

## An investigation of genetic variation among Australian isolates of *Bipolaris sorokiniana* from different cereal tissues and comparison of their abilities to cause spot blotch on barley

N. L. Knight<sup>A</sup>, G. J. Platz<sup>B</sup>, A. Lehmensiek<sup>A</sup> and M. W. Sutherland<sup>A,C</sup>

<sup>A</sup>Centre for Systems Biology, Faculty of Sciences, University of Southern Queensland, Toowoomba, Qld 4350, Australia.

<sup>B</sup>Hermitage Research Station, Queensland Primary Industries and Fisheries, Department of Employment, Economic Development and Innovation, Warwick, Qld 4370, Australia.

<sup>C</sup>Corresponding author. Email: marksuth@usq.edu.au

### ABSTRACT:

*Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*), the causal agent of common root rot (CRR) and foliar spot blotch (SB) diseases in barley and wheat, is an economically important fungal pathogen worldwide. However, the relationship between these two diseases is poorly understood. Differences within Australian *B. sorokiniana* populations were revealed by cluster analysis of amplified fragment length polymorphisms in genomic DNA of 48 *B. sorokiniana* isolates collected from the northern grain-growing region of Australia. Isolates collected from SB infections clustered apart from isolates collected from CRR infections. A subset of 31 *B. sorokiniana* isolates was assessed for their abilities to cause SB infections on barley leaves using a differential set of 15 barley genotypes and three other cereal species. The pathogen samples included 14 isolates from CRR infections of either wheat or barley and 14 isolates from SB infections of barley. Phenotypic experiments revealed that isolates of *B. sorokiniana* collected from barley SB infections showed a high level of pathogenic variability across the differential set. In contrast, isolates from CRR infections produced significantly less SB disease on inoculated barley leaves. Cluster analysis of the phenotypic infection response scores grouped isolates into three pathogenicity clusters demonstrating low, intermediate or high pathogenicity. The results of this study suggest divergence within Australian populations of *B. sorokiniana* in relation to host tissue specificity.

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Genetic variation in *Bipolaris sorokiniana*

N. L. Knight *et al.*

### Introduction

The hemibiotrophic fungus *Bipolaris sorokiniana* (*Bs*) (teleomorph: *Cochliobolus sativus*) is an important pathogen of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) globally (Kumar *et al.* 2002). *Bs* causes the disease common root rot (CRR) and the foliar disease spot blotch (SB). In Australia, SB of wheat is rare, whereas CRR is widespread (Butler 1961; Wildermuth 1986; Murray and Brown 1987; Murray and Brennan 2009a). In barley crops, both diseases can be significant constraints on yield (Murray and Brennan 2009b). Under favourable conditions SB can lead to greater losses, however, it is confined to the warmer and more humid growing areas of northern New South Wales and Queensland (Meldrum *et al.* 1999, 2004). Understanding the inter-relationship between these diseases is important in terms of disease control measures and for predicting disease incidence in subsequent seasons and changing climate.

Molecular markers such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphisms (AFLP) have been utilised to characterise *Bs* populations in North America, South America and Syria (Zhong and Steffenson 2001; Oliveira *et al.* 2002; Ghazvini *et al.* 2006; Arabi and Jawhar 2007). Zhong and Steffenson (2001) employed AFLP markers to analyse *Bs* isolates (from barley leaves and barley and wheat kernels) originating predominantly from North America and found that while isolates did cluster into groups, these groups did not correlate with the observed pathotypes. Arabi and Jawhar (2007) utilised RAPD markers to compare a group of *Bs* isolates collected from both barley leaves and roots but found no genetic distinction between isolates in relation to tissue source or pathogenicity.

Phenotypic studies of *Bs* isolates from North America, Syria and Australia have tested SB virulence on leaves of a range of barley genotypes, typically assigning pathotypes using coded triplet nomenclature (Valjavec-Gratian and Steffenson 1997; Arabi and Jawhar 2003; Meldrum *et al.* 2004). The numbers of distinct pathotypes detected vary between these studies, which differed in the number of isolates tested and the size and composition of the host differential set employed. Recently, hierarchical cluster analysis has been utilised as an alternative approach to pathotype designation (Ghazvini and Tekauz 2008). This study indicated that a wide selection of *Bs* isolates, in which eight pathotypes had been delineated by coded triplet nomenclature, condensed into only three distinct pathogenic clusters (PC) defined by low virulence, differential virulence or virulence with varying levels of aggressiveness.

There is also evidence of changes in pathogen virulence in response to selection pressure. Valjavec-Gratian and Steffenson (1997) detected virulence in local USA isolates of *Bs* on the previously resistant and widely grown cultivar Bowman. These observations demonstrate that *Bs* populations have the potential to overcome resistance in historically resistant barley sources, highlighting the desirability of host lines with durable multigenic resistance.

The first aim of the present study was to examine the genotypic variation within Australian *Bs* populations using an AFLP-based cluster analysis. The second aim was to test the ability of *Bs* isolates from CRR-infected roots to cause SB disease on barley leaves. Finally, we compared the virulence of Australian *Bs* isolates across an extensive differential set of host genotypes to extend our understanding of pathotype structure in Australian populations of the pathogen.

## Materials and methods

### *Fungal isolates*

Forty-eight isolates of *Bs*, isolated from either SB or CRR infections, were collected for use in this study (Table 1). The SB and CRR isolates, collected primarily from south-east Queensland and northern New South Wales, were provided from collections at the Queensland Primary Industries and Fisheries (QPIF) Hermitage Research Station, Warwick and the QPIF Leslie Research Centre, Toowoomba. All of the SB isolates were collected from barley tissues, with the exception of isolate 20004, which was collected from infected prairie grass (*Bromus willdenowii*). The CRR isolates were collected from either wheat or barley tissues. Two additional isolates, SB37i and 07003, were collected from barley seed.

