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Characterisation and diversity of *Pyrenophora teres* f. *maculata* isolates in Western Australia

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Abstract

Spot type net blotch caused by *Pyrenophora teres* f. *maculata* (Ptm) has become a prominent disease in Western Australia, as has also occurred elsewhere. The disease has a negative impact on both grain yield and quality resulting from reduced grain size. Lack of resistance and stubble retention are the likely factors in the increased severity of the disease in barley growing areas of Western Australia. Because of the increasing importance of spot type net blotch and the need to improve barley resistance, understanding pathogen virulence is a high priority as this has direct impact on the identification and utilization of resistance genes in breeding programs. Ninety nine isolates of Ptm were collected from geographically dispersed barley fields of Western Australia during 2001 and 2002. Forty nine sporulating isolates of Ptm were classified into seven isolate groups (IGs) on the basis of their infection responses on 26 differential barley lines. The 26 lines were likewise classified into four line groups (LGs) based on their distinguishing response to the spot type net blotch isolates. The varied infection responses among the differential barley lines demonstrated a wide geographic dispersal of IGs, as well as previously undetected virulence, in Ptm in Western Australia. The commercially grown barley cultivars Baudin and Gairdner are regarded as susceptible to spot type net blotch, but showed a range of reactions to the various Ptm isolates as seedling plants. The variability in the pathogen and the resistance identified in some genotypes used in this study are being investigated further to develop superior, adapted germplasm for use in barley breeding programs in Australia.

Additional keywords: Barley differential set, pathogen variability, virulence, cluster analysis

Introduction

Spot type net blotch (STNB) caused by *Pyrenophora teres* f. *maculata* Drechs. Smedeg. (anamorph: *Drechslera teres* [Sacc.] Shoem.) is a prominent foliar disease of barley (*Hordeum vulgare* L. emend. Bowden) in Australia (Young and Loughman 1995; Jayasena et al. 2007; McLean et al. 2009) and other parts of the world (Bockelman et al. 1983; Karki and Sharp 1986; Tekauz 1990; Steffenson 1997; Leisova et al. 2005; Serenius et al. 2007). In Western Australia (WA), the disease was first reported from Chapman Research Station in 1977 (Khan and Tekauz 1982). Leaf symptoms are associated with dark brown circular or elliptical spots accompanied by chlorosis of the surrounding leaf tissue (Smedegård-Petersen 1971; Khan and Tekauz 1982). These can reduce grain yield up to 44% in severe epidemics (Jayasena et al. 2002, 2007) and varied from 3-22% in cultivars Beecher and O'Conner in WA (Khan 1989).

Studies on the occurrence and distribution of different virulence types of *P. teres* f. *maculata* are essential to identify useful forms of resistance and assist in the development of future barley breeding strategies (McDonald and Linde 2002). Pathogen variation in STNB has been studied using differential barley sets (Khan and Tekauz 1982; Bockelman et al. 1983; Karki and Sharp 1986; Tekauz 1990; Arabi et al. 1992; McLean et al. 2010) and molecular markers, such as amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD), sequence-tagged microsatellite primers (Jonsson et al. 2000; Rau et al. 2003; Leisova et al. 2005; Serenius et al. 2007; Bogacki et al. 2010; Lehmensiek et al. 2010). These studies reveal high genetic diversity with a possibility of sexual recombination.

In WA, a 1995-96 crop survey identified net type net blotch (NTNB) as the predominant form of the disease in barley. Only a small number of STNB isolates in south coastal areas were identified (Gupta and Loughman 2001). This was the first indication that this disease could occur outside its previously recorded northern wheat-belt range in WA (Khan and Tekauz 1982). Isolates of STNB were differentiated on a barley cultivar Herta which indicated variability in this pathogen.

STNB outbreaks have occurred seasonally in recent years and growers depend primarily on fungicides and crop rotation practices for its control and management. The increased prevalence in WA followed the release of the susceptible malting cultivar Gairdner which became widely grown in the south coastal region. The spread of STNB in other parts of WA could be associated with the release of susceptible cultivars such as Hamelin, Baudin and Vlamingh, which have replaced Stirling in high yielding environments of WA .

Current practices of stubble retention and the lack of cultivar resistance to STNB indicated that this disease should be an important breeding target. A successful resistance breeding program depends upon a thorough understanding of the diversity in the pathogen, which requires regular sampling of the population to monitor changes in virulence, distribution and isolate composition. Pathogen studies ensure future success for control with resistant cultivars. Jayasena et al. (2004) identified the teleomorph stage of *P. teres* f. *maculata* from infected stubble collected from South Stirling, WA. The release of susceptible cultivars, wide use of conservation tillage practices and occurrence of the teleomorph stage has increased the possibility of

development of new virulence in the STNB pathogen as a threat to barley production. The objective of this study was to establish the distribution and pathogenic variations in *P. teres* f. *maculata* in WA.

