Final Report to the Australian Flora Foundation

Project Aim

Development of molecular markers for resistance to myrtle rust in Australian Myrtaceae.

Background

Most Australian myrtaceous species have been shown to be vulnerable to infection by the fungal pathogen *Austropuccinia psidii* (Beenken, 2017), causal agent of myrtle rust. Although a species may be susceptible to infection by *A. psidii*, a small proportion of resistant individuals are usually found in genetically diverse wild populations when tested in controlled inoculations (Morin et al., 2012). These variable responses suggest that resistant individuals, when identified, may be useful for replanting and breeding programs and to develop molecular markers for germplasm screening (Butler et al., 2016; Mamani et al., 2010).

A previous transcriptome analysis identified candidate genes that are associated with myrtle rust resistance within the east coast Australian littoral rainforest tree, *Syzygium luehmannii* (F. Muell.) L.A.S. Johnson (Tobias et al., 2018). While field specimens of *S. luehmannii* are generally resistant to disease from *A. psidii*, individual plants show variable responses under controlled inoculation. The identified candidate genes, both recognition receptors, seemed promising to investigate further as a molecular marker for resistance, both within species and potentially the wider Myrtaceae family. The receptor genes were homologs for a *Eucalyptus grandis* annotated nucleotide binding site leucine rich repeat-type resistance gene (NLR) and a receptor-like kinase (RLK) (Myburg et al., 2014). However, with no reference genome for the *S. luehmannii* available, and the well-known problems in assembling immune receptor genes due to their repetitive structure (Steuernagel et al., 2020), it was determined to resolve the full sequences computationally before designing primers to test as molecular markers.

1. Computational characterization (based on available sequence data)

Existing RNA-seq data from inoculated plants, (National Center for Biotechnology Information BioProject PRJNA356336) was used to make targeted new assemblies of the currently identified genes for resistance within *Syzygium luehmannii*. Custom developed python scripts were used to identify all reads that matched with the closest translated gene homolog from *E. grandis* (Eucgr.C01968 and Eucgr.K03223) and then assembled with the reads into 'genes' with trinity/2.5.1 (Haas et al., 2013). Outputs were multi-sequence fasta files for both putative NLR-type resistance gene (*APR* for *Austropuccinia psidii* resistance) and receptor-like kinases (*RLK*) previously noted for differential expression in resistant plants. The

primers could then be tested across a range of Myrtaceae plants that were either resistant or susceptible to the rust disease.

2. Primer development and testing.

Based on the comprehensive assembly of the receptor genes and using the longest assembled 'transcript' in resistant plants, primers were developed and tested on cDNA (Figure 1). Primers were designed to span the conserved NBARC domain and the variable leucine-rich repeat (LRR) region for the receptor thereby spanning intronic regions. The expected amplicon size from DNA amplification should be larger than for the cDNA (712 bp), though without a genome for the plant, the size was not possible to predict. RNA-seq differential expression previously indicated that the putative RLK was absent in susceptible plants pre-inoculation and that the APR gene was upregulated in resistant plants at 24 and 48 hour post inoculation. The PCR amplification of cDNA, however, showed that the putative RLK gene was present in all plants, while the APR gene only amplified in two resistant plants, R1 and R4 (Figure 1). As the original experimental plants were not able to be retained in 2017, the primers were not tested on plant DNA so further validation was not completed. Successful primers for APR were; TGATGTAGATCATGCGAGCCAA (forward), AACGGTTTCGGTTCCCTTCTTTTG (reverse) and for RLK; GGGCAATGAGACTCCTAATACT (forward), and ACCACGCCAAAGCTATACACA (reverse).

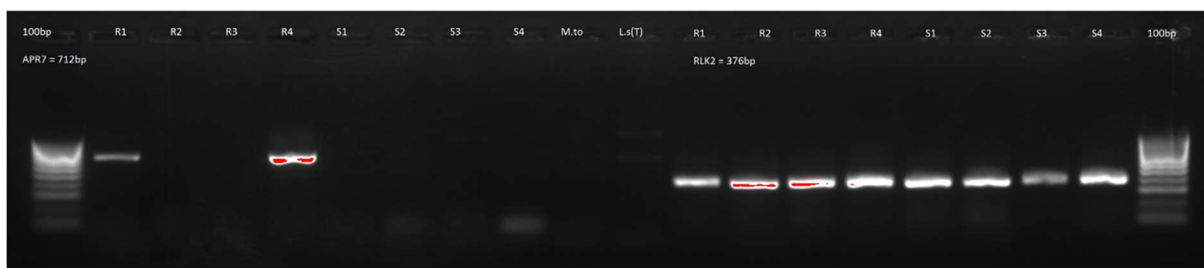

Figure 1. Primer testing for putative resistance (APR) and receptor-like kinase (RLK) genes were tested on cDNA from susceptible (S) and resistant (R) *Syzygium luehmannii* plants showing that RLK was present in all samples and APR was present in only two resistant samples (R1 and R4).

