



British Mycological
Society promoting fungal science

journal homepage: www.elsevier.com/locate/funbio



Genetic analysis of growth, morphology and pathogenicity in the F₁ progeny of an interspecific cross between *Fusarium circinatum* and *Fusarium subglutinans*

Lieschen DE VOS*, Magriet A. VAN DER NEST, Nicolaas A. VAN DER MERWE, Alexander A. MYBURG, Michael J. WINGFIELD, Brenda D. WINGFIELD

Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Lunnon Road, Hillcrest, Pretoria 0001, South Africa

ARTICLE INFO

Article history:

Received 5 April 2011

Received in revised form

4 July 2011

Accepted 6 July 2011

Available online 19 July 2011

Corresponding Editor: Michael Lorenz

Keywords:

Fusarium circinatum

Fusarium subglutinans

Interspecific cross

Mycelial growth

Pathogenicity

QTLs

ABSTRACT

Fusarium circinatum and *Fusarium subglutinans* are two distinct species in the *Gibberella fujikuroi* species complex. A genetic linkage map produced from an interspecific cross between these species was used to identify quantitative trait loci (QTLs) associated with variation in mycelial growth and morphology of colony margins (CMs) in the 94 F₁ progeny. Mycelial growth was assessed by measuring culture size at 25 °C and 30 °C, while CM morphology was characterized in the parents and assessed in their F₁ progeny. In order to test the pathogenicity of the progeny, *Pinus patula* seedlings were inoculated and lesion lengths were measured after 3 weeks. Seven putative QTLs were associated with mycelial growth, three for growth at 25 °C and four at 30 °C. One highly significant QTL ($P < 0.001$) was present at both growth temperatures. For CM morphology, a QTL was identified at the same position ($P < 0.001$) as the QTL responsible for growth at the two temperatures. The putative QTLs accounted for 45 and 41 % of the total mycelial growth variation at 25 °C and 30 °C, respectively, and for 21 % of the variation in CM morphology. Only one of the 94 F₁ progeny was pathogenic on *P. patula* seedlings. This observation could be explained by the genetic constitution of this F₁ isolate, namely that ~96 % of its genome originated from the *F. circinatum* parent. This F₁ individual also grew significantly faster at 25 °C than the *F. circinatum* parent ($P < 0.05$), as well as more rapidly than the average growth for the remaining 93 F₁ progeny ($P < 0.05$). However, no association was found between mycelial growth and pathogenicity at 25 °C. The highly significant QTL associated with growth at two temperatures, suggests that this is a principal genomic region involved in mycelial growth at both temperatures, and that the same region is also responsible for CM morphology.

© 2011 British Mycological Society. Published by Elsevier Ltd. All rights reserved.

Introduction

Fusarium circinatum and *Fusarium subglutinans* are distinct fungal taxa that reside in the *Gibberella fujikuroi* species complex (Nelson *et al.* 1983; Nirenberg & O'Donnell 1998). This complex

includes economically important pathogens of crops and trees. Based on the biological species concept, *F. circinatum* resides in mating population H (Nirenberg & O'Donnell 1998; Britz *et al.* 1999) and *F. subglutinans* in mating population E (Nelson *et al.* 1983).

* Corresponding author. Tel.: +27 12 420 3948; fax: +27 12 420 3947.

E-mail addresses: lbahlman@fabi.up.ac.za, magriet.vandernest@fabi.up.ac.za, albe.vdmerwe@fabi.up.ac.za, zander.myburg@fabi.up.ac.za, mike.wingfield@fabi.up.ac.za, brenda.wingfield@fabi.up.ac.za

1878-6146/\$ – see front matter © 2011 British Mycological Society. Published by Elsevier Ltd. All rights reserved.

doi:10.1016/j.funbio.2011.07.003

In a study of *F. subglutinans* isolates from maize and teosinte, one strain of *F. subglutinans* isolated from teosinte was moderately interfertile with a strain of the pine pitch canker pathogen, *F. circinatum* (Desjardins *et al.* 2000). This interspecific cross was the basis for a study by De Vos *et al.* (2007), who used genetic linkage mapping to study the genetic differentiation of the two parental genomes. That study placed 248 AFLP markers and two gene-based markers (the mating type idiomorphs (MAT) and the histone (H3) gene) onto a genetic linkage map that was organized into 12 major linkage groups. Of these markers, 55 % showed significant transmission ratio distortion from the expected 1:1 transmission ratio of a haploid cross ($P < 0.05$). All but 12 favoured alleles of the *F. subglutinans* parent. This unusually high percentage of markers displaying transmission ratio distortion could be attributed to various factors. One is the presence of linkage between markers and distorting genetic factor(s), which could affect the fitness of gametes leading to a biased transmission of parental alleles to the next generation (Zamir & Tadmor 1986). Another contributing factor could be an association between the genetic divergence of the parental isolates and the levels of transmission ratio distortion (Paterson *et al.* 1991; Grandillo & Tanksley 1996). In this regard, interspecific crosses, such as those treated in this study, tend to display higher levels of segregation distortion.

