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## **Genetic analysis of seedling resistance to crown rust in five diploid oat (*Avena strigosa*) accessions**

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### **Abstract**

Crown rust, caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks., is a serious menace in oats, for which resistance is an effective means of control. Wild diploid oat accessions are a source of novel resistances that first need to be characterized prior to introgression into locally adapted oat cultivars. A genetic analysis of resistance to crown rust was carried out in three diverse diploid oat accessions (CIav6956, CIav9020, PI292226) and two cultivars (Saia and Glabrota) of *A. strigosa*. A single major gene conditioning resistance to Australian crown rust pathotype (Pt) 0000-2 was identified in each of the three accessions. Allelism tests suggested that these genes are either the same, allelic, or tightly linked with less than 1% recombination. Similarly, a single gene was identified in Glabrota, and possibly two genes in Saia; both cultivars previously reported to carry two and three crown rust resistance genes, respectively. The identified seedling resistance genes could be deployed in combination with other resistance gene(s) to enhance durability of resistance to crown rust in hexaploid oat. Current diploid and hexaploid linkage maps and molecular anchor markers (simple sequence repeat [SSR] and diversity array technology [DArT] markers) should facilitate their mapping and introgression into hexaploid oat.

**Keywords:** allelism, genetic analyses, *Puccinia coronata*, seedling resistance

## **Introduction**

Crown rust, caused by *Puccinia coronata* Corda f. sp. *avenae* Eriks., is a major disease of oat, occurring across nearly all oat growing areas of the world (Simons, 1970). In Australia, the pathogen is restricted to reproducing asexually due to the absence of its alternate host, *Rhamnus* spp. The year-round presence of wild oat, particularly in northern parts of New South Wales and in Queensland (Brouwer & Oates, 1986), where prevailing summer-dominant rainfall patterns favour their growth, enables continuous survival of the pathogen (Park, 2008). In wheat, this continuity in the life cycle of the leaf rust pathogen increases the chance of new pathotypes evolving through rare events including mutation (Watson 1981; Park *et al.* 1995; Brake *et al.*, 2001) and somatic hybridisation (Park *et al.*, 1999). Cultivars with major gene resistances that remain effective for only a few years (Carson, 2009) have not been successful in controlling crown rust in Australia (R.F Park, unpublished). However, single major gene resistances are more effective when deployed in areas where inoculum levels are low (Park, 2008). Of the 97 *Pc* genes identified thus far, 91 are effective at all growth stages, whereas six condition adult plant resistance (APR) (CDL, 2006). While a majority of the all stage resistance genes were identified in the wild hexaploid oat species *A. sterilis* (Simons *et al.*, 1978), some have come from the diploid species *A. strigosa*, which was also reported as a potential source of stem rust resistance (Steinberg *et al.*, 2005).

Genetic analyses of rust resistance involve studies on the inheritance of resistance in progeny resulting from crosses between resistant and susceptible genotypes. Further, crosses between resistant varieties will determine if the resistance genes thus identified are allelic or different. To be successful, a genetic study to identify the number of gene(s) present in a given accession requires testing of a minimum number of progeny

that can be determined from any genetic hypothesis. For this, Hanson (1959) described formulae to determine minimum family sizes in terms of numbers of progeny required to carry out a genetic analysis of crosses in which different numbers of genes are segregating.

The present study involved a genetic analyses of resistance to crown rust Pt. 0000-2 in three accessions from diverse geographical regions (CIav6956, CIav9020, PI292226) and the cultivars Saia and Glabrota, all belonging to the diploid species *A. strigosa*. Pathotype 0000-2 was chosen for its broader virulence on diploids, when compared to other pathotypes of our collection. The objective of the experiment was to determine the number of genes present in each genotype. The three accessions were shown previously to be resistant to eight Australian crown rust pathotypes (Cabral & Park, 2014). While virulence to Saia exists in Australia, no isolate with virulence for Glabrota has been detected (R.F Park, unpublished). Genetic analyses of Saia and Glabrota were conducted to verify earlier reports of the presence of the resistance genes *Pc15*, *Pc16* and *Pc17* in the former and *Pc18* and *Pc29* in the latter (CDL, 2006). Because these earlier studies did not generate single gene stocks for each of these genes, it was hoped that this could also be achieved in the present work.

## **Materials and Methods**

### ***Plant materials***

A genetic analysis of resistance to crown rust was carried out in three diploid *Avena strigosa* accessions CIav6956, CIav9020 and PI292226, from a USDA National Small Grains Collection, and in two diploid *Avena strigosa* cultivars Saia and Glabrota, maintained at the Plant Breeding Institute Cobbitty (PBIC). Each of these five genotypes was crossed separately to a susceptible accession CIav9112, also taken from the same USDA National Small Grains Collection.