Materials and methods

Collection of P. teres f. maculata isolates

During 2001 and 2002, STNB symptomatic leaf samples were collected from 99 barley crops distributed across all barley growing regions of WA (Fig. 1). The infected leaves from each location were placed in paper envelopes, dried and stored at room temperature (20-25^{o}C).

Single spore isolation and inoculum production

Leaf tissue with STNB lesions was cut into 5-10 mm segments, surface sterilized in 0.5% sodium hypochlorite solution for two minutes and then rinsed twice in sterile deionised water. Leaf segments were blotted dry and aseptically transferred to 2% water agar plates, and incubated at 15-18^{o}C with 12 hours near UV light/12 hours dark. After 3-5 days, where sporulation occurred, a single conidium representing the collection was aseptically transferred to peanut oatmeal agar (POA) medium plates (Speakman and Pommer 1986) and incubated for two weeks to produce a colony. Forty nine single spore isolates were successfully established. These isolates were catalogued and stored in the Department of Agriculture and Food, Western Australia Plant Pathogen Collection (WAC) in Perth as lyophilised mycelia.

Differential barley set

A set of 26 barley lines was used as a differential set to characterise the *P. teres* f. *maculata* population. The lines were selected on the basis of their previous use in Australia (Khan and Tekauz 1982; Platz et al. 2000; Gupta and Loughman 2001; McLean et al. 2010) and elsewhere (Bockelman et al. 1983; Karki and Sharp 1986; Tekauz 1990; Arabi et al. 1992; Steffenson and Webster 1992; Afanasenko et al. 2009) to detect pathogen variation in net blotch studies (Table 1). The differential set also included commercial barley cultivars Baudin, Gairdner, Stirling and Yagan released and grown in WA.

Differential lines were sown in 10 cm diameter plastic pots in clumps of 10 seeds per line and two lines per pot containing pasteurized soil mix (2 parts river sand and 1 part peat moss with nutrients and trace

elements. pH ranging from 6.8-7). The plants were grown in the glasshouse at 18-22^{o}C for two weeks with an average day length of 12 hours, or until the second leaf was fully unfurled.

Inoculum and inoculation of host plants

Conidia were harvested from POA plates by adding sterile distilled water and dislodging these with a rubber spatula. The spore suspension was filtered through gauze and adjusted to 2 x 10^4conidia/ml using a hemocytometer. Two ml of this suspension was applied per pot of 20 seedlings (approximately 2 x 10^3 conidia per plant) using an airbrush sprayer. The plants were placed in a mist chamber at 16-18^{o}C and leaf wetness was maintained for 24 hours. Subsequently, plants were returned to the glasshouse to allow for symptom development. Fifteen isolates were tested in duplicate to determine reproducibility of the infection types produced.

Scoring Infection types

The most common infection types on the second leaves of the barley lines were scored 11 days post-inoculation using the modified scale (G. Platz, unpublished) of Tekauz (1985). Any significant differences among the infection types of the second leaves of the same barley line were also recorded. Plants with infection types of 1-3 were considered resistant, 4-5 as intermediate in reaction and 6-9 as susceptible.

Statistical Analysis

Seedling responses of 26 barley lines to *P. teres* f. *maculata* isolates were analysed using a number of methods. An imputation of missing data (4%) of the Genotype x Isolate (G x I) matrix using the MULTMISSING procedure in GenStat (2009) was done to complete this matrix of means. A hierarchical cluster analysis using the *hclust* function in the "R" (R Development Core Team Australia 2010) program with a complete linkage method was used to cluster the isolates and the genotypes to form a dendrogram. A bi-plot method (singular-value-decomposition function *svd* in R program) based on Gabriel (1971) was used to examine the interaction of the G x I sub-matrix formed from the cluster analysis groupings. The matrix was centred for genotype and isolates groupings. Principal component scores for the first 2 dimensions were plotted for the genotype and isolate vectors. Maps of Western Australia and Australia were done in the R program using the *shapefiles* obtained from GeoScience Australia < http:///www.ga.gov.au>.

Results

The distribution of the STNB isolates indicated that the disease is widely distributed in the barley growing regions of WA (Fig. 1). The variance component analysis of the raw infection scores were estimated to be 1.63 between lines (σ_L^2), 0.28 between isolates (σ_I^2), 0.63 for the interaction between line x isolate (σ_{IL}^2). Fifteen of the 49 isolates were screened in duplicate to gauge the random variation in response of barley lines to isolates, resulting in an estimated value of 0.38 for the replication variation (σr^2). The line main effect, isolate main effect and line x isolate interaction were 56%, 11% and 33% respectively of the total sum-of-squares of the mean disease scores. The G x I mean scores were subjected to the cluster analysis to delineate isolate and line groupings.

