Hort Innovation

Final Report

Development of molecular markers for fusarium wilt resistance in banana

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Delivery partner:

The University of Queensland

Project code:

BA17006

Project:

Development of molecular markers for fusarium wilt resistance in banana (BA17006)

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Summary

Fusarium wilt, in particular that caused by Tropical Race 4 (TR4) of the soil borne fungal pathogen *Fusarium oxysporum* f.sp. *cubense* (*Foc*), threatens the viability of commercial banana production in Australia, as it does worldwide. Once present in a plantation, *Foc* cannot be eradicated; the fungus persists in the soil either as resting spores or as a saprophyte on alternative hosts. There is no known effective chemical control. Genetic resistance is the only viable option for control and consequently the identification of disease resistant varieties is of the greatest priority for the Australian Banana Industry. The objective of this study was to initiate work to develop closely linked molecular markers associated with sources of genetic resistance to Fusarium wilt obtained from wild diploid banana. Such molecular markers will enhance the Australian banana industry's interaction with international banana breeding programs and allow efficient selection of suitable resistant germplasm for introduction into Australia.

Previous genetic studies at the University of Queensland have used Malaccensis as a source of resistance to Fusarium wilt TR4 and also subtropical race 4 (SR4). This allowed the development of a molecular marker that has been used to screen germplasm in international breeding programs to predict lines carrying resistance. In this current study known diploid resistance lines Calcutta 4, Pisang Jari Buaya (PJB) and SH3362 were investigated to ascertain the genetics of resistance and to initiate segregating populations for use in the development of molecular markers.

Results in this study initially indicate that the resistance in the diploid line Calcutta 4 is heterozygous, as a cross with a known susceptible Malaccensis line produced a F_1 population that segregated 1:1 for resistance to susceptibility when challenged in pot trials to *Foc* SR4. The resistant F_1 plants were subsequently placed in the field and self-pollinated; seed is currently developing.

Attempts to produce a F_1 population from a cross between the resistant diploid PJB and susceptible Malaccensis have so far been unsuccessful because of infrequent production of female flowers and limited production of pollen on the male flowers. However, a cross using the improved diploid line SH3662, a line from the Honduran (FHIA) breeding program that is derived from PJB and Malaccensis progenitors, has produced F_1 seed. The F_1 plants of this cross with SH3362 also segregated in response to *Foc* SR4 in pot trials in a ratio of 12 resistant to 8 susceptible plants. The F_2 population will be assessed before predictions of the underlying genetics can be made. The F_1 resistant plants have been placed in the field and will be self-pollinated in the coming months when flowering occurs.

Both populations of F_2 plants will be screened with *Foc* SR4 and *Foc* TR4 to obtain a better understanding of the genetics controlling the resistance. This will allow the development of molecular markers associated with the resistance from these diverse sources.

Meanwhile studies with the original Malaccensis resistance line that allowed the identification of the original molecular marker are continuing. A more defined region, than before, on banana chromosome 3 has been identified as the location of the resistance trait and through international collaborations this work is ongoing with a view to identifying the gene/s conferring resistance; such genes would be the ultimate and most accurate molecular marker.

The research conducted in this project was supported by additional resources made available through a ranged of funding sources including the Bill and Melinda Gates Foundation through the International Institute for Tropical Agriculture.

Public summary

Fusarium wilt, in particular that caused by Tropical Race 4 (TR4) of the soil borne fungal pathogen *Fusarium oxysporum* f.sp. *cubense* (*Foc*), threatens the viability of commercial banana production in Australia, as it does worldwide. Once present in a plantation, *Foc* cannot be eradicated; the fungus persists in the soil either as resting spores or as a saprophyte on alternative hosts. There is no known effective chemical control. Genetic resistance is the only viable option for control and consequently the identification of disease resistant varieties is of the greatest priority for the Australian Banana Industry.

The objective of this study was to initiate work to develop closely linked molecular markers associated with sources of genetic resistance to Fusarium wilt obtained from wild diploid banana. Such molecular markers will enhance the Australian banana industry's interaction with international banana breeding programs and allow efficient selection of suitable resistant germplasm for introduction into Australia.

Results in this study has determined that the resistance in the diploid line Calcutta 4 is heterozygous, as a cross with a known susceptible Malaccensis line produced a F_1 population that segregated 1:1 for resistance to susceptibility when challenged in pot trials to *Foc* SR4.

The study has also determined that the F_1 plants from a cross of SH3662, a line from the Honduran (FHIA) breeding program that is derived from PJB, and Malaccensis progenitors, has segregated in response to *Foc* SR4 in pot trials in a ratio of 12 resistant to 8 susceptible plants.

Meanwhile in studies with the original Malaccensis resistance line, a more defined region on banana chromosome 3 has been identified as the location of the resistance trait. Through international collaborations, this work is ongoing with a view to identifying the gene/s conferring resistance and developing an associated gene-specific molecular marker.