Friel *et al.* (2007) also made an interspecific cross between the same parental isolates of *F. circinatum* and *F. subglutinans* (De Vos *et al.* 2007) and showed that the MAT idiomorphs displayed no transmission ratio distortion, while this was present in the H3 gene. These results were consistent with those of De Vos *et al.* (2007) using the gene-based markers. Also, none of the F₁ progeny displayed pathogenicity on *Pinus radiata* and it was hypothesized that this could be due to a very low probability of finding viable F₁ progeny with all the *F. circinatum* genes necessary for pathogenicity to pines (Friel *et al.* 2007). The bias against the genome of *F. circinatum* suggests a general fitness benefit for F₁ progeny that have inherited *F. subglutinans* alleles (De Vos *et al.* 2007).

In the Basidiomycete *Heterobasidion annosum* species complex, hybrid progeny placed on a substrate favouring only one parent were less competitive than this parental strain (Garbelotto *et al.* 2007). When inoculated onto a substrate that is favourable to both parents, the hybrid progeny were as fit as the parental genotypes. The fact that none of the F₁ progeny from a cross between *F. circinatum* and *F. subglutinans* displayed pathogenicity to pines (Friel *et al.* 2007) suggests that this was indicative of the effect that the substrate has on the fitness of fungal hybrids. Thus, *P. radiata* would represent an unfavourable substrate for the F₁ progeny of the cross between *F. subglutinans* that occurs on maize, and *F. circinatum*, a pathogen of pines. In the present study, we considered mycelial growth on agar, representing a substrate that is favourable to both parents. Mycelial growth in *Fusarium* spp. has been hypothesized to be correlated with isolate pathogenicity (Doohan *et al.* 2003) where rapid growth is usually associated with high levels of pathogenicity. To test this hypothesis, we also investigated whether there might be an association between pathogenicity and fitness typified by mycelial growth at 25 °C.

The genetic linkage map of the *F. circinatum* × *F. subglutinans* interspecific cross can be used to identify quantitative

trait loci (QTLs) for any quantitative traits that are polymorphic in the F₁ progeny. So far, only one study has reported on the mapping of QTLs in the genus *Fusarium*, where QTLs for pathogenicity and aggressiveness of *Fusarium graminearum* towards wheat was mapped (Cumagun *et al.* 2004). In the present study we mapped regions of the genome involved in the expression of morphological traits such as mycelial growth and colony margin (CM) morphology. Mycelial growth was studied at two different temperatures, to consider differences in mycelial growths between the two parental species. In addition, this would make it possible to determine whether individual genes or a combination of genes are involved in the variation in growth observed in the F₁ population, and to determine the genomic origin of these QTLs. Furthermore, pathogenicity in the F₁ progeny of the interspecific cross was considered in order to verify whether there was a bias against the *F. circinatum* genome, as reported by Friel *et al.* (2007).

Materials and methods

Fungal isolates and mycelial growth studies

Isolates used in this study included the parents of an interspecific cross between *Fusarium circinatum* and *Fusarium subglutinans* (Desjardins *et al.* 2000) and 94 isolates from the F₁ progeny. The progeny of this cross represented the same isolates used for genetic linkage analysis in De Vos *et al.* (2007). All isolates were grown on half strength Potato dextrose agar (PDA; 20 % w/v PDA and 5 % w/v agar). For mycelial growth and CM studies, a mycelial plug was removed from the edge of an actively growing culture for each isolate, and placed at the centre of a Petri plate (90 mm in diameter).

The two parental isolates were tested for growth at a range of temperatures, from 10 °C to 35 °C at 5 °C intervals, with five replicate plates for each isolate. After incubation in the dark for 7 d, mycelial growth was measured along two perpendicular axes of the colonies at right angles to each other. Two growth temperatures, 25 °C and 30 °C, were identified at which the parental isolates displayed differential mycelial growth. Thereafter, growth was assessed at these temperatures for the 94 F₁ progeny with five replicate plates per isolate. In addition CM morphology was characterized for the parental isolates as well as for the 94 F₁ progeny when grown at 25 °C for 7 d in the dark. The two parents had an observable difference in CM morphology. *Fusarium circinatum* had a smooth colony edge and *F. subglutinans* had an irregular (lacinate) edge (Fig 1A and B). The progeny displayed either of these, or an intermediate morphotype (crenate) (Fig 1C). The phenotypes were scored as '1' for smooth, '2' for crenate, and '3' for lacinate.

Pathogenicity studies

All 94 F₁ isolates were grown on 1/2 PDA for 7 d at 25 °C in the dark. Spores were washed from the cultures with 15 % (v/v) glycerol. Spore concentration was determined using a haemocytometer and adjusted to 5×10^4 spores ml⁻¹ for each isolate, using sterile distilled water.