P. teres f. maculata isolate classification

Seven isolate groups (IGs) were delineated among the 49 of 99 isolates that sporulated by cluster analysis (Fig. 2). At the level of truncation chosen, a relatively high proportion of the variation was due to differences in isolate means across barley lines.

Based on the isolates that were characterised, the isolates belonging to five IGs (IG1, IG2, IG3, IG4 and IG6) were randomly distributed throughout the barley growing regions of WA (Fig. 1). The single IG5isolate came from the southern coastal region, whereas two isolates of IG7 were from near the northern towns of Moora and Wongan Hills, WA.

The isolate groups (IGs) were composed of 1 to 20 isolates which were relatively homogenous within each group (Table 2, Fig. 2). The nodes of the dendrogram and the seven terminal groups were labelled using the fusion point that they represented in the sequence of classification. In the dendrogram, more similar groups fused earlier.

Barley differential set classification

The 26 barley differential lines had mean infection values that ranged from 1.9 to 7.5 against the 49 *P. teres* f. *maculata* isolates (Table 2). Four line groups (LG1, LG2, LG3 and LG4) were delineated from the cluster analysis based on their responses to the barley lines (Fig. 3). These LGs had 2 to 12 barley lines which

showed differential infection responses on the isolates belonging to different IGs. The LG1 barley lines Baudin and Stirling showed lower (more resistant) responses to isolates in IG1 and IG6, intermediate responses to IG2 and IG4, and higher responses on IG3, IG5 and IG7, thus depicting genetic variation in the line x isolate interactions. The 12 lines of LG2 showed a narrow range of mean values (3.8 to 5.6) against all IGs. Seven lines of LG3 showed lower general mean values (1.9 to 4.0) against all IGs and were thus regarded as mostly resistant. The five lines of LG4 showed intermediate responses against IG2 and IG5, but had susceptible mean values within the narrow range of 6.0 to 6.7 to the remainder of IGs.

The mean values of isolates belonging to IG1 followed a similar response pattern but showed somewhat higher mean infection values compared to isolates of IG6 against all barley lines grouped into the four LGs (Table 2).

Barley x Isolate groupings

The bi-plot which explained 96% (PC1 79% + PC2 17%) of the total variation of the centered LG x IG data from Table 2, revealed that the IG1 and IG6 isolate groups are represented in the same quarter, indicating less variation compared to the other IGs (Fig. 4). Similarly, IG3, IG5 and IG7 had similar mean values, but showed high mean values against Baudin and Stirling (LG1) compared to IG1 and IG6. The higher responses of IG3, IG5 and IG7 on LG1 as compared to those of IG1 and IG6, resulted in these IGs being plotted in the opposite quarter of the biplot.

Isolates belonging to IG2 and IG4 had intermediate responses against barley lines in LG1 as compared to the lower mean response of IG1 and IG6, but higher mean responses than IG3, IG5 and IG7. This resulted in these IGs appearing in the lower half of the bi-plot, showing their distinguishing feature. Further, the mean values of IG4 were higher on line groups LG2, LG3 and LG4 compared to those of IG2 and thus appeared in the different quarters of the lower half of the bi-plot. The principal components 1 and 2 analysis indicated that isolate groups IG2, IG3, IG5 and IG7 can be considered as one larger cluster, IG4 a second, and IG1 and IG6 the third.

Similarly, using the principal components 1 and 2 analysis, the line groups LG2, LG3 and LG4 which showed a narrow range of responses to *P. teres* f. *maculata* isolates were plotted on the left side of the bi-plot, whereas LG1 which showed a broader range of responses appeared on the right side (Fig. 4).

Discussion

STNB was first reported in Australia in the northern agricultural areas of Western Australia in 1977 (Khan and Tekauz 1982). Later, Gupta and Loughman (2001) reported the first southern occurrence of this disease in agricultural regions of WA, but observed that the distribution of the pathogen was limited relative to NTNB. The yield loss and disease control studies of Jayasena et al. (2002, 2007) indicate that *P. teres* f. *maculata* is currently a widespread pathogen of significance to the barley industry in WA. This is due to stubble retention and cultivation of susceptible cultivars in WA. The current study reveals a wide distribution of the pathogen throughout agricultural regions of WA, indicating the growing importance of the disease in the region in the past two decades. Estimates of the negative impact of STNB indicate that this is also an important pathogen in other barley growing regions of Australia, resulting in *P. teres* f. *maculata* being considered among the three most important barley pathogens in Australia (Murray and Brennan 2010).

