Genomics tools for healthier grapevines

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Outline:
1. Define genomics and introduce next generation sequencing
2. Describe the genomics tools available to viticulture and their application to diagnose diseases, monitor disease risks, and accelerate marker assisted breeding

Genomics

Genomics = the study of an organism’s entire genome.
Genome = the entirety of an organism's hereditary information encoded in its DNA.

The main difference between genomics and genetics is that genetics scrutinizes the inheritance, composition, and function of the single gene where as genomics addresses all genes in the genome and their inter relationships in order to identify their combined influence on the organism.

The grapevine genome

*Vitis vinifera* var. PN40024 (487Mb; 29,791 protein coding genes)

*Vitis vinifera* cv. Pinot Noir (504.6 Mb; 29,585 protein coding genes)
Next Generation DNA sequencing

Radical improvement of our sequencing capability in a short period of time

"Cost per Genome" - the cost of sequencing a human-sized genome (~5 times a grapevine genome)

The costs associated with the following activities are not reflected in the graph:
- Development of bioinformatics/computational tools for sequence analysis
- Management of individual sequencing projects
- Informatics equipment
- Data analysis downstream of initial data processing (e.g., sequence assembly, sequence alignments, identifying variants, and interpretation of results)

A genomic revolution in plant research

Exponential increase of sequenced plant genomes after the introduction of next generation sequencing technologies

http://genomevolution.org

Illumina's Cheap New Gene Machine

Market share (2013):
~70% Illumina
16% Life
10% Roche
3% PacBio

Illumina's new machine slashes genome sequencing cost to $1,000
...

A genomic revolution in plant research

Whole genome comparison between table and wine grapes reveals a comprehensive catalog of structural variants

LARGE-SCALE PROJECT ARTICLES

De novo transcriptome characterization of Vitis vinifera cv. Corinna unveils varietal diversity

- What are the genes in the grape genome responsible for wine flavor differences between grape varieties (i.e. what genes make a Riesling a Riesling and a Cabernet Sauvignon a Cabernet Sauvignon)?
- The expression of which genes should we target to enhance or fine-tune wine grape quality?
- What functions can we manipulate to improve quality in less-than-optimal environments?
### Genomes of grapevine pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdery mildew</td>
<td><img src="image" alt="Powdery Mildew" /></td>
</tr>
<tr>
<td>Botryosphaeria dieback</td>
<td><img src="image" alt="Botryosphaeria Dieback" /></td>
</tr>
<tr>
<td>Botrytis bunch rot</td>
<td><img src="image" alt="Botrytis Bunch Rot" /></td>
</tr>
<tr>
<td>Eutypa dieback</td>
<td><img src="image" alt="Eutypa Dieback" /></td>
</tr>
<tr>
<td>Esca</td>
<td><img src="image" alt="Esca" /></td>
</tr>
</tbody>
</table>

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### Genomics tools in viticulture

#### Application of genomic tools in viticulture

1. **Diagnostics**
   - Discovery of disease causing organisms
   - Early detection of diseases on asymptomatic vines
   - Monitor pathogens in the field

2. **Breeding**
   - **Genotyping** (marker development, genetic maps, markers for marker assisted breeding)
   - **“Deep phenotyping”** (functional characterization of complex traits to accelerate breeding)

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### Deep sequencing as diagnostic tool

**Why use next generation sequencing for disease diagnostics?**

- **Extremely rapid** generates millions of reads in a few hours
- **Cost effective** more than 200 million sequences for less than $2,000
- **Very efficient** sequences produced from an infected plant will include sequences from any pathogens present (viruses, viroids, fungi and bacteria)
- **Very sensitive** can detect unknown pathogens or very low titer pathogens in asymptomatic vines

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This method expedites the entire process of novel pathogen discovery, identification, and, subsequently, the development of more routine assays for new pathogens.
**Deep sequencing as diagnostic tool for grape viruses**

**Characterize undiagnosed diseases**

- Deep sequencing of cDNA libraries obtained from double-stranded RNA enriched nucleic acid (NA) preparations from bark scrapings of dormant canes from symptomatic plants.

**Grapevine red blotch on Cabernet Franc**

**Other undiagnosed diseases**

- Syrah decline diseases
- Graft-incompatibility symptoms
- Leafroll-like diseases
- Grapevine vein-clearing

**Deep sequencing as diagnostic tool for grape viruses**

**Trunk diseases**

**Esca disease**

- Phaeomoniella chlamydospora
- Phaeoacremonium spp.
- Togninia minima

**Eutypa dieback**

- Eutypa lata
- Eutypa apiculata
- Diatrype prominens
- Diatrype alpina
- Diatrype whitmanensis
- Phytophthora spp.

