Molecular Breeding & Crop Improvement of Jatropha and Guayule Hybrid Cultivars

Eric J. Mathur, Chief Science Officer & Senior Vice President
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Plant domestication dates back to 8000 BC.
Crop improvement requires diverse germplasm

- Present day crops are improved versions of their wild ancestors
- Diverse germplasm is required for crop domestication
- Jatropha & Guayule landraces lacked genetic diversity

Tomato     100X
Rubber     400X
Maize      25X
Jatropha    50X
Guayule    10X
The era of plant genomics has arrived

**Modern Genome Tools**
- Physical & genetic maps
- Germplasm phylogeny (GBS)
- Plant re-sequencing
- SNP & gene discovery
- RNA-Seq & proteomics
- Trait: marker association (GWAS)
- Genomic Selection (GS)

**Commercial Impact**
- Accelerate improvements
- Maximize genetic potential
- Yield preservation
- Consistent productivity
- Uniform plant architecture
- IP protection - DNA barcodes
- Predictive breeding

The DNA sequencing explosion

**The Economist**

$3,000,000,000

$1,000
Jatropha produces tri-carpelate fruits with high oilseed content

- Exceptional fatty acid profile
- Excellent oxidative stability
- Good cold flow properties
- High value specialty chemicals
- No competition with food security

<table>
<thead>
<tr>
<th>Oilseed</th>
<th>Cloudpoint</th>
<th>Stability (hrs@110°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canola</td>
<td>-3°C</td>
<td>14.1</td>
</tr>
<tr>
<td>Jatropha</td>
<td>3°C</td>
<td>13.1</td>
</tr>
<tr>
<td>Soy</td>
<td>1°C</td>
<td>5.3</td>
</tr>
<tr>
<td>Palm</td>
<td>13°C</td>
<td>13.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Jatropha</th>
<th>Palm</th>
<th>Canola</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>19%</td>
<td>49%</td>
<td>6%</td>
</tr>
<tr>
<td>Unsaturated</td>
<td>81%</td>
<td>51%</td>
<td>94%</td>
</tr>
<tr>
<td>18:1</td>
<td>36%</td>
<td>42%</td>
<td>64%</td>
</tr>
<tr>
<td>18:2</td>
<td>45%</td>
<td>9%</td>
<td>19%</td>
</tr>
</tbody>
</table>
Jatropha domestication: case study

A genetic bottleneck in *Jatropha* prevented early domestication

Jatropha oil applications:
biodiesel, jet fuel, specialty oils & chemicals

soaps
candles
medicinals
living fence
fertilizers

Jatropha has been cultivated for centuries; the Portuguese recognized it’s value
Jatropha germplasm from Central America exhibits high phenotypic diversity

- Extreme branching
- Apical dominance
- Dwarfs
- Fungal & rust resistance
- Dehiscent
- Indehiscent
- Female only flowers

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DNA barcoding: High throughput digital genotyping

- Leaf Punches
- Tissue Lysis
- HTP DNA Extraction
- DNA Marker Sequencing
- High Resolution Melt
- Liquid Handling

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DNA barcoding: HRM genotyping with SSR markers

PyroMark Sequencing
Population structure deduced from SSR markers with HRM

- 12 distinct clades
- Identifies best parental lines
- Maximizes hybrid vigor
- Increases plant productivity
- Improves plant health
- Assures yield preservation
- Provides IP protection
Jatropha hybrid technology improves profitability

**Higher Yields**
Hybrid vigor; enhanced seed, oil & biomass yield

**Increased Profitability**
Agronomic efficiencies better economics & higher productivity

**Stress Tolerance**
Improved WUE & NUE disease & pest resistance

**Preserve Yield Potential**
Maintain yield under seasonally adverse conditions

Introduction of hybrid corn resulted in 400% yield gain

**Commercial Variety**  |  **SGB Hybrid**
50-fold oilseed yield improvement in Jatropha by maximizing heterosis

800 accession families form 18 heterotic clades with filtered SNPs
Yulex is a fully integrated business combining patented technology, platform for rapid crop improvement, and product demand across multiple vertical markets.

**CROP SCIENCE-AG**
- Proven crop improvement platform
  - Molecular Breeding & Agrigenomics
  - Hybrid testing & development
  - Agronomic & Crop production research
  - Seed multiplication & processing
  - Sustainable commercial production

**BIOPROCESSING**
- Patented extraction technologies
  - Clean bioprocessing
  - GEN I Aqueous-based extraction
    - Latex emulsion
  - GEN II Solvent-based extraction
    - Biorubber, Resins, Bagasse

**MATERIALS SCIENCE**
- Value creation and market pull
  - Application development
  - Material enhancement
  - Co-branded products
  - Yulex Brand for high-value markets
Yulex emulsion & solid rubber applications

Yulex provides a safer, natural rubber for latex-allergy sensitive individuals.

Latex-Allergy Safe
Marginal genetic improvement over 60 years of breeding

- Narrow germplasm base
- Perennial crop
- High rubber yields require 18 to 24 months
- Multi-year trialing to evaluate and realize yields
- Landraces lack uniformity
- Complicated genetics
Guayule bottleneck: AZ lineage arose from only 5 bulked plants.
Germplasm diversity exists at the center of origin of *Parthenium argentatum*

A severe genetic bottleneck exists in commercial Guayule germplasm

Selected wild germplasm is highly divergent

Guayule center of origin is in Northern Mexico
Yulex harnessed guayule genetics to produce pure-line hybrids

- Traditional breeding methods have been flawed
  - low trait heritability
  - variability in germplasm
  - 30% genetic dilution with each cycle
  - apomixis restricts genetic gain
  - ploidy & aneuploidy complicate breeding

- Yulex leveraged facultative apomixis to produce true hybrids
  - Hybrids are uniform plants with consistent productivity
  - Hybrids can produce clonal progeny
  - Molecular breeding tools maximize hybrid yield & vigor

- Guayule hybrids represent disruptive technology
  - 15-fold improvement in rubber yield uniformity
  - IP portfolio on hybrid creation, selection & seed multiplication

<table>
<thead>
<tr>
<th>Hybrid Plant Number</th>
<th>Triplicate Extractions</th>
<th>Average % Total Rubber</th>
</tr>
</thead>
<tbody>
<tr>
<td>F15-9</td>
<td>4.1</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>F15-11</td>
<td>4.6</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>F15-12</td>
<td>4.2</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

*1 yr old plants

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Four distinct clades
Yulex Guayule hybrids demonstrate high yield performance

Three factors driving higher yield potential of Guayule:

1) Plant rubber content
2) Plant Weight
3) Total Biomass

<table>
<thead>
<tr>
<th>Yulex Hybrids</th>
<th>Plant Rubber Content (%)</th>
<th>Total Rubber/plant (g)</th>
<th>Total Biomass* (MT/Ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Performer</td>
<td>9.5</td>
<td>52.5</td>
<td>54.3</td>
</tr>
<tr>
<td>Top 10</td>
<td>8.4</td>
<td>30.0</td>
<td>49.1</td>
</tr>
<tr>
<td>AZ-6 Check</td>
<td>6.0</td>
<td>18.5</td>
<td>24.8</td>
</tr>
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</table>

*Fresh Weight of defoliated 24 month-old plants
Acknowledgements

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