Mitch McGrath
Sugar Beet Research Geneticist with USDA-ARS since 1996
& Adjunct Professor, Plant, Soil, and Microbial Sciences
Michigan State University
East Lansing, Michigan, USA

“Sugarbeet Genetics, Genomics, and Germplasm Enhancement”
NAPB Webinar Series 2015
April 22, 2015

Mitch.McGrath@ars.usda.gov

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Introduction to the crop

an industrial crop...

World growing regions

Average regional sugar beet output (kg/ha)
Sucrose ~ 8 million tons
55% beet (green), 45% cane (red)
HFCS (yellow) ~ 9 million tons

Sugarbeets for Sugar, Harvested Acres: 2007
1.25 million acres (0.5 million hectares)
$1.34 billion to growers
Sugar Beet Field Operations
65+ year history at MSU
Saginaw Bean & Beet Farm

Sugar Beet Root

<table>
<thead>
<tr>
<th>Water</th>
<th>Dry Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>75%</td>
<td>25%</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Sucrose</th>
<th>Non-Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.5%</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insoluble</th>
<th>Soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nitrogenous Organic</th>
<th>N-Free Organic</th>
<th>Mineral Matter</th>
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</thead>
<tbody>
<tr>
<td>1.1%</td>
<td>0.9%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Non-Sucrose DM
SucDM
Water
Sucrose yield = root yield \times sucrose percent – loss to molasses

Sucrose accumulation over time

Yield accumulation over time

Water content has huge influence on sugar content & yield

\text{Yield water} \times \text{yield sucrose (FW)}

\text{Percent water} \times \text{percent sucrose (FW)}

- no correlation between yield and percent ($R^2 = 0.09$)
The crop

\[ \text{Beta vulgaris L. spp. vulgaris} \]
\[ 2n = 2x = 18 \]

Leaf beet / chard

Red / Table / Garden / Beetroot

Sugar / Energy

Fodder / Mangel

Pigments are betalains (\( R \) & \( Y \))
not anthocyanins

---

**Germplasm enhancement: 2008 data**

<table>
<thead>
<tr>
<th>Accession</th>
<th>Lineage &amp; Description</th>
<th>T/A %</th>
<th>%SucFW</th>
<th>SucA %</th>
<th>%DM %</th>
<th>%water %</th>
<th>%sucDM %</th>
<th>APH Rhizoctonia Fusarium RZM</th>
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<tr>
<td>EL-A021744</td>
<td>Low water elites</td>
<td>67.3</td>
<td>20.2</td>
<td>18.4</td>
<td>7467.2</td>
<td>23.2</td>
<td>76.8</td>
<td>79.5 1.7 3.4 2.0 2.5</td>
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<tr>
<td>EL-A022463</td>
<td>FS SR Comp x OTRangeA</td>
<td>32.0</td>
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<td>18.4</td>
<td>8132.3</td>
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<td>77.2</td>
<td>80.6 1.9</td>
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<td>61.8</td>
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<td>21.5</td>
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<td>20.5</td>
<td>18.4</td>
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<td>22.5</td>
<td>77.5</td>
<td>81.8 2.3 1.8</td>
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<td>Rhizoc elites</td>
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<td>78.8 1.4 3.5 2.1 4.2</td>
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<tr>
<td>EL-A021725</td>
<td>(95HS2/sal) x 07-5E</td>
<td>41.3</td>
<td>18.6</td>
<td>17.8</td>
<td>6670.6</td>
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<td>77.6</td>
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<td>EL-A016102</td>
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<td>23.1</td>
<td>18.1</td>
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<td>21.4</td>
<td>17.9</td>
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<tr>
<td>EL-A022462</td>
<td>low water IC1 - B</td>
<td>83.0</td>
<td>23.5</td>
<td>18.3</td>
<td>8567.5</td>
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<td>77.9</td>
<td>82.6 1.5 1.8</td>
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<td>EL-A015019</td>
<td>SR Comp F4 (unselected)</td>
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<td>17.9</td>
<td>8099.8</td>
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<td>HS elites</td>
<td>93.8</td>
<td>24.9</td>
<td>17.9</td>
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<td>77.9</td>
<td>81.0 2.6 4.3 2.1 3.0</td>
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<tr>
<td>EL-A015020</td>
<td>SR Comp F4 (14%)</td>
<td>83.3</td>
<td>26.4</td>
<td>17.2</td>
<td>9086.5</td>
<td>22.1</td>
<td>77.9</td>
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<tr>
<td>EL-A015022</td>
<td>SR Comp F4 (16-17%)</td>
<td>76.5</td>
<td>19.0</td>
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<td>C40 high sucrose x SR</td>
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<td>21.9</td>
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<td>low water IC - C</td>
<td>60.0</td>
<td>22.4</td>
<td>18.0</td>
<td>8051.0</td>
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<td>78.2</td>
<td>82.7 2.5 2.2</td>
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<td>EL-A021734</td>
<td>SR96/sal</td>
<td>76.5</td>
<td>24.0</td>
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<td>78.3</td>
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<td>Mix EL0224 x SR-Suc-2003</td>
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<td>RZM RZC Hero IC</td>
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<td>23.6</td>
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<td>EL-A015029</td>
<td>EL53 (1)</td>
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<td>20.5</td>
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<td>7746.0</td>
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<td>24.1</td>
<td>19.2</td>
<td>9237.5</td>
<td>24.6</td>
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<td>22.2</td>
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<td>24.4</td>
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<td>80.3</td>
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<td>Beta 5930R</td>
<td>commercial</td>
<td>67.5</td>
<td>22.6</td>
<td>18.6</td>
<td>8460.0</td>
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<td>78.5</td>
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<td>23.4</td>
<td>76.6</td>
<td>76.7</td>
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<td>HM276Rr</td>
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<td>59.5</td>
<td>19.1</td>
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<td>23.4</td>
<td>76.6</td>
<td>79.5</td>
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<td>E17</td>
<td>commercial</td>
<td>69.8</td>
<td>27.9</td>
<td>17.8</td>
<td>9861.7</td>
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<td>76.9</td>
<td>77.0</td>
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<td>Beta 5451</td>
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<td>50.8</td>
<td>22.0</td>
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<td>7879.4</td>
<td>23.0</td>
<td>77.0</td>
<td>77.9</td>
</tr>
</tbody>
</table>