**Significant increase in management costs**

**Botryosphaeria canker**

- Botryosphaeria spp.
- Neofusicoccum parvum

**Trunk diseases**

- Application of pruning wound protectants
- Delayed pruning or double pruning

**Preventative methods**

- Adoption of preventative practices and their timing can be improved by measuring the disease risk in the vineyard

**Trunk diseases**

**Significant increase in management costs**

**Eutypa and Botryosphaeria cost growers $260 million/year in CA**


**Trunk diseases are caused by fungi that infect grapevines through wounds and openings**

- Most commonly through pruning wounds
- Wounds caused by mechanical damage, re-training, etc.
- Endophytes? and/or latent pathogens

“A healthy vine is fundamental to the successful beginning and sustainability of all grape vineyards. Growers depend on commercial grapevine nurseries for vine stock that is free of known pathogens and serious viruses and true to type. This is not an easy task. At present, it is not possible for nurseries to ensure fungal trunk pathogen-free stock.”

(Granpasso and Arrangal, 2011. *Plant Disease* 95(10):1049-1055)
Monitor risk of trunk diseases

Detection of Spores in the Field

- Set spore trap in the vineyard
- 2. Diagnostic lab conducts traps after rain
- 3. High system from high
- 4. DNA-based spore detection

Requires pathogen specific assay

development of routine assays for "new" pathogens

Identification of new pathogens
(species, more aggressive or fungicide resistant strains)

Sequencing Data

- Genome assembly
- Fungal purification

Genomes of grapevine pathogens

Strain  | Species  | Host  | Heat Tolerance  | Country  | Agressiveness  | Total Assembly (Mb)  | Number of Serotypes
--- | --- | --- | --- | --- | --- | --- | ---
E015E  | E. late | Grape | high | Napa Co.  | 84.42 | 7875 | 295.15 |
E016E  | E. late | Grape | high | Napa Co.  | 84.48 | 9100 | 294.24 |
E017D  | A. ribis | Prunus armeniaca | high | Napa Co.  | 84.27 | 7215 | 294.29 |
E028E  | A. ribis | Prunus armeniaca | high | Napa Co.  | 84.45 | 7003 | 294.47 |
E029E  | A. ribis | Prunus armeniaca | high | Napa Co.  | 84.45 | 7003 | 294.47 |
E030E  | A. ribis | Prunus armeniaca | high | Napa Co.  | 84.45 | 7003 | 294.47 |
E031E  | A. ribis | Prunus armeniaca | high | Napa Co.  | 84.45 | 7003 | 294.47 |
E032E  | A. ribis | Prunus armeniaca | high | Napa Co.  | 84.45 | 7003 | 294.47 |
E033E  | A. ribis | Prunus armeniaca | high | Napa Co.  | 84.45 | 7003 | 294.47 |
E034E  | A. ribis | Prunus armeniaca | high | Napa Co.  | 84.45 | 7003 | 294.47 |
Genes of grapevine pathogens

Early and non-destructive diagnosis

Early detection of trunk diseases (field or nursery)

Wood cankers are internal.

- Sampling leaves is non-destructive.
- Test for grape genes expressed in early stage of infection.

Classification of grapevine pathogens

Grapevine trunk disease genomics

- Eutypa lata (Eutypa dieback)
- Togninia minima (Esca)
- Neofusicoccum parvum (Botryosphaeria canker)

Genome assembly

- Total size
- N50
- Coverage
- Completeness

- Total size
- N50
- Coverage
- Completeness

Sampling the cordon, spurs, canes, or trunk is destructive.

- Sampling leaves is non-destructive.

Neofusicoccum parvum (Botryosphaeria canker)

Eutypa lata (Eutypa dieback)

Togninia minima (Esca)

Baumgartner lab
Early and non-destructive diagnosis

1. Fungal inoculation
2. Leaf sampling

Genetic markers for early detection

Data analysis

Diagnostic marker for early and non-destructive detection of trunk diseases

Genomics tools and breeding

Genome-wide genetic marker discovery and genotyping using next-generation sequencing

Traditional marker discovery
- Costly and can not be parallelized
- Time consuming cloning and primer design
- Scoring is expensive and labourious

NGS Based Marker Discovery
- Discovering, sequencing, and genotyping of large number of markers
- Fast

Methods for high-throughput marker development
1. Reduced-representation libraries (RRLs)
2. Complexity reduction of polymorphic sequences (CRoPS)
3. Restriction-site-associated DNA sequencing (RAD-seq)
4. Shotgun sequencing

Genetic resistance

Genetic resistance is the most cost-effective and environmentally-friendly method for controlling powdery mildew

Prioritization of genes for breeding

There is growing number of identified and mapped resistance genes

Long-term durable resistance can be achieved by combining together multiple resistance genes (pyramiding)
Multiple resistant gene stacking

Stacking (combining) multiple resistance genes to:
1. enhance resistance
2. reduce the likelihood of development of new virulent strains

Expanding the sources of genetic resistance

Prioritization of genes for breeding resistance

Take home messages

1. Genomics (genome/transcriptome/metagenome sequencing and analyses) can be used as a fast approach to characterized biological systems for which there is little information.
2. Next generation sequencing can be used to identify new pathogens, to detect infections on asymptomatic vines, and monitor disease pressure and epidemics of new aggressive or fungicide resistance strains.
3. The cost of sequencing is declining rapidly, soon it will be used routinely as diagnostic tool in nurseries, vineyards, and wineries.
4. Next generation sequencing technologies help accelerate breeding by rapid genome-wide marker discovery and quantitative characterization of complex traits.
Acknowledgments

Our team

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