3. Further screening for phenotype in response to inoculation with *A. psidii*

In 2018, additional *Syzygium luehmannii* plants (n=50) were purchased, inoculated under controlled conditions and scored for response after 10 days with 74 % susceptible and 26 % resistant. The APR primers that were promising in cDNA resistant plants were tested against DNA from three highly susceptible (Syz 6, 37 and 50) and four highly resistant *S. luehmannii* plants (Syz 3, 5, 9 and 36), as well as from two *Melaleuca quinquenervia* (Mq1 152 and 48) plants that had been screened and phenotyped (Figure 2). The results were inconclusive and

indicate that this class of resistance genes are highly polymorphic across individuals both within the species of *S. luehmannii* and across another myrtaceous species.

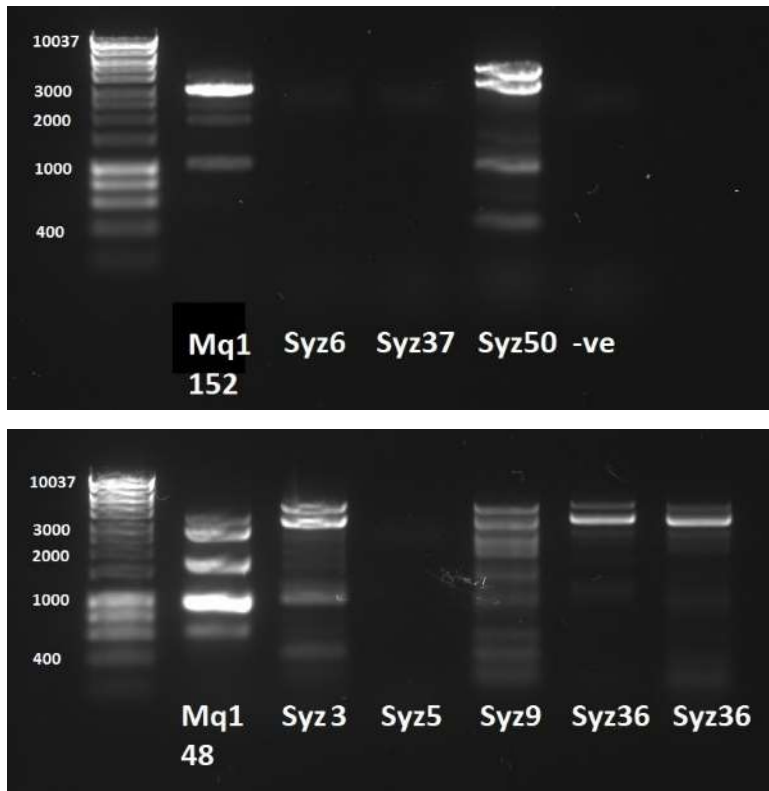

Figure 2. Primer testing for putative resistance (APR) gene were tested on DNA from susceptible (above) and resistant *Syzygium luehmannii* (Syz) (below) and *Melaleuca quinquenervia* (Mq1) plants. Ladder is bp.

4. Results and discussion

The results of testing molecular markers for putative resistance gene markers against *A. psidii* infection in Myrtaceae were inconclusive. The primers indicated that a specific resistance receptor (APR) was present in gene expression from two of four resistant *Syzygium luehmannii* plants and absent in all the susceptible plants, based on amplification of cDNA (Figure 1). While the putative gene may indeed be upregulated only in these resistant plants, it is not confirmed as a key indicator for resistance as the transcript is absent for the other two resistant plants. The putative RLK receptor amplified in all tested plant cDNA and was therefore not a useful indicator going forward. PCR amplification of the APR primers conducted on the DNA from additional phenotyped plants (Figure 2) was also inconclusive. The non-specific binding and amplification show that the primers are complementary to several regions of DNA in many of the samples. Future work may investigate these markers in greater detail however their use as molecular markers to indicate resistance to the pathogen causing myrtle rust is not currently viable.

5. Additional notes

The Australian Flora Foundation's (AFF) generous support was acknowledged at the following conferences:

2018 Queensland Molecular Biology Conference, New Zealand

2021 NSW DPI SoS Conference

2021 The Myrtle rust Symposium, Ballina

2022 The Mycological Society

The AFF were included as industry partners in a successful ARC Linkage Grant (LP190100093) that further developed strategies to understand the impacts of myrtle rust. Additionally the AFF is acknowledged as a funder for a new myrtle rust genome to aid plant:pathogen investigations. The related manuscript is a preprint due to ongoing work, however the authors wished to make the data public for other researchers and therefore it is currently available on BioRxiv.

https://www.biorxiv.org/content/10.1101/2022.04.22.489119v1.full

6. Acknowledgements

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7. References

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