*resistant check*  
*susceptible check*
A view to the evolution of sugar beets

**Beta vulgaris ssp. maritima**

$2n = 2x = 18$
Historically important US public sugar beet germplasm releases (~1940-2000)

12 plants per accession
69 RAPD alleles scored

Within population heterozygosity has decreased with local breeding over time
Sugar beet (from the Atlas des Plantes de France, 1891)

1747 – Marggraf
Beet crystals = cane crystals

1784 – Achard
Selected first sugar beet

1830’s – Vilmorin
Selected high sugar mother roots & tested progeny


for flowering:
(greenhouse protocol)

Req’s vernalization:
(5° C, Oct 1 – Dec 30°)

Bolting
B- annual, bb biennial
(Jan – Feb)

Flowering
(March-April)

Seed harvest & processing
(June-July)

Mulitgerm

MM or Mm

Monogerm

mm

Kern: Am.Crystal Sugar Co.
Pollen control is key to beet breeding

Fertile anther
- normal cytoplasm

Cytoplasmic MS anther
- sterile cytoplasm

Expression of O-type CMS requires 3 recessive genes: xx, (yy), zz (2 are linked)

Commercial hybrid seed production

2-row pollinator
6-row CMS
Experimental hybrid seed production

Numerous CMS Seed Parents (monogerm)

Single Pollen Parent (multigerm)

Experimental seed production -> complex self-incompatibility system

OP seed production

Self Fertile: greenhouse (RILs)

Sib & Pair Cross

SF : field
Breeding in open pollinated crops:
Manipulating gene frequency where gene function(s) unknown

Mother root selection (mass selection with or without progeny testing)
Sib-mating (Pair crosses)
Inbreeding via dominant self-fertility allele ($S^f$; suppressor of self-incompatibility?)
Hybrids enforced with nuclear or cytoplasmic male sterility

Predominant diseases of U.S. growing regions and USDA-ARS breeding station disease responsibilities

- Viruses
  - Curly Top
  - Yellows Complex
  - Rhizomania
- Cyst nematode
- Rhizoctonia
- Cercospora
- Aphanomyces
- Curly Top
- Rhizomania
- Cyst nematode
- Aphanomyces
- Cercospora
- Rhizoctonia
- Cyst nematode
Cercospora leaf spot resistance (mass selection)

Cercospora tolerance variety trial
Saginaw, MI (2005)

July 27

August 15

Mother root selection for Rhizoctonia Crown & Root Rot and damping-off resistance

July 7, 2008

August 21, 2008

East Lansing, MI
Using self-fertility ($S'$) to examine segregation
- dominant suppressor of self-incompatibility

Sucrose content in MSR $F_2$ sugar x red population
(greenhouse grown plants)

Sucrose concentration (mg/g DW)

F1

Sugar beet parent

Red beet parent

Individual roots

Sugar x Red = MSR RIL population

Within MSR $F_3$ family variation
Sugar x Red beet (MSR) F₅ inbred population (showing just green segregants)

- Low within family variation
- High between family

Root Weight
0.3 - 2.5 Kg

Sucrose content
11.5 - 23.3%

Water content
71.4 - 81.1%

Dry Matter = biomass
18.9 - 28.6%

154 Sugar x Red MSR F5 RIls
(average of 5 roots)
Partitioning phenotypic variability across the chromosomal landscape

Yield Traits

Physiological Traits

Water (1 – biomass)

