Genomics Based Approaches to Genetic Improvement in Sugarcane

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Genetics

Life Enriched by Plant Biodiversity
Plant Resources
FOR FOOD, FUEL OR CONSERVATION

ROBERT HENRY
Food
Biomass options

- Sorghum
- Sugarcane
- Grasses
- Shrubs
- Trees
Special Journal Issues- Biofuels
Special Journal Issues-SNP
Genotyping by mass analysis

Allele 1
EXTEND Primer (23-mer)

CTA

extended Primer (24-mer)

+ Enzyme
+ ddGTP/ddATP
+ dCTP/dTTP

Allele 2
EXTEND Primer (23-mer)

GTA

eXtended Primer (25-mer)

21 22 23 24 25 26 27 28
Example of SNP genotyping for breeding selection

Rice quality
Endonucleolytic mutation analysis by internal labelling (EMAIL)∗

Technologies for different applications

- **RT-PCR**: 100,000 SNPs, 1,000 samples
- **MassARRAY®**: 10,000 SNPs, 100 samples
- **WGS**: 1,000 SNPs, 10 samples
SNP Discovery

454 Sequences of sugarcane genes
NGS applications for SNP discovery

- Amplicon sequencing
- cDNA sequencing
- Sequencing of gene rich regions
- Whole genome sequencing
SNP discovery in sugarcane
Summary of SNP detection in 454 sequences of 300 amplicons

<table>
<thead>
<tr>
<th></th>
<th>Female (S. officinarum) parent</th>
<th>Male (offic. X spont.) Parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Products with one consensus seq.</td>
<td>247</td>
<td>242</td>
</tr>
<tr>
<td>Total length of amplicons</td>
<td>58 kb</td>
<td>57 kb</td>
</tr>
<tr>
<td>Products with one or more SNPs</td>
<td>213</td>
<td>227</td>
</tr>
<tr>
<td>Products SNPs absent</td>
<td>34</td>
<td>15</td>
</tr>
<tr>
<td>SNPs with rare allele frequency ≥ 4%</td>
<td>1,013</td>
<td>1,632</td>
</tr>
<tr>
<td>Mean SNPs per amplicon</td>
<td>4.76</td>
<td>7.19</td>
</tr>
<tr>
<td>Average sequence depth at SNP sites</td>
<td>279</td>
<td>257</td>
</tr>
<tr>
<td>Candidate SD SNPs (4% to 15% freq.)</td>
<td>216</td>
<td>788</td>
</tr>
</tbody>
</table>
Frequency of rare SNP allele

Minor allele proportion in 454 sequences

No. of SNP sites
454 SNP discovery

• One SNP every: 35/58 bases
• Developed SNP identification macros that also produce a consensus sequence with SNP sites coded ready for MassARRAY Assay Design software
• ~ 90% of assays validated a SNP
• Published in Plant Biotechnology Journal
Pipeline for mapping genes of interest using SNPs

Discover & evaluate SNPs

Develop/test SNP marker assays

Genotype mapping population

454 re-sequencing

Sequenom MassARRAY platform
Impact of NGS

• Gene discovery
• Promoter analysis
• Expression profiling
• Conventional breeding
• GM sugarcane
Classical Approaches

Example:
Sucrose Phosphate Synthase
Southern Cross Plant Genomics

- NGS sequencing facility (ARC)
- Bioplatforms Australia (NCRIS)
- Intersect (NSW)
THE SEQUENCING RACE

The increasingly crowded market for genome-sequencing machines includes new entrants looking to push the boundaries in both speed and accuracy.

SOLiD 3 R&D, now and future direction

- Currently exceeding 40Gb
- Sequencing to 100 bp
- 1B tags through greater bead density
- 100Gb by end of year
- Road map for 250Gb
- $10K genome at high coverage by end of year
NGS experiments

- Wheat genome sequencing
- *Oryza* reference genome sequencing
- Sugarcane gene sequencing
- Wheat transcriptome analysis
- Rainforest biodiversity
- Eucalypt genome re-sequencing
Next Generation DNA sequencing accelerated discovery of genes for food and energy traits
Sugarcane genome sequencing
Relationships within the Sorghum Genus

- S. exstans [ S ]
- S. intrans [ S ]
- S. angustum [ S ]
- S. interjectum [ S ]
- S. ecarinatum [ S ]
- S. brachypodum [ S ]
- S. matarankense [ P ]
- S. stipoideum [ S ]
- S. timorense [ P ]
- S. amplus [ S ]
- S. bulbosum [ S ]
- S. plumosum [ S ]
- S. grande [ P ]
- S. leiocladum [ P ]
- S. nitidum [ P ]
- S. purpureo-sericeum [ P ]
- S. versicolor [ P ]
- S. X alnus [ E ]
- S. arundinaceum [ E ]
- S. bicolor [ E ]
- S. X drummondi [ E ]
- S. halepense [ E ]
- S. propinquum [ E ]
- S. macroporum [ C ]
- S. laxiflorum [ H ]
- Cleistachne sorghoides
- Zea mays
- Hordeum vulgare
Sugarcane Genome Sequencing-CRC Project

- SCU/CSIRO
- Focus on gene rich parts of genome
- Establishes allelic diversity within and between genotypes
- Assignment of alleles to genomes
- Target SNP mining
- Facilitates gene mapping
- International collaboration
Sugarcane volume

• GENETICS, GENOMICS AND BREEDING IN CROP PLANTS

• Volume 10
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Biofuels CRC

Mission:
Transport energy self sufficiency and reduced carbon emissions from Australian transport
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