Six-month-old *Pinus patula* seedlings were wounded by removing the growth tips and wounds were inoculated with a 10 µl

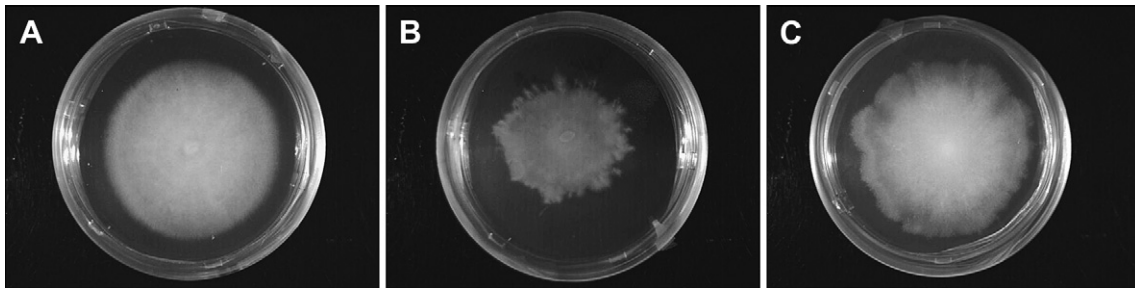


Fig 1 – CM morphology at 25 °C after 7 d of growth in the dark. (A) *F. circinatum* displays a smooth edge. (B) *F. subglutinans* displays an irregular (lacinate) edge. (C) Example of F_1 isolate that showed an intermediate (crenate) edge morphology to (A) and (B).

drop of spore suspension. In order to minimize the effect of genetically variable seedlings on the pathogenicity of the fungus, 10 biological replicates were used for each isolate. Ten seedlings were inoculated with sterile distilled water to serve as a negative control. Inoculated seedlings were allowed to grow in the greenhouse at 25 °C for 3 weeks, after which lesion lengths were measured from the point of inoculation along the seedling stem.

Statistical analyses

Statistical analyses were performed using Statistica V8.0 (StatSoft, Inc.). The frequency distribution was determined and analysis of variance (ANOVA) performed for mycelial growth at 25 °C and 30 °C. Individual observed broad sense heritability (H^2), i.e. the proportion of genotypic to phenotypic variance ($H^2 = \sigma_G^2 / \sigma_P^2$), was calculated for the *in vitro* studies. To determine whether there were significant differences ($P < 0.05$) in mycelial growth of parental isolates and the 94 F_1 progeny, t-tests were performed.

QTL detection

Map Manager QTXb15 V0.25 (Manly et al. 2001) was used to identify markers linked to mycelial growth at two temperatures and to the CM morphology. The ‘Hide redundant loci’ option was chosen to remove markers that were associated with identical genotypes (duplicate markers) as well as those closer than 10 cM, in order to minimize interference due to background segregation of these markers. The cross type was selected as ‘Arbitrary cross’ as this option allows for the most accurate analysis of haploid data. De Vos et al. (2007) included markers (55 %) that displayed transmission ratio distortion ($P < 0.05$) in their map. Therefore, to allow Map Manager QTX to analyze data containing markers showing transmission ratio distortion, the ‘Allow for segregation distortion’ function was chosen.

A permutation test (1000 permutations at 1 cM intervals using the additive model) was performed to empirically determine the experiment-wise significance levels for significant ($\alpha = 0.05$) and highly significant ($\alpha = 0.001$) QTLs. Analyses of QTLs for mycelial growth and CM morphology suggested that log-of-the-odds (LOD) values of 2.96–4.57 were significant ($\alpha = 0.05$) and those higher than 4.57 were highly significant ($\alpha = 0.001$) at 25 °C. Similarly, LOD values of 3.07–4.54 were

significant and those higher than 4.54 were highly significant for QTLs at 30 °C. For CM morphology, LOD values of 3.07–4.72 were significant and values above 4.72 were highly significant. Simple interval mapping (SIM) was used to test for the presence of a putative QTL every 1 cM throughout the genetic linkage map. Composite interval mapping (CIM) was performed to control for the effect of background segregating QTLs, at the location of target QTLs. The marker from each linkage group with the highest association to a QTL was added as a background locus and mapping was performed to more precisely establish the interval position of the target QTL (Manly & Olson 1999). QTLs were recorded when the SIM and CIM likelihood ratio (LR) values were equal to, or greater than, the experiment-wise significance levels for significant and highly significant QTLs. LR values were converted to LOD values by using $LR = 4.6 \times LOD$ (Liu 1998). Epistatic interactions were detected using the ‘Interactions’ option.