Keywords

banana, *Fusarium oxysporum*, resistance, germplasm, TR4, breeding, molecular marker

Introduction

Genetic resistance offers a long-term means of control of Fusarium wilt in banana, but truly resistant commercially viable varieties are not yet available. Resistance is present in several wild banana lines but introgression into agronomically favourable lines through breeding is complex. Molecular markers provide plant-breeding programs with a means of identifying germplasm that carry favourable traits. If these traits determine disease-resistance, it is then possible to quickly marker-select suitable germplasm and/or varieties and then selectively screen these for pathogen resistance thereby increasing efficiency and reducing the workload in the breeding pipeline. However, to develop molecular markers closely linked to resistance in the first place, fundamental genetic studies must be carried out and that does involve screening with the pathogen.

It is the polyploid nature of edible banana and the ultimate desire to have sterile fruit that renders banana-breeding complex. However, breeding programs do exist, located in regions around the world but in areas generally free of Fusarium wilt, especially that caused by *Foc* TR4. To facilitate a breeding program for *Foc* TR4 resistance, a large collection of germplasm will have to be assessed for variation in host resistance against *Foc* TR4 at a suitable identified location. Potential screening sites for both *Foc* TR4 and sub-tropical race 4 (SR4) exist within Australia, in Northern Territory and SE Qld/NSW respectively, but introducing banana germplasm into Australia involves strict quarantine controls. It takes a minimum of 18 months for an individual accession to clear the quarantine procedures including all the various assessments to determine the presence of exotic viruses. Consequently, only a limited number of accessions are imported and processed at any time. The availability of molecular markers associated with Fusarium wilt resistance enables the screening of DNA and subsequent selection of resistant varieties for potential introduction into Australia.

The use of such markers will allow for rapid identification of resistant varieties allowing effective use of resources in the post entry quarantine processing rather than on varieties that may later prove to be susceptible. In other words, the use of molecular markers will allow varieties likely to be Fusarium resistant to be prioritised through the quarantine screening process, thus avoiding the potential subsequent time and financial waste of importing and processing of varieties that later prove to be susceptible.

The overarching objective of this project was to expedite the screening process for identifying Fusarium wilt resistant lines in banana. Specifically, the aims within BA17006 were to continue an ongoing program at the University of Queensland to undertake crosses between resistant and susceptible wild diploid banana lines to generate populations that can be screened with the Fusarium wilt fungus with the ultimate objective of identifying molecular markers associated with resistance. In generating these potential segregating populations, an increased understanding of the genetics behind Fusarium resistance would be gained and therefore assist in understanding the disease process and formulating good management practices.

Sources of resistance to Fusarium wilt exist amongst available *Musa* germplasm. Ongoing research by the project team in a program led by the International Institute for Tropical Agriculture (IITA) with funding from the Bill and Melinda Gates Foundation (BMGF) identified a molecular marker in a diploid Malaccensis line associated with Fusarium wilt resistance (Chen et al. 2021 in prep). Studies are continuing on this Malaccenis population with the aim of identifying the actual gene or genes responsible for the resistance. In the meanwhile, the molecular marker is being used to screen germplasm from the FHIA (Honduran) and EMBRAPA (Brazilian) banana breeding lines. However, it is evident that some of the FHIA lines as well as the EMBRAPA lines were developed using sources from more than one resistant progenitor. The Malaccensis molecular marker is effective in identifying the single resistance locus in Malaccensis. It detects this locus-specific resistance in germplasm derived from Malaccensis. It does not detect resistance at other genome regions (loci) and/or the same locus in other *M. accuminata* subspecies where sequence divergence might prevent it from being detected. For example, Calcutta 4 or Pisang Jari Buaya (PJB) are both resistant to *Foc* TR4 but the marker screen showed negative for both lines. This indicates several likely scenarios. One is that these two lines carry the same resistance locus as Malaccensis but due to linkage disequilibrium between the marker and the resistance gene, and/or sequence divergence in the *M. accuminata* subspecies, this locus failed to be detected. Alternatively, the resistance maybe controlled by unidentified genes at genome locations other than the Malaccensis locus. Performing genetic studies on Calcutta4 and PJB would allow us to potentially mine new sources of resistance. The benefit of multiple resistance sources being available is that combining such (pyramiding) in a single line leads to more durable resistance (Pilet-Nayel et al. 2017).

In this project studies assessing the underlying genetics of resistance in Calcutta 4 and PJB were initiated. The aim being to obtain molecular markers for additional sources of resistance for use in marker assisted selection for banana breeding programs. Crossing these diploid lines onto a known universally susceptible Malaccensis accession was the

first step in understanding the genetics behind these resistance sources. PJB is however known to be notoriously infertile and for this reason, the line of SH3362 was also used. The SH3362 line is a product of the Honduran banana breeding program and is understood to have both Malaccensis and PJB as its progenitors. SH3362 in turn is a progenitor of FHIA 01 otherwise known as the cultivar Goldfinger. Calcutta 4, PJB and SH3362 had been previously assessed in pot trials against *Foc* SR4 and *Foc* TR4 and shown to be resistant (Chen et al 2019).