All 48 isolates were analysed in a genetic diversity study while a subset of 31 isolates was tested for the ability to cause SB symptoms on a range of barley genotypes. This subset was chosen based on differing disease, host and geographic origins. All available barley CRR isolates were tested. Cultures of each isolate were grown from single conidia.

### *AFLP analysis*

Forty to sixty milligrams of fungal mycelium were harvested from cultures grown in starch nitrate broth (Dodman and Reinke 1982) at 25°C for 1 week. A Wizard Genomic DNA Extraction kit (Promega, Sydney, Australia) was used to extract the fungal DNA, following the supplied plant DNA extraction protocol. Extracted DNA was visualised using a Gel Documentation system (BioRad, Gladesville, Australia) and quantified using the standard DNA provided in the AFLP Core Reagent kit (Invitrogen, Mulgrave, Australia).

An AFLP procedure similar to that described by Vos *et al.* (1995) was employed. Approximately 300 ng of DNA was restricted using *EcoRI* and *MseI* restriction enzymes. After restriction, specific adaptors were ligated onto the cut sites using T4 DNA ligase. Preselective PCR contained 5 µL of restricted-ligated DNA, 0.2 U of *Taq* DNA polymerase (BIOTAQ, Boline, Alexandria, Australia), 2.5 µL of Boline 10× NH<sub>4</sub><sup>+</sup> PCR reaction buffer, 100 µM dNTPs, 1.5 mM MgCl<sub>2</sub> and 0.25 µM of *EcoRI* (E-A or E-G) and *MseI* (M-A or M-C) primers with one selective nucleotide, in a total volume of 25 µL. The preselective amplification PCR cycling conditions were 20 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 1 min. Selective amplification was then performed in a 20-µL reaction mix containing 2 µL of preselective amplified DNA, 0.2 U of *Taq* DNA polymerase (Boline), 2 µL of Boline 10× NH<sub>4</sub><sup>+</sup> PCR reaction buffer, 100 µM dNTPs, 1.5 mM MgCl<sub>2</sub> and 0.25 µM of *EcoRI* and *MseI* primers with two selective nucleotides. The PCR cycling conditions were 12 cycles of 94°C for 30 s, 65°C for 30 s and 72°C for 1 min, followed by 23 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min. After addition of 4 µL of formamide, the PCR products were denatured at 95°C for 5 min, snap-cooled on ice and fractionated for 3 h at 80 W in 8% polyacrylamide gels in TRIS-borate-EDTA buffer. The DNA silver-staining method (Sourdille *et al.* 1998) was used to observe the AFLP amplicons produced by the primer pairs. Only polymorphic amplicons between 100 and 1000 bp were scored. Four isolates representing the major genetic clusters were reextracted and retested using each primer combination.

Amplicons produced by each primer pair were scored as binary data and a similarity matrix was constructed using the Dice coefficient (Dice 1945) in the Qualitative Data program within the NTSYS-pc software package. Cluster analysis of the matrix values was performed by employing the Unweighted Pair Group Method with Arithmetic Means (UPGMA) algorithm provided in the SAHN program of NTSYS-pc 2.2 and a dendrogram was produced. A two-way Mantel test was also applied to this data by NTSYS to test for association. The clade support was assessed through a 1000-replicate bootstrap test in WINBOOT (<http://www.irri.org/science/software/winboot.asp>, accessed 10/10/2009) to define confidence intervals (Felsenstein 1985).

All 48 *Bs* isolates were tested for the presence of pathotype 2 (defined as having high virulence on the genotype Bowman). The pathotype 2-specific PCR contained 1 µL of DNA, 0.05 U of *Taq* DNA polymerase (Boline), 1 µL of Boline 10× NH<sub>4</sub><sup>+</sup> PCR reaction buffer, 100 µM dNTPs, 1.5 mM MgCl<sub>2</sub> and 0.25 µM of the unique pathotype 2 primers E-AG/M-CA-207 (Zhong and Steffenson 2001, 2002), in a total volume of 10 µL. The PCR cycling conditions were 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 55°C for 1 min and 72°C for 1 min, followed by 72°C for 10 min. DNA from a North American isolate of pathotype 2 (isolate ND90Pr) was provided by Professor Brian Steffenson (University of Minnesota) as a positive control. The samples were run on a polyacrylamide gel using a Gel-Scan 2000 instrument (Corbett Research, Sydney, Australia).

### *Plant materials*

Fifteen barley genotypes and single wheat, rye (*Secale cereale*) and triticale (*Triticale hexaploide*) genotypes (Table 2) were grown as a differential set for this study. Genotype selection was based on results reported in previous studies (Valjavec-Gratian and Steffenson 1997; Zhong and Steffenson 2001; Meldrum *et al.* 2004) and identification of new lines of interest during disease screening at QPIF Hermitage Research Station.

The 18 line differential set was grown in six 10-cm pots. Each pot contained three genotypes, each located in separate sectors. Three to six seeds were planted for each genotype. A replicated differential set was grown for each isolate phenotypically tested. Before inoculation, the positions of the 12 pots were randomised using Microsoft Excel. Pots were maintained in a glasshouse with a temperature range of 14–30°C, watered daily and fertilised weekly with Flowfeed EX7 (Grow Force, Acacia Ridge, Australia) after seedling emergence.

