Red raspberry (Rubus idaeus)

Outline of Presentation

- Background
- Resources
- Adoption of NGS & Arrays

Background

- Red raspberry is a rich source of nutrition with established health benefits.
- Greater consumer interest in fruit and promotions for healthier lifestyles.
- Enormous scope for market expansion.

- Increase consumption:
  - improve consistency in taste, appearance, appropriate firmness and shelf life of fruit.
- Industry relies on small number of varieties, and a decreasing number of chemicals for pest and disease control.

Solution

- New high quality varieties with resistance to major pests and diseases.

Resources

‘Latham’ x ‘Glen Moy’ segregating population used to identify QTLs for fruit quality traits, plant architecture and a range of pest and disease resistances.

- Fruit ripening (Graham et al., 2010)
- Colour (McCallum et al., 2010)
- Anthocyanins (Kassim et al., 2009)
- Aroma Volatiles (Patterson et al., 2013)
- Size
- Sugars
- Acids
- Flavour components
- Root rot (Graham et al., 2011)
- Cane diseases (Graham et al., 2006)

‘Latham’ x ‘Glen Moy’ mapping population (188 progeny).

- North American ‘Latham’
  - Sweet and aromatic
  - Small rounded fruit
  - Dark red
  - Resistant to root rot
  - Late ripening
  - Released 1930’s

- European ‘Glen Moy’
  - Moderately sweet
  - Large conical shaped fruit
  - Pale red
  - Susceptible to root rot
  - Early ripening
  - Released 1980’s

Fruit ripening (Graham et al., 2010)

Colour (McCallum et al., 2010)

Anthocyanins

Aroma Volatiles (Patterson et al., 2013)

Size

Sugars

Acids

Flavour components

Root rot (Graham et al., 2011)

Cane diseases (Graham et al., 2006)
**Mapping Anthocyanin Pigments**

- Pigments: Impart deep vibrant coloration to berries
- Elucidate factors that influence anthocyanin production in raspberry.
- Quantified (HPLC) the eight anthocyanin pigments: Cyanidin & Pelargonidin glucosides: Cyanidin 3-glucoside (C3G), 3-sophoroside (C3S), 3-rutinoside (C3R), glyconutinoside (CGR).
- Pelargonidin 3-glucoside (P3G), 3-sophoroside (P3S), 3-rutinoside (P3R), glyconutinoside (PGR).
- Across two seasons and from two different environments (open field and tunnel).

**Anthocyanin Results**

- Seasonal and Environmental Effects on Anthocyanins Production:
- Higher total anthocyanin pigments in 2006 (higher temps and sunshine hours, less rainfall than 2007).
- Higher total anthocyanin pigments in open field.

(Kassim et al., 2009: Woodhead et al., 2008)

**Resources**

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- Colour (McCallum et al., 2010)
- Anthocyanin (Kassim et al., 2009)

**Aroma Volatiles**

- Size
- Sugars
- Acids
- Flavor components
- Pest (Graham et al., 2011)
- Cane diseases (Graham et al., 2006)

**Mapping Raspberry Aroma Volatiles**

- Compositional make up of food (fingerprint).
- 213 volatiles identified in red raspberry.
- 12 important to aroma:
  - e.g. Raspberry ketone, a-ionone, a-ionol, b-ionone, b-damascenone, linalool, geraniol benzyl alcohol
  - Hexanoic, acetic acid
  - Acetoin, 3-3-hexanol

**QTL Mapping Raspberry Aroma Volatiles**

- Results:
  - 4 QTLs controlling key volatiles were identified.
  - Volatiles from the same pathway mapped to same locus.
  - Specific variation has significant effect on volatiles concentration produced (2006–2007).
Adoption of Next Generation Sequencing (NGS) Technology

RNA extracted from white and red fruit stages of Latham and Glen Moy - 454 sequencing.

**Raspberry**
- 275Mbp
- 14 chromosomes
- Diploid

**Result**
- Over 1.3 million sequences were generated with similar size distributions (400-500 bases).
- Assembly containing 63,811 contigs, including 936,487 reads.
- Database of genes expressed in raspberry fruit (‘fruit transcriptome’) was compared to available genomes and mined for genes involved in fruit quality traits.

Rubus Microarray Analysis

- Two arrays (8 x 60k format) run to determine relative abundance of nucleic acid sequences in various fruit developmental stages collected from 3 biological replicates of Moy and Latham.

**Results**
- Around 36,000 (65%) probes showed an expression signal in fruit.
- Significant gene expression changes:
  - 20,000 on the basis of fruit stage,
  - 8,000 on the basis of genotype, and
  - 700 have interaction between stage & genotype.

Fruit Softening Traits

Definitions:
Loss of firmness during fruit ripening in raspberry is associated with:
- Loss of skin strength,
- Separation of drupelets from the receptacle,
- Breakdown of cell walls in the mesocarp.

