Genetic analysis of growth, morphology and pathogenicity in the F_1 progeny of an interspecific cross between *Fusarium circinatum* and *Fusarium subglutinans*

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Summary

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 (CM) in the 94 F_1 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 F_1 progeny. In order to test the pathogenicity of the progeny, *Pinus patula* seedlings were inoculated and lesion lengths were measured after three 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 F_1 progeny was pathogenic on *P. patula* seedlings. This observation could be explained by the genetic constitution of this F_1 isolate, namely that ~96% of its genome originated from the *F. circinatum* parent. This F_1 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 F_1 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.

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).

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 F_1 progeny displayed pathogenicity on *Pinus radiata* and it was hypothesized that this could be due to a very low probability of finding viable F_1 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 F_1 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 F_1 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 F_1 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* x *F. subglutinans* interspecific cross can be used to identify QTLs for any quantitative traits that are polymorphic in the F_1 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 F_1 population, and to determine the genomic origin of these QTLs. Furthermore, pathogenicity in the F_1 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 *F. circinatum* and *F. subglutinans* (Desjardins *et al.,* 2000) and 94 isolates from the F_1 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 PDA (potato dextrose agar; 20% w/v potato dextrose agar 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 seven days, 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 F_1 progeny with five replicate plates per isolate. In addition CM morphology was characterized for the parental isolates as well as for the 94 F_1 progeny when grown at 25˚C for 7 days 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 (laciniate) edge (Figure 1A and B). The progeny displayed either of these, or an

intermediate morphotype (crenate) (Figure 1C). The phenotypes were scored as '1' for smooth, '2' for crenate and '3' for laciniate.

Pathogenicity studies

All 94 F_1 isolates were grown on ½ PDA for 7 days at 25˚C in the dark. Spores were washed from the cultures with 15% (v/v) glycerol. Spore concentration was determined using a haemacytometer and adjusted to 5 x 10^4 spores/ml for each isolate, using sterile distilled water.

Six month old *P. patula* seedlings were wounded by removing the growth tips and wounds were inoculated with a 10µl drop of spore suspension. In order to minimize the effect of genetically variable seedlings on the pathogenicity of the fungus, ten 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 three 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 ($H^2 = \sigma^2_G / \sigma^2_P$), 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 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 Log-of-the-odds (LOD) values by using LR = 4.6 x LOD (Liu, 1998). Epistatic interactions were detected using the "Interactions" option.

Results

Mycelial growth studies and statistical analyses

The parental isolates, *F. circinatum* and *F. 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 (Figure 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 x 10^{-6}) (Table 1). The opposite was true at 30˚C (P = 3.58 x 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 25ºC (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.

CM morphology of the progeny displayed either of the two parental species phenotypes, or an intermediate phenotype. Of the 94 F_1 progeny, 36.17% had smooth, 41.49% had laciniate and 22.34% had an intermediate (crenate) colony margin morphology. This was normally distributed (P = 0.00).

Pathogenicity

Inoculation of pine seedlings with the 94 F_1 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 F_1 progeny were not significantly different from the *F. 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 F_1 individual grew significantly faster *in vitro* at 25˚C in comparison to the two parental isolates. Growth in this isolate (66.90mm ± 2.28) was also more rapid than the average growth for the remaining 93 F_1 progeny ($P < 0.05$). However, no association was found between

mycelial growth and pathogenicity at 25˚C as there were instances of other F_1 individuals that also grew significantly faster than the pathogenic F_1 at 25ºC, yet were not pathogenic.

QTL detection

Three QTLs were detected for mycelial growth at 25˚C (Figure 3A + B), four at 30˚C (Figure 3A + C), and only one for CM morphology (Figure 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 231cM (Figure 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 *F. circinatum* and *F. 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 F_1 progeny. Our results showed that only one isolate from 94 F_1 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 *F. 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 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 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 (χ^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 45.1cM 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, MapManager 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 F_1 progeny did not display a continuous distribution with only one individual (FCC 2025) pathogenic to *P. patula* seedlings. Therefore QTL analysis could not be performed for pathogenicity. When compared to the lesion length produced by the *F. circinatum* parent, the F_1 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 F_1 progeny that was highly pathogenic. This might be explained by the genetic constitution of the isolate. Data from the F_1 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* (Figure S1; see Table 4 of De Vos *et al.* (2007) for the number and origin of intact linkage groups). Interestingly, of the 94 F_1 progeny selected in this study, the F_1 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% (Figure S1). Only $^{1}/_{94}$ F_1 progeny (FCC 2025) showed a *F. circinatum* genomic constitution > 90%, whereas $^{13}/_{94}$ F_1 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 F_1 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 $^{13}/_{94}$ F_1 individuals having a genomic constitution of > 90%.

Friel *et al.* (2007) found that of 178 F_1 progeny isolates of the same cross, none were pathogenic on *Pinus radiata* trees. They speculated that the complete absence of pathogenicity in the F_1 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 F_1 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 (Figure S1). It is also possible that the gene-for-gene relationship could account for the lack of pathogenicity in the majority of the F_1 individuals (Flor, 1942). The F_1 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 F_1 individuals to cause disease.

In this study we identified QTLs involved in mycelial growth, CM morphology as well as a pathogenic F_1 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 10X coverage (www.genomesonline.org). The potential applications of this genomic sequence are great and could, for instance, provide insights into *Fusarium 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.

