ElizabethProgress Narrative/technical report

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1. Executive Summary

The parental line 848 and a homozygous resistant line from the parent 852 were re-sequenced using the Illumina HiSeq400 platform. A total of 173 and 197 millions of paired end reads were generated for 848 and 852 respectively. After QC procedures, reads were aligned to the reference Malaccensis genome DH Pahang from which SNPs and INDELs were identified. In the chromosome 3 region surrounding the putative Foc-TR4 resistance locus, deletions and insertions are located in the introns and exons of some predicted gene models. Some of these include known resistance gene homologs. These structural variations are currently being analysed for mapping.Embryo-culture of an additional 200 individuals from a self-cross of the resistant parent (852)has been undertaken to help to better-define and validate the candidate region. New SNP markers have also been developed to delimit the region. The Foc-TR4 screen in pot trials produced some valuable results at the Coastal PlainsResearch Station (Darwin). In addition to the Malaccensis population, assessment was made of some germplasm available in Australia. Several of the lines did appear to be resistant to *Foc*TR4. A total of 380 tissue culture derived plants (123 lines) of the segregating 852 family were sent to IITA (Tanzania). These will be used as part of the PhD project of Mohamed Mpinafor mapping genes conferring resistance to *Foc* race 1.

2. Primary Outcomes, Intermediate Outcomes, Outputs and milestones

Primary Outcome 3 Genetics of *Fusariumoxysporum* f. sp. *cubense* (*Foc*), burrowing nematode (*Radopholussimilis*) and weevil resistance in banana understood serving the future development of molecular markers for banana breeding

Intermediate outcome 9 Three SNP-based linkage maps developed from 3 unrelated mapping diploid populations segregating for *Foc*

Output 273 diploid mapping populations (each 200 genotypes) segregating for *Foc* imported

Target for period 1: *Foc* diploid mapping population imported from UQ to Arusha (IITA)

Target for Period 2: 200 genotypes from each population plantedin the field

Target for Period 3:A total of 75 lines of the *Maa* population have been sent to IITA Tanzania. Each line consists of three clones. Additionally, 60 lines have been sent to Tanzania during April 2017.

Target for Period 4 :A total of 380 tissue culture derived plants (123 lines) of the segregating 852 family were sent to IITA (Tanzania). These will be used as part of a PhD (MohamedMpina) project for mapping genes conferring resistance to Foc race 1.

0% variance; A full population of Malaccensis plants have now been sent to IITA, although in some cases the number of clones was limited. It is expected that these can be multiplied in the field in Arusha.

Output 29 Identification within *Maa* population (UQ) of the location of *Foc* resistance trait

Target for period 1: DNA of extended Maa-UQ population extracted

Target for period 2: SNP analysis conducted

To date, 27 candidate genes have been analysed for gene expression and SNP variations between the resistant and susceptible parents. Further mapping and validation of SNPs will be performed to reduce the genetic interval of *Foc* SR4 and TR4 resistance.

Target for period 3:SNP based markers were developed to refine the genetic interval. A number of candidate genes have been reduced based on cross-over events associated with resistance or susceptibility. Candidate genes are being analyzed at the expression levels. WRKY20 has been successfully transformed into tomato.

No Target planned for Period 4: However fine mapping of the region on chromosome 3 is continuing. Additionally other lines have been planted in the field with a view to conducting crosses and generating populations that potentially segregate for alternative sources resistance to Fusarium wilt.

Intermediate outcome 10 The three mapping populations segregating for *Foc* are quantitatively phenotyped

Output 31 Phenotypic data on resistance to Fusarium wilt race 1 and race 4 in the Maa population (UQ)

Target for period 1: Glasshouse pot trials with Maa population with race 1 and 4

Target for Period 2: Further glasshouse pot trials on Maa population with race 1 and 4

Foc TR4 testing has been arrangedand will be performed at the Coastal Plains Station in Northern Territory (Dr Bob Williams). We have a large shade house setup with enough room for 200 plants to be tested. PCR markers will be used to pre-select lines carrying cross over events in the TR4 resistance locus for testing. A combined technique of applying spore suspension and infested millet as inoculum in pot trials has worked and produced reliable and reproducible phenotypic data. This was performed using the subtropical race 4 VCG0120 on the progeny of a F1 cross between the resistant (850) and susceptible (848) plants.