### ***Experimental design***

The four accessions used in *Experiment 1* were chosen from among an initial set of 20 accessions, which were first tested as seedlings with crown rust Pt. 0000-2 (PBI rust isolate accession no. 982774) and Pt. 4473-4,6,10 (PBI rust isolate accession no. 013535) (Cabral and Park 2014) and later categorised as either resistant or susceptible to both pathotypes. This resulted in the identification of 10 resistant and 10 susceptible accessions, which were crossed in various combinations. Florets on panicles of the female parent were emasculated and bagged to prevent contamination from foreign/undesired pollen. The following season, genotypes were selected based on flowering synchrony, pollen production and plant height/crossing-suitability. Three resistant pollen parents CIav6956, CIav9020 and PI292226 and a single susceptible female parent, CIav9112, were thus selected for *Experiment 1*. In *Experiment 2*, cultivars Saia and Glabrota, both resistant to the above two pathotypes, were used as pollen parents and accession CIav9112 was used as the susceptible female parent.

The F<sub>2</sub> populations for all five crosses were each derived from two F<sub>1</sub> plants (Fig1.). Seedling-progeny from all crosses were tested with Pt. 0000-2 in the F<sub>2</sub> and F<sub>3</sub> generations. For the *CIav9112/Saia* cross, a third Pt. 0207-5,6,10 (PBI rust isolate accession no. 962510) was tested on F<sub>4</sub> seedlings (as F<sub>2</sub> and F<sub>3</sub> seed was used up) in order to confirm/determine the number of resistance genes effective against Pt. 0207-5,6,10 in cultivar Saia. Approximately 80–100 seeds of a single plant were taken from among the 20–30 F<sub>3</sub> plants previously phenotyped at the seedling stage with Pt. 0000-2.

### ***Embryo rescue of F<sub>1</sub> seeds***

Although the parental genotypes used in the two crossing experiments belonged to the same species, *A. strigosa*, their diverse origins and difficulty in crossing prompted the use of embryo rescue to hasten the recovery of healthy F<sub>1</sub> seedlings. Immature F<sub>1</sub> seeds harvested 15–18 days after crossing were subjected to the rescue procedure described by Sidhu *et al.* (2006). The caryopsis was washed initially in 95% ethanol for 30 seconds and then in 10–15 ml of bleach for five minutes, followed by a final rinse (2–3 times) in sterile water. Rescued embryos were placed in test tubes containing Gamborg's medium, stored in darkness for two days at 4°C, and later kept at room temperature for the next two days before being placed in a culture room. Two weeks later, seedlings were transplanted into potting mix and left to harden in an illuminated cold room maintained at 14°C. Two to three weeks later, F<sub>1</sub> plants were transferred to growth rooms, where they remained until maturity.

### ***Seedling inoculation and disease scoring***

The parental accessions were tested for response to crown rust pathotypes 0000-2 and 4473-4,6,10 obtained from the PBI culture collection. A third pathotype, 0207-5,6,10 was also used to test F<sub>4</sub> progeny from the cross Clav9112/Saia. Fourteen day-old F<sub>2</sub> and F<sub>3</sub> seedlings from each of the five crosses were inoculated separately with Pt. 0000-2, as described in Cabral & Park (2014). Urediniospores suspended in a light mineral oil were sprayed over the seedlings using an atomiser. The seedlings were then placed in a misting chamber at room temperature for 24 hours, and later transferred to a growth room maintained at 23°C. Two weeks later, seedlings were scored for crown rust response using an IT scale of “0”–“4” described by Murphy (1935), with minor modifications (refer Cabral and Park, 2014). While IT scores between “0” and “2” were considered resistant, those between “2–3” and “3” were indicative of moderate resistance, and scores of “3–3+” and “3+” indicated susceptibility. The letters “c” and “n” denote chlorosis and necrosis respectively, while “+” or “-” signs following an

IT score indicate the level of intensity of infection or an intermediate IT response. All F<sub>2</sub> plants were selfed and grown to maturity for F<sub>3</sub> seed. At harvest, 20–30 F<sub>3</sub> seeds from each plant were planted separately, inoculated with Pt. 0000-2, and scored two weeks later.

### ***Tests of allelism***

The F<sub>2</sub> and F<sub>3</sub> progenies from each of the three resistant parent intercrosses (CIav6956/PI292226, CIav6956/CIav9020, PI292226/CIav9020) were used to study allelism between the resistance genes detected in the resistant by susceptible crosses. One hundred and fifty F<sub>2</sub> seedlings from each cross were inoculated with Pt. 0000-2.

### ***Statistical analyses***

Chi-squared ( $\chi^2$ ) analyses of the data from F<sub>2</sub> and F<sub>3</sub> progeny were conducted for all crosses, in order to test the goodness-of-fit of observed to expected segregation ratios. The  $\chi^2$  statistic was calculated using the formula  $\chi^2 = (O - E)^2 / E$ , where O and E represent the respective observed and expected frequencies of resistant and susceptible F<sub>2</sub> individuals, and homozygous or non-segregating resistant (NSR), segregating (SEG) and homozygous or non-segregating susceptible (NSS) F<sub>3</sub> families. The maximum recombination value ( $p$ ) was calculated using the formula given by Hanson (1959):  $P_{RC} = [1 - \sqrt[n]{P}]$ ; where 'P<sub>RC</sub>' (probability of observing recombinant types) =  $2p - p^2$  (for complete F<sub>2</sub> & F<sub>3</sub> data), 'n' is the number of F<sub>2</sub> individuals and  $P = 0.05$ . The  $p$  value is determined from the quadratic formula:  $p = -b \pm \sqrt{b^2 - 4ac} / 2a$ , given  $ap^2 + bp + c = 0$  (Kramer & Burnham, 1947; Adhikari, 1996).