The previous identification of variation in virulence of STNB in a small sample of the pathogen population (Gupta and Loughman 2001), higher yield losses in susceptible barley cultivars (Jayasena et al. 2002), and the identification of the pathogen teleomorph (Jayasena et al. 2004) warranted the current study to understand the genetic variation in the pathogen, prior to defining resistance targets for breeding resistant barley.

Among the 49 geographically dispersed *P. teres* f. *maculata* isolates, variation in virulence was identified throughout the survey region of WA. The varied reaction of barley lines to infection with this range of isolates arose from both genetic and random effects. When the size of these components were estimated from a subset of 15 isolates, the component of variance for the isolate x line interaction was larger than the error variance one . Certain barley lines gave very distinctive responses to different *P. teres* f. *maculata* isolates. In other instances the degree of variation in barley line response among isolates was narrow and similar to the variation induced by random effects. We have concluded that the majority of pathogen variation represented in this pathogen isolate collection exists as three broad isolate virulence clusters, as identified by

their pattern of responses to four distinguishing LGs (Fig. 4). These results concur with, and expand upon the previously reported variation in virulence of *P. teres* f. *maculata* on barley line Herta, detected in a broader study of overall *P. teres* virulence (Gupta and Loughman 2001). Since half the isolates could not be characterised and the survey reported in this study was undertaken nearly 10 years ago, further sampling of the *P. teres* f. *maculata* isolates may indicate that the distribution of some of the IGs (IG5 and IG7) may be broader (or different) than reported here. Due to the lack of standard differential set or non-availability of near-isogenic lines, the selection of barley differential lines used in this study was based on previous studies in Australia and overseas (Khan and Tekauz 1982; Bockelman et al. 1983; Karbi and Sharp 1986; Tekauz 1990; Arabi et al. 1992; Gupta and Loughman 2001; McLean et al. 2010). Although, the composition of the differential barley set was not the same in the various studies, but this provides a basis for some comparison to study the variation in pathogen virulences reported in WA and elsewhere. For example, a collection of 42 Canadian *P. teres* f. *maculata* isolates were classified into seven pathotype groups using 11 barley differentials (Tekauz 1990). This represents a breadth of virulence diversity comparable to the current situation in WA. By contrast, only two broadly avirulent isolates (03-0022 and 03-0089) among a collection of 44 Victorian isolates tested on 21 barley lines were identified (McLean et al. 2010). Fifteen out of 26 barley lines from the present study were also used in the study from Victoria. . The pathogen population from Victoria induced predominantly susceptible response on lines Betzes and Herta, whereas the WA pathogen population induced mainly resistant responses on Betzes, and somewhat more susceptible responses on Herta. These responses indicate different virulence spectra between Victoria and WA isolates of *P. teres* f. *maculata*, which appear to be genetically distinct, likely because of the geographic separation between eastern and western barley growing regions of Australia.

Based on the common responses and historical usage, two lines from each line group can characterise *P. teres* f. *maculata* isolates in WA (Table 3). The reduced differential set can also be used in the similar studies elsewhere. Baudin and Stirling, classified in LG1, were selected as they are commercially-grown WA cultivars and showed variable response to the isolates in the study. Herta and Beecher were selected to represent the 12 lines of LG2 on the basis of their previous virulence studies in WA. Apart from spot type isolates, Beecher also distinguishes net type virulences present in WA (Gupta and Loughman 2001). CI9214 was mostly resistant and had been genetically characterised (Williams et al. 2003) whereas Yagan also remained resistant and is a feed cultivar grown in WA. Cape and Coast are commonly used susceptible spot type checks in WA. Coast is resistant to net type isolates and thus distinguishes from spot type ones. Characterisation of barley

lines would greatly assist to determine the pathogen variation against known resistance genes. One or more STNB seedling and adult plant resistance genes have been identified on chromosomes 3H, 4H, 5H, 6H and 7H in barley lines Chebec, CI 9214, CI 9819, CI 9831, Galleon, Keel, Q21861, Tilga, TR 250, TR 251 and VB 9104 (Molnar et al. 2000; Williams et al. 2003; Friesen et al. 2006; Gupta et al. 2006; Manninen et al. 2006; Grewal et al. 2007). Barley lines, Betzes, Golf, Harbin, Manchuria, Rojo and Yagan from LG3, which were resistant to most of the isolates in this study, may also be useful sources of resistance. These resistant barley lines can be further characterised to find new resistance genes compared to the ones already known, which can assist in detection of STNB virulence against known resistance genes.

Baudin and Stirling (group LG1), which are widely grown cultivars in WA, are susceptible to STNB as adult plants in commercial production, but showed variable reactions to *P. teres* f. *maculata* isolates of different IGs at the seedling stage . Differential responses at the seedling and adult stages were also observed by Williams et al. (2003) in barley genotypes Chebec, Tilga and WI3141. Seedling resistance in the cultivar susceptible at adult stage is useful for early crop establishment for its commercial production.

