check, TAM-O-301, were 6.5 and 16.3%, respectively, while the susceptible check, Otana, had a CI and average disease severity of 52.5 and 52.5%, respectively (Table 2). Standard errors of mean disease severity and CI in BR-2016, BR-2017, SP-2016, and SP-2017 on Otana and TAM-O-301 are presented in Table 3.

Analysis of variance showed highly significant differences ($P < 0.0001$) for disease severity and CI (Table 2). In all, 33 of 36

Table 5. Reaction of 36 oat accessions to eight *Puccinia coronata* f. sp. *avenae* races at seedling stage

Accession	Origin	Status ^z	Seedling resistance ^y							
			MGBH	LLMG	LCBB	LQBG	MGLB	NSGG	LSGG	LBBB
Otana	...	Cultivar	3	43+	3+	3	3	3	3	3
TAM-O-301	...	Cultivar	;0	;	;	;1	0	;	0;	;0
PI 137599	Argentina	Landrace	1+	;	;1+	3	1	2N	3+	0;
PI 140902	Australia	Cultivar	1+	2C	3C	4	3	2+N	3	2C
PI 140903	Australia	Uncertain	3	2C	3	43C	3	4	3+	3
PI 163404	Argentina	Unknown	3	2+	3	43C	1	2N	3	2
PI 168091	Turkey	Landrace	;	;1	2C	1+	2	4	4	;
PI 168092	Turkey	Landrace	0;	;1-	1+	2C	3+	3N	3N	;1+
PI 175023	India	Landrace	1+	3N	4	3N	;1	4	2	;1+
PI 177842	Turkey	Landrace	;12-	;1	1;	11+	3	4	34	;
PI 186268	Argentina	Cultivar	1+	3N	3N	3	1+2N	4	2	;1-
PI 186270	Argentina	Uncertain	3	3-	3	3C	;1	1	;1	1
PI 186619	Brazil	Cultivar	2	2-	3N	3C	;	1	;	1
PI 189628	Brazil	Uncertain	1	2	3N	43	11+	1	2	1
PI 193040	Argentina	Unknown	0	;	1+ ^a	;1+	;1-	2N	2	0
PI 194201	Uruguay	Uncertain	0	1;	;1	;1-	;0	0;	;0	;1
PI 197790	Australia	Uncertain	1+	2	23	3N	3	3	22+	1
PI 198224	Argentina	Cultivar	;1	2	3	3N	21+	4	2	1lif
PI 219765	Greece	Uncertain	3	2	3	3	3	4	34	2
PI 237090	Brazil	Uncertain	2	2	2	22+	2	2	2	2
PI 244465	Unknown	Unknown	;1+	;C	3+	;1	;	2N	21	11+
PI 247930	Brazil	Uncertain	0	0;	;	2	;1 lif	0;	;1	;
PI 247932	Brazil	Cultivar	3-	3-N	3-	3N	;	;1	;0	1lif
PI 285583	Poland	Cultivar	34-	3	3	3	3	3-	3	3
PI 287288	Greece	Unknown	22+	2-	2N	2+N	3N	4	3	;
PI 287295	Italy	Landrace	0;	2	3	21+	34-	34-	22+	0;
PI 287296	Italy	Landrace	3-	2	4	43	3	34	34	3
PI 287298	Italy	Landrace	3	2	4	4	3	43+	4	23
PI 287300	Greece	Landrace	0;	2	3+4	3+	2	3	2C	2 lif
PI 287302	Greece	Landrace	0;	;	4	3N ^b	32+	4	3+	11+
PI 287306	Albania	Landrace	;	2	3N	32+	3	3+	2N	2
PI 287307	Italy	Landrace	1+	2	3	3	3	3	3	2+
CIav 2272	United States	Uncertain	3	3	4	3	3	3	3	3
CIav 3390	United States	Cultivar	3	3	3+	4	3	3	3	3
CIav 3412	United States	Cultivar	34	3	3	4	2	3+	3	3
CIav 4042	United States	Cultivar	3	2	3	3	2N	11+	3	2
CIav 4097	United States	Cultivar	34	3	4	3C	2	1+	2	2
CIav 4318	United States	Cultivar	3	3	4	3C	2	2N	34	4

^y Symbols: + or - after infection type (0-to-4 scale) denotes the level of intensity of infection, C = chlorosis, N = necrosis, and lif = low infection frequency.