## **Results**

### ***CIav9112/CIav6956***

A total of 128 F<sub>2</sub> seedlings were tested with Pt. 0000-2 (Suppl Table S1). Of these, 46 F<sub>2</sub> seedlings in Family 1 segregated 37 resistant and 9 susceptible, fitting a 3:1 segregation ratio ( $\chi^2_{(1df)} = 0.72$ ;  $P = 0.5 - 0.3$ ), and 82 F<sub>2</sub> seedlings in Family 2 segregated 62 resistant and 20 susceptible, also fitting a 3:1 ratio ( $\chi^2_{(1df)} = 0.02$ ;  $P = 0.9 - 0.5$ ). A Chi-squared test for heterogeneity involving Families 1 and 2 returned a non-significant value ( $\chi^2_{(1df)} = 0.45$ ;  $P = 0.9 - 0.5$ ), and thus data from both families were pooled i.e. 99 resistant (ITs ranging between “;cn” & “1-2cn”) and 29 susceptible F<sub>2</sub> seedlings (Table 1), fitting a 3:1 segregation ratio ( $\chi^2_{(1df)} = 0.29$ ;  $P = 0.9 - 0.5$ ).

Of the 128 F<sub>2</sub> seedlings that were transplanted for subsequent seed production, 94 survived. The 94 F<sub>3</sub> families generated were tested with Pt. 0000-2 and segregated 51 non-segregating resistant (NSR): 23 segregating (SEG): 20 non-segregating susceptible (NSS) (Suppl Table S2), which did not fit a 1:2:1 ratio ( $\chi^2_{(2df)} = 45.30$ ;  $P < 0.05$ ). However, on grouping the NSR and SEG classes, an F<sub>3</sub> ratio of 74:20 (resistant:susceptible) was obtained (Table 1), in agreement with a 3:1 ratio ( $\chi^2_{(1df)} = 0.69$ ;  $P = 0.5 - 0.3$ ) for segregation of a single dominant locus. Individual Chi-squared values of the 23 segregating families are given in Suppl Table S3.

### ***Clav9112/PI292226***

A total of 131 F<sub>2</sub> seedlings were tested with Pt. 0000-2 (Suppl Table S4). Of these, 71 seedlings (Family 1) segregated 57 resistant and 14 susceptible, ( $\chi^2_{(3:1) (1df)} = 1.05$ ;  $P = 0.5 - 0.3$ ), and 60 seedlings (Family 2) segregated as 48 resistant and 12 susceptible ( $\chi^2_{(3:1) (1df)} = 0.80$ ;  $P = 0.5 - 0.3$ ). A Chi-squared test for heterogeneity involving Families 1 and 2 returned a non-significant value ( $\chi^2_{(1df)} = 0.0$ ;  $P = 1.0$ ) (Table 2). Of the 131 F<sub>2</sub> seedlings transplanted, 87 survived and their corresponding 87 F<sub>3</sub> families segregated 46 NSR: 27 SEG: 14 NSS (Suppl Table S5), which did not fit a 1:2:1 ratio ( $\chi^2_{(2df)} = 37.13$ ;  $P < 0.05$ ). However on grouping the NSR and SEG classes, an F<sub>3</sub> ratio



of 73R:14S was obtained in agreement with a 3:1 ratio ( $\chi^2_{(1df)} = 3.68$ ;  $P = 0.1 - 0.05$ ) for segregation at a single dominant locus (Table 2). Individual chi-squared values of the 27 segregating families are given in Suppl Table S6.

### ***CIav9112/CIav9020***

A total of 83 F<sub>2</sub> seedlings were tested with Pt. 0000-2 (Suppl Table S7). A Chi-squared test for heterogeneity involving Families 1 and 2 returned a non-significant value ( $\chi^2_{(1df)} = 0.06$ ;  $P = 0.9 - 0.5$ ), and thus data from both families were pooled i.e. 64 resistant (ITs ranging between “;cn” & “1-2cn”) and 19 susceptible F<sub>2</sub> seedlings (Table 3), fitting a 3:1 segregation ratio ( $\chi^2_{(1df)} = 0.20$ ;  $P = 0.9 - 0.5$ ). Due to poor recovery of seed, F<sub>3</sub> tests were not carried out using Pt. 0000-2.

### ***Tests of allelism***

One hundred and fifty F<sub>2</sub> seedlings from each of the three resistant parent intercrosses were inoculated with Pt. 0000-2. All F<sub>2</sub> seedling populations failed to segregate, producing ITs of “;n” to “;1n”. Further, 100 F<sub>2</sub> seedlings from each cross were transplanted and grown to maturity to generate F<sub>3</sub> seed. Twenty F<sub>3</sub> seeds from each of the 100 F<sub>2</sub> plants were inoculated with Pt. 0000-2. No segregation was observed among or within the F<sub>3</sub> progeny (Table 4). Further, taking into consideration complete numbers of F<sub>2</sub> individuals and F<sub>3</sub> families, and assuming the genes for resistance in any two parents were different, the probability of observing recombinant types was calculated using the maximum recombination value ( $p$ ) between two loci. The  $p$  value was found to be less than 0.01, at  $P = 0.05$ , suggesting less than 1% recombination, or that fewer than 10 of 1,000 F<sub>3</sub> lines would be segregating or homozygous susceptible.