Results

Mycelial growth studies and statistical analyses

The parental isolates, *Fusarium circinatum* and *Fusarium subglutinans*, have different hosts (*Pinus* spp. and teosinte, respectively). Therefore mycelial growth at a range of temperatures was expected to be different for the two species (Fig 2). In comparison to *F. circinatum*, *F. subglutinans* showed a greater range of temperatures at which it could grow. At 25 °C, *F. circinatum* grew significantly faster than *F. subglutinans* ($P = 1.08 \times 10^{-6}$) (Table 1). The opposite was true at 30 °C ($P = 3.58 \times 10^{-6}$), with *F. circinatum* growing significantly more slowly than *F. subglutinans* (Table 1). These two temperatures were thus selected for mycelial growth studies of the F_1 progeny of a cross between *F. circinatum* and *F. subglutinans*.

The average mycelial growth of the 94 F_1 progeny was significantly less than growth for *F. circinatum* ($P = 0.0088$), but not for *F. subglutinans* ($P = 0.46$), at 25 °C (Table 1). The average mycelial growth of the 94 F_1 progeny at 30 °C was not significantly different to that of *F. circinatum* ($P = 0.19$), but was different to that of *F. subglutinans* ($P = 0.015$) (Table 1). Mycelial growth at 25 °C was normally distributed ($P = 0.033$) and the broad sense heritability was 0.98. Similarly, at 30 °C the frequency distribution was normal ($P = 0.0497$) and the broad sense heritability was 0.99.

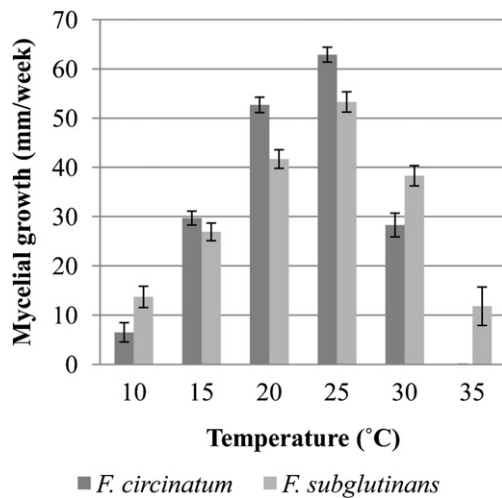


Fig 2 – Measurements of the mycelial growth of *F. circinatum* and *F. subglutinans* (five replicates) at a range of temperatures for 7 d in the dark on 1/2 PDA. Error bars represent the standard deviation.

CM morphology of the progeny displayed either of the two parental species phenotypes, or an intermediate phenotype. Of the 94 F₁ progeny, 36.17 % had smooth, 41.49 % had lacinate and 22.34 % had an intermediate (crenate) CM morphology. This was normally distributed ($P = 0.00$).

Pathogenicity

Inoculation of pine seedlings with the 94 F₁ isolates revealed that only one isolate (FCC 2025; *Fusarium* Culture Collection, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa) was pathogenic (11.80 ± 2.39 mm). The other 93 F₁ progeny were not significantly different from the *Fusarium subglutinans* parental isolate or from the negative control (distilled water) ($P > 0.05$; results not shown). Therefore the lesion length data did not display a continuous distribution.

The pathogenic F₁ individual grew significantly faster *in vitro* at 25 °C in comparison to the two parental isolates. Growth in this isolate (66.90 ± 2.28 mm) was also more rapid

Table 1 – Average mycelial growth of the parental isolates, 94 F₁ progeny and the F₁ isolate FCC 2025 at 25 °C and 30 °C for 7 d in the dark on 1/2 PDA.

Isolate	25 °C ^a	30 °C ^b
<i>F. circinatum</i>	62.90 ± 1.52 (a)	28.30 ± 2.41 (a)
<i>F. subglutinans</i>	53.30 ± 2.06 (b)	38.30 ± 2.06 (b)
94 F ₁ progeny ^c	53.66 ± 12.28 (b)	31.22 ± 10.23 (a)

a *In vitro* mycelial growth at 25 °C measured in mm/week. Numbers followed by different letters in the same column are significantly different at $P = 0.05$.

b *In vitro* mycelial growth at 30 °C measured in mm/week. Numbers followed by different letters in the same column are significantly different at $P = 0.05$.

c Mycelial growth as measured for the mean of the 94 F₁ progeny.

than the average growth for the remaining 93 F₁ progeny ($P < 0.05$). However, no association was found between mycelial growth and pathogenicity at 25 °C as there were instances of other F₁ individuals that also grew significantly faster than the pathogenic F₁ at 25 °C, yet were not pathogenic.

QTL detection

Three QTLs were detected for mycelial growth at 25 °C (Fig 3A and B), four at 30 °C (Fig 3A and C), and only one for CM morphology (Fig 3A). Only one QTL, namely the one for mycelial growth at 30 °C that lies nearest to marker AA/TC-121bh, displayed transmission ratio distortion ($\chi^2 = 12.30$, $P = 0.00048$). No epistatic interactions were detected in mycelial growth, suggesting that these loci act independently. One QTL appeared in all three mapped traits, namely AT/AC-625bh on linkage group 2 at position 231 cM (Fig 3A). This QTL was highly significant ($P < 0.001$) in all three cases.