Methodology

Banana Diploid Lines

Up to four clumps of clonal accessions of Calcutta 4, PJB and SH3362 were already established at the Queensland Department of Agriculture and Fisheries (QDAF) research facility at Redlands, Queensland. Also growing in the field at Redlands were two known sources of susceptible diploid *Musa* spp. These susceptible diploids were of Malaccensis and Malaccensis /Banksii origin and had been used in the previous program to obtain the molecular markers for the Malaccensis resistance.

This frost-free facility at Redlands on the coast in the southern suburbs of Brisbane has proven to be conducive for production of seed within diploid banana lines. The protocols for undertaking the pollinations had been developed by Prof. Aitken over several years. Use of multiple clones, and allowing multiple suckering, enabled a staggering of floral development resulting in the availability over time of receptive female flowers and donor male flowers for pollination. Crosses were undertaken between the resistant lines and the susceptible Malaccensis. The direction of the cross depending on the availability of suitable flowers at the time.

As flowers emerged from the pseudostem, the entire bell was enclosed within a microfibre cloth banana bag. The bag was secured at the peduncle with wire preventing bird or flying insect entry. Bags were placed on the emerging inflorescences 2-7 days prior to the opening of the first bract. Meanwhile, inflorescences (bells) of potential pollen donors were enclosed in the microfibre cloth bags. Male flowers generally develop on the inflorescences of these diploid lines from around the eighth hand. Bagging of the flowers of the male donor minimized the risk of any extraneous pollen being introduced by flying insects onto the anthers of the male flowers.

Calcutta 4 is a relatively short stature line. The inflorescences were produced on a short peduncle which when first emerged needed to be teased out from the pseudostem to allow the bag to cover the bell before bract opening. PJB is a tall stature line, taking up to 15 months for the first inflorescence to emerge and when it did so it produced an abundance of hands. SH3362 is also a tall stature plant taking 16 months to produce inflorescences. The inflorescences of SH3362 were excessively long extending 4-5metrers in length, were parthenocarpic with multiple hands and with the bell reaching down to the ground in some cases.

The clumps of the Malaccensis susceptible lines produced abundant thin pseudostems with small bells. These plants were particularly small as the stand had been established several years previously and was in decline due to the presence of *Foc* SR4 in the plot to which these lines were susceptible.

Pollen production

Pollinations were undertaken generally during the morning 2- 7 days after enclosing the newly emerging inflorescence. Weather conditions impacted the quality of the pollen. The most suitable pollen was that which appeared mealy and abundant on the anthers. Timing was critical but in the case of Calcutta 4 and SH3362 apparent viable pollen (mealy in texture) was obtained. However very limited pollen was observed on PJB. The anthers of PJB were waxy, a limited amount of pollen could be prized from a few anthers on a few occasional hands. Attempts to obtain pollen at different times of the day did not seem to improve the availability of pollen from the PJB plants.

Female flower production

In general, up to the first six hands per bunch appeared to bare exclusively female flowers with the SH3362 and Calcutta lines. However, the Malaccensis susceptible lines occasionally produced pollen at an earlier stage and so care had to be taken when using Malaccensis as the female parent to ensure that no inadvertent self-pollination occurred. Of the few inflorescences of PJB that developed, the stigmata appeared withered.

Pollination to seed harvest took 4-8 months; bunches were harvested once the first fruit started to turn yellow. All seed was recovered using embryo culture.

Embryo culture

Embryo culture is labour-intensive but facilitates the production of multiple clones of each accession. The seed were surface sterilised and then individually prized open, the embryo removed and placed onto culture media. After 8 weeks the tissue culture plantlets were separated out and sub-cultured onto fresh media to promote the production of multiple clones of each accession. After a further 12 weeks minimum, the plants were transferred from tissue culture initially into seedling trays for acclimatization and then transferred to a heated glasshouse.

F_1 plant production and pathogenicity testing

Where multiple clones were available for a F_1 accession, one to two plants were prepared for field planting and the remainder used for pathogenicity tests. It should be noted that not all accessions proliferate in tissue culture limiting the numbers that could be utilized further. Twelve weeks post tissue culture, plants in the glasshouse had reached the stage suitable for pathogenicity testing.

Pathogenicity testing was carried out as previously described (Chen et al. 2019) by applying a standard quantity of *Foc* SR4 infested millet to the potting mix in which the banana plants were placed. When available, up to five replicate clones were used for each accession. Plants were maintained in a glasshouse with a temperature range of 20-28C. Twelve weeks after inoculation plants were destructively sampled and assessed for the presence of corm discolouration on a 1-8 scale (Chen et al. 2019), with 1 being the absence of discolouration and 8 being a dead plant.

For field plantings, F_1 plants were first transferred to a shade house and after approx. 6 weeks were placed in the field at the QDAF Research Facility at Redlands, Queensland. From 14 months later pollinations were undertaken as before but, in this case, using pollen from within the same accession (i.e., self-pollination).

Outputs

Enhanced Knowledge of the Genetics of Fusarium Wilt Resistance

Generation of F_1 populations with Calcutta 4 as a Fusarium resistant parent

Seed was obtained using Calcutta 4 both as a male and as a female parent in crosses with a Fusarium susceptible line of Malaccensis.