### ***CIav9112/Saia***

**Tests with Pt. 0000-2:** Due to a severe aphid infestation of the bagged F<sub>1</sub> panicles, only 17 viable F<sub>2</sub> seeds were recovered from two F<sub>1</sub> plants. The resultant F<sub>2</sub> ITs, reactions of F<sub>3</sub> families, with Chi-squared values for individual segregating families, and a heterogeneity Chi-squared value, are given in Table 6.

Tests of the 17 F<sub>2</sub> seedlings ( $\chi^2_{(3:1)} = 0.71$ ;  $P = 0.5 - 0.3$ ) fitted a 3:1 ratio implying segregation at a single locus (Table 5). For the F<sub>3</sub> tests, 20–30 seedlings from each of the 17 F<sub>2</sub> plants were tested with Pt. 0000-2. The 17 F<sub>3</sub> families segregated as 3 NSR: 9 SEG: 5 NSS, which fitted a 3:1 ratio ( $\chi^2$  value of 0.2;  $P = 0.9 - 0.5$ ) (Tables 5), on pooling the NSR and SEG families.

**Tests with Pt. 0207-5,6,10:** The above test with Pt. 0000-2 detected a single seedling resistance gene in Saia, previously reported to carry three genes. Therefore, a second Pt. 0207-5,6,10 was tested on F<sub>4</sub> seedlings (as F<sub>2</sub> and F<sub>3</sub> seed was used up) derived from selfed seed of a single plant taken from among the 20–30 F<sub>3</sub> plants previously phenotyped at the seedling stage with Pt. 0000-2. Approximately 80–100 F<sub>4</sub> seedlings from each of these 17 families were tested with Pt. 0207-5,6,10, to determine the number of resistance genes effective against Pt. 0207-5,6,10 in cultivar Saia.

The 17 F<sub>4</sub> families segregated 6 NSR: 8 SEG: 3 NSS ( $\chi^2_{(1:2:1)} = 0.99$ ;  $P = 0.7 - 0.5$ ). On grouping NSR and SEG classes, a 3:1 segregation ratio ( $\chi^2 = 0.02$ ;  $P = 0.9$ ), suggesting resistance to Pt. 0207-5,6,10 was also conditioned by a single dominant gene. However, the eight SEG families showed significant deviations from a 3:1 ratio (data not shown). Further, the observed ratios of the 17 F<sub>4</sub> families (6 NSR: 8 SEG: 3 NSS) were also a good fit for a two-gene model ( $\chi^2_{(7:8:1)} = 3.6$ ;  $P = 0.2 - 0.1$ ) (Table 5). Of the eight SEG families, comprising a total of 651 plants, four were a fit for a 9:7 ratio suggestive of complimentary gene action, with one family (#8) returning a

significant P value of  $< 0.05$ . Three families (#3,4,13) were a fit for a 7:9 ratio, of which one (#3) had a significant P value of  $< 0.01$ . Finally, a 15:1 segregation was observed for a single family (#2; Table 6). The resistant ITs of “;1” to “2–3” observed in response to Pt. 0207-5,6,10 (in F<sub>4</sub> populations) were slightly higher than those of “;” to “1–2” with Pt. 0000-2 (in F<sub>3</sub> populations). Additionally, tests with Pt. 0000-2 identified three families to be NSR, while only two of these three families were confirmed to be NSR when tested with Pt. 0207-5,6,10. This suggests that the gene conditioning resistance to Pt. 0000-2 in Saia is different from the genes conditioning resistance to Pt. 0207-5,6,10.

### ***CIav9112/Glabrota***

A test of 77 F<sub>2</sub> seedlings (Suppl Table S8) with Pt. 0000-2 confirmed segregation at a single locus (Table 7). Due to a poor recovery of seed, testing of the F<sub>3</sub> generation with Pt. 0000-2 could not be carried out.

## **Discussion**

Genetic analyses of resistance to crown rust Pt. 0000-2 in F<sub>2</sub> and F<sub>3</sub> seedling progenies crosses CIav9112/CIav6956, CIav9112/PI292226 and CIav9112/CIav9020 established that the respective resistant parental lines each carried a single dominant gene. Allelism tests suggested that these accessions carried the same gene, or if different, the genes were linked at less than 1 cM. Cross CIav9112/Glabrota was tested with Pt. 0000-2, and resistance was conferred by a single dominant gene. Tests of CIav9112/Saia with Pt. 0000-2 also implicated a single dominant gene in cultivar Saia effective against Pt. 0000-2, with the possibility of two genes segregating in response to Pt. 0207-5,6,10.