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Figure 1: Colony margin morphology at 25˚C after seven days of growth in the dark. (A) *F. circinatum* displays a smooth edge. (B) *F. subglutinans* displays an irregular (laciniate) edge. (C) Example of F_1 isolate that showed an intermediate (crenate) edge morphology to (A) and (B).

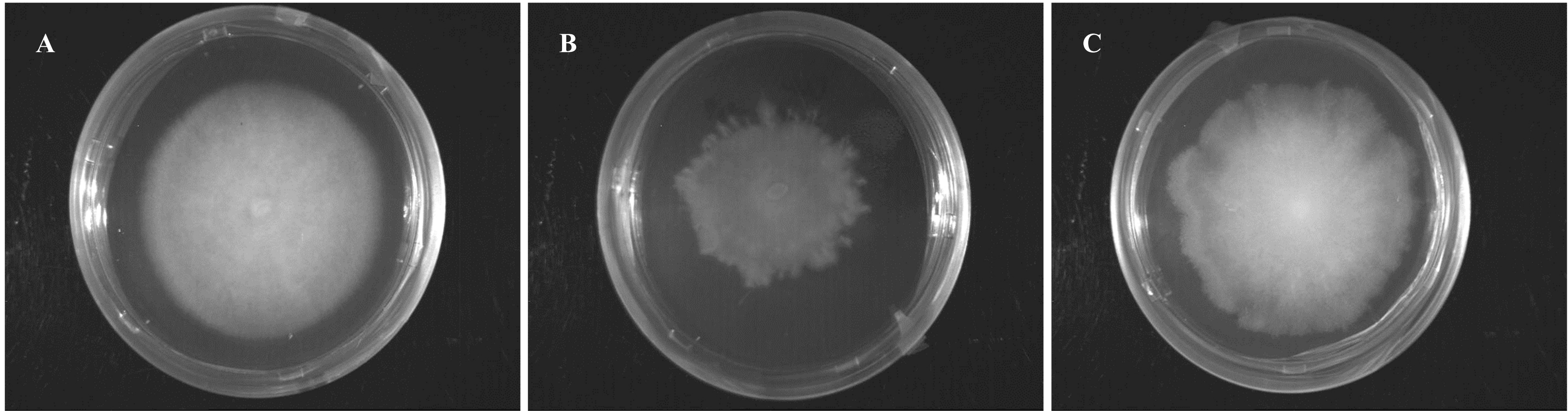


Figure 2: Measurements of the mycelial growth of *F. circinatum* and *F. subglutinans* (five replicates) at a range of temperatures for seven days in the dark on ½ PDA. Error bars represent the standard deviation.

