Identification of candidate genes involved in resistance to myrtle rust in Riberry

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Myrtle rust – causal agent - a biotrophic fungal pathogen, *Austropuccinia psidii*
Australian Myrtaceae plants and myrtle rust

Of Australian species tested 94% were susceptible (108/115) (Morin et al. 2012)

http://www.anbg.gov.au
Riberry is a commercially grown and harvested Australian Myrtaceae

Natural distribution of *Syzygium luehmannii* (Riberry / Lilly Pilly)
http://www.ala.org.au/

Fresh, dried or frozen it is used in sauces, jams, desserts, salads
Inoculated 103 plants: 75% resistant (29% hypersensitive response)

Fungal penetration (at 48 hrs) in both resistant and susceptible

But the pathogen is inhibited in resistant plants – programmed cell death at entry point and surrounding cells
Disease resistance determined by early recognition of pathogen

- Transcript studies identify genes expressed at a precise moment in time
- Expect that the plant trait is related to the expressed genes
- Look for differential gene expression between resistant and susceptible individuals
- Specifically look for recognition receptors that initiate global gene expression change for disease resistance

## RNAseq Trinity *de novo* assembly results

Used CD-EST-2D-HIT to merge transcriptomes - **69,736 transcripts** (*Eucalyptus grandis* - 46,280 protein coding transcripts)

<table>
<thead>
<tr>
<th>Plant ID</th>
<th>Total trinity 'genes'</th>
<th>Total trinity transcripts</th>
<th>% GC</th>
<th>N10</th>
<th>N50</th>
<th>Median contig length</th>
<th>Total assembled bases</th>
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</table>
Expression differences in Resistant versus Susceptible plants

DE with EdgR (significance cut-off at FDR 0.01)

Pre-inoc.

n = 33

24h

n = 63

48h

n = 187
Susceptible (A) and Resistant (B) DE at 0 versus 48hrs

\[ a = \text{zinc finger protein}, \ b,d = \text{carboxylesterase 12}, \ c = \text{Puccinia psidii ITS}, \ e = \text{zinc finger CCCH domain-containing protein}, \ f = \text{myosin heavy chain kinase}, \ g = \text{metalloendoproteinase}, \ h = \text{strigolactone esterase D14}, \ i = \text{auxin-regulated gene involved in organ size}, \ j = \text{uncharacterized}. \]
Resistant versus susceptible receptor expression

Lectin RLK homologs

NBS-LRR homologs
I-Tasser protein model (left): TIR-NBS-LRR receptor homologue **up-regulated** in **resistant** plants (closest homolog in *E. grandis* is within the *Ppr1* locus, chr 3)

ExPASy Prosites (below): Lectin Receptor-like Kinase homologue **only** present in **resistant** plants pre-inoculation. A transmembrane receptor with three domains; bulb lectin, PAN and protein kinase.
A. D14

B. PAL

C. R burst oxidase

D. Chitinase

E. Thaumatin-like

F. DRR

G. RLK

H. MybTF

I. LRR extensin
Results

• Resistant plants respond rapidly to inoculation.

• Susceptible plants appear to not recognise pathogen.

• Differential expression largely comprised of genes coding for transcription factors, enzymes in the secondary metabolite pathway, receptor-like kinases and defence.

• Two recognition receptors that look interesting.
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