In annual cereal rust surveys conducted by the PBI, Pt. 0000-2 was first reported to be prevalent in Western Australia and South Australia during 2003-2004, and was found

to be avirulent on all genotypes in the crown rust differential set except the genotype carrying *Pc46* (Park & Kavanagh, 2004). The same pathotype was collected in New South Wales and Queensland during 2005–06, and has steadily prevailed since, in eastern and Western Australia (Park & Kavanagh, 2008). The broad avirulence and continued prevalence of Pt. 0000-2 in Australia were factors that prompted its use in the genetic analyses reported here. The detection of a single resistance gene in Saia with this pathotype and possibly two with Pt. 0207-5,6,10 was therefore unexpected, as was the detection of a single resistance gene in Glabrota, previously reported to carry resistance genes *Pc18* and *Pc29*.

The susceptible parent CIav9112, collected in Ontario Canada (GRIN, 2009), is less vigorous, weedy and shorter in stature compared to the three resistant parental accessions. It was susceptible to all eight crown rust pathotypes tested by Cabral & Park (2014). The resistant accessions CIav6956, PI292226 and CIav9020, collected from Canada, Israel and Argentina, respectively, were all very similar in morphology and had identical ITs to the eight pathotypes used despite the large distances between their origins (Cabral & Park, 2014). This could suggest that all three resistant accessions might actually be identical or derivatives of a common parental accession. Comparative tests of the genotypes using molecular markers could help resolve this.

In *Experiment 1*, the F<sub>2</sub> progeny resulting from each of the three crosses CIav9112/CIav6956, CIav9112/PI292226 and CIav9112/CIav9020 were tested with Pt. 0000-2. The resistant: susceptible F<sub>2</sub> data fitted an expected 3:1 segregation ratio (Tables 1, 2 & 3), indicating a single gene segregation in each cross. These results suggested that seedling resistance to Pt. 0000-2 in each wild oat accession was governed by a single dominant gene. Further, Chi-squared tests of data from F<sub>3</sub> progeny of crosses CIav9112/CIav6956 and CIav9112/PI292226, with the above

pathotype returned significant values for an expected 1:2:1 segregation ratio. However, on grouping NSR and SEG classes, non-significant chi-squared values were observed, consistent with the presence of a single dominant gene (Tables 1 & 2). The deviation from a 1:2:1 segregation ratio among F<sub>3</sub> progeny of the crosses CIav9112/CIav6956 and CIav9112/PI292226 appeared to be due to an excess of NSR families. A possible explanation for the comparatively lower number of SEG families might be that the resistance genes displayed partial dominance, which coupled with temperature sensitivity (Bonnett *et al.*, 2002), could have led to the misclassification of at least some heterozygous resistant plants as susceptible. However, this might not explain the lower numbers of NSS F<sub>3</sub> families observed for both crosses. Therefore, an alternative explanation for an excess of NSR families, and a relatively fewer number of SEG families might be cooler night-time temperatures at testing, resulting in heterozygous resistant plants being misclassified as resistant.

Tests of allelism were conducted among the three resistant parental accessions (CIav6956, PI292226, CIav9020). All F<sub>2</sub> and F<sub>3</sub> populations from the resistant intercrosses failed to segregate (Table 4), indicating that the resistance gene in each of the three parents was the same, allelic, or tightly linked. Given that similar disease ITs of “;n” and “;1n” were obtained for the three parental accessions and all their respective F<sub>2</sub> and F<sub>3</sub> progeny populations, it would appear that the genotypes carry a common gene. If the genes for resistance in any two parents were different, the maximum recombination value (for complete F<sub>2</sub> and F<sub>3</sub> data) at  $P = 0.05$  would be less than 0.01 (Hanson, 1959). In this case, the probability of observing double recessive recombinants among F<sub>2</sub> progeny populations of the respective three crosses or the P<sub>RC</sub> value was 0.000625, and the corresponding F<sub>2</sub> population size thus required, was calculated to be approximately 7,380 plants. Given the difficulties in making crosses among the wild oat accessions, and the extremely low seed-sets, the calculated

population sizes would have been beyond the resources of the present study. In *Experiment 2*, results of separate tests of progeny from the cross CIav9112/Saia confirmed the presence of a single resistance gene in response to Pt. 0000-2, and possibly two genes in response to Pt. 0207-5,6,10, in Saia. The resistance gene(s) detected with each pathotype are probably different, based upon differences in the respective low ITs and numbers of NSR families identified in tests with the two pathotypes. In a genetic analysis of crown rust resistance in F<sub>2</sub> progeny of reciprocal crosses between accessions CI 4748 and CD 3820 to two North American races, Murphy *et al.* (1958) postulated three resistance genes in accession CD 3820 and further reported that this accession was identical to cultivar Saia based on similar responses to a series of North American pathotypes. However, in the current literature (CDL, 2006), the three genes *Pc15*, *Pc16* and *Pc17* are reported in Saia but not in CD 3820. While the origin of this discrepancy is unknown, it is possible that the tentative system of gene nomenclature proposed by the Committee of Genetic Nomenclature in Oats (Dyck & Zillinsky, 1963) may have contributed. Although there are currently 97 designations for *Pc* genes, Park (2008) mentioned the difficulty in identifying many of the genes due to a lack of single gene reference stocks. The present studies provide good examples to illustrate this difficulty as only one or at most two genes could be detected in Saia, and it is not known if these genes represent *Pc15*, *Pc16*, *Pc17* or a different undescribed gene. Also, the fact that resistance gene characterization in oat is solely dependent on pathotypes known to undergo shifts in virulence should highlight an urgent need for the development and maintenance of single gene reference stocks.