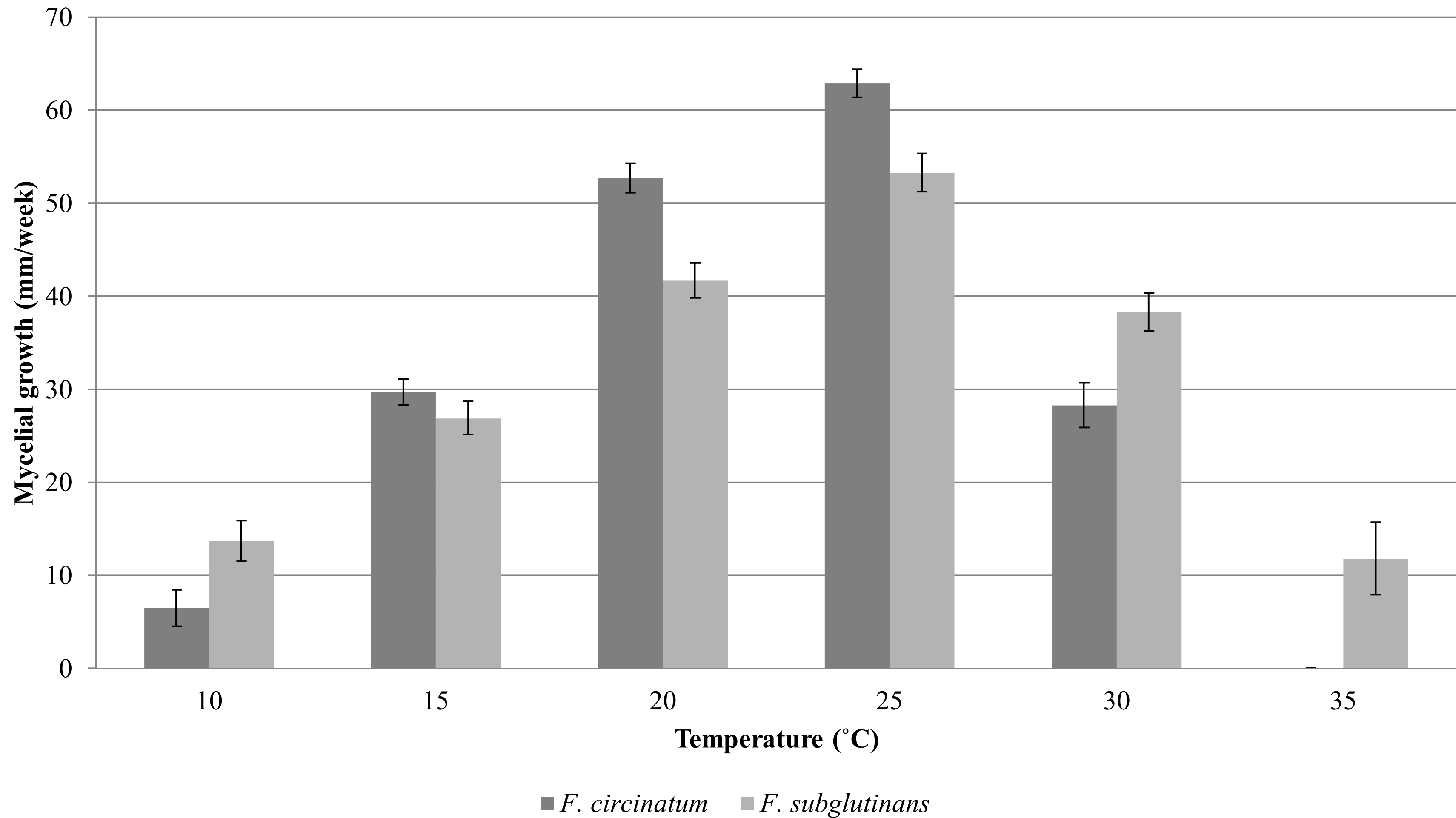
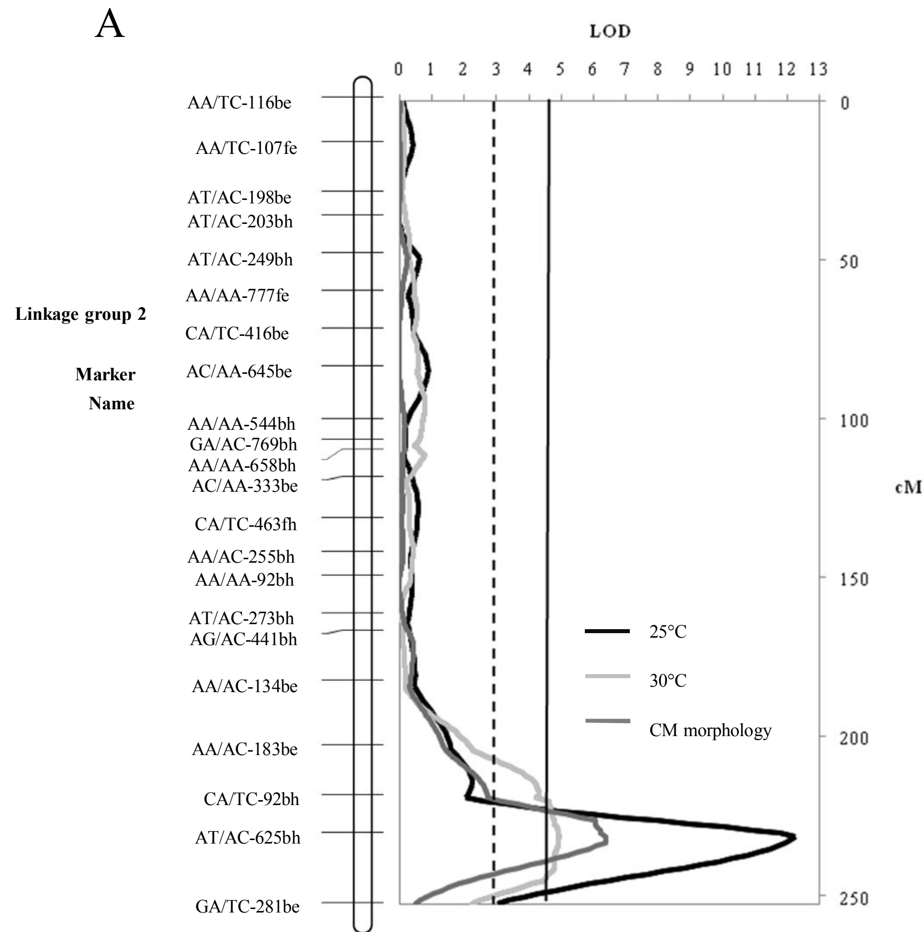