In conclusion, the virulence spectrum of *P. teres* f. *maculata* was found to be more geographically dispersed in WA than previously reported. The greater variability and new virulence found in *P. teres* f. *maculata* in this study needs to be considered in barley breeding programs targeting resistance as the optimal disease management strategy. This could best be achieved by pyramiding diverse seedling and adult plant resistance genes to counter the current threat posed by STNB in WA and elsewhere in Australia.

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Table 1. Twenty six barley differential lines used to determine pathogen variation of *Pyrenophora teres* f. *maculata* isolates from Western Australia

Barley line	Reference	Source
Baudin	Cultivar, Western Australia	DAFWA*
Beecher	Khan and Tekauz (1982), Steffenson and Webster (1992), Platz *et al.* (2000), Gupta and Loughman (2001), Afanasenko et al. (2009), McLean *et al*.(2010)	DAFWA
Betzes	Tekauz (1990), Gupta and Loughman (2001), McLean *et al*. (2010)	DEEDI
Cape	Steffenson and Webster (1992), Platz *et al*. (2000), McLean *et al*. (2010)	DEEDI
CI 11458	Steffenson and Webster (1992), Platz *et al*. (2000)	DEEDI
CI 5822	Steffenson and Webster (1992)	DEEDI
CI 7584	Khan and Tekauz (1982), Bockelman *et al*. (1983), Karki and Sharp (1986), Steffenson and Webster (1992), McLean *et al*.(2010)	DEEDI
CI 9214	Karki and Sharp (1986), Tekauz (1990), Gupta and Loughman (2001), Afanasenko *et al*. (2009), McLean *et al.* (2010)	DEEDI
CI 9819	Bockelman *et al*.(1983), Steffenson and Webster (1992), Afanasenko *et al*. (2009)	DEEDI
Coast	Steffenson and Webster (1992), McLean *et al.* (2010)	DEEDI
Dampier	Khan and Tekauz (1982), McLean *et al*. (2010)	DAFWA
Gairdner	Cultivar, Western Australia	DAFWA
Gilbert	Platz *et al*. (2000), Gupta and Loughman (2001), McLean *et al*. (2010)	DEEDI
Golf	Arabi *et al*. (1992), Platz *et al*. (2000), Gupta and Loughman (2001)	DEEDI
Grimmett	Platz *et al*. (2000), Gupta and Loughman (2001), McLean *et al*. (2010)	DEEDI
Harbin	Steffenson and Webster (1992), Gupta and Loughman (2001), Afanasenko *et al*. (2009)	DEEDI
Hazera	Platz *et al*. (2000), Gupta and Loughman (2001), McLean *et al*. (2010)	DEEDI
Herta	Tekauz (1990), Platz *et al*. (2000), Gupta and Loughman (2001), McLean *et al*. (2010)	DEEDI
Kombar	Steffenson and Webster (1992), Platz *et al*. (2000), Gupta and Loughman (2001), McLean *et al*. (2010)	DEEDI
Manchuria	Steffenson and Webster (1992)	DEEDI
Prior	Platz *et al*. (2000), Afanasenko *et al*. (2009), McLean *et al*. (2010)	DEEDI
Rika	Steffenson and Webster (1992), Platz *et al.* (2000), Gupta and Loughman (2001), McLean *et al.* (2010)	DEEDI
Rojo	Steffenson and Webster (1992)	DEEDI
Skiff	Platz *et al.* (2000), Afanasenko *et al*. (2009), McLean *et al*. (2010)	DEEDI
Stirling	Cultivar, Western Australia	DAFWA
Yagan	Cultivar, Western Australia	DAFWA

*DAFWA, Department of Agriculture and Food Western Australia; DEEDI, Department of Employment, Economic Development and Innovation.

Table 2. Mean responses of 26 barley lines classified into four line groups (LGs) against 49 isolates of *Pyrenophora teres* f. *maculata* classified into seven isolate groups (IGs).