Because the cultivar Saia was reported to carry three genes, *Pc15*, *Pc16* and *Pc17* (Murphy *et al.*, 1958) for resistance to crown rust, selected F<sub>4</sub> lines from CIav9112/Saia were tested with a second pathotype (Pt. 0207-5,6,10) which produced a different infection type on Saia (“;++n”) compared to Pt. 0000-2 (“;n”). Results of

this test gave no unambiguous gene numbers for resistance to Pt. 0207-5,6,10 in Saia. The genes conferring resistance to both these pathotypes were apparently different because one of the three NSR F<sub>3</sub> families homozygous to Pt. 0000-2, segregated in response to Pt. 0207-5,6,10 in the F<sub>4</sub> generation (Table 6). Therefore, neither can the possibility of additional resistance genes in Saia, against other pathotypes be ruled out, nor can the hypothesis of the presence of three resistance genes in Saia be rejected.

A pooled Chi-squared analysis of data from tests of F<sub>2</sub> progeny of cross Clav9112/Glabrota with Pt. 0000-2 supported the presence of a single dominant resistance gene. Individual Chi-squared values for Families 1 and 2 were also non-significant, hence strengthening the conclusion of segregation at a single locus (Table 7). Because confirmation tests of the F<sub>3</sub> progeny could not be carried out due to a lack of seed, the assumption of a single gene for resistance to Pt. 0000-2 in Glabrota, based only on F<sub>2</sub> seedling data and a single isolate, is not entirely conclusive. Cultivar Glabrota is reported to carry genes *Pc18* and *Pc29* (CDL, 2006). Upon tests of F<sub>2</sub> progeny of crosses CI2630/CI3214 and CI2630/CI7010 with races 205 and 264, and further tests of F<sub>2</sub> and F<sub>3</sub> progeny of the cross CI2630/CI1994 with races 216 and 264, Simons *et al.* (1959) concluded that a single dominant gene was present in accession CI2630 (Glabrota). In studies involving two accessions of *A. strigosa glabrescence*, Marshall & Myers (1961) reported a single partially dominant gene for resistance in accession CI2835, and two independent dominant genes in CI2524, either or both conditioning resistance to crown rust. In the present study, the resistant ITs of cultivar Glabrota (“0” & “1–2”), and those of accessions CI2835 (“0;” & “1”) and CI2630 (“I”- no macroscopic evidence of infection), were nearly identical. This strongly suggests the possibility of a single gene for resistance to crown rust in cultivar Glabrota, or more likely that Pt. 0000-2 was only able to detect a single resistance gene in this genotype.

Based on results of the allelism tests and multi-pathotype seedling tests (Cabral & Park, 2014), the three resistant parental accessions CIav6956, CIav9020 and PI292226 might be assumed to carry an identical crown rust resistance gene. Studies reported in Cabral *et al.* (2013) showed that accessions CIav6956 and CIav9020 were identical and accession PI292226 distinct when tested with 11 SSR markers. It is hence possible that accessions CIav6956 and CIav9020 are selections of a single parental accession, despite their distant collection sites. This is possible because oat accessions have often been distributed around the world in nursery sets and hence the country of collection might not necessarily be the place from which a given accession originated (G. J. Scoles, personal communication).

The use of F<sub>2</sub> derived populations for studies of allelism in wild oat was found to be unsuitable, given the difficulties involved in hybridisation, and subsequent generation of large progeny populations. Although future experiments of a similar nature should ideally involve backcross populations derived from each of the three accessions, obtaining the required number of progeny will likely remain a challenge, besides the complicated task of introgression of resistance from *A. strigosa* to *A. sativa*. A single gene or possibly two genes for resistance to crown rust was identified in Saia, whereas an earlier report (Murphy *et al.*, 1958) suggested the presence of three genes. As the current results are based on an analysis of a mere 17 F<sub>2</sub> individuals and their derived F<sub>3</sub> families, further confirmation should be made. However, it is unlikely that more than two genes were effective against the pathotypes used, because the number of susceptible F<sub>2</sub> plants was greater than a third of the total of 17 plants. Additionally, a single gene was detected in cultivar Glabrota, which was reported to carry two genes for resistance to crown rust. Because only a small F<sub>2</sub> population from CIav9112/Glabrota was tested with Pt. 0000-2, the experiment should be repeated in



order to obtain a correct estimate of the number of genes present in Glabrota. The fact that a different set of crown rust pathotypes was used in the earlier study might account for the discrepancies in the number of genes in Saia, an explanation that could also be extended to results for Glabrota.

The current system of nomenclature for *Pc* genes is not based on single gene reference stocks or rigorous tests of allelism with previously designated loci, making it next to impossible to accurately identify them or to discriminate new resistance genes from them. The value of these current *Pc* gene designations is therefore questionable and needs to be revisited. This might involve replacing the entire resistance gene nomenclature system with a new one in which resistance genes are designated strictly on the basis of single gene reference stocks, a defined set of pathogen isolates and mapped chromosomal positions.