### *Inoculation*

Inoculum preparation and inoculation methods were based on those described by [Meldrum \*et al.\* \(1999\)](#) and [Fetch and Steffenson \(1994\)](#). Seedlings were inoculated 14 days after sowing at Zadoks' growth stage 13 ([Zadoks \*et al.\* 1974](#)). Inoculum was produced from cultures grown on starch nitrate agar ([Dodman and Reinke 1982](#)) in the dark at 25°C for ~14 days to allow sufficient conidial production. A concentration of 4800 conidia/mL was produced for each fungal isolate separately, resulting in each replicated differential set (12 pots) being inoculated with 50 mL. The conidial solutions were applied using a Krebs airless sprayer. Viability of the conidial suspensions was confirmed via germination counts after 6–8 h of growth at 25°C on water agar. After inoculation and a 24-h incubation period in a dew chamber (14 h dark followed by 10 h light at 22°C), the plants were placed in growth rooms with 12 h light at 25°C followed by 12 h dark at 15°C for 11 days. Infection responses were recorded 12 days after inoculation (growth stage 15). Six fungal isolates of varied source were tested in parallel in the same period. These were selected to ensure that a full range of responses from resistant to highly susceptible was produced in each experiment. Infection with isolate 05050 was repeated on four separate occasions, while isolates 05047 and SB37i were applied on two separate occasions to gauge consistency of disease symptom expression between experiments conducted at different times.

### *Pathotype and virulence group designation*

Infection response scoring, coded triplet nomenclature and cluster analysis were applied as previously described ([Limpert and Müller 1994](#); [Fetch and Steffenson 1999](#); [Meldrum \*et al.\* 2004](#); [Ghazvini and Tekauz 2007](#)). Infection responses were given ratings of 0–9 according to the type (presence of necrosis and chlorosis) and relative size of lesions on the second leaves of barley seedlings, as described by [Fetch and Steffenson \(1999\)](#). A score of 0–4.5 was classed as resistant [minute necrotic lesions ranging up to small necrotic lesions (<2 mm<sup>2</sup>) with restricted chlorotic margins], whereas a score of >4.5 was classed as susceptible [medium necrotic lesions (>2 mm<sup>2</sup>) with restricted chlorotic margins ranging up to large oval lesions with chlorotic margins and expanding diffuse chlorosis]. A control inoculation was performed using distilled water. An average SB infection response score on barley was calculated for each isolate using the respective isolate scores across selected lines of the replicated differential set (only lines with at least one score >4.5). A one-way ANOVA was used to compare average scores of each host/infection source group (barley CRR, wheat CRR and barley SB) using SPSS (version 6.1) ([Coakes and Steed 1997](#)). A total of 31 different isolates was screened in this manner. These included all the CRR isolates from barley, barley seed and Prairie grass. A selection of isolates from barley SB and wheat CRR was included to represent a wide variety of the geographical locations sampled.

Hierarchical cluster analysis, using the NTSYS-pc software package (version 2.20 g; Exeter Software, NY), was based on the average virulence scores of each isolate across each line of the differential set which showed susceptibility to at least one isolate. Lines which were resistant to all isolates were excluded from the analysis due to lack of variation. A similarity matrix based on the average taxonomic distance coefficient ([Sneath and Sokal 1973](#)), produced using the SIMINT function, was examined using the UPGMA algorithm provided in the SAHN program of NTSYS. In addition, a three-category method was applied where SB reactions were divided into resistant (score 1–3.5), intermediate (score >3.5–6) and susceptible (score >6–9) groups for comparison with the aforementioned pathotype ranking methods.

## Results

### *AFLP analysis*

DNA of 48 *Bs* isolates underwent AFLP analysis using 22 primer pairs. The primer pairs produced varying numbers of scorable polymorphic amplicons, with each pair producing unique banding patterns. A total of 134 polymorphic amplicons was recorded. Forty-eight different AFLP phenotypes were observed using the combined results of all the primer pairs, indicating the genetic uniqueness of each *Bs* isolate. The isolates formed four distinct groups, linked by a similarity coefficient greater than 0.5 (Fig. 1). The fit within these groups indicated a matrix correlation value of 0.8.

The inclusion of isolate 20004 (group 1) as an outlier allowed the AFLP groupings of barley and wheat isolates to be more clearly observed. The CRR isolates grouped separately from the SB isolates (group 3), forming two distinctly different branches represented by groups 2 and 4. Results for the independently reextracted samples mirrored those of the initial screens on these samples, indicating the reproducibility of the analysis.

Amplification with the primer set for the AFLP marker E-AG/M-CA-207, specific for pathotype 2 (virulent on Bowman), gave a positive band for the sample of pathotype 2 DNA from the USA. However, these primers failed to amplify this fragment in any of the 48 Australian isolates tested.

### *Average infection responses*

Average SB infection response scores on barley are presented in Fig. 2, excluding the results from the non-barley differential genotypes and the five barley genotypes, which were resistant to all isolates: Bowman, ND B112, Larker, ND11231-12, and WPG8412-9-2-1. The remaining genotypes were susceptible to at least one isolate. Although the SB isolates showed a wide range of pathogenicity scores on the selection of barley genotypes, CRR isolates failed to induce susceptible lesions (scores <4.5). The lesions produced in response to either avirulent SB isolates or to CRR isolates were similar in both size and appearance. The control plants sprayed with water showed no sign of infection on the second seedling leaves, indicating no cross-infection between neighbouring treatment blocks. The phenotypic results displayed a high level of correlation between replicates within single experiments, with differences in scoring of only one rating point or less. This high correlation was also seen in replicate experiments repeated at different times [isolates 05047, SB37i (2×) and 05050 (4×)]. Isolates in common with the study by Meldrum *et al.* (2004) produced comparable reactions.