For mycelial growth at 25 °C, the QTLs were located on two linkage groups (LG 2 and 12), and accounted for 45 % of the total phenotypic variance (Table 2). Four QTLs were identified for mycelial growth at 30 °C. Three of these QTLs spanned LG1, and the fourth QTL was on LG2. Together, they accounted for 41 % of the total trait variance. The QTL on LG2 was shared, indicating that for mycelial growth at 25 °C and 30 °C, this QTL is the only common factor. Only one QTL was identified for CM morphology. This QTL was present at the same location as the shared QTL for mycelial growth at 25 °C and 30 °C. This QTL accounted for 21 % of the total phenotypic variance.

Discussion

The interspecific cross between *Fusarium circinatum* and *Fusarium subglutinans* (Desjardins *et al.* 2000), and the genetic linkage map derived from it (De Vos *et al.* 2007), provided a unique opportunity to determine the genetic basis of mycelial growth and pathogenicity of the F₁ progeny. Our results showed that only one isolate from 94 F₁ progeny was pathogenic, and that this result could be explained by the genetic constitution of this particular isolate. Mycelial growth was investigated at two temperatures (25 °C and 30 °C) and an area of the genome was found that was associated with variation in mycelial growth at both of these temperatures. One highly significant QTL ($P < 0.001$) was present at both growth temperatures as well as for CM morphology. This suggests that this QTL is involved in mycelial growth at both temperatures and that the same region is also involved in CM morphology. Furthermore, there was no association between mycelial growth and pathogenicity at 25 °C. This study is only the second after the study of *Fusarium graminearum* to genetically map QTLs in the genus *Fusarium* (Cumagun *et al.* 2004).

The fact that *F. circinatum* grew significantly faster at 25 °C than *F. subglutinans* and the opposite was observed for growth at 30 °C provided a useful basis for comparison of growth at these two temperatures. The high heritability (0.98 and 0.99 at 25 °C and 30 °C, respectively) gave an indication of the low environmental variation of mycelial growth in Petri dishes. Similar heritability values have been observed in growth studies with