^z Improvement status.

accessions had significantly lower disease severity than Otana. Only three accessions (namely, Clav 4318, PI 197790, and PI 287298) were not significantly different from Otana based on disease severity. On the other hand, all 36 oat accessions and TAM-O-301 had significantly lower CI than Otana. However, there was no significant difference in severity and CI between the 36 accessions and TAM-O-301 (Table 2).

South America had the highest proportion of accessions with infection responses of MS, MR, and R with 77.3%; followed by Australia, North America, Asia, Africa, and Europe with 47.1, 22.1, 13, 10.5, and 7.4%, respectively (Table 1).

Seedling resistance. Thirty-six accessions that were not susceptible in the first round of tests at Baton Rouge and St. Paul, with mean CI ≤ 20 , and 31 *Pc* differential lines were evaluated using eight *P. coronata* f. sp. *avenae* races. The seedling IT of the *Pc* differential lines to the eight *P. coronata* f. sp. *avenae* races is described in Table 4. Three accessions (Clav 2272, Clav 3390, and PI 285583) produced compatible IT to all races, indicating a lack of seedling resistance to the races used in this study. The remaining 33 accessions exhibited resistance to at least one race. We categorized these into two groups: (i) accessions with IT patterns similar to one or more of the *Pc* differential lines and (ii) accessions with IT patterns not similar to any of the *Pc* differential lines.

One accession, PI 177842, originating from the Mediterranean region of Turkey, had an IT pattern similar to the *Pc71* differential line, suggesting the possible presence of this seedling resistance gene (Tables 4 and 5). Three accessions (PI 193040, PI 194201, and PI 247930) produced incompatible IT to all the *P. coronata* f. sp. *avenae* races used in the study. In terms of susceptibility and resistance, these three accessions had similar disease reaction with four *Pc* differential lines (*Pc54*, *Pc58*, *Pc91*, and *Pc94*).

There were 28 accessions that exhibited compatible reactions with one or more races but their IT patterns did not match any of the differential lines (Table 5).

APR in the growth chamber. The three accessions that had crown rust resistance in the field tests but that were susceptible to all races at the seedling stage had MR to MS disease reactions when inoculated in growth chambers at the adult plant stage (Table 6). Accession Clav 3390 was MR against all of the *P. coronata* f. sp. *avenae* races used in the study, while accessions Clav 2272 and PI 285583 were MS to six races and MR to two races (Table 6). Otana, the susceptible check, had disease reactions of S to MS, while TAM-O-301, the resistant check, was R or MR to all the races as adult plants in the growth chambers in two independent tests (Table 6).

Discussion

Field resistance tests were conducted in two locations for two seasons. APR is selected in the field and, to reliably identify resistance lines, disease assays must optimize either natural infections or induce epidemics (Ellis et al. 2014). The St. Paul location is ideal to test oat genotypes for crown rust resistance because the nursery is surrounded by the alternate host of *P. coronata* f. sp. *avenae* in order to create maximum disease pressure and race diversity. As expected,

there was high disease pressure in both locations, as shown by high disease severity on the susceptible check cultivar throughout both fields (range: 40S to 60S). Statistical tests also showed low standard error for disease severity and CI on Otana and TAM-O-301 in all location-years (Table 3). These disease-conducive environments enabled reliable resistance testing in both years.

Of the 607 accessions tested against naturally occurring inoculum in the field, 97 were identified with some level of field resistance, having infection responses ranging from MS to R. Although 1 accession selected as R in Baton Rouge was S in St. Paul, 11 of 20 randomly selected S accessions in Baton Rouge were R in St. Paul (data not shown). In addition, of the 25 accessions that showed a disease reaction of R in Baton Rouge, only 2 (PI 137599 and PI 193040) had a similar disease reaction in St. Paul. Such variability was expected because the virulence of *P. coronata* f. sp. *avenae* varies from location to location (Carson 2011).