A one-way ANOVA demonstrated a significant difference ( $P < 0.01$ ) between the infection responses caused by SB isolates from barley and the infection responses produced by CRR isolates from barley and wheat. A significant difference in infection response was not evident between barley and wheat CRR isolates. The isolates 20004, collected from prairie grass leaf, and SB37i, collected from barley seed, showed significantly lower mean virulence scores than the other SB isolates and were in the same range as scores for the CRR isolates. The non-barley differential genotypes Ryesun (rye), Hartog (wheat) and Madonna (triticale) displayed SB-resistant reactions towards all 31 fungal isolates. Scores on the 1-9 scale were 3 or less for all these particular host/isolate combinations.

### *Pathotype detection*

Coded triplet nomenclature (Limpert and Müller 1994) revealed 11 apparent pathotypes present in the 31 isolates phenotypically tested across 12 genotypes of the differential set

(Table 3). Bowman and ND B112 were included in the analysis to allow comparison of results with previous studies, which solely used Bowman, ND B112 and ND 5883. The 16 isolates in pathotype 0.0.0.0 exhibited low pathogenicity on all lines of the differential set. This low pathogenicity group consisted of the entire selection of CRR isolates as well as isolates SB37i and 20004. Six SB isolates were rated as virulent pathotype 1.7.7.7, the only pathotype represented by more than one barley SB isolate. The current method classified resistant as a score of 0–4.5 and susceptible as 5–9. If this is arbitrarily altered (e.g. resistant as 0–4 and susceptible as 4.5–9, or resistant as 0–5 and susceptible as 5.5–9) the pathotype classifications alter for some isolates but the total number of different pathotypes remains similar.

Hierarchical cluster analysis revealed three major PC of isolates (Fig. 3) using the average infection response scores across the 10 differential lines that were susceptible to at least one isolate. These PC were characterised by either low pathogenicity (PC1), high pathogenicity (PC2) or intermediate pathogenicity (PC3) and were represented, respectively (but not exclusively), by pathotype 0.0.0.0, pathotype 1.7.7.7 and pathotypes inducing a resistant response on ND 5883. The three-category method produced three groups similar to those defined by hierarchical cluster analysis (Table 4). No apparent relationship occurred between the geographic origin of isolates and either their pathotype or AFLP similarity groupings.

## Discussion

Australian *Bs* isolates causing either SB or CRR have a similar genetic relatedness to that reported for overseas populations (Zhong and Steffenson 2001; Moura Nascimento and Van Der Sand 2008), however, there are distinct genetic subgroups which correspond to host-tissue specificities. This separation has not previously been reported. AFLP analysis also provides preliminary evidence, in the case of isolates from CRR infections, for the presence of two further distinct subgroups. This potentially reflects a degree of specialisation based on host identity. Host specificity of *Bs* causing CRR has previously been demonstrated by Conner and Atkinson (1989), where isolates from CRR of wheat were highly virulent on wheat roots, yet weakly virulent on barley roots and vice versa. The degree of CRR cross-infection between species has major implications for planning of crop rotations. Our observation that CRR isolates from barley and wheat form two distinct genetic clusters and cause similarly low disease scores on barley leaves suggests not only the potential for host specificity but also a second level of specificity based on tissue specialisation.

Studies in Syria, Mexico and Sweden comparing root and leaf reactions of barley and wheat in response to isolates originating from barley or wheat root or leaf infections reported no physiological specialisation of *Bs* isolates (Almgren *et al.* 1999; Duveiller and García Altamirano 2000; Arabi and Jawhar 2007; Persson *et al.* 2008). However, Australian root isolates in this study and in Meldrum *et al.* (2004) caused a much lower level of leaf symptoms than isolates previously isolated from leaves. It appears that these CRR isolates retain the ability to initiate infection, but lack the ability to grow aggressively in leaf tissue. These low disease scores may be due to a lack of virulence factors for successful SB infection and suggest a divergent shift towards tissue specialisation in the populations of *Bs* in Australia that requires further investigation.

SB infection responses on the non-barley hosts indicated that isolates which were virulent on barley were not able to produce virulent infection responses on wheat, rye or triticale. These observations, together with the non-pathogenicity of the Prairie grass isolate, suggest that *Bs* isolates from SB infections across multiple host species may also contain host-specific

subgroups. However, a report by [Sampson and Watson \(1985\)](#) used a single *Bs* isolate from quack grass (*Agropyron repens*) leaf spot in Canada to demonstrate significant leaf infection of 47 out of 51 grass species inoculated. A more extensive study of grass host specificity of Australian *Bs* isolates is warranted.

Assessment of SB causing isolates using this extensive barley differential set indicates a degree of pathotype complexity that is not revealed when smaller differential sets are employed (e.g. [Valjavec-Gratian and Steffenson 1997](#)). Pathotype designation allows isolates to be classed into groups based on an ability to infect subsets of the differential lines. Significantly, the most virulent group as defined by coded triplet nomenclature, pathotype 1.7.7.7, was the only designation to include multiple isolates. The other identified pathotypes exhibited a wide range of differential pathogenicity. Significant levels of pathotypic variation have been previously observed in *Bs* populations ([Fetch and Steffenson 1994](#); [Arabi and Jawhar 2003](#); [Meldrum et al. 2004](#); [Ghazvini and Tekauz 2007](#)). The major weakness with the coded triplet nomenclature system of classifying pathotypes is the arbitrary distinction of resistant versus susceptible responses based on dividing a 9-point scale into two classes.

[Ghazvini and Tekauz \(2008\)](#) attempted to overcome this problem by using hierarchical cluster analysis to group isolates according to their infection response scores across the differential set. This analysis does not classify isolates of varying virulence into numerous distinct pathotypes, since the analysis also detects variations in aggressiveness in addition to variation in virulence class. Our hierarchical cluster analysis parallels that reported by [Ghazvini and Tekauz \(2008\)](#), with three PC being defined that reflect PC1, PC2 and PC3 disease responses across the differential set.