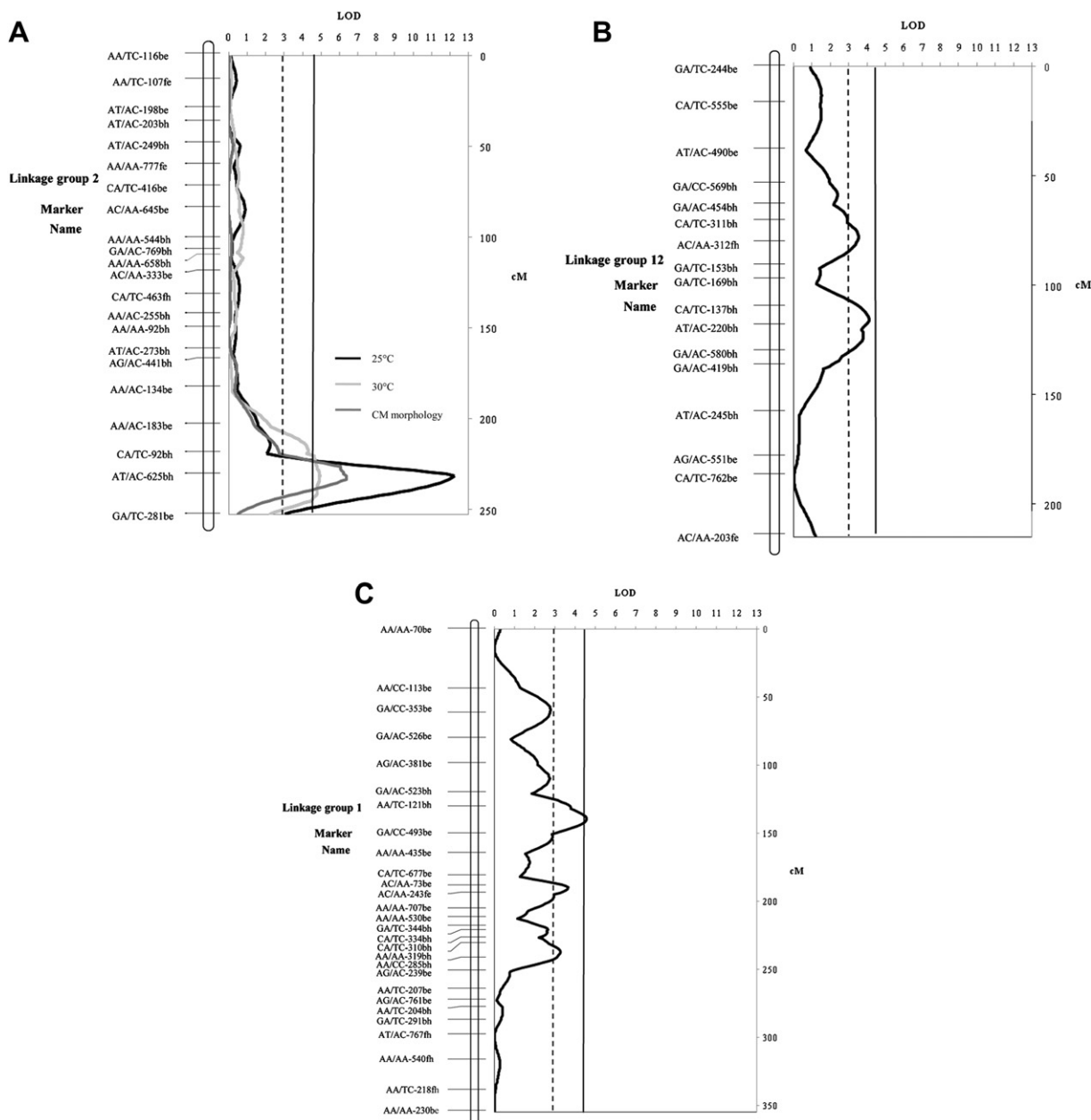


Fig 3 – (A) Location of a QTL on linkage group 2. The LOD significance levels for significant ($\alpha = 0.05$) and highly significant ($\alpha = 0.001$) QTLs are indicated by a dashed and solid line, respectively. (B) Location of a QTL for mycelial growth at 25 °C on linkage group 12. The LOD significance level ($\alpha = 0.05$) is indicated with a dashed line. (C) Location of a QTL for mycelial growth at 30 °C on linkage group 1. The LOD significance level ($\alpha = 0.05$) is indicated with a dashed line.

other fungi (Olson 2006; Van der Nest et al. 2009). Furthermore, one highly significant QTL, positioned at marker AT/AC-625bh on LG 2, was detected at both growth temperatures, indicating that this genomic region is important for mycelial growth.

For CM morphology, only one QTL was identified and it was highly significant ($P < 0.001$). This QTL was present at the same location as the shared QTL for mycelial growth at 25 °C and 30 °C. As the putative QTLs only accounted for 45 % and 41 % of the total mycelial growth variation at 25 °C and 30 °C, respectively, and 21 % for the variation in CM morphology, there are likely additional QTLs that are associated with these traits that were not detected. Possible reasons for this

discrepancy could be the presence of additional small effect QTLs that were not detected, as has been shown by Olson (2006) in a *Heterobasidion* interspecific cross. QTLs that were not expressed under the growth conditions used in this study, as well as those that were not polymorphic in nature (and hence not detectable), could also account for additional QTLs.

In the genetic map of De Vos et al. (2007), a large proportion of the markers displayed transmission ratio distortion (55 %, $P < 0.05$). Ninety-six percent of the markers exhibiting transmission ratio distortion were skewed towards the *F. subglutinans* parent. The estimated genome coverage of this map showed that 89 % of loci were within 10 cM of a framework

Table 2 – QTLs for mycelial growth detected at 25 °C and 30 °C, as well as for CM morphology at 25 °C.

Trait	Linkage group	Nearest upstream marker ^a	QTL position (cM) ^b	LOD ^c	R ² ^d
25 °C	LG2	AT/AC-625bh	231	12.26**	32 %
	LG12	CA/TC-311bh	77	3.52*	6 %
		CA/TC-137bh	117	4.09*	7 %
30 °C	LG1	AA/TC-121bh	148	4.48*	12 %
		AC/AA-73be	190	3.48*	9 %
		AA/AA-319bh	238	3.24*	9 %
	LG2	AT/AC-625bh	236	4.96**	11 %
CM	LG2	AT/AC-625bh	231	6.37**	21 %

*Experiment-wise significance level of $P = 0.05$ determined using Map Manager QTX.
**Experiment-wise significance level of $P = 0.001$ determined using Map Manager QTX.
a Nearest marker upstream to QTL position on the map of De Vos et al. (2007).
b Based on De Vos et al. (2007). Values indicate the map position of the QTL towards the bottom of the linkage group.
c LOD values were obtained using the equation $LR = 4.6 \times LOD$ (Liu 1998).
d The percentage of the total trait variance that can be explained by a QTL being present at this locus.

marker, so this bias was not due to genome coverage. In the present study, only one QTL, the one for mycelial growth at 30 °C that lies nearest to marker AA/TC-121bh, displayed transmission ratio distortion ($\chi^2 = 12.30$, $P = 0.00048$). This marker also displays bias towards the *F. subglutinans* genome (De Vos et al. 2007). The QTL lies in a 45.1 cM area with four markers displaying highly significant transmission ratio distortion ($P < 0.001$). To account for the effect (beneficial or detrimental) of the distorting loci on QTL detection, Map Manager QTX has an 'Allow for segregation distortion' function. This allows the program to use the contingency analysis (G-statistic), which is not sensitive to the effects of segregation distortion (García-Dorado & Gallego 1992).