	Isolate group						
	IG1 (20*)	**IG2 (6)**	**IG3 (10)**	**IG4 (6)**	**IG5 (1)**	**IG6 (4)**	**IG7 (2)**
Line group	11016**, 11039, 11041, 11151, 11156, 11159, 11163, 11169, 11170, 11173, 11177, 11178, 11179, 11184, 11188, 11189, 11195, 11199, 11202, 11203	11040, 11046, 11158, 11180, 11181, 11198	11042, 11043, 11044, 11045, 11047, 11050, 11052, 11053, 11058, 11152	11049, 11051, 11056, 11153, 11160, 11183	11182	11190, 11200, 11201, 11214	11209, 11212
LG1 (2*) Baudin, Stirling	3.2 (0.10)	5.4 (0.34)	6.4 (0.14)	5.0 (0.37)	7.0 (0.00)	1.9 (0.13)	7.5 (0.29)
LG2 (12) Beecher, CI 11458, CI 5822, CI 7584, Dampier, Gilbert, Grimmett, Hazera, Herta, Prior, Rika, Skiff	5.2 (0.05)	3.8 (0.14)	4.8 (0.09)	5.6 (0.12)	3.8 (0.51)	4.4 (0.18)	4.6 (0.28)
LG3 (7) Betzes, CI 9214, Golf, Harbin, Manchuria, Rojo, Yagan	3.0 (0.07)	2.0 (0.13)	2.6 (0.10)	4.0 (0.20)	1.9 (0.26)	1.9 (0.15)	2.6 (0.32)
LG4 (5) Cape, CI 9819, Coast, Gairdner, Kombar	6.7 (0.05)	4.9 (0.23)	6.0 (0.12)	6.3 (0.18)	5.0 (0.55)	6.3 (0.14)	6.4 (0.31)

*Number of isolates and lines; **DAFWA reference collection number; Values in parenthesis are the standard error for each mean value.

Table 3. Seedling response of the reduced differential set of barley lines which distinguishes isolate groups (IGs) representing 49 *Pyrenophora teres* f. *maculata* isolates.

	Barley line			
Isolate group	Baudin/Stirling	Beecher/Herta	CI9214/Yagan	Cape/Coast
IG1/IG6	R – I	I	R	S
IG2	I	I	R	I
IG3/IG7	S	I	R	S
IG4	I	I – S	I	S
IG5	S	R – I	R	I

R = Resistant (infection response 3 or less); I = Intermediate (infection response between >3 and < 6); S = Susceptible (infection response 6 or higher).

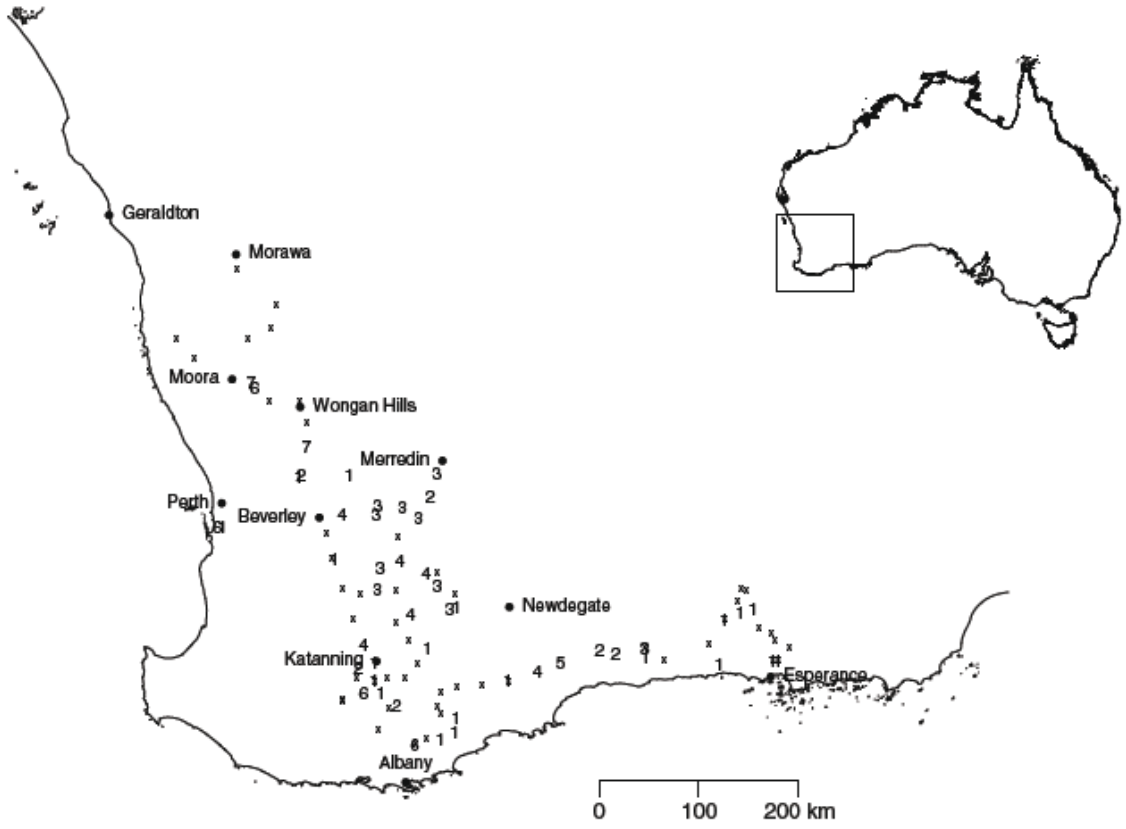

Fig. 1. Distribution of 99 isolates of *Pyrenophora teres* f. *maculata* obtained from barley crops in Western Australia. x = Isolates with no sporulation. The numerals 1 to 7 depict the seven isolate groups (IGs) delineated by cluster analysis from the 49 isolates that sporulated.