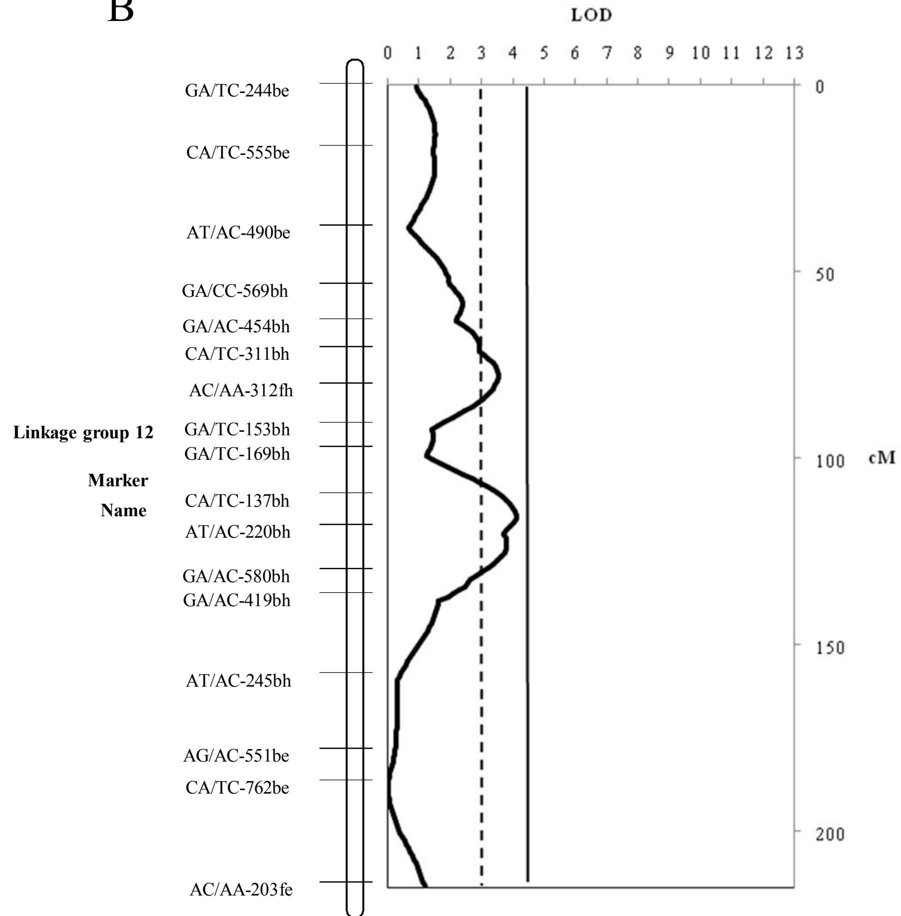
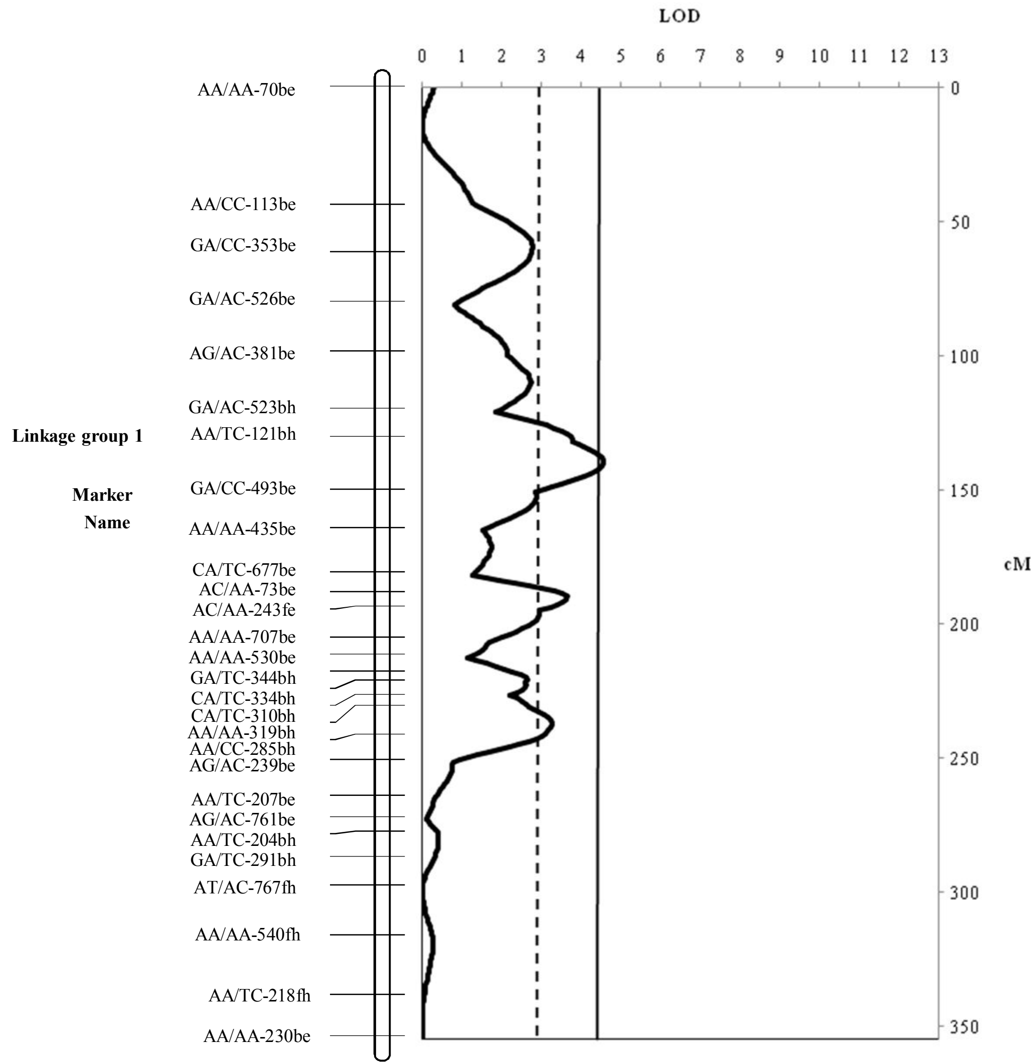


