Exploring the use of transposon mobilisation to produce a gene-tagged population for grapevine

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The Role of Transposons (TE’s) in Crop Improvement

- **Maize** (*Zea mays*): A Mite TE insertion near the *tb1* gene was a key event in the domestication of maize (Studer et al. 2011).

- **Nectarines** (*Prunus persica*) are hairless peaches. Vendramin et al. (2014) found that the fuzzy-less nectarine phenotype is caused by a TE in exon 3 of the *PpeMYB25* gene.

- **Cauliflower** (*Brassica oleracea* var botrytis):
  - The purple mutant was found to be caused by a TE upregulating a DNA regulatory region controlling anthocyanin production (Chiu, Zhou et al. 2010).
  - The orange coloured mutant accumulates high levels of b-carotene in the curd, was found to be caused by a TE insertion into the *Or* gene (Lu, Van Eck et al. 2006).
Transposon-induced Colour Change in Grape

The Reiterated Reproductive Meristem ‘RRM’ Mutant

The result of a transposed hAT element into the promoter of *VvTFL1A*, a meristem identity factor in the variety Carignan.

Grape TE Programme Goals

- Determine the TE diversity in grape
- Establish reference genomes for our key varietals
- Establish tissue culture systems for TE mutagenesis
- Determine treatments that mobilise TE’s in grape
  - Induction of transcription
  - Achievement of integration
  - Tools required to measure these events accurately
- Genotyping and Phenotyping
  - Build a web-based browser for data visualisation
- Make the data available for forward and reverse genetic approaches
Transposable Elements in Grape

TE’s make up 40-50% of the grape genome, 32,500 different types identified, over 220,000 copies per cell.
Transposon Movements are Cell-autonomous Events. They Result in Chimerism

Periclinal chimeras

Anticlinal chimeras
Chimerism – Genetic Variation Within a Plant

Pinot gris is a Periclinal chimera

Pinot meunier is a Periclinal chimera

An anticlinal chimera of Sauvignon blanc

To minimise chimeras we use somatic embryogenesis
Optimising Somatic Embryogenesis in Grape

Embryo formation and germination can both be stimulated by cold and GA3 treatment.
Quantification of TE Mobilisation (‘TE Fingerprint’)

Aim: To identify new insertions in each individual

**TE Fingerprint** (Tim Millar and Susan Thomson)
- A computer programme written in Python

Input data:
- Paired end sequence data (Illumina)
- Library of transposon sequences
- Grape genome sequence

Output
- Identify paired reads at TE boundaries
- Map those and identify differences between individuals
hAT Element Relocation (using ‘TE Fingerprint’)
New Gypsy Insertion (using ‘TE Fingerprint’)

Parent
Regenerant
Regenerant
Regenerant
Regenerant
Regenerant
Regenerant
Regenerant
Field Planting

- 1,300 somaclones planted to-date
- Another 1,200 ready to plant next season
- The first 100 fully genotyped
- The first fruit was seen in the 2017/18 season
Somaclonal Mutants

- One PN clone has altered dormancy. It breaks buds in mid winter and goes dormant in mid summer
- Three PN clones that are more susceptible to powdery mildew infection
- Two PN clones with a bushy habit (reduced apical dominance)
- Several plants with reduced vigour
- Fruit and bunch changes? (too soon)
Thank You
UNCOVERING THE GENETIC AND EPIGENETIC IMPACT OF A TRANSPOSON BURST IN GRAPEVINE

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Introduction

Endogenous transposable elements (TEs) can be stimulated, through a combination of tissue culture and stress treatments, to produce novel genetic diversity in plants. Using this approach, we are generating a population of novel grapevine somaclonal mutants as a resource for gene function studies and as novel germplasm for and the wine industry.

Novel molecular and bioinformatic tools enable semi-automated, high throughput genotyping of genomic TE insertion sites in each vine, so that a searchable database of genetic variation accompanies the population.

Whole genome bisulphite sequencing of a subset of these plants will also reveal the genome-wide epigenetic impact of a temporary loss of TE silencing and the consequent TE mutagenesis, a fundamental process in eukaryotic evolution.

Results & Discussion

Transposon-enriched sequencing requires less than 1% of the genome be sequenced to genotype greater than 85% of transposon insertion sites. Approximately 60,000 transposon insertion sites are scored per genome.

TEFingerprint allows rapid comparisons of transposon loci among enriched sequence libraries.