Several major effect loci for SB resistance in seedlings have been identified, particularly on chromosomes 1H and 7H ([Steffenson et al. 1996](#); [Steffenson 2000](#)). Current studies indicate the presence of other major and minor resistance loci in some host lines ([Bovill et al. 2010](#)). These loci may be responsible for the intermediate disease responses in some genotype/isolate interactions in the differential set, leading to a high number of apparent pathotypes using coded triplet nomenclature and to considerable variation within PC3 as defined by hierarchical cluster analysis ([Fig. 2](#)). Epistatic and genotype by environment interactions involving these genes may also contribute to the range of phenotypic responses observed in PC3. From these experiments it appears that the quantitative nature of SB resistance in some host genotypes renders problematic the allocation of a classical 'pathotype' concept to individual fungal isolates. The generation of differential host sets, in particular for international comparisons of pathogenicity, will be challenging.

Three differentials (ND 5883, Bowman and ND B112) were employed by [Valjavec-Gratian and Steffenson \(1997\)](#) and [Zhong and Steffenson \(2001\)](#) to describe three pathotypes: 0 (exhibits virulence on all three differentials), 1 (exhibits virulence only on ND 5883) and 2 (exhibits virulence only on Bowman), in predominantly North American *Bs* populations. Infection responses on these lines indicated that only pathotypes 0 and 1 were observed among the *Bs* isolates tested in this study. Our failure to detect any pathotype 2 isolates, as defined by virulence on genotype Bowman and by a specific PCR test ([Zhong and Steffenson 2001, 2002](#)), suggests that virulence on Bowman is rare or absent in Australian populations. This possibly reflects a lack of selection pressure, as Bowman, or lines with closely related pedigrees, have not been deployed in commercial Australian germplasm.

The disease responses of the barley genotypes of the differential set usefully separated the *Bs* isolates into as many as 11 putative pathotypes within three PC, except for the North

American lines Bowman, Larker, ND B112, ND11231–12 and WPG8412–9-2–1, which were resistant to all isolates. The susceptibility of each of the Australian barley genotypes (Sloop, Skiff, Stirling, Lindwall, Gilbert and VB9524) to a varying subset of the fungal isolates tested highlights the importance of current attempts to incorporate resistant germplasm identified by international programs, into Australian breeding lines.

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**Fig. 1.** Dendrogram derived from AFLP analysis of 48 *Bipolaris sorokiniana* isolates from spot blotch and common root rot infections. The bootstrap values of the subgroups are indicated. (–) = not tested.

**Fig. 2.** Average infection responses induced by *Bipolaris sorokiniana* isolates from spot blotch and common root rot infections of barley and wheat, across 10 barley lines. Bars represent the standard error.

**Fig. 3.** Dendrogram of similarity of the average spot blotch infection response scores of 30 *Bipolaris sorokiniana* isolates, collected from wheat or barley, on 10 barley differential lines. The coded triplet nomenclature pathotypes are shown on the right.

**Table 1. Characteristics of *Bipolaris sorokiniana* isolates used in phenotypic and genotypic diversity analysis**

Isolate	Location	Host source	Infection source	Year collected	
20004 <sup>A</sup>	Casino	NSW	Prairie Grass	Spot blotch	2000
98043 <sup>B</sup>	Biloela	Qld	Barley	Spot blotch	2000
98051 <sup>B</sup>	Logan Point	Qld	Barley	Spot blotch	2000
98137	Cobbitty	NSW	Barley	Spot blotch	1999
99108 <sup>B</sup>	Hermitage	Qld	Barley	Spot blotch	1999
SB60 <sup>B</sup>	Hermitage	Qld	Barley	Spot blotch	1999
05047 <sup>A</sup>	Gatton	Qld	Barley	Spot blotch	2005
05050 <sup>A</sup>	Pilton	Qld	Barley	Spot blotch	2005
06001 <sup>A</sup>	Kingaroy	Qld	Barley	Spot blotch	2006
98036 <sup>A</sup>	Grafton	NSW	Barley	Spot blotch	1999
98042 <sup>AB</sup>	Monto	Qld	Barley	Spot blotch	1999
98052 <sup>AB</sup>	Bauhinia Downs	Qld	Barley	Spot blotch	1999
98068 <sup>AB</sup>	Gindie	Qld	Barley	Spot blotch	1999
98114 <sup>A</sup>	Croppa Creek	NSW	Barley	Spot blotch	1999
98121 <sup>AB</sup>	Tamworth	NSW	Barley	Spot blotch	1999
98129 <sup>A</sup>	Moree	NSW	Barley	Spot blotch	1999
99034 <sup>AB</sup>	Jandowae	Qld	Barley	Spot blotch	1999
99109 <sup>AB</sup>	Aratula	Qld	Barley	Spot blotch	1999
SB61 <sup>A</sup>	Monto	Qld	Barley	Spot blotch	2001
SB63 <sup>A</sup>	Hermitage	Qld	Barley	Spot blotch	2001
SB37i <sup>A</sup>	Woomelang	Vic	Barley	Seedborne	1999
07003 <sup>A</sup>	Bundaberg	Qld	Barley	Seedborne	2007
95#11 <sup>A</sup>	Millmerran	Qld	Barley	Common root rot	1995
96#14 <sup>A</sup>	Nindigully	Qld	Barley	Common root rot	1996
A04#36 <sup>A</sup>	Tummaville	Qld	Barley	Common root rot	2004
A05#7 <sup>A</sup>	Billa Billa	Qld	Barley	Common root rot	2005
96#15	Nindigully	Qld	Wheat	Common root rot	1996
A01#29	Weemelah	NSW	Wheat	Common root rot	2001
A01#32	Blackville	NSW	Wheat	Common root rot	2001
A01#36	Bullarah	NSW	Wheat	Common root rot	2001
A02#18	Nindigully	Qld	Wheat	Common root rot	2002