The seed from a particular cross between Malaccensis 848 (susceptible) and Calcutta 4 (resistant) undertaken in 2018, was used to generate a F_1 population. Using embryo culture, multiple clones were produced in tissue culture for each of 22 F_1 plants obtained. Two clones of each accession were prepared for field planting, whilst the remaining clones were set aside for glasshouse pot plant trials with *Foc* SR4.

Results of the pot plant trials of the F_1 plants were obtained in early 2020 showing that 11 accessions were resistant (a score of 3 or less) and 11 were susceptible (a score of 4 or more) (see Figure 1). Although F_2 assessments are still required, this 1:1 segregation pattern indicates that Calcutta 4 could be heterozygous for resistance *Foc* SR4.

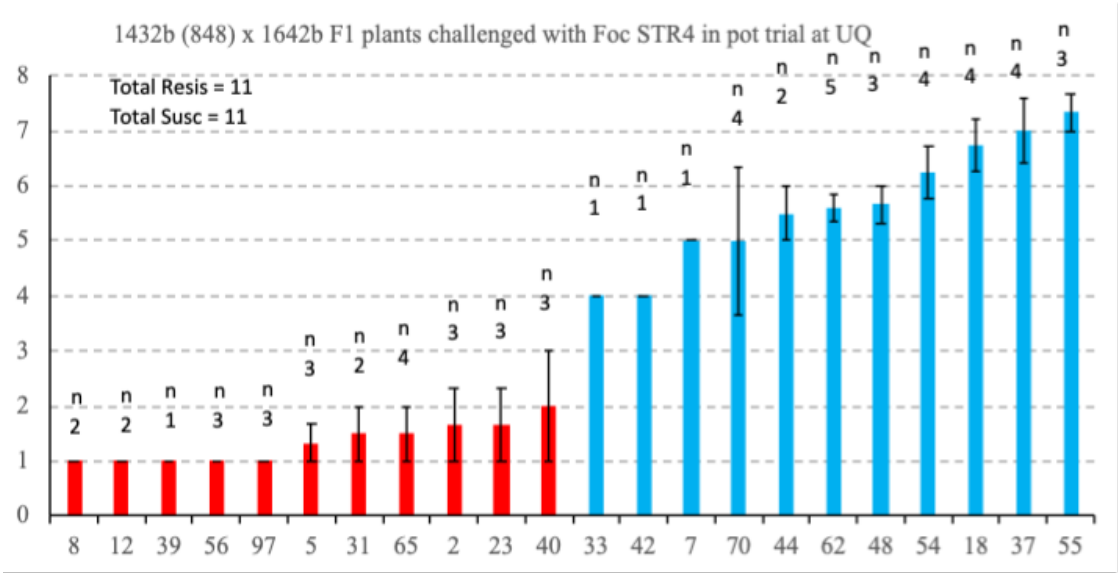

Figure 1. Internal disease score on scale 1-8 of F_1 plants from a Malaccensis susceptible x Calcutta 4 cross. The number of clones (n) assessed for each line was dependent on availability.

The F_1 plants were placed in the field at Redlands in September 2019. The F_1 plants in the field at Redlands are mostly of a small stature, between 2-3m in height (see Figure 2). The first accession to flower did so after 9 months but most have taken 14 months to flower. Self-pollinations have been undertaken commencing November 2020; these were dependent on female and male flowers of the same accession being available at the same time. Seed has not yet been harvested. The minimum successful generation time (from pollination of the original cross to self-pollination of the F_1 plants) was 20 months (see Appendix 1).

Figure 2. F_1 plant number 23 (from the Mal848 x Calcutta 4 cross) at Redlands field site (top right). First hand from a newly opened bract after self-pollination using donor male flowers from a clone of the same accession top left), close-up of the stigmata with pollen grains on surface following pollination (bottom image).

Generation of F_1 populations with PJB as a Fusarium resistant parent

Of the two clumps of PJB in the field, the first flowering stem was produced on one clump after 14 months and on the second clump after 20 months. These very tall plants produced multiple hands with parthenocarpic fruit in the first few hands.

Attempts to use PJB as a female parent in the crosses were unsuccessful and when left to allow open pollination to occur no seed was produced. The anthers of the male flowers appeared waxy (see Figure 3) regardless of the time of day observed and only a few pollen grains were observed on occasional flowers. Pollination attempts onto susceptible Malaccensis lines were not successful, although only a few attempts were possible because of lack of available female Malaccensis flowers at the time.

PJB is known to have poor fertility but the production of some pollen grains, albeit infrequently, does support the case for further attempts to be undertaken. PJB is genetically very distinct from Malaccensis and thus more likely to have a distinct source of resistance and for that reason further attempts to generate a segregating population for potential marker development are warranted.