Softening is dependent on the co-ordinated, interdependent activities of many genes and their regulation and action under differing environmental conditions.

To identify the genetics of fruit softening, utilized the ‘Latham’ ‘Glen Moy’ mapping population together with the latest generation of genomic tools.

‘Glen Moy twice as firm as Latham’

Development of an Agilent Dual Mode Gene Expression Rubus Microarray

- Identify genes that determine cultivar differences in fruit quality by comparing expression profiles of raspberry cultivars.
- Design unigene set assembled from existing sequence resources, different tissues and stages.
- In total, 176,323 sequences were assembled generating 41,155 contigs and 22,098 singletons.
- Sequences were BLASTx searched and a total of 55,708 unigene probes were designed and utilised in a 8 x 60k format.

Rubus Fruit Development Array

**Fruit development time course**

- Examples of both gene expression increasing or decreasing during fruit development.
- Genotypic differences.

Mapping Softening Phenotype

- Ripen fruit samples from field and polytunnel production (2 years) using:
  - ‘Breeder score’ of firmness on a 1 - 4 (Firm – Soft) scale,
  - 16 measurements with a QTS-25 Texture Analyzer.

**Results**
- Calculations for Hardness, Rigidity, Final load, and Force/Mass.
- Breeder score and Mass (10 berry weights) were significantly (P< 0.001) more heritable and QTLs located on linkage groups (LG) 1, 3 and 5.

QTS-25 Texture Analyzer validated:
- Reliable quantitative measurement of fruit firmness that is comparable with a ‘breeder score’.
- Identify chromosomal regions responsible for trait variation.
Candidate Softening Genes

- Candidate genes with expected roles in fruit softening were identified using databases together with the 454 datasets.
- Data for 20 different genes associated with cell wall hydrolysis were mapped onto the JHI Rubus genetic linkage maps 1-7.
- Candidate genes distributed across all 7 Rubus linkage groups, the majority on LG 3.
- Xylo and pectinase activity (PME, LG1, LG3) are two activities occurring in the softening process and are key genes.
- Key:
  - databases
  - RF, ripe fruit
  - WR, white/red fruit
  - MG, mature green
  - IG, immature green

RNA quality control and RT-qPCR validation

- The minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines (Bustin et al. 2009) were followed.
- Robust pipeline for extracting RNA, designing and evaluating PCR assays for RT-qPCR experiments using geNorm software.
- Total RNA from 5 different stages of raspberry fruit from 3 biological replicates.
- Novel reference genes Claithr, YLS8, and TIP41 were most suitable for accurate normalization with raspberry fruit samples.

RT-qPCR validation

Mean normalized gene expression levels in Moy (M) and Latham (L)

- Relative expression levels for SAMDC show a steady increase up to the ripe fruit stage.
- XL, there is a decrease in expression from the immature green stage to white fruit, an increase in whitened fruit, followed by a significant rise in ripe fruit.
- SAMDC LG3
- XL LG5
- XL = β-1,4 xylosidase

Coordinated expression between candidate genes

- Scatter plots - stage of fruit and normalized expression levels (Aquaporin, SAMDC, PME, and XL).
- Very strong positive relationship between PME and Aquaporin, and XL and SAMDC.
- Strong negative relationship between SAMDC and Aquaporin, SAMDC and PME, XL and Aquaporin, and XL and PME.
- Coordinated expression:
  - The inhibition of PME activity coincided with the increased levels of SAMDC and XL.
  - Together these enzymes may help coordinate the process of fruit ripening with cell wall degradation processes.
The Future

- Candidate genes will be taken forward into breeding populations for confirmation of the link between various SNP/indel markers and fruit quality traits.
- Multiplex genotyping platforms to develop rapid and cost-effective screening of wide range of germplasm for many markers, as well as to further validate the association of the markers/genes identified with a trait.
- Markers deployed in marker assisted selection (MAS) programs.

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  - Robust pipeline for designing and evaluating PCR assays for RT-qPCR experiments using geNorm software.
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Coordinated expression between candidate genes

- PME enzymes involved in cell wall disassembly by increasing the in vivo susceptibility of pectins to hydrolysis.
  1. High expression of PME at early stages, decrease coincides with increased levels of PME, followed by increased levels of PL and then PG enzymes to break down the accumulation of pectin substances.
  2. SAMDC is involved in the ethylene biosynthesis pathway and shows a positive correlation with levels of XL, hydrolases of glycosidic linkages in cellulose and hemocellulose.
  3. Together these enzymes may help co-ordinate the process of fruit ripening with the cell wall degradation process.
  4. Fruit cells regulate their turgor pressure, and as cell wall integrity as they ripen and this requires aquaporins, which regulate water flow and hence turgor pressure.
  5. A positive correlation between levels of PME, XL, and a decrease in hypoxia and thus water movement during fruit development should allow exposure of substrates to the actions of hydrolyases.

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‘New high quality raspberry varieties’