A02#86	Dulacca	Qld	Wheat	Common rot	root	2002
A03#5	Goondiwindi	Qld	Wheat	Common rot	root	2003
A04#11	Wallumbilla	Qld	Wheat	Common rot	root	2004
A05#34	Moree	NSW	Wheat	Common rot	root	2005
A05#35	Moree	NSW	Wheat	Common rot	root	2005
A05#49	Inglestone	Qld	Wheat	Common rot	root	2005
A05#57	Nindigully	Qld	Wheat	Common rot	root	2005
A02#19 <sub>A</sub>	Mulga View	Qld	Wheat	Common rot	root	2002
A03#18 <sub>A</sub>	Tallwood	NSW	Wheat	Common rot	root	2003
A03#36 <sub>A</sub>	Moree	NSW	Wheat	Common rot	root	2003
A03#47 <sub>A</sub>	Spring Ridge	NSW	Wheat	Common rot	root	2003
A03#6 <sup>A</sup>	Goondiwindi	Qld	Wheat	Common rot	root	2003
A04#17 <sub>A</sub>	North Bungunya	Qld	Wheat	Common rot	root	2004
A04#4 <sup>A</sup>	Wellcamp	Qld	Wheat	Common rot	root	2004
A04#51 <sub>A</sub>	Tara	Qld	Wheat	Common rot	root	2004
A04#56 <sub>A</sub>	Dulacca	Qld	Wheat	Common rot	root	2004
A05#47 <sub>A</sub>	Wandoan	Qld	Wheat	Common rot	root	2005

<sup>A</sup>Isolates phenotypically tested.

<sup>B</sup>Isolates used by [Meldrum \*et al.\* \(2004\)](#).

**Table 2. Differential set consisting of 18 different genotypes of barley and other cereal species**

Genotype	Crop type	Origin
Bowman <sup>ABC</sup>	Barley	USA
Larker <sup>BC</sup>	Barley	USA
ND B112 <sup>ABC</sup>	Barley	USA
CI 1227 <sup>B</sup>	Barley	USA
CI 6311 <sup>B</sup>	Barley	USA
ND 5883 <sup>AB</sup>	Barley	USA
ND11231-12 <sup>C</sup>	Barley	USA
WPG8412-9-2-1 <sup>C</sup>	Barley	Canada
Delta	Barley	United Kingdom
Stirling <sup>B</sup>	Barley	Australia
Gilbert <sup>B</sup>	Barley	Australia
Lindwall <sup>B</sup>	Barley	Australia
Skiff <sup>B</sup>	Barley	Australia
VB9524	Barley	Australia
Sloop	Barley	Australia
Ryesun <sup>BC</sup>	Rye	Australia
Hartog <sup>BC</sup>	Wheat	Australia
Madonna <sup>BC</sup>	Triticale	Australia

<sup>A</sup>Genotypes used by Valjavec-Gratian and Steffenson (1997) and Zhong and Steffenson (2001).

<sup>B</sup>Genotypes used by Meldrum *et al.* (2004).

<sup>C</sup>Indicates genotypes resistant to all isolates in this study.

**Table 3. Disease reactions induced by 11 pathotypes of *Bipolaris sorokiniana* among 31 isolates on a differential set of 12 barley lines<sup>A</sup> based on the classical binary [R (resistant) or S (susceptible)] method of pathogenicity designation using coded triplet nomenclature**

Genotype	<i>B. sorokiniana</i> pathotype										
	0.0.1.3	0.0.1.6	0.0.5.4	1.0.2.6	1.5.1.7	1.5.7.7	1.6.5.6	1.6.5.7	1.6.7.6	1.7.7.7	0.0.0.0
ND 5883	R	R	R	S	S	S	S	S	S	S	R
Bowman	R	R	R	R	R	R	R	R	R	R	R
ND B112	R	R	R	R	R	R	R	R	R	R	R
Stirling	R	R	R	R	S	S	R	R	R	S	R
Gilbert	R	R	R	R	R	R	S	S	S	S	R
Lindwall	R	R	R	R	S	S	S	S	S	S	R
Skiff	S	S	S	R	S	S	S	S	S	S	R
Delta	R	R	R	S	R	S	R	R	S	S	R
VB9524	R	R	S	R	R	S	S	S	S	S	R
CI 1227	S	R	R	R	S	S	R	S	R	S	R
CI 6311	S	S	R	S	S	S	S	S	S	S	R
Sloop	R	S	S	S	S	S	S	S	S	S	R
No. Isolates <sup>B</sup>	1	1	1	1	1	1	1	1	1	6	16

<sup>A</sup>Resistant genotypes Larker, ND11231–12 and WPG8412–9-2–1 and non-barley genotypes Ryesun, Madonna and Hartog have been omitted.

<sup>B</sup>Number of isolates within each pathotype.