Figure 3. The waxy anthers on the male flowers of a Pisang Jari Buaya (PJB) inflorescence

Generation of F_1 populations with SH3362 as a resistant parent

Seed was obtained using SH3362 both as a male and as a female parent in crosses with a Fusarium susceptible line of Malaccensis. The seed from a particular cross between a susceptible Malaccensis line C1-1 and the resistant SH3362 undertaken in 2019, was used to generate a F_1 population (see Appendix 2). Malaccensis line C1-1 was previously generated from a cross between Malaccensis 850Rr x Malaccensis 848rr, Rr = a resistant plant carrying a dominant R and recessive r allele for *Foc* resistance, i.e. heterozygous for the locus, and rr = homozygous alleles at the same locus for a *Foc* susceptible individual.

Using embryo culture, multiple clones were produced in tissue culture for each of 20 F_1 plants obtained. Single clones of each accession were prepared for field planting, whilst the remaining clones were set aside for glasshouse pot plants trials with *Foc* SR4.

The F_1 plants known to be resistant were placed in the field at Redlands in August 2020 in a new plot where banana had not been previously grown and where it is hoped that *Foc* SR4 was not present (Figure 4).

As of April 2021, after 8 months in the field, flowers had not yet to developed on the F_1 plants. It should be noted that a stand of Malaccensis 848 planted in the same plot had flowered after 5 months.

The pot plant trial results following inoculation with *Foc* SR4 on these F_1 plants were obtained in August 2020 showed that 12 accessions were resistant and eight were susceptible.

Figure 4. F_1 plants (Malaccensis susceptible x SH3362) in a shade house prior to planting (left) and in the field (right) at Redlands following planting in August 2020

Generation of F_1 populations with other Potential diploid lines

Additional diploid plants known to have resistance to Fusarium wilt have been planted in the field at Redlands with aim of carrying out further crosses with the susceptible Malaccensis line. These include the lines known as Tjau lagada; Tuu gia and Pisang Gajih Merah (Figure 5). Additionally, three further clones of Pisang Jari Buaya have been planted. Five clones of the original susceptible Malaccensis line 848 have also been planted in this plot and will be used as the susceptible parent in crosses with the aforementioned resistant diploid lines.

Figure 5. Diploid lines in the field plot at Redlands, 5 months after planting

Molecular markers for use in breeding programs

Ongoing Studies on the Malaccensis Resistance

As stated previously a molecular marker associated with Fusarium resistance, to both *Foc* SR4 and *Foc* TR4, has been generated using a segregating Malaccensis population.

Ongoing studies using this population are being conducted to identify the actual gene or genes that are conferring resistance. This has involved creation of a larger segregating population, pathogenicity testing and bioinformatic analysis. This work has been supported by the IITA/BMGF funding that recommenced in January 2020 (Figure 6). It is also being supported by extensive international collaborations with colleagues at CIRAD, Bayer Crop Sciences (U.S.A) and the State University of Colorado. A more defined region on banana chromosome 3 has now been identified compared with previously available data. This may allow the development of a new molecular marker and/or lead to actual identification of the resistance gene

Outcomes

This project has generated new knowledge on the genetics of TR4 and SR4 resistance. Through this project, we have identified that both Calcutta 4 and SH3362 are heterozygous for *Foc SR4* resistance. Throughout this project, the research team has also strengthened Australia participation in international collaboration and knowledge transfer activities, including the participation in international banana breeding and genetics programs. These are detailed below.

Increased understanding of the genetics of resistance in wild diploid lines

The diploid lines Calcutta 4, Pisang Jari Buaya (PJB) and SH3362 had previously been shown to be resistant to *Foc* SR4 and *Foc* TR4. The genetics controlling the resistance in these lines was not known. By crossing both Calcutta 4 and SH3362 onto a Fusarium susceptible line we obtained populations segregating for resistance and susceptibility in the F_1 when challenged with *Foc* SR4. This indicates that both Calcutta 4 and SH3362 are likely heterozygous for resistance to *Foc* SR4 and not homozygous for resistance.

Assessment of the F_2 generation (self-pollination of the F_1) against *Foc* SR4, and *Foc* TR4, will provide confirmation of the underlying genetics of resistance in these lines.

Crosses with PJB were not obtained due to infrequent occurrence of flowering and poor pollen production. However, the observation that some pollen was produced warrants further attempts to make crosses using this useful line.

Enhancement of genetic understanding and continuity of Fusarium wilt resistance research that strengthens Australian participation in international banana-breeding programs.

Funding- BMGF

The Bill and Melinda Gates Foundation (BMGF) initially supported the University of Queensland study on developing a molecular marker for resistance from a Malaccensis line through the International Institute for Tropical Agriculture (IITA) led program for the "Improvement of Banana for Smallholder Farmers in the Great Lakes Region of Africa". The University of Queensland component of that program ran from 2014 to 2018. A second program with further funding from BMGF with IITA as the lead, "Accelerated Breeding for Better Bananas", commenced in October 2019. The University of Queensland molecular marker project has been included in this second program, with continuity of research and personnel (Dr Andy Chen) made possible with the intervening funding from Hort Innovation through BA17006. Figure 6 summarises the funding received from IITA/BMGF and Hort Innovation in support of the ongoing molecular marker program over the period 2014-2022.