Elements of Modern Plant Improvement

Populations

‘real’ breeding

“Markers”

Phenotypes
The phenotype: seed with 95% germination consistently average 60 established beets

Germination in solution
Constitutive gene expression (95%)

Induced gene expression (5%)

Induced:
- Filter Paper (0.9%)
- NaCl (0.4%)
- Mannitol (0.25%)
- H₂O₂ (0.6%)
- NaCl + H₂O₂ (0.4%)
- Water+ (2.45%)

Phenotypic markers & gene discovery

Differential gene expression between low- and high-vigor germinating sugar beet seedlings (96 hr germination)

BC4

807 cDNA/bands surveyed
50 primer dd-PCR combinations
40 cDNA fragments cloned & sequenced

PCR confirmation

Germin-like Protein -> putative oxalate oxidase
oxalalic acid -> CO₂ + H₂O₂

Model for initiation of seedling vigor via hydrogen peroxide

beet seed & favorable germination conditions

stressed-induced Germin-like Protein expression
or, exogenous hydrogen peroxide

H₂O₂ acts as inter-cellular ‘vigor signal’

Stress-responsive (e.g. ‘vigor’) gene expression (induction of glyoxylate cycle for lipid catabolism)

intra-cellular ‘vigor signal’ is recognized and transduced (via a MAP Kinase cascade?)

activation of gene expression via specific Transcription Factors (currently unknown)

growth under a wider range of favorable conditions
‘transcriptomes for traits?’
-> gene / process / marker discovery

Germinating seedlings

<table>
<thead>
<tr>
<th>Process</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis</td>
<td>11%</td>
</tr>
<tr>
<td>Redox</td>
<td>5%</td>
</tr>
<tr>
<td>Secondary</td>
<td>7%</td>
</tr>
<tr>
<td>Hormone Metal</td>
<td>7%</td>
</tr>
<tr>
<td>Phosphorus Biochemistry</td>
<td>5%</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>7%</td>
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<td>Nucleic acids</td>
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<td>Lipid</td>
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<tr>
<td>Carbohydrate</td>
<td>16%</td>
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<tr>
<td>Amino Acid</td>
<td>10%</td>
</tr>
<tr>
<td>Oxidative Phosphorylation</td>
<td>4%</td>
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<tr>
<td>TCA cycle</td>
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<tr>
<td>ATP Synthase</td>
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</tr>
<tr>
<td>Light harvesting</td>
<td>1%</td>
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</table>

Count transcripts devoted to catabolic functions:
Germinating seedlings  ->  ~ 26%
Developing seedlings   ->  ~ 15%

GO - Biological Process
- cellular metabolic process
- organic substance metabolic process
- primary metabolic process
- single-organism metabolic process
- nitrogen compound metabolic process
- single-organism cellular process
- biosynthetic process
- establishment of localization
- cellular response to stimulus
- response to chemical stimulus
- single-multicellular organism process
- anatomical structure development
- single organism signaling
- multicellular organismal development
- cellular process
- single-organism developmental process

Need a genome sequence to better inter-relate genes and phenotypes

2n = 2x = 18
750 Mb

100x T. Schmidt

3-10 week developing roots
## Current status of beet genome sequence(s)

**Good gene-space coverage**

**Too many contigs!**

<table>
<thead>
<tr>
<th>Genome</th>
<th>Version</th>
<th>Derivation</th>
<th>Coverage</th>
<th>Contigs &gt; 1kb</th>
<th>Total Length (Mb)</th>
<th>% of genome</th>
<th># predicted genes</th>
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<td>C869</td>
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<td>535.3</td>
<td>71.4</td>
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<td>MSR-F7 (pool)</td>
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<td>bi-allelic</td>
<td>114 x</td>
<td>104,202</td>
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<td>33.5</td>
<td>nd</td>
</tr>
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</table>

* Dohm et al. (2014) Nature 505:546–549

Currently pursuing reference genome quality genome sequence:

- Next-next-generation sequencing
- Hi-C scaffolding libraries
- Optical mapping
- Hybrid next-gen sequencing & BAC library integration
- Genetic map integration

Background slide: Network of beet root development genes

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**Thanks to many current and former members of the Sugarbeet Research Program, USDA-ARS, East Lansing, Michigan**

**Thanks to many, many colleagues around the world**

**Funding provided by USDA-ARS base funds with direct and in-kind assistance from the member companies of the Beet Sugar Development Foundation, Michigan Sugar Company, & Michigan State University**

**Thanks to the organizers (and presenters) of these NAPB plant breeding webinars**

**And, especially, thanks to you, for your time, interest, and attention!**