The*Foc* race 1 phenotyping is currently being carried out in Arusha by PhD candidate Mohamed Mpina.

Target for Period 3: Phenotypic data for subtropical race 4 collected at UQ and phenotypic data for tropical race 4 to be collected at Northern Territory Coastal Plains Research Station.

Target for Period 4: The Foc TR4 screen of the recombinant individuals (852 family) also produced consistent phenotypes which suggest that the genetic location of the candidate region is correctly defined by the corresponding marker loci.

0% variance. However, further progeny lines with apparent cross over events are being screened versus TR4 for fine mapping.

Output 32 Genomic regions associated with Foc resistance identified on the banana linkage maps and on the integrated map

Target for Period 4: We re-sequenced the parental line 848 and a homozygous resistant line from the parent 852 on the Illumina HiSeq400 platform. A total of 173 and 197 millions of paired end reads were generated for 848 and 852 respectively. After QC procedures, reads were aligned to the reference malaccensis genome DH Pahang from which SNPs and INDELs were identified. In the chr3 region surrounding the Foc-TR4 resistance locus, deletions and insertions are located in the introns and exons of some predicted gene models. Some of these include known resistance gene homologs. We are currently analysing these structural variations for mapping.

0% variance although a QTL is available no clear target gene has yet been identified and for that purpose re sequencing is being conducted

3. Results to date

The parental line 848 and a homozygous resistant line from the parent 852have been re-sequenced using the Illumina HiSeq400 platform. A total of 173 and 197 millions of paired end reads were generated for 848 and 852 respectively. After QC procedures, reads were aligned to the reference malaccensis genome DH Pahang from which SNPs and INDELs were identified. In the chromosome 3 region surrounding the predicted Foc-TR4 resistance locus, deletions and insertions are located in the introns and exons of some predicted gene models. Some of these include known resistance gene homologs. We are currently analysing these structural variations for mapping.

Understanding of the Malaccensis Population

Samples of the Malaccensis DNA was sent to theInstitute of Experimental Botany, Czech Republic, for determination of ploidy and also for placement within the phylogeny based on SSR markers. This placed the susceptibleMalaccensis line 848 actually outside that considered to be Malaccensisand the resistant Malaccensis line 852 somewhaton the periphery of the Malaccensisaccessions(see below)

Figure 1 Subset of SSR Tree of *Musa* spp. including lines 848 and 852 provided by Pavla*et al.*

Further analysis of 848 and 852has been undertaken by G. Martin *et al.* at CIRAD, Montpellier where they found thatline 848, although mostly Malaccensis, had what could be considered a more mixed heritage including Banksii and some Zebrina .Both 848 and 852 were consideredhighly heterozygous particularly in the zone of intereston chromosome 3. This opens up possibilities of variation compared with the current Pahang sequence data.

Further Mapping

We have also embryo-cultured an additional 200 individuals from a self-cross of the resistant parent (852). The DNA of these plants have been extracted and PCR markers have been used to screen these lines. This will help us to better-define and validate the candidate region. New SNP markers have also been developed to delimit the region. Accessions where crossing-over events have occurred near the region of interest in chromosome 3 are being assessed in glasshouse trials using *Foc*- SR4 in Brisbane and in some cases Foc-TR4 in the Northern Territory.

TR4 Pot Trial Assessments at Coastal Plains Research Station, Northern Territory (*this work on the assessment of different germplasm is funded by the current Horticulture Australia BA14014 program but has been included as it is relevant to the use of the marker generated from the Malaccensispopulation which is funded by IITA)*

The *Foc*-TR4 screen produced some valuable results at the Coastal Research Station (Darwin). The disease severity score is based on the extent of discolouration in a cross-sectioned rhizome based on Mak*et al.*http://www.fao.org/docrep/007/ae216e/ae216e0k.htm (this visual scale of 1 to 8 does differ slightly from the scale of 1 to 6 used by Viljoen *et al*. ; we have used the 1-8 scale from the onset and so are doing so for consistency across our screens). The highly susceptible genotypes have a score of greater than 3 (>20% rhizome discoloured). These include the Lady Finger, Williams, FHIA26 and Malaccensis#848. Lines with a completely clean rhizome have a score of 1. These lines are resistant to *Foc*TR4 and they include FHIA2, FHIA21, FHIA25, M61, IV9 Calcutta4, SH3142, Pahang, PJB, Madang and CAM020. The lines that show slight degree of symptoms fall anywhere between 2 and 3. These lines would possibly develop more symptoms if sufficient time is given. They include the FHIA18, Calcutta4 (a different accessions from IV9), GCTCV119, PGM, FHIA3 and FHIA23. These results are summarised in Figure 2