Figure 3: (A) Location of a QTL on Linkage Group 2. The LOD significance levels for significant (α = 0.05) and highly significant (α = 0.001) QTLs are indicated by a dashed and solid line, respectively. (B) Location of a QTL for mycelial growth at 25˚C on Linkage Group 12. The LOD significance level (α = 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 (α = 0.05) is indicated with a dashed line.

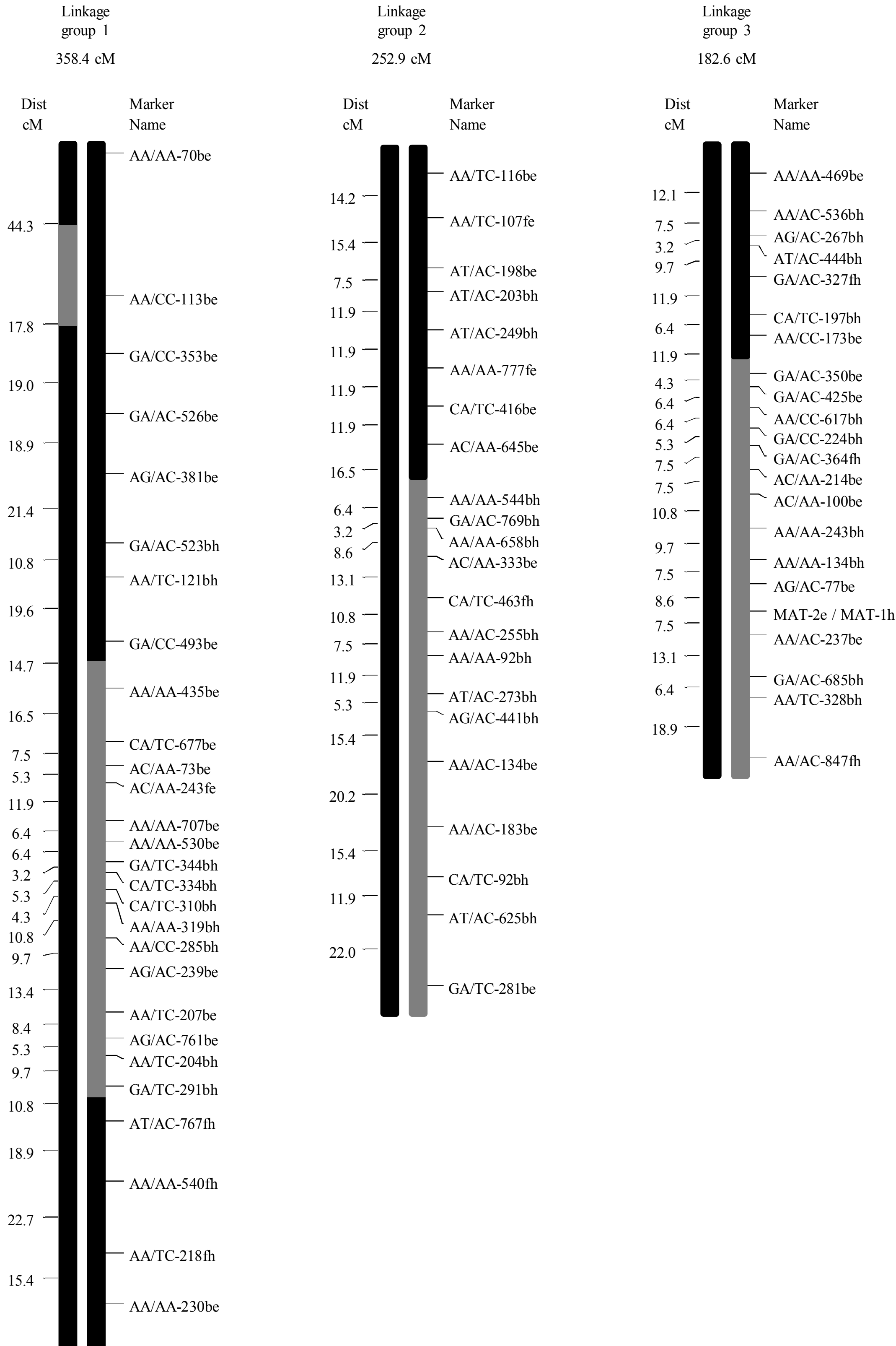


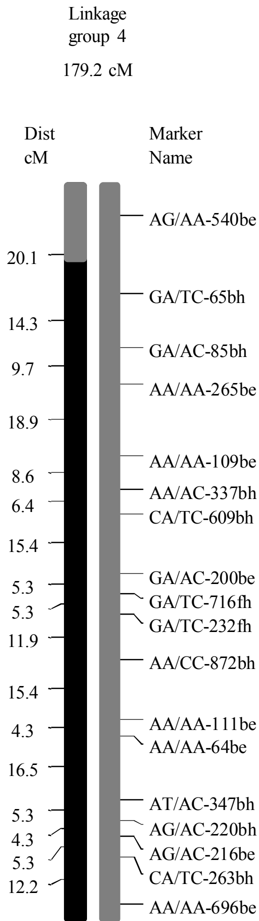

B
LOD
0 1 2 3 4 5 6 7 8 9 10 11 12 13
GA/TC-244be
CA/TC-555be
AT/AC-490be
GA/CC-569bh
GA/AC-454bh
CA/TC-311bh
AC/AA-312fh
Linkage group 12
GA/TC-153bh
GA/TC-169bh
Marker
Name
CA/TC-137bh
AT/AC-220bh
GA/AC-580bh
GA/AC-419bh
AT/AC-245bh
AG/AC-551be
CA/TC-762be
AC/AA-203fe
0
50
100
cM
150
200