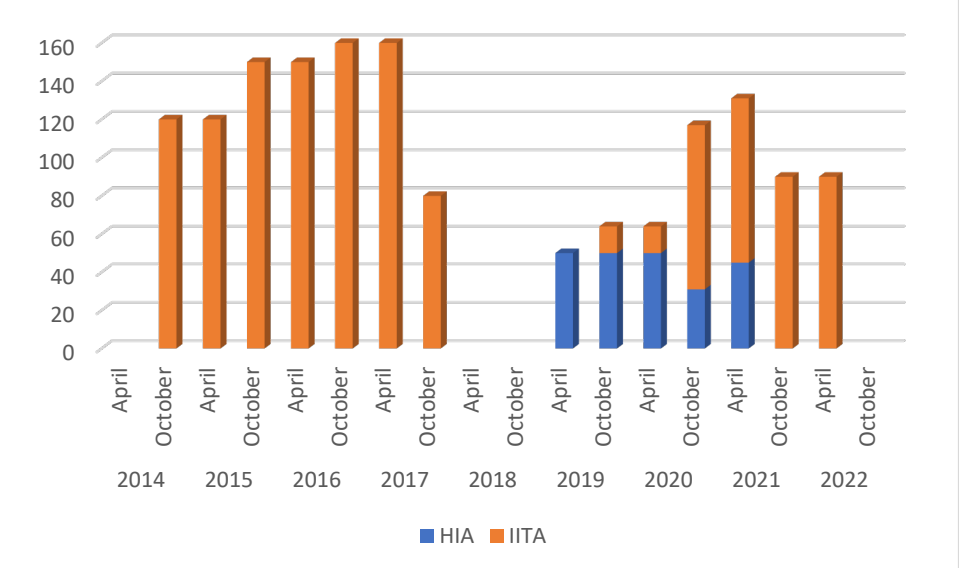

Figure 6. Funding ($1000s) received for the banana marker project per 6 month period from 2014 to 2022 from IITA/BMGF (orange) and Hort Innovation (blue). Continuity of the project in 2018 was maintained due to an agreed carry-over of funds.

Collaborations with Banana Breeding and Genetics Programs

CIRAD

Dr Andy Chen was successful in being awarded a fully funded fellowship through the Montpellier University of Excellence scheme to visit the CIRAD labs at Montpelier, France to allow further bioinformatic analysis of the Malaccensis genotypes. Unfortunately, due to Covid-19 restrictions he has not yet been able to take up this option. However, regular collaboration with CIRAD is ongoing particularly regarding the genome map of banana, which is based on a Pahang, a diploid line related to Malaccensis. The aim being to better define the map in the region where the Fusarium resistance gene is located.

Bayer Crop Science

In January 2020 Prof. Aitken was invited to attend a meeting in Germany initiated by Bayer Crop Science involving several international organisations with interests in developing means of combatting *Foc* TR4. Prof. Aitken presented an outline of the molecular marker development work. Collaborations have continued since with a view to seeking further international funding following on from the BMGF/IITA program. In the meanwhile, personnel at Bayer Crop Science USA have freely made available their time to help analyse bioinformatic data associated with the Malaccensis mapping project; this has assisted in better defining the proposed region of the resistance trait.

International Breeding Programs

The Malaccensis marker has already been used by breeding programs at IITA and EMBRAPA; more recently CIRAD has requested its use.

Stakeholder Meetings and Forums Attended

Australian Banana Congress

April 2019 Poster: Analyzing Resistance to Fusarium wilt in the wild banana sub species Malaccensis.

May 2021 Poster will be presented

Guangxi Academy of Agricultural Sciences, Nanning, China

September 2019 Visit by Prof Aitken and Dr Chen and presentation given

International Conference on Banana, Tamil Nadu India

February 2020 Prof. Aitken invited speaker (declined due to Covid-19 concerns);

Australian Banana Scientific Symposium, Brisbane

April 2021 Dr Chen will present on: “Dissection of genetic resistance to *Fusarium oxysporum* f. sp. *cubense* in *Musa accuminata* subsp. Malaccensis”

Malaysia National Banana Congress

September 2021 Prof. Aitken Invited as plenary speaker (will be presented most likely in a virtual format)

Publications

Chen A, Sun J, Matthews A, Armas-Egas L, Chen N, Hamill S, Mintoff S, Tran-Nguyen LTT, Batley J, Aitken EAB (2019). Assessing variations in host resistance to *Fusarium oxysporum* f sp. *cubens*e race 4 in *Musa* species, with a focus on the subtropical race 4. ***Frontiers in Microbiology*** 15 10:1062. doi: 10.3389/fmicb.2019.01062.

Monitoring and evaluation

This two-year project funded by Hort Innovation is part of ongoing research receiving international funding. The proposed outcomes can therefore be considered in the short, medium, and long term. At the conclusion of BA17006 these short-term outcomes have been met.