In some Basidiomycetes, an association between mycelial growth and the MAT locus has been given as a possible explanation of transmission ratio distortion at the MAT loci and markers surrounding them (Simchen 1966; Larraya et al. 2001; Van der Nest et al. 2009). In contrast, no association was found between a specific MAT idiomorph and mycelial growth for the *Fusarium* spp. used in this study. Also, the MAT locus did not display transmission segregation distortion in this study, which is similar to the results of Friel et al. (2007). The *Fusarium* spp. used in this study are Ascomycetes and it appears that they do not display the same genetic determinants that influence mycelial growth and sexual recognition that have been shown for certain Basidiomycetes (Simchen 1966; Larraya et al. 2001; Van der Nest et al. 2009).

The inoculation data for the F₁ progeny did not display a continuous distribution with only one individual (FCC 2025) pathogenic to *Pinus patula* seedlings. Therefore QTL analysis could not be performed for pathogenicity. When compared to the lesion length produced by the *F. circinatum* parent, the F₁ isolate FCC 2025 was equally pathogenic with no significant difference ($P = 1.00$) found between the two isolates. Doohan et al. (2003) hypothesized that faster growing

Fusarium species on cereals are more pathogenic than those that grow slowly. In contrast, results of this study showed there was no association between mycelial growth and pathogenicity. This is similar to the results of a study using an interspecific cross between host-specific species of *Heterobasidion* (Olson 2006; Lind et al. 2007). Results of the present study add evidence to suggest that, mycelial growth and pathogenicity are traits apparently not controlled by the same loci.

It was unusual to find only a single isolate amongst the F₁ progeny that was highly pathogenic. This might be explained by the genetic constitution of the isolate. Data from the F₁ map of De Vos et al. (2007) were subjected to the Graphical Genotyping program (GGT; Van Berloo 1999). It was found that approximately 96.3 % of this individual's genome was descended from *F. circinatum*, i.e. the maternal parent. The six putative QTLs identified in this study were not found in regions inherited from the *F. subglutinans* parent. Linkage groups 2, 3, 5, 7, 9, and 11 (6/12 linkage groups) were intact (non-recombinant) linkage groups inherited from *F. circinatum* (Fig S1; see Table 4 of De Vos et al. (2007) for the number and origin of intact linkage groups). Interestingly, of the 94 F₁ progeny selected in this study, the F₁ isolate displaying a genomic constitution closest to the *F. circinatum* genomic constitution of the pathogenic isolate, was FCC 2020, with a *F. circinatum* genomic contribution of ~61.7 % (Fig S1). Only 1/94 F₁ progeny (FCC 2025) showed a *F. circinatum* genomic constitution >90 %, whereas 13/94 F₁ progeny had a *F. subglutinans* genomic constitution >90 %. This could be explained by the fact that the interspecific cross showed a clear bias towards the transmission of *F. subglutinans* alleles, with F₁ individuals receiving an estimated 59.8 % of their genomes from this parent (De Vos et al. 2007). Although not tested in this study, we hypothesize that a greater number of progeny should be pathogenic on teosinte, as seen from the 13/94 F₁ individuals having a genomic constitution of >90 %.

Friel et al. (2007) found that of 178 F₁ progeny isolates of the same cross, none were pathogenic on *P. radiata* trees. They speculated that the complete absence of pathogenicity in the F₁ progeny implied a bias towards the genome of the *F. subglutinans* (or nonpathogenic) parent. Our results suggest two possible reasons for this. One is that multiple genes may be required for pathogenesis, and these genes were possibly incompletely represented in the F₁ progeny, other than in isolate FCC 2025. This also implies that *F. circinatum* alleles that are essential for pathogenicity are located in the 35 % of the *F. circinatum* genome present in FCC 2025, but absent in FCC 2020 (Fig S1). It is also possible that the gene-for-gene relationship could account for the lack of pathogenicity in the majority of the F₁ individuals (Flor 1942). The F₁ individuals in this study (except FCC 2025) and in that of Friel et al. (2007) inherited various avirulence genes from the *F. subglutinans* parent. These were recognized by the host (*P. patula*), which resulted in resistance as seen by the inability of the F₁ individuals to cause disease.

In this study we identified QTLs involved in mycelial growth, CM morphology as well as a pathogenic F₁ isolate that displayed pathogenicity to *P. patula* due to its highly conserved genomic constitution to *F. circinatum*. These results are important to our understanding of the apparent lack of correlation between fitness traits such as pathogenesis and morphological traits such as mycelial growth. Specifically, variation in pathogenicity and mycelial growth variation may involve different genomic

loci in *Fusarium* spp. To identify these genes as well as other genes of interest, the *F. circinatum* parental strain used in this study, has been sequenced with a 10× coverage (www.genome-online.org). The potential applications of this genomic sequence are great and could, for instance, provide insights into *F. circinatum* genomic architecture, the identification of host-specific genes and could aid in the elucidation of the molecular mechanisms of pathogenicity, as well as aid in studies regarding different *Fusarium* species.