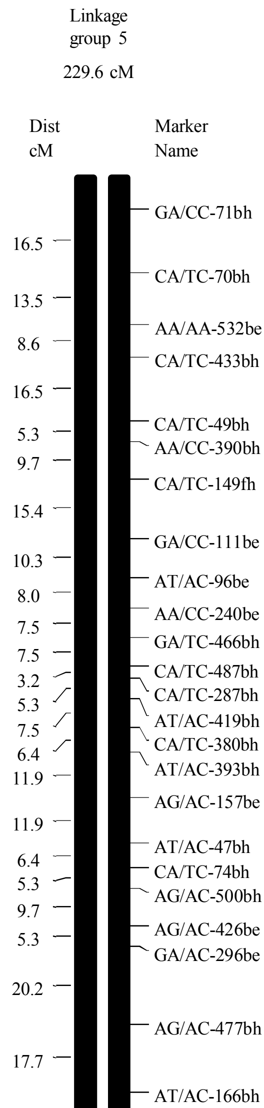
C

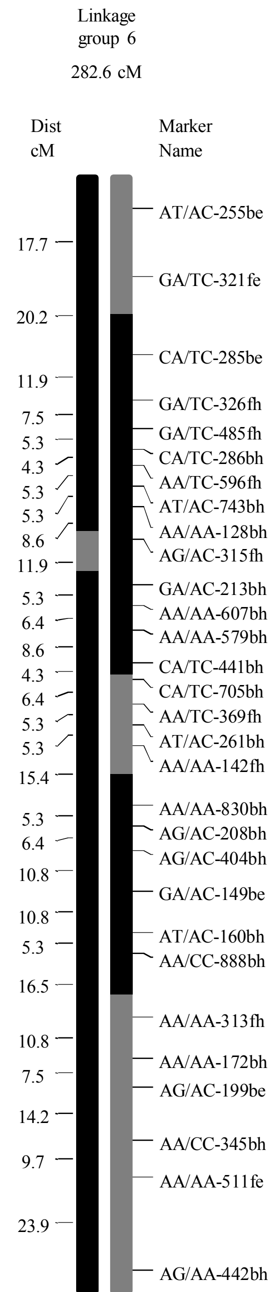


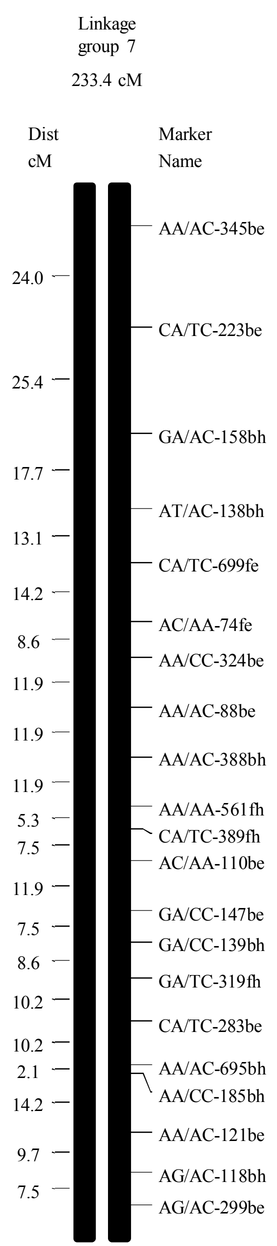
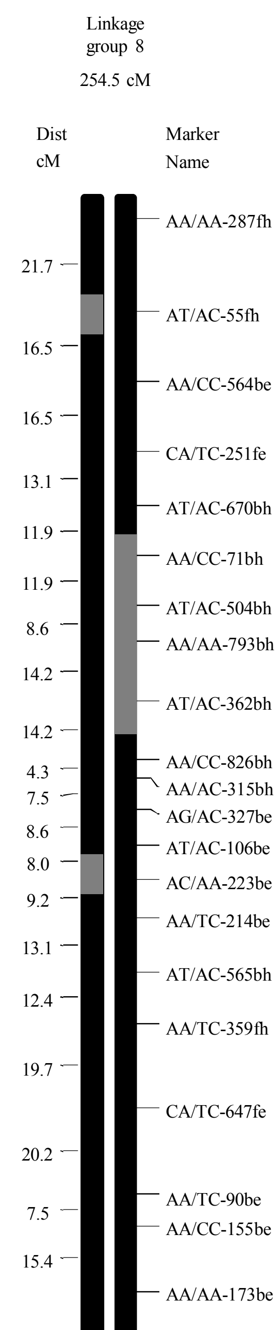
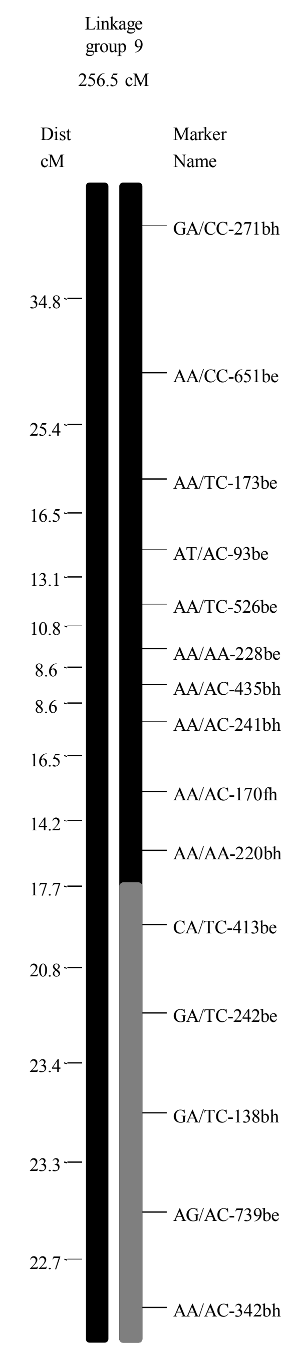
Supplementary Figure 1: Comparison of the graphical representation of the genome of FCC 2025 (linkage group to the left) and FCC 2020 (linkage group to the right). Twelve linkage groups are shown with the black bars representing the genome originating from the F. circinatum parent and the grey bars that originating from the F. subglutinans parent.