In the short term (end of BA17006 project)

- An increased understanding of the genetics of resistance in wild diploid banana line that will be used in the development of molecular markers associated with resistance.
- Enhancement of genetic understanding and continuity of Fusarium wilt resistance research that will strengthen Australian participation in international banana-breeding programs including that funded by the Bill and Melinda Gates Foundation for the benefit of the Australian Banana Industry.

Key Evaluation Questions

Proposed Question	Project Teams' Response
Effectiveness	
To what extent has/have • F_2 plants been screened for Fusarium wilt • Increased understanding of genetics of resistance been obtained • Calcutta 4 and/or PJB resistance been compared with that of known resistance source in Malaccensis.	• F_2 seed in the progeny of the Malaccensis x Calcutta 4 cross are developing and should be harvested mid 2021 with expected F_2 plant screening in early 2022 • Segregation of resistance and susceptibility in the F_1 of both crosses undertaken illustrates that resistance in Calcutta 4 and SH3362 is likely heterozygous • It is not yet known if the resistance in Calcutta 4 and SH3362 is distinct from that in Malaccensis.
Relevance	
To what extent has the project: • Developed information for additional and/or improved molecular markers for use in the screening of germplasm to Fusarium wilt for ultimate introduction into Australia. • Shared research outputs with international research community allowing increased access to participation in international banana breeding efforts for the benefit of the Australian Banana Industry. • Continued to develop research outputs to leverage funding and research exchange from and with international agencies	• The Malaccensis molecular marker has been used by EMBRAPA and CIRAD; both these organisations send material to Australia via QDAF. It is not known however if they have prioritised the material available based on the markers. Dr Edson from EMBRAPA had intended to spend a sabbatical at the University of Queensland and would have worked specifically on this but this has been delayed due Covid-19 related travel restrictions • Both EMBRAPA and CIRAD have access to the University of Queensland Malaccensis molecular maker and can select material based on that. • Continued international interactions are ongoing. Additional *Foc* TR4 trials on our populations will be undertaken at the Univ. of Stellenbosch under the current IITA program and possibly at Univ. of Wageningen related to proposed future funding.
Process Appropriateness	
• To what extent were target levels of engagement of levy payers achieved?	• Australian Banana Congress poster presentation in 2019 and intended in 2021. • For this stage in the project the level of

• Was the range of communication and engagement processes used to the target audience appropriate? • Was content regularly provided through the communications project?	communications is considered appropriate • Project milestone updates communicated to the Australian Banana Gowers Council R&D manager
• To what extent and how was interaction with international breeding programs achieved? • Were peer reviewed scientific publications produced and published?	• Interaction with international breeding programs has been with EMPRAPA by email and includes a proposed joint publication; CIRAD by regular Skype meetings, grant application and also proposed joint publications; IITA by on-going funding from BMGF, Zoom meetings and email. All these organisations have used or intend to use the University of Queensland molecular marker on banana germplasm. • One publication has been published in Frontiers in Microbiology and another is in draft form awaiting final data
Efficiency	
To what degree has this project • Effectively interacted with international banana researchers and breeding programs for more cost effective and faster achievements towards exploring *Foc* TR4 resistance • Has student participation assisted in the project achievements? • Have additional funds been injected to the project from other sources?	• The interaction with international banana researchers has been excellent and very productive allowing better prediction of the location of the Malaccensis resistance gene. This includes: ▪ CIRAD's banana genomics group headed by Dr Angelique D'Hont and the bioinformatics group at Bayer Crop Science. ▪ Screening with the Malaccensis marker EMBRAPA and IITA ▪ Assistance in screening from Dr Jiaman Sun from Guangxi Academy of Agricultural Sciences whilst on sabbatical at the University of Queensland. • Several students including Masters' coursework and Honours students have participated in the study • As indicated funds for BMGF/IITA have been secured until 2022.

Recommendations

Identification of genetic resistance and integration into agronomically suitable banana varieties, whether by conventional breeding or via the use of genetic manipulation is imperative for the continued viability of banana production in Australia, as well as globally.

The existing Malaccensis molecular marker developed by the project team has already been used to select germplasm predicted to be resistant to Fusarium wilt.

1. *It is recommended that this marker should be used to select potential new varieties for entry into Australia.*

   Calcutta 4 and PJB potentially offer alternative sources of resistance from that found in Malaccensis.

2. *It is recommended that research should continue to characterise these resistance traits and if determined to be different from that found in Malaccensis, then molecular markers associated with the resistance should be identified for use in germplasm selection.*

   For all potential resistance sources, the best molecular marker would be the actual resistance gene/s.

3. *It is recommended that work continues in the identification and cloning of such genes for use in marker selective conventional breeding or in gene manipulation protocols.*

   Breeding in banana is complex and unlike many other major crop plant species, genetic and bioinformatic resources are limited. Progress towards identifying resistance genes in banana has greatly benefitted from international collaborations.

4. *It is recommended that the Australian banana research community continues to support such collaborative research efforts for the benefit of the Australian banana industry.*

At the onset it was recognised that the desired outcomes of this research would extend beyond BA17006. These previously itemised medium- and long-term outcomes are listed below. Through international collaborations and funding these outcomes may be achievable.