Acknowledgements

We thank the University of Pretoria, members of the Tree Protection Cooperative Program (TPCP), the Mellon Foundation, the National Research Foundation (NRF)/Department of Science and Technology (DST), Centre of Excellence in Tree Health Biotechnology and the THRIP initiative of the Department of Trade and Industry (DTI) in South Africa for financial assistance.

Supplementary material

Supplementary material related to this article can be found online at [doi:10.1016/j.funbio.2011.07.003](https://doi.org/10.1016/j.funbio.2011.07.003).

REFERENCES

- Britz H, Coutinho TA, Wingfield MJ, Marasas WFO, Gordon TR, Leslie JF, 1999. *Fusarium subglutinans* f. sp. *pini* represents a distinct mating population in the *Gibberella fujikuroi* species complex. *Applied and Environmental Microbiology* **65**: 1198–1201.
- Cumagun CJR, Bowden RL, Jurgenson JE, Leslie JF, Miedaner T, 2004. Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat. *Phytopathology* **94**: 520–526.
- Desjardins AE, Plattner RD, Gordon TR, 2000. *Gibberella fujikuroi* mating population A and *Fusarium subglutinans* from teosinte species and maize from Mexico and Central America. *Mycological Research* **104**: 865–872.
- De Vos L, Myburg AA, Wingfield MJ, Desjardins AE, Gordon TR, Wingfield BD, 2007. Complete genetic linkage maps for an interspecific cross between *F. circinatum* and *F. subglutinans*. *Fungal Genetics and Biology* **44**: 701–714.
- Doohan FM, Brennan J, Cooke BM, 2003. Influence of climatic factors on *Fusarium* species pathogenic on cereals. *European Journal of Plant Pathology* **109**: 755–768.
- Flor, 1942. Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* **32**: 653–669.
- Friel CJ, Desjardins AE, Kirkpatrick SC, Gordon TR, 2007. Evidence for recombination and segregation of pathogenicity to pine in a hybrid cross between *Gibberella circinata* and *G. subglutinans*. *Mycological Research* **111**: 827–831.
- Garbelotto M, Gonthier P, Nicolotti G, 2007. Ecological constraints limit the fitness of fungal hybrids in the *Heterobasidion annosum* species complex. *Applied and Environmental Microbiology* **73**: 6106–6111.
- García-Dorado A, Gallego A, 1992. On the use of the classical tests for detecting linkage. *The Journal of Heredity* **83**: 143–146.
- Grandillo S, Tanksley SD, 1996. Genetic analysis of RFLPs, GATA microsatellites and RAPDs in a cross between *L. esculentum* and *L. pimpinillifolium*. *Theoretical and Applied Genetics* **92**: 957–965.
- Larraya LM, Pérez G, Iribarren I, Blanco JA, Alfonso M, Pisabarro AG, Ramírez L, 2001. Relationship between monokaryotic colony growth and mating type in the edible basidiomycete *Pleurotus ostreatus*. *Applied and Environmental Microbiology* **67**: 3385–3390.
- Lind M, Dalman K, Stenlid J, Karlsson B, Olson Å, 2007. Identification of quantitative trait loci affecting virulence in the basidiomycete *Heterobasidion annosum* s.l. *Current Genetics* **52**: 35–44.
- Liu B, 1998. *Statistical Genomics: linkage, mapping and QTL analysis*. CRC Press, Florida, USA.
- Manly KF, Olson JM, 1999. Overview of QTL mapping software and introduction to Map Manager QT. *Mammalian Genome* **10**: 327–334.
- Manly KF, Cudmore RH, Meer JM, 2001. Map Manager QTX, cross platform software for genetic mapping. *Mammalian Genome* **12**: 930–932.
- Nelson PE, Toussoun TA, Marasas WFO, 1983. *Fusarium Species: an illustrated manual for identification*, 1st edn. The Pennsylvania State University Press, University Park & London.
- Nirenberg HI, O'Donnell K, 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* **90**: 434–458.
- Olson Å, 2006. Genetic linkage between colony growth and the intersterility genes S and P in the basidiomycete *Heterobasidion annosum* s. lat. *Mycological Research* **110**: 979–984.
- Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD, 1991. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. *Genetics* **127**: 181–197.
- Simchen G, 1966. Monokaryotic variation and haploid selection in *Schizophyllum commune*. *Heredity* **21**: 241–263.
- Van Berloo R, 1999. GGT: software for the display of graphical genotypes. *Journal of Heredity* **90**: 328–329.
- Van der Nest MA, Slippers B, Steenkamp ET, De Vos L, Van Zyl K, Stenlid J, Wingfield MJ, Wingfield BD, 2009. Genetic linkage map for *Amylostereum areolatum* reveals an association between vegetative growth and sexual and self-recognition. *Fungal Genetics and Biology* **46**: 632–641.
- Zamir D, Tadmor Y, 1986. Unequal segregation of nuclear genes in plants. *Botanical Gazette* **147**: 355–358.