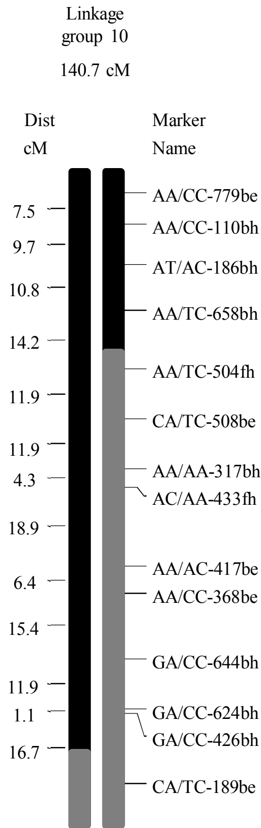


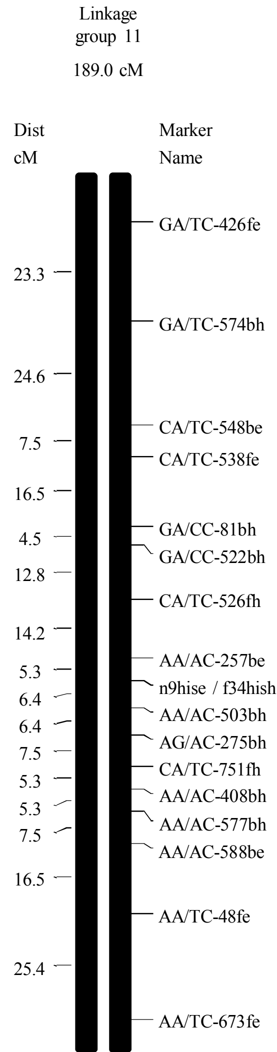

Linkage group 4
179.2 cM
Dist cM
Marker Name
AG/AA-540be
20.1
GA/TC-65bh
14.3
GA/AC-85bh
9.7
AA/AA-265be
18.9
AA/AA-109be
8.6
AA/AC-337bh
6.4
CA/TC-609bh
15.4
GA/AC-200be
5.3
GA/TC-716fh
5.3
GA/TC-232fh
11.9
AA/CC-872bh
15.4
AA/AA-111be
4.3
AA/AA-64be
16.5
AT/AC-347bh
5.3
AG/AC-220bh
4.3
AG/AC-216be
5.3
CA/TC-263bh
12.2
AA/AA-696be


Linkage group 5
229.6 cM
Dist cM
Marker Name
GA/CC-71bh
16.5
CA/TC-70bh
13.5
AA/AA-532be
8.6
CA/TC-433bh
16.5
CA/TC-49bh
5.3
AA/CC-390bh
9.7
CA/TC-149fh
15.4
GA/CC-111be
10.3
AT/AC-96be
8.0
AA/CC-240be
7.5
GA/TC-466bh
7.5
CA/TC-487bh
3.2
CA/TC-287bh
5.3
AT/AC-419bh
7.5
CA/TC-380bh
6.4
AT/AC-393bh
11.9
AG/AC-157be
11.9
AT/AC-47bh
6.4
CA/TC-74bh
5.3
AG/AC-500bh
9.7
AG/AC-426be
5.3
GA/AC-296be
20.2
AG/AC-477bh
17.7
AT/AC-166bh


Linkage group 6
282.6 cM
Dist cM
Marker Name
AT/AC-255be
17.7
GA/TC-321fe
20.2
CA/TC-285be
11.9
GA/TC-326fh
7.5
GA/TC-485fh
5.3
CA/TC-286bh
4.3
AA/TC-596fh
5.3
AT/AC-743bh
5.3
AA/AA-128bh
8.6
AG/AC-315fh
11.9
GA/AC-213bh
5.3
AA/AA-607bh
6.4
AA/AA-579bh
8.6
CA/TC-441bh
4.3
CA/TC-705bh
6.4
AA/TC-369fh
5.3
AT/AC-261bh
5.3
AA/AA-142fh
15.4
AA/AA-830bh
5.3
AG/AC-208bh
6.4
AG/AC-404bh
10.8
GA/AC-149be
10.8
AT/AC-160bh
5.3
AA/CC-888bh
16.5
AA/AA-313fh
10.8
AA/AA-172bh
7.5
AG/AC-199be
14.2
AA/CC-345bh
9.7
AA/AA-511fe
23.9
AG/AA-442bh