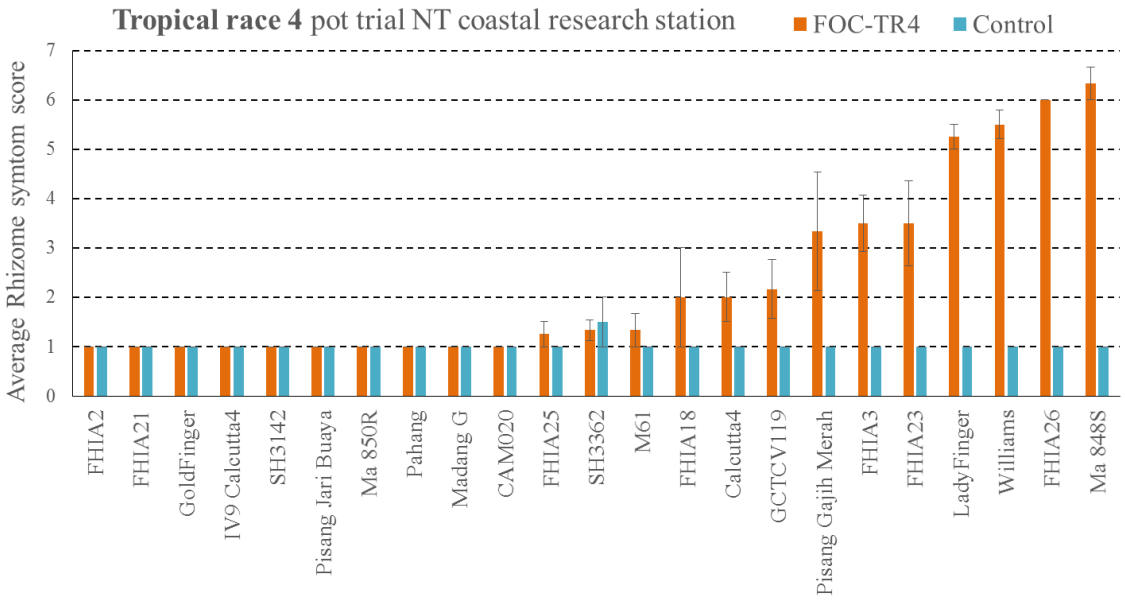

Figure 2. Assessment of different banana accessions inoculated at 12 weeks post tissue culture with*Foc*TR4-infested millet and assessed 12 weeks post inoculation using the 1-8 scale based on Mak*et al*. A replication of five plants per accession was used in most cases. A rhizome score of 1 indicates a healthy plant, scores above 3 indicate highly diseased.