Medium Term (2-5 years): The identification of additional molecular markers associated with Fusarium wilt resistance traits which will be used to develop and support efforts of continual introduction of resistant varieties into Australia that possess resistance to the Fusarium wilt fungus strains TR4 and SR4.

Long Term (greater than 3 years): The outcome of this research will lead to the more rapid progress towards the identification of commercially resistant banana varieties for the benefit of the Australian banana industry.

Refereed scientific publications

Journal article

Chen A, Sun J, Matthews A, Armas-Egas L, Chen N, Hamill S, Mintoff S, Tran-Nguyen LTT, Batley J, Aitken EAB. (2019) Assessing Variations in Host Resistance to *Fusarium oxysporum* f sp. *cubense* Race 4 in *Musa* species, With a Focus on the Subtropical Race 4. ***Frontiers in Microbiology.*** **10**:1062. doi: 10.3389/fmicb.2019.01062.

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Chen A, Sun J, Matthews A, Armas-Egas L, Chen N, Hamill S, Mintoff S, Tran-Nguyen LTT, Batley J, Aitken EAB. Assessing Variations in Host Resistance to *Fusarium oxysporum* f sp*. cubense* Race 4 in Musa Species, With a Focus on the Subtropical Race 4. ***Frontiers in Microbiology*** **10**:1062. doi: 10.3389/fmicb.2019.01062.

Pilet-Nayel ML, Moury B, Caffier V, Montarry J, Kerlan MC, Fournet S, Durel CE, Delourme R. (2017) Quantitative Resistance to Plant Pathogens in Pyramiding Strategies for Durable Crop Protection. ***Frontiers in Plant Science.*** **8**:1838. doi: 10.3389/fpls.2017.01838.

Intellectual property, commercialisation and confidentiality

An Intellectual Property Register has been developed with Hort Innovation. This register contains details regarding project outputs and project background intellectual property.

No project outputs have been identified for commercialisation from this project.

There are no intellectual property, commercialisation or confidentiality issues to report.

Acknowledgements

In developing and screening populations with Calcutta 4 and PJB

At the University of Queensland: Dr Andy Chen (Research Fellow); with technical support from Ms Ning Chen, Ms Andrea Matthews and Mr Brendan Fu

At Queensland Department of Agriculture and Fisheries: Ms Sharon Hamill (Maroochy Research Station); Mr Dave Flatley and colleagues (Redlands Farm)

Ongoing work in refining the Malaccensis molecular marker

Dr Andy Chen; CIRAD (Drs Angelique d'Hont; Nabila Yahiaoui, Guillaume Martin); Bayer Crop Science USA; State University of Colorado; Dr Jiaman Sun from the Guangxi Academy Agricultural Sciences.

Appendices

Appendix 1. Timeline of Malaccensis (susceptible) x Calcutta 4 F_1 production and progeny testing

Female Parent		Cross		Male Parent	
August 2017	Malaccensis rr 848 (clone 1432(-20) b) planted			August 2017	Calcutta 4 (clone 1642b) planted
				March 2018	First flower produced Used as pollen donor*
				July 2018	(open pollinated seed harvested)
		9/3/18	Ma848 H1,2 x Calcutta 4 Cross undertaken		
		31/7/18	Seed harvested		
			F_1 embryo cultured 22 accessions maintained in glasshouse		
		24/9/19	two clones (a and b) each of 13 accessions planted in field		
				22/1/20 July 2020	F_1 accessions screened v *Foc* SR4 = 7R: 7S Repeat screen + 8 more accessions = 11R: 11:S
		21/1/20	Pathogenicity tests known; seven accession susceptible leaving accessions #2,5,8,23,31,39,56 for potential self-seed production		
		30/6/20	#2 flowered, small stature plant, no synchronous flowering		
		3/11/20	#8 flowered		
		6/11/20	#8 H1,2 x #2b		
		9/11/20	#8 H3-5 x #8 (H5 pollen)		
		8/1/21	#23b H1 x #23a		
		11/1/21	#23b H2-4 x #23a		

*Reciprocal cross also produced seed, currently in store

H#= hand number; R=resistant; S= susceptible

Appendix 2. Timeline of Malaccensis (susceptible) x SH3362 F_1 production and progeny testing

Female Parent		Cross		Male Parent	
August 2017	Malaccensis rr (850x848 H3F1E14C1-1) planted			August 2017	SH3362 planted
April 2018	First flower			December 2018	First flower* Used as pollen donor
18/1/19	Emerging flower bagged				
		21/1/19	Mal H1 x SH3362 cross undertaken		
		2/8/19	Seed harvested		
		2/1/20	F_1 deflasked from tissue culture		
		11/5/20	23 F_1 transferred to shade house at Redlands		
				20/8/20	F_1 screened in glasshouse v *Foc* SR4 = 12R:8S
		21/8/20	16 F_1 (12 known resistant) planted at Redlands		
		19/3/21	Healthy plants but not yet flowering		

*Reciprocal cross also produced seed, currently in store

H#= hand number; R=resistant; S= susceptible