Linkage
group 7
233.4 cM
Dist
cM
Marker
Name
24.0
25.4
17.7
13.1
14.2
8.6
11.9
11.9
11.9
5.3
7.5
11.9
7.5
8.6
10.2
10.2
2.1
14.2
9.7
7.5
AA/AC-345be
CA/TC-223be
GA/AC-158bh
AT/AC-138bh
CA/TC-699fe
AC/AA-74fe
AA/CC-324be
AA/AC-88be
AA/AC-388bh
AA/AA-561fh
CA/TC-389fh
AC/AA-110be
GA/CC-147be
GA/CC-139bh
GA/TC-319fh
CA/TC-283be
AA/AC-695bh
AA/CC-185bh
AA/AC-121be
AG/AC-118bh
AG/AC-299be

Linkage
group 8
254.5 cM
Dist
cM
Marker
Name
21.7
16.5
16.5
13.1
11.9
11.9
8.6
14.2
14.2
4.3
7.5
8.6
8.0
9.2
13.1
12.4
19.7
20.2
7.5
15.4
AA/AA-287fh
AT/AC-55fh
AA/CC-564be
CA/TC-251fe
AT/AC-670bh
AA/CC-71bh
AT/AC-504bh
AA/AA-793bh
AT/AC-362bh
AA/CC-826bh
AA/AC-315bh
AG/AC-327be
AT/AC-106be
AC/AA-223be
AA/TC-214be
AT/AC-565bh
AA/TC-359fh
CA/TC-647fe
AA/TC-90be
AA/CC-155be
AA/AA-173be

Linkage
group 9
256.5 cM
Dist
cM
Marker
Name
34.8
25.4
16.5
13.1
10.8
8.6
8.6
16.5
14.2
17.7
20.8
23.4
23.3
22.7
GA/CC-271bh
AA/CC-651be
AA/TC-173be
AT/AC-93be
AA/TC-526be
AA/AA-228be
AA/AC-435bh
AA/AC-241bh
AA/AC-170fh
AA/AA-220bh
CA/TC-413be
GA/TC-242be
GA/TC-138bh
AG/AC-739be
AA/AC-342bh


Linkage
group 10
140.7 cM
Dist
cM
Marker
Name
7.5
9.7
10.8
14.2
11.9
11.9
4.3
18.9
6.4
15.4
11.9
1.1
16.7
AA/CC-779be
AA/CC-110bh
AT/AC-186bh
AA/TC-658bh
AA/TC-504fh
CA/TC-508be
AA/AA-317bh
AC/AA-433fh
AA/AC-417be
AA/CC-368be
GA/CC-644bh
GA/CC-624bh
GA/CC-426bh
CA/TC-189be


Linkage
group 11
189.0 cM
Dist
cM
Marker
Name
23.3
24.6
7.5
16.5
4.5
12.8
14.2
5.3
6.4
6.4
7.5
5.3
5.3
7.5
16.5
25.4
GA/TC-426fe
GA/TC-574bh
CA/TC-548be
CA/TC-538fe
GA/CC-81bh
GA/CC-522bh
CA/TC-526fh
AA/AC-257be
n9hise / f34hish
AA/AC-503bh
AG/AC-275bh
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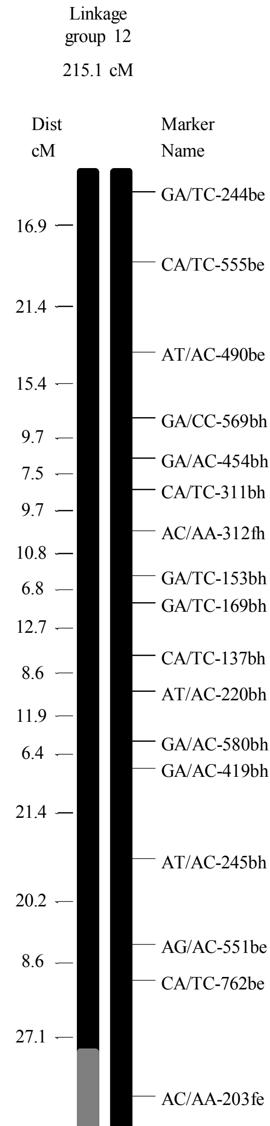
AA/AC-588be
AA/TC-48fe
AA/TC-673fe


Linkage
group 12
215.1 cM
Dist
cM
Marker
Name
16.9
21.4
15.4
9.7
7.5
9.7
10.8
6.8
12.7
8.6
11.9
6.4
21.4
20.2
8.6
27.1
GA/TC-244be
CA/TC-555be
AT/AC-490be
GA/CC-569bh
GA/AC-454bh
CA/TC-311bh
AC/AA-312fh
GA/TC-153bh
GA/TC-169bh
CA/TC-137bh
AT/AC-220bh
GA/AC-580bh
GA/AC-419bh
AT/AC-245bh
AG/AC-551be
CA/TC-762be
AC/AA-203fe

Isolate	25°C[a]	30ºC[b]
F. circinatum	62.90 ± 1.52 (a)	28.30 ± 2.41 (a)
F. subglutinans	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 25ºC 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 30ºC 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 F_1 progeny.

Table 1: Average mycelial growth of the parental isolates, 94 F_1 progeny and the F_1 isolate FCC 2025 at 25ºC and 30ºC for seven days in the dark on ½ PDA.

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 x LOD (Liu, 1998).

[d] The percentage of the total trait variance that can be explained by a QTL being present at this locus.

Table 2: QTLs for mycelial growth detected at 25˚C and 30˚C, as well as for colony margin morphology at 25ºC.