The development of increasing numbers of molecular markers in oat such as genomic SSRs (Li *et al.*, 2000; Pal *et al.*, 2002), EST-derived SSRs (Becher, 2007) and DArTs (Tinker *et al.*, 2009) should aid in characterising such genes more fully. Furthermore, the difficulties associated with generating large populations to identify and characterise resistance genes accurately in diploid genotypes could be reduced by using doubled haploid (DH) populations (Rines, 1983; Rines & Dahleen, 1990; Kiviharju *et al.*, 2000; Kiviharju, 2009). However, currently, DH populations are only available for hexaploid oat (Tanhuanpää *et al.*, 2008 & 2012).

The current availability of diploid oat linkage maps (O'Donoghue *et al.*, 1992; Rayapati, *et al.*, 1994; Yu *et al.*, 2000; Kremer *et al.*, 2001) and molecular anchor markers (DArTs, SSRs) might enable tagging of the genes identified in our study. Although major seedling resistance genes have short life spans (3–5 years), they can be

highly effective in combating crown rust. Therefore, the seedling resistance genes identified in our study are significant and could also be novel given their wild diploid sources. However, these single seedling resistances might only be useful if deployed in combination with other resistance gene(s).

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Table 1 Segregation of response to *Puccinia coronata* f. sp. *avenae* pathotype 0000-2 among F<sub>2</sub> seedlings, and non-segregating resistant (NSR), segregating (SEG) and non-segregating susceptible (NSS) F<sub>3</sub> families from the cross Clav9112/Clav6956

F <sub>2</sub> families	Res ITs “;cn” to “1-2”	Sus ITs “3-3+”&“3+”	NS	SEG	NS	df	$\chi^2_{(3:1)}$	P value
1	37	9	-	-	-	1	0.72	0.5 – 0.3
2	62	20	-	-	-	1	0.02	0.9 – 0.5
Sum $\chi^2$	-	-	-	-	-	2	0.74	0.9 – 0.5
Pooled	99	29	-	-	-	1	0.29	0.9 – 0.5
Heterogeneity $\chi^2$	-	-	-	-	-	1	0.45	0.9 – 0.5
<b><u>F<sub>3</sub> families</u></b>								
94	-	-	51	23	20	2	44.95 <sup>a</sup>	< 0.01*
94	-	-	74	-	20	1	0.69	0.5–0.3

a-  $\chi^2_{(1:2:1)}(2df)$ ; \*Chi-squared values significant at P < 0.05

Table 2 Segregation for response to *Puccinia coronata* f. sp. *avenae* pathotype 0000-2 among F<sub>2</sub> seedlings, and non-segregating resistant (NSR), segregating (SEG) and non-segregating susceptible (NSS) F<sub>3</sub> families from the cross Clav9112/PI292226

F <sub>2</sub> families	Res ITs “;cn” to “1-2”	Sus ITs “3-3+”&“3”	N	SEG	NS	df	$\chi^2_{(3:1)}$	<i>P</i> value
1	57	14	-	-	-	1	1.05	0.5 – 0.3
2	48	12	-	-	-	1	0.80	0.5 – 0.3
Sum $\chi^2$	-	-	-	-	-	2	1.85	0.5 – 0.3
Pooled	105	26	-	-	-	1	1.85	0.5 – 0.3
Heterogeneity $\chi^2$	-	-	-	-	-	1	0.00	1.0
<u>F3 families</u>								
87	-	-	4	27	14	2	37.13 <sup>a</sup>	< 0.05*
87	-	-	$\frac{7}{3}$	-	14	1	3.68	0.1 – 0.05

a-  $\chi^2_{(1:2:1)}(2df)$ ; \*Chi-squared values significant at  $P < 0.05$



Table 3 Segregation for response to *Puccinia coronata* f. sp. *avenae* pathotype 0000-2 among F<sub>2</sub> seedlings from the cross Clav9112/Clav9020

Family	Resistant IT (“;cn” to “cn”)	Susceptible IT (“3-3+” & “3+”)	df	$\chi^2$ value	<i>P</i> value
1	32	10	1	0.04	0.9 – 0.5
2	32	9	1	0.22	0.9 – 0.5
Sum $\chi^2$	-	-	2	0.26	0.9 – 0.5
Pooled	64	19	1	0.20	0.9 – 0.5
Heterogeneity $\chi^2$	-	-	1	0.06	0.9 – 0.5

Table 4 Segregation for infection type (IT) response to *Puccinia coronata* f. sp. *avenae* pathotype 0000-2 of F<sub>2</sub> seedlings and F<sub>2:3</sub> families of resistant parent intercrosses between three wild oat accessions

Cross	No. of F <sub>2</sub> progeny	Seedling ITs	No. of F <sub>2:3</sub> families	Seedling ITs
CIav6956/PI292226	150	;n to ;ln	≤ 100	;n to ;ln
CIav6956/CIav9020	150	;n to ;ln	≤ 100	;n to ;ln
PI292226/CIav9020	150	;n to ;ln	≤ 100	;n to ;ln