**Table 4. Disease reactions induced by 15 isolates of *Bipolaris sorokiniana* identified in PC2 and PC3 (Fig. 3) on a differential set of 12 barley lines. Reactions defined by the triple category method (R = resistant, I = intermediate, S = susceptible)**

Genotype	<i>B. sorokiniana</i> isolate												
	05047	99034	98068	98114	07003	98129	05050	98036	99109	SB61	06001	98042	98052
ND 5883	I	I	I	I	I	S	S	S	S	I	I	S	S
Bowman	R	R	R	R	R	R	R	R	R	R	R	R	R
ND B112	R	R	R	R	R	R	R	R	R	R	R	R	R
Stirling	I	I	I	R	I	I	I	I	I	I	I	I	I
Gilbert	I	I	R	R	I	R	I	I	I	I	S	S	I
Lindwall	I	I	I	I	R	I	S	S	S	S	S	S	S
Skiff	I	I	I	I	I	I	S	S	S	S	S	S	S
Delta	I	I	R	I	I	I	I	I	I	I	S	S	S
VB9524	I	I	I	I	I	I	I	S	I	I	I	I	I
CI 1227	I	I	I	I	I	I	S	S	I	I	S	S	S
CI 6311	I	I	I	I	I	S	S	I	S	I	S	S	S
Sloop	I	I	I	I	I	S	S	S	S	S	S	S	S

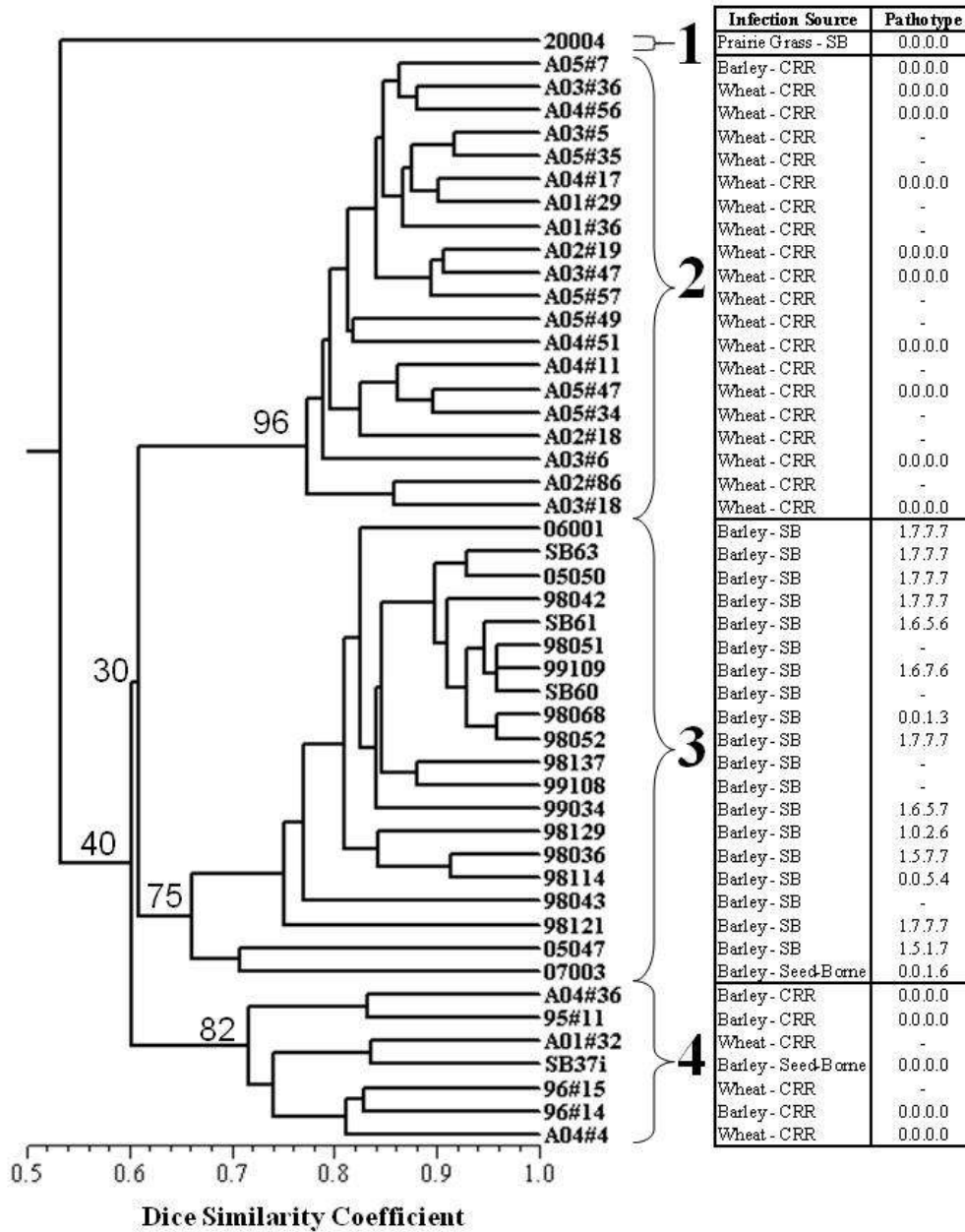


Fig. 1.