The seedling tests on 36 field-resistant accessions identified Clav 2272, Clav 3390, and PI 285583 as being susceptible to all races, suggesting that the resistance in these accessions was not due to major genes that are effective at the seedling stage. This result, coupled with their field reactions of MR with reduced pustule size, suggested APR. Their four location-year mean CI values were also in the low range and similar to the resistant check, TAM-O-301 (Fig. 1). The field observation was further supported by growth chamber tests at the flag leaf stage, where Clav 3390 showed moderate APR. The other two accessions (Clav 2272 and PI 285583) were MR to MS in adult plant growth chamber tests. According to Portyanko et al. (2005), APR in oat to crown rust is expressed as a type of quantitative resistance with reduced pustule size, reduced spore production, and

Fig. 1. Distribution of coefficient of infection values for 607 oat accessions based on an adult plant evaluation conducted at Baton Rouge, LA in 2016. The coefficient of infection values shown by the arrows for TAM-O-301, Clav 2272, Clav 3390, PI 285583, and Otana are the means of four location-years.

Table 6. Mean disease severity and infection response of suspected adult plant resistance accessions, Clav 3390, Clav 2272, and PI 285583; susceptible check cultivar Otana; and resistant check cultivar TAM-O-301 against eight *Puccinia coronata* f. sp. *avenae* races after inoculation at the adult plant stage and incubation in a growth chamber for 14 days²

Race	Experiment 1					Experiment 2				
	Clav 3390	Clav 2272	PI 285583	TAM-O-301	Otana	Clav 3390	Clav 2272	PI 285583	TAM-O-301	Otana
MGBH	5MR	5MR	TrMR	TrMR	5MS	10MR	10MR	10MR	5MR	25MS
LLMG	20MR	50MS	10MS	5RMR	35MSS	20MR	40MS	10MS	TrMR	30MSS
LCBB	20MR	35MS	20MS	10RMR	40MSS	15MR	30MS	15MS	5RMR	45MSS
LQBG	30MR	40MS	30MS	5RMR	20MS	25MR	40MS	30MS	5RMR	25MS
MGLB	15MR	10MS	10MS	15MR	35MS	20MR	20MS	15MS	10MR	40MS
NSGG	30MR	40MS	20MS	10MR	40MSS	25MR	30MS	20MS	10MR	35MSS
LSGG	30MR	30MS	20MS	10RMR	30MSS	20MR	30MS	20MS	10RMR	35MSS
LBBB	20MR	10MR	10MR	10RMR	20MS	20MR	10MR	10MR	5MR	30MS

² Abbreviations: S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant.

extended latent period. Quantitative resistance is usually preferred over major gene resistance to achieve durable resistance against various cereal rusts (Ellis et al. 2014; Lin et al. 2014; Portyanko et al. 2005). Quantitative resistance is likely more important in the case of oat, given the fact that major gene resistance often loses its effectiveness in less than 5 years (Carson 2011; Chong and Kolmer 1993; Leonard et al. 2004; McCallum et al. 2007). Hence, the three landraces in general, and Clav 3390 in particular, could be potential sources of APR to crown rust. The genes responsible were not characterized in the present study, other than to show that they were not effective at the seedling stage. For all other accessions, further investigation is required to determine the basis of the field resistance because it could be race specific, as has been shown in wheat against yellow rust and leaf rust (Dyck et al. 1966; Milus et al. 2015), due to either the additive effects of minor genes or to the combined presence of several major genes, each effective against part of the pathogen population, resulting in less disease in the field when exposed to a mixture of races.

Four accessions (PI 193040, PI 194201, PI 237090, and PI 247930) were resistant to all eight *P. coronata* f. sp. *avenae* races used in the present study, indicating broad-spectrum resistance similar to four of the *Pc* gene differentials. The resistance in these accessions may be due to either one or more of these *Pc* genes or to combinations of other *Pc* genes. On the other hand, 28 accessions had unique virulence patterns not similar to any of the *Pc* differential lines but with some similarities to each other. Sometimes the accessions differed by only one single-race reaction. For example, PI 287295 was similar to PI 197790, except for additional effectiveness to race LQBG. Clav 4318 was similar to Clav 3412, except for additional effectiveness to race NSGG. Also, PI 186270 was similar to Clav 4318 and PI 186619 to PI 175023, except for additional effectiveness to two races each. These accessions may carry *Pc* genes that are not currently characterized. However, further investigation is needed to confirm whether novel genes are present and to determine how they can be deployed most effectively by breeders.