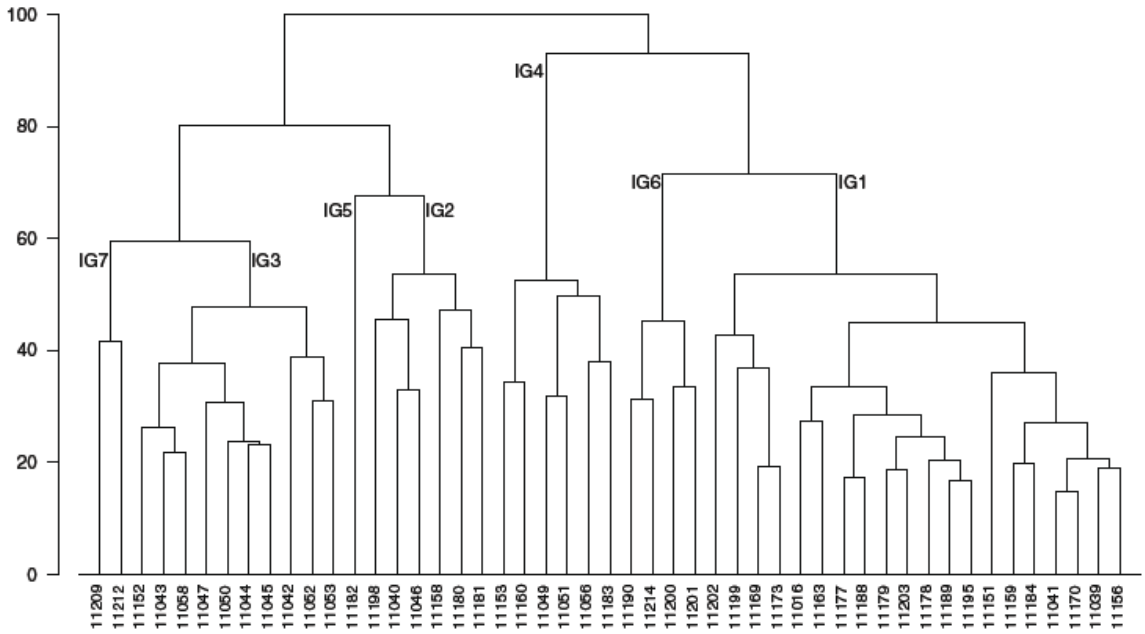

Fig. 2. Dendrogram delineated by cluster analysis. IG1 to IG7 identify the isolate groupings among 49 isolates of *Pyrenophora teres* f. *maculata.* The Euclidean distance, shown as the percentage, represents the measure of similarity.

