The Search for Gene H

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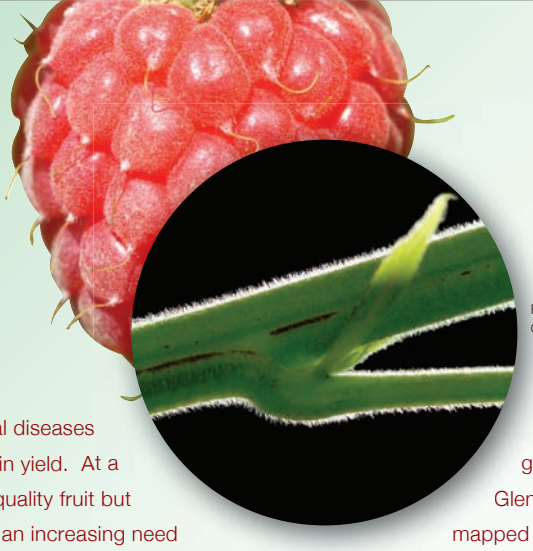


Figure 1.
Cane hairs in Rubus

Raspberry is prone to infection by several fungal diseases which can potentially cause serious reductions in yield. At a time when there is consumer demand for high quality fruit but resistance to chemical disease control, there is an increasing need to breed disease resistant raspberry cultivars. Resistance to some fungal diseases is associated with morphological characters, particularly cane pubescence (fine hairs (Figure 1)), an epidermal cell trait which is determined by gene H (HH or Hh giving hairs; hh giving glabrous canes). Utilising a cross between Glen Moy (Hh) and Latham (hh) this trait has been mapped to linkage group 2 and has been shown to be closely associated with resistance to cane botrytis and spur blight but not rust or cane spot[1]. The challenge now is to identify what gene H is and how it contributes to resistance to these diseases.

Materials and Methods

The existing markers lying closest to gene H are small AFLP fragments or were present only in the Latham parent.
To get closer to gene H in raspberry, two approaches are being used:

A. Identifying New Markers Close to Gene H

- DNA from progeny from a cross between Moy and Latham plants with a known hairy or non-hairy phenotype were bulked and subjected to AFLP analysis using 56 *Mse*I/*Eco*RI primer combinations.
- Products were analysed on an ABI3730 automated sequencer to determine the presence or absence of bands.
- AFLPs yielding bands that were present in bulked hairy samples but absent in the non-hairy samples were repeated radioactively and the bands excised, cloned and sequenced.
- AFLPs were run on the whole 188 progeny from the Moy x Latham cross to allow the bands to be mapped.

B. Targeted Gene Approach

- In plants a common set of proteins (WD40, bHLH and MYB) interact to form a regulatory complex that controls epidermal cell identity, including hairs, and ensures proper spatial and temporal distribution of specialised epidermal cells[2].
- Primers were designed to these proteins and used to amplify the corresponding genes in raspberry.
- Sequences to MYB and WD40 have been used to screen the raspberry BAC library and positive BAC clones are being sequenced and mapped in the Moy x Latham population.

Results

A. Identifying New Markers Close to Gene H

- A 256 bp band was identified in hairy but not in non-hairy samples from one AFLP combination (Figure 2).
- The AFLP was run on the mapping population and lies within 5 cM of gene H (Figure 3).
- The band was sequenced but showed no homology to sequences in the database.
- Using the sequence of the AFLP band a gene walking technique was used and a 500 bp fragment obtained. Amplification of this band in Moy and Latham has led to the identification of a single nucleotide polymorphism (SNP) (Figure 4).
- This has been mapped to the same location as the AFLP fragment and lies close to Gene H.

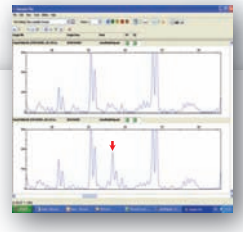


Figure 2. Eectrophero-gram of the AFLP products from hairy (H) /non-hairy (NH) samples.

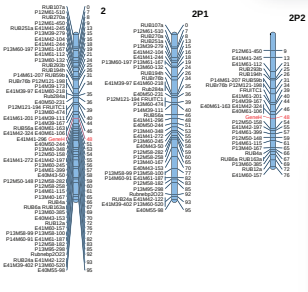
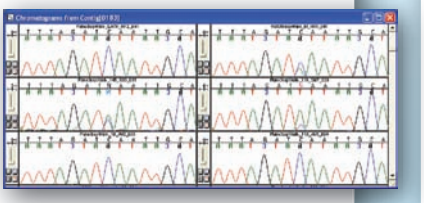


Figure 3. Linkage group 2 in raspberry

Figure 4. SNP in Moy and Latham sequences derived from the AFLP E40M12-256 band



B. Targeted Gene Approach

- WD40, bHLH and MYB PCR products have been amplified and sequenced.
- WD40 and MYB sequences have been used to identify a total of 20 clones from the raspberry BAC library which are being placed onto the genetic linkage map.
- One WD40 clone (Ri25D10) contains an SSR which gives a complex pattern but can be scored as an AFLP. Fragment 25D10_235 maps to linkage group 2 and is significantly associated with gene H.
- Ri9O22 contains a MYB gene and is located on linkage group 3.

Discussion

Using two different approaches we are moving closer to the gene H region in raspberry. The 256 bp AFLP fragment has been mapped close to gene H in Glen Moy and has been validated using a SNP approach. Now, the larger fragment obtained by gene walking will be used to probe the BAC library, allowing us to move closer to the sequences underlying this trait.

Targeting candidate genes involved in epidermal cell fate allows functional genes to be placed on the raspberry linkage map and using these to identify the corresponding BACs permits the physical map to be anchored to the genetic map. In addition, as many of the genes involved in epidermal cell fate are also involved in anthocyanin synthesis, this approach means that some fruit quality-related genes may also be identified and mapped.

References

1. Graham, J., et al., (2006). Mapping gene *H* controlling cane pubescence in raspberry and its association with resistance to cane botrytis and spur blight, rust and cane spot. *Theoretical and Applied Genetics*, **112**: 818-831.

2. Ramsay, NA & Glover BJ (2005) MYB–bHLH–WD40 protein complex and the evolution of cellular diversity. *Trends in Plant Science*, **10**: 63-70.

Acknowledgements
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