The high proportion of resistance in accessions from South America may be associated with coevolution of the accessions with the high virulence complexity and diversity of *P. coronata* f. sp. *avenae* isolates in the continent (Leonard and Martinelli 2005). PI 194201, originating from Uruguay, is of particular interest because, according to the GRIN database (<https://www.ars-grin.gov/npgs/>), this accession has resistance to multiple diseases, including stem rust, barley yellow dwarf, and smut, which are all important diseases of oat. This accession could prove valuable for oat breeding programs.

The proportion of resistant accessions in the *A. sativa* gene pool evaluated (16%) was low compared with the resistance in the gene pool of wild oat relatives. For example, Tan and Carson (2013) reported resistance at the seedling as well as the adult plant stage in 45% of 332 accessions of 11 wild oat species originating from Morocco. Though at the seedling stage, Sowa et al. (2016) and Cabral and Park (2014) also reported that 25, 68, 56, 68, and 25% of accessions of *A. magna*, *A. murphyi*, *A. insularis*, *A. strigosa*, and *A. barbata*, respectively, had resistance against crown rust when tested using *P. coronata* f. sp. *avenae* isolates from Poland and Australia. Most of the *Pc* genes currently deployed in cultivated oat were derived from the diploid species *A. strigosa* and the wild hexaploid oat *A. sterilis* (Simons et al. 1978). Other species such as *A. barbata*, *A. vavilova*, *A. abyssinica*, *A. magna*, and *A. longiglumis* have also been identified as sources of crown rust resistance (Cabral and Park 2014; Tan and Carson 2013). The transfer of genes from wild relatives to cultivated oat is difficult and sometimes impossible (Sowa et al. 2016; Tan and Carson 2013) due to barriers arising from ploidy level differences (Jellen and Leggett 2006). Of the many *Pc* genes identified from wild relatives, only a few have been transferred to cultivated hexaploid oat due to interspecies crossing barriers. These include the first successful transfer of a resistance gene from the wild hexaploid *A. sterilis* to cultivated oat (Simons 1985), the transfer of *Pc94* from *A. strigosa* to cultivars Leggett and Stride (Aung et al. 1996), and the transfer of *Pc91* from *A. magna* to cultivar HiFi (McMullen et al. 2005). Obtaining resistance sources from the cultivated hexaploid gene pool for a major disease such as crown rust

eliminates problems associated with gene transfer from a different species, as well as overcomes the obstacle associated with suppression or inhibition of gene expression after interspecific gene transfer (Hegarty et al. 2008; Rines et al. 2007). Because of these challenges, Sowa et al. (2016) recommended the use of accessions whose ploidy level was verified as hexaploid as a promising alternative to using tetraploid oat as a source of resistance. The present study showed that the hexaploid gene pool is a rich source of resistance to crown rust, possibly enabling the unhindered transfer of genes to locally adapted germplasm and facilitating the development of new disease resistant cultivars. Although sourcing resistance within the species overcomes the technical barrier, linkage drag will remain a challenge in any hybridization program.

Comparison of accessions for their field response to crown rust with previous evaluations (GRIN, <https://www.ars-grin.gov/npgs/>) showed that, of the 225 accessions 190 (84.4%) and 12 (5.3%) had the same susceptible and resistance field responses, respectively. However, 21 accessions that were previously susceptible exhibited some level of resistance in the current study, and two accessions that were resistant in the earlier studies had a susceptible response now. Change in the susceptibility of accessions against *P. coronata* f. sp. *avenae* is expected because of the continuous emergence of new races on the alternate host (Carson 2011). Accession PI 247930, from Brazil, showed a particularly consistent field response of R and MR across locations and over time: Ames, IA in 1983 (1R), St. Paul, MN in 1994 (20R), Baton Rouge, LA in 2015–16 (1R/5R), and St. Paul, MN in 2016–17 (30MR/10MR).

Accessions that were identified in the present study with APR and seedling resistance will diversify the resistance sources for crown rust resistance breeding. With a high rate of virulence evolution in the *P. coronata* f. sp. *avenae* population, emphasis should be given to breeding cultivars with APR and pyramiding of seedling resistance *Pc* genes to develop cultivars more likely to exhibit durable resistance against crown rust. The accessions identified in this study will contribute to such work.

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