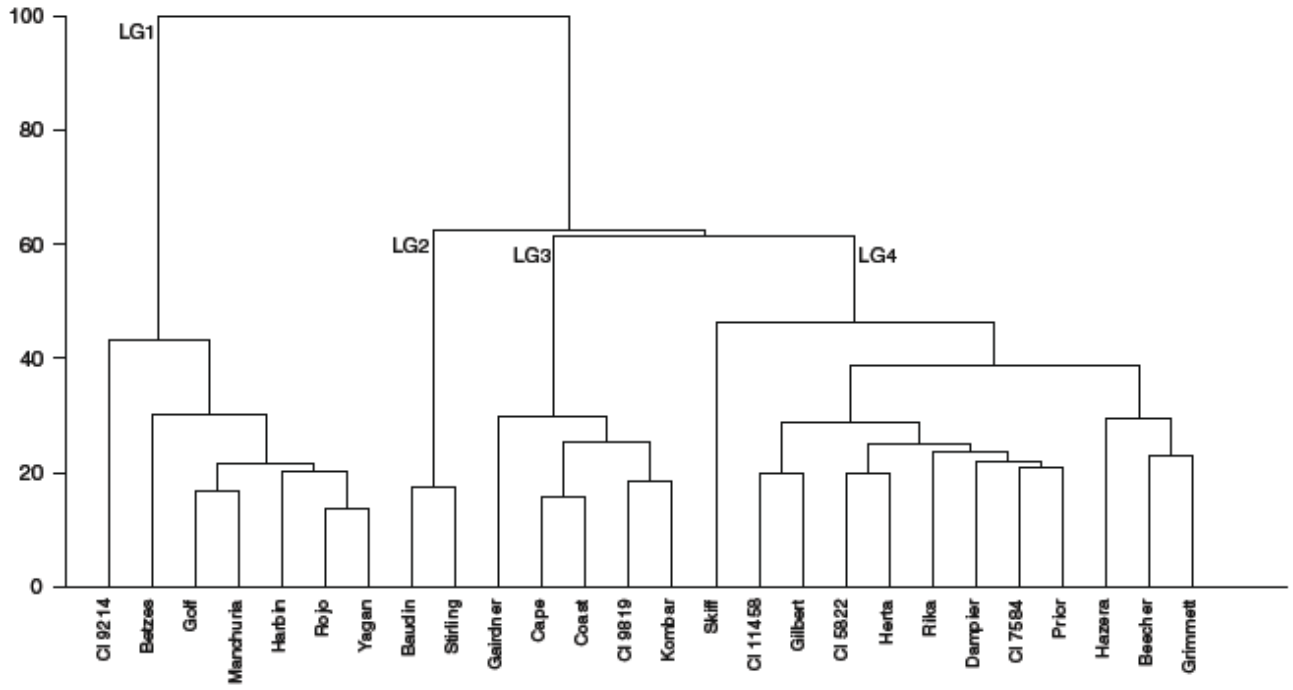

Fig. 3. Dendrogram delineated by cluster analysis. LG1 to LG4 identify the line groupings among 26 barley lines. The Euclidean distance, shown as a percentage, represents the measure of similarity.

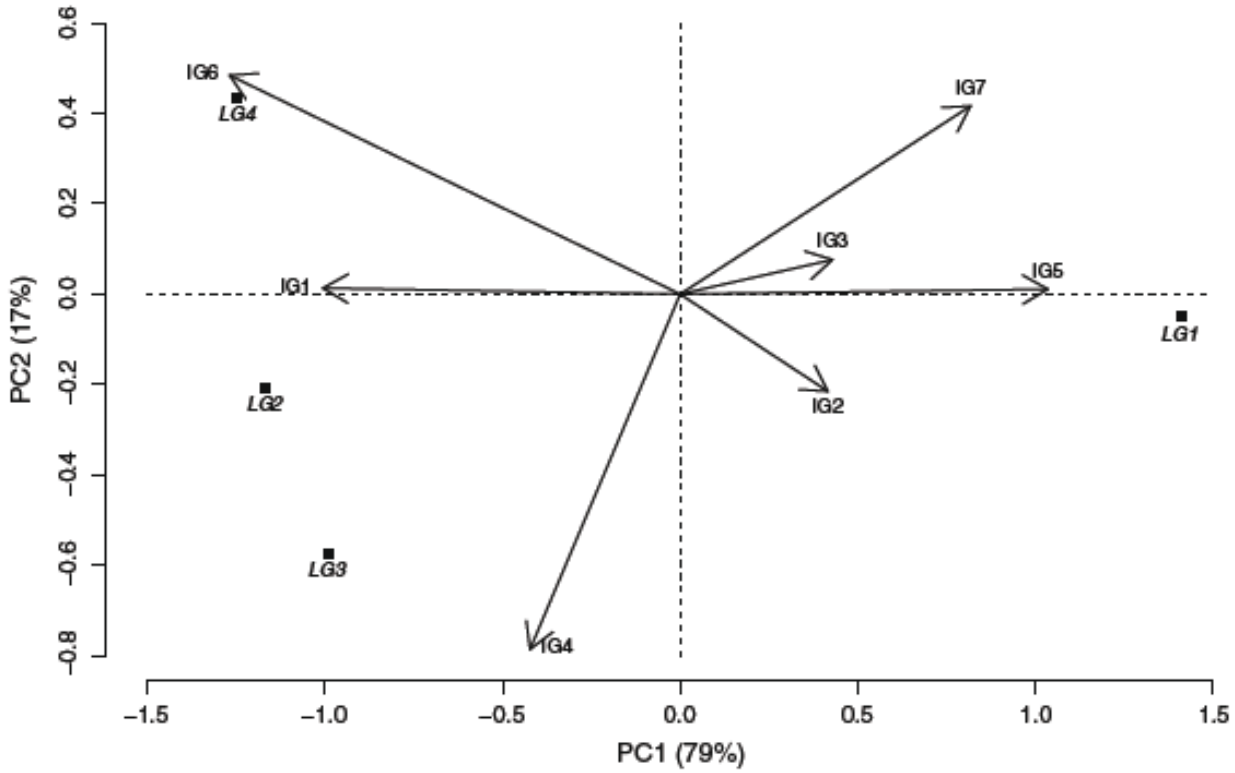

Fig. 4. Bi-plot of the LG x IG matrix showing the *Pyrenophora teres* f. *maculata* Isolate Group vectors with the barley Line Group vectors for the 2 major principal components (PC1 and PC2) with the percentage of variance accounted for by each of the components.