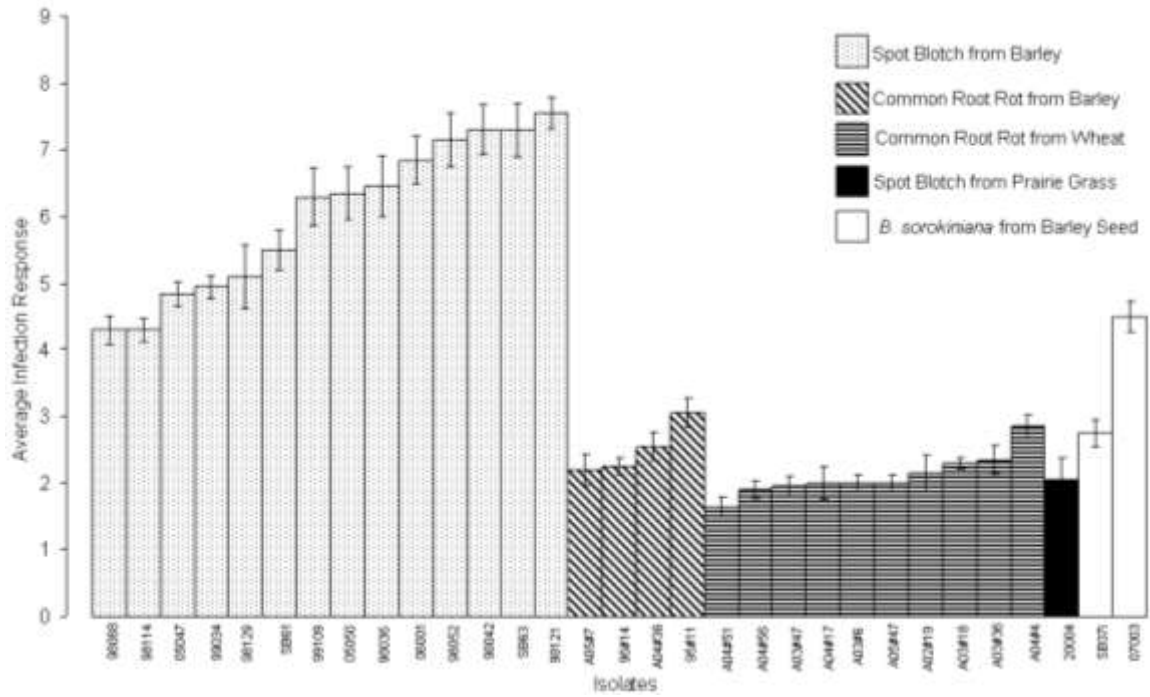


Fig. 2.

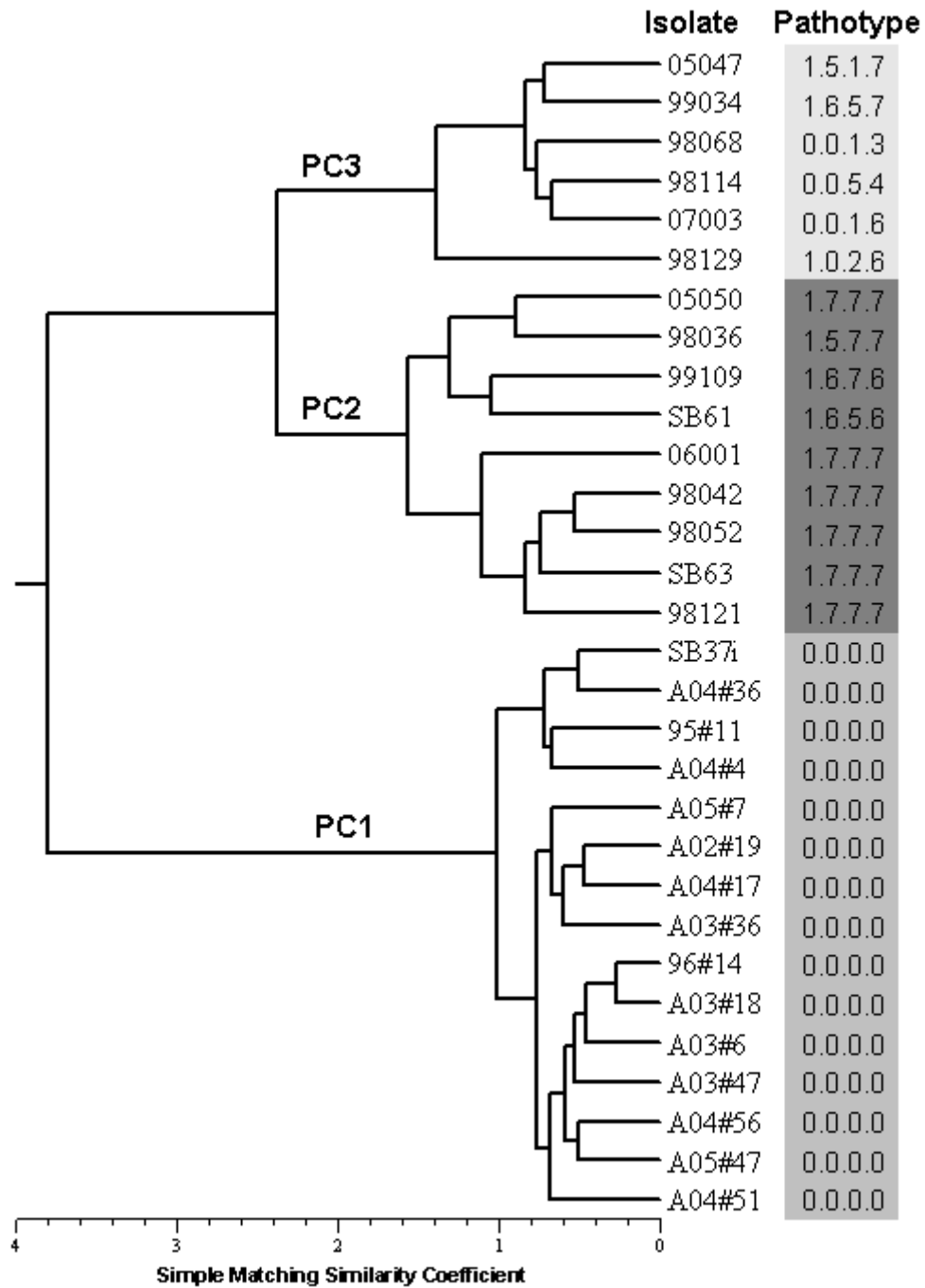


Fig. 3.