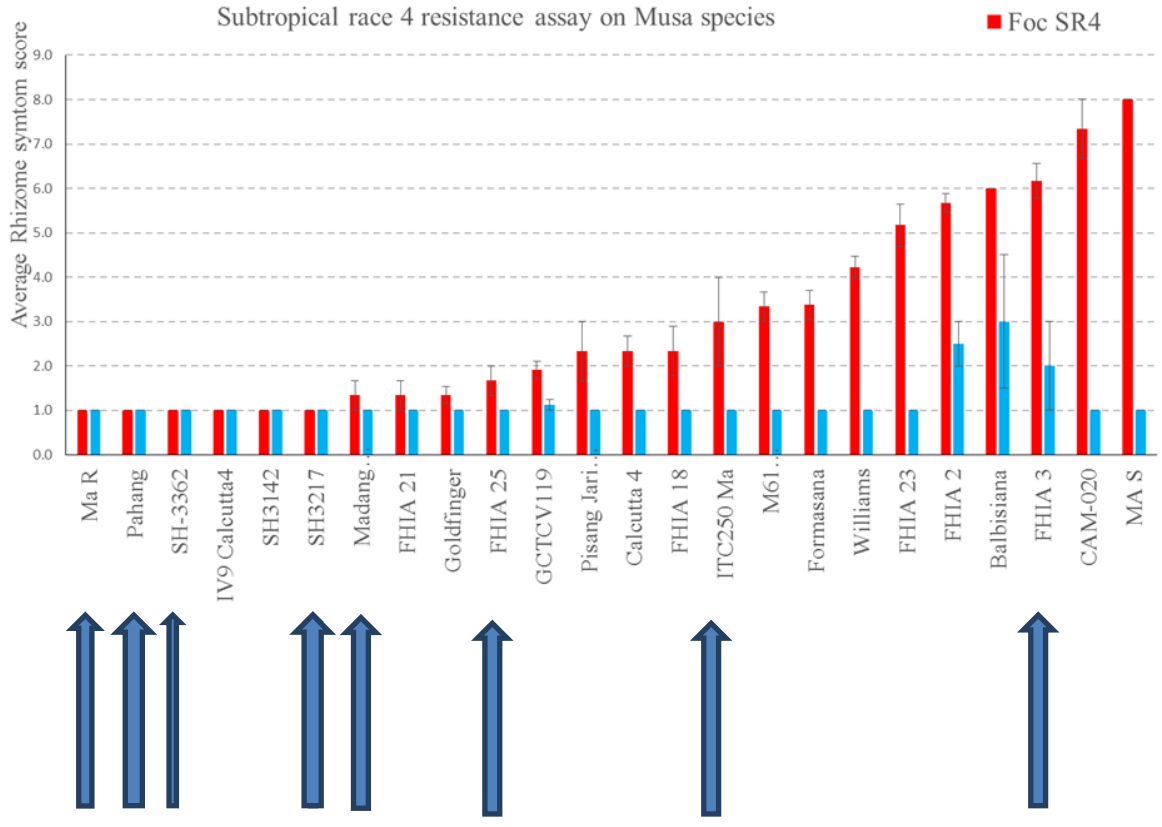

Figure 3. As for figure 2, except that *Foc*SR4 was used as inoculum. Blue arrows indicate a positive result for the current molecular marker. The thinner arrow indicates heterozygous for the marker.

Note the FHIA line SH3362 appears to be heterozygous for the marker. FHIA03 is the only exception where the marker has given a positive result yet the plants are susceptible to both*Foc* SR4 and TR4.

CAM20 and FHIA2 are the only instances so far where the results for*Foc*TR4 and*Foc* SR4 differ. Further assessments need to be made to verify these results although observatiosn on differences with FHIA02 have been made previously.

The pot trial data is consistent with the data obtained from the field trials at the same location. This suggests that the pot trial method can reliably detect*Foc* TR4 resistance in different genotypes. The *Foc* TR4 screen of the recombinant individuals (852 family) also produced consistent phenotypes.

The pot trials undertaken in the Northern Territory were done so in collaboration with NT Department of Primary Industries allowing the use of their facilities and assistance from their staff.

Malaccensis Population Assessment versus Foc race 1

A total of 380 tissue culture derived plants (123 lines) of the segregating 852 family were sent to IITA (Tanzania). These will be used as part of MohamadMpina'sPhD project for mapping genes conferring resistance to Foc race 1. Additionally, a subset of the population (25 clones) have been assessed against a race 1 VCG present in Australia. This has been undertaken in the glasshouse facility on a separate campus of the University of Queensland inorder to minimise the possibility of mixing the races of*Foc*. However, the growing conditions have not been ideal. Symptoms have developed but the final data still needs to be analysed.

4. Challenges Encountered(Variance)

As far as the objectives of the IITA/BMGF program no major challenges have been encountered. With the completion offundingof “Improvement of banana for smallholders in the Great Lakes Region of Africa” to UQ as of November 2018, further funds are currently be sought via a tender known as a “Request for Proposal “ from Hort Innovation Australia. If successful, this will allow continuation of this research for markers associated with Fusarium resistance.

5. Lessons learned

The original SNP map generated for the segregating Malaccensis population included data from 848 and the other susceptible Malaccesnis lines 846 and 845 which were not actual parents in the segregating population but at the time were considered siblings of the heterozygous parent. In view of their diverse heritage of 848, it is suspected this may have slightly skewed the location of the marker; these data are now being excluded from new analyses.

6. Work Plan

a. Screen more progeny in the region of the predicted cross over events
b. Complete the expression analysis of candidate genes using quantitative PCR.
c. Initiate crosses using different germplasm

7. Budget Summary

To follow

8. Other relevant project information

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