Table 5 Segregation of response to *Puccinia coronata* f. sp. *avenae* pathotypes 0000-2 and 0207-5,6,10 among F<sub>2</sub>, non-segregating resistant (NSR), segregating (SEG) and non-segregating susceptible (NSS) F<sub>3</sub> and F<sub>4</sub> families derived from the cross CIav9112/Saia

F <sub>2</sub> Families	Res	Sus	NSR	SEG	NSS	df	$\chi^2_{(3;1)}$	P value
1	7	2	-	-	-	1	0.04	0.9 – 0.8
2	5	3	-	-	-	1	0.67	0.5 – 0.3
Sum $\chi^2$	-	-	-	-	-	2	0.71	0.9 – 0.5
Pooled	12	5	-	-	-	1	0.71	0.5 – 0.3
Heterogeneity $\chi^2$	-	-	-	-	-	1	0.00	1.0
<u>F<sub>3</sub> families (Pt. 0000-2)</u>								
17	-	-	3	9	5	2	1.60	0.5 – 0.1
17	-	-	12	-	5	1	0.71	0.5 – 0.1
<u>F<sub>4</sub> families (Pt. 0207-5,6,10)</u>								
17	-	-	6	8	3	2	0.99 <sup>a</sup> /3.6 <sup>b</sup>	0.7 – 0.5 <sup>a</sup> /0.2 – 0.1 <sup>b</sup>
17	-	-	14	-	3	1	0.02	0.9

a-  $\chi^2_{(1;2;1)} (2df)$ ; b-  $\chi^2_{(7;8;1)} (2df)$  \*Chi-squared values significant at P < 0.05

Table 6 Infection types (ITs) of 17 F<sub>2</sub> plants and their respective F<sub>3</sub> and F<sub>4</sub> families from the cross Clav9112/Saia tested with *Puccinia coronata* f. sp. *avenae* pathotypes (Pts.) 0000-2 and 0207-5,6,10 along with the heterogeneity Chi- squared values for eight segregating (SEG) families

Plant No.	F <sub>2</sub> IT	Reaction of F <sub>3</sub> families (Pt. 0000-2)		df	$\chi^2_{(3:1)}$ SEG families	P value	Reaction of F <sub>4</sub> families (Pt. 0207-5,6,10)		df	$\chi^2_{(9:7)}$ SEG families	P value
		IT “;” to “1-2”	IT “3+”				IT “;1” to “2-3”	IT “3+”			
		1	3+				-	NSS <sup>2</sup>			
2	;1-2n	14	5	1	0.02	0.9 – 0.5	81	5	1	0.25 <sup>a</sup>	0.7 – 0.5
3	;1cn	15	5	1	0.00	> 0.95	28	83	1	15.4 <sup>b</sup>	< 0.01*
4	;1n	15	5	1	0.00	> 0.95	31	50	1	0.8 <sup>b</sup>	0.5 – 0.3
5	3+	-	NSS	-	-	-	0	NSS	-	-	-
6	;1-2n	14	2	1	1.33	0.5 – 0.1	NSR	0	-	-	-
7	;n+	7	8	1	6.41	< 0.05*	40	37	1	0.68	0.5 – 0.3
8	;1-n	NSR <sup>1</sup>	0	-	-	-	26	5	1	5.36	0.05 – 0.01*
9	;1-n	NSR	0	-	-	-	NSR	0	-	-	-
10	3+	-	NSS	-	-	-	NSR	0	-	-	-
11	;1-n	15	6	1	0.15	0.9 – 0.5	60	44	1	0.09	0.8 – 0.7
12	;1-n	14	9	1	2.45	0.5 – 0.1	0	NSS	-	-	-
13	;1n	19	8	1	0.31	0.9 – 0.5	31	50	1	0.8 <sup>b</sup>	0.5 – 0.3
14	;n	NSR	0	-	-	-	NSR	0	-	-	-
15	3+	-	NSS	-	-	-	NSR	0	-	-	-
16	3+	-	NSS	-	-	-	NSR	0	-	-	-
17	;1cn	15	5	1	0.00	> 0.95	44	36	1	0.05	0.9 – 0.8
Pooled		128	53	1	1.76	0.5 – 0.1	341	310	1	3.96	0.05 – 0.01*
		Heterogeneity $\chi^2$		8	8.91	0.5 – 0.1	Heterogeneity $\chi^2$		8	19.47	0.05 – 0.01*

a-  $\chi^2_{(15:1)(1df)}$ ; b-  $\chi^2_{(7:9)(1df)}$ ; <sup>1</sup>NSR- non-segregating resistant; <sup>2</sup>NSS- non-segregating susceptible; \*Chi-squared values significant at P < 0.05

Table 7 Segregation for response to *Puccinia coronata* f. sp. *avenae* pathotype 0000-2 among F<sub>2</sub> individuals derived from the cross CIav9112/Glabrota

Family	F <sub>2</sub>		df	$\chi^2_{(3:1)}$	<i>P</i> value
	Resistant	Susceptible			
1	28	8	1	0.15	0.9 – 0.5
2	33	8	1	0.65	0.5 – 0.3
Sum $\chi^2$	-	-	2	0.80	0.9 – 0.5
Pooled	61	16	1	0.73	0.5 – 0.3
Heterogeneity $\chi^2$	-	-	1	0.07	0.9 – 0.5