Sweet Sorghum

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Overview

- What is sweet sorghum
- Potential as a biofuel
- Transformation with bombardment
- My project

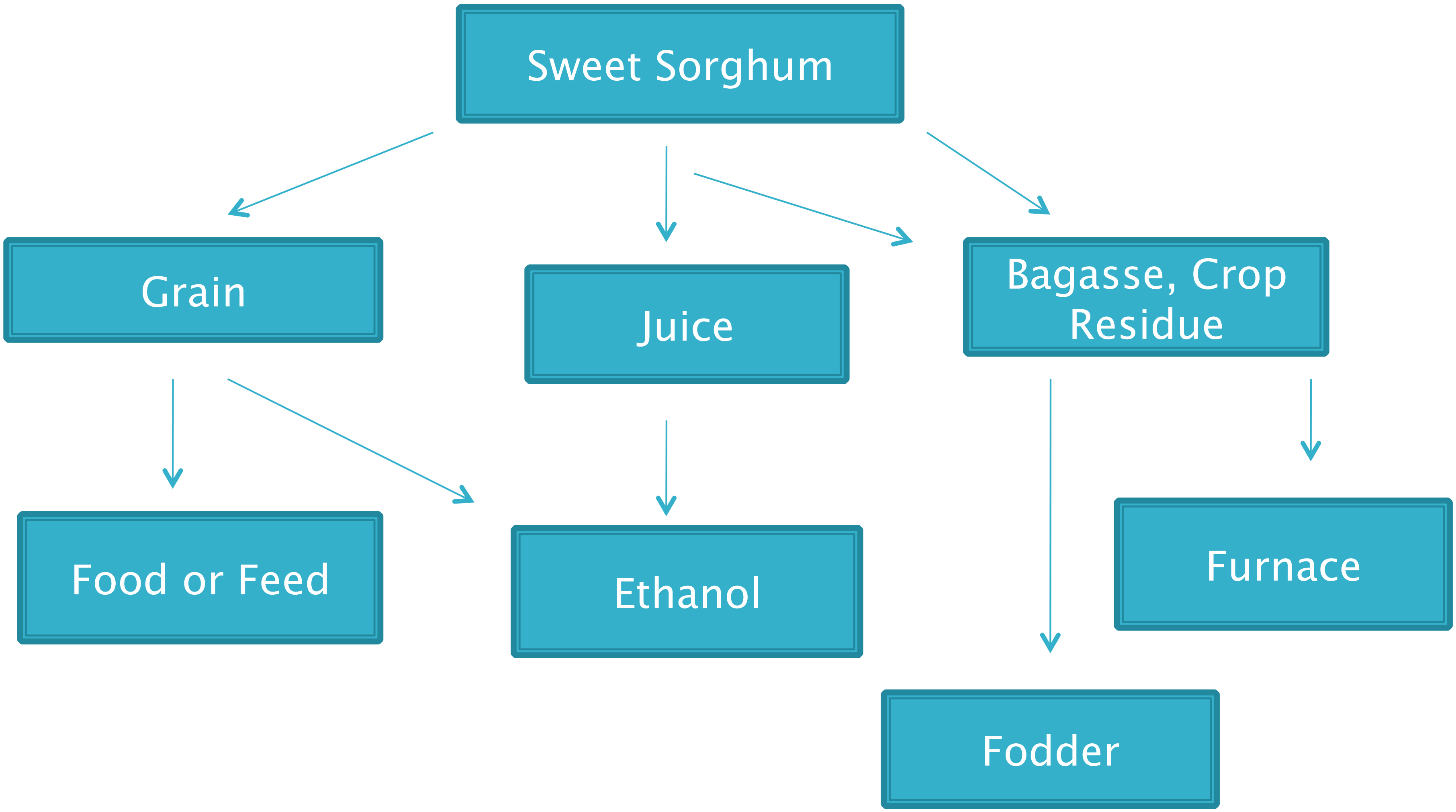
Sweet Sorghum

- Accumulates high levels of sugar, mostly sucrose, in stalk
- Closely related to sugarcane
- Differs from grain and forage but still produces grain and high biomass

Potential as a Biofuel

- Has the appropriate composition and yield for a potential biofuel
- Juice, grain, fodder and bagasse
- Sugar and starch: easiest ways to produce ethanol
- Grain: feed or ethanol production
- Leftover stalk and bagasse: fodder or burned

Sorghum Processing



Stress Tolerant and Efficient

- Ability to grow on marginal land
- No food–fuel issues
- Drought tolerance
- Low nutrient inputs
- Short duration (4 mo.)
- Efficient (low cost; high output)
- Annual, responsive to supply and demand changes

Diverse

- Widely adaptable/flexible
  - Land races/cultivars
  - Biomass production (sugar, grain, biomass)
- Relatively well studied genome
- Existing genetic variation

Existing Genetic Variation

- Diversity of cultivars
- Brown midrib mutants (*bmr*)
  - Reduced lignin
  - Greater digestibility
  - Mutations in genes for enzymes of the lignin biosynthesis pathway
- Waxy mutation (*wx*)
  - Low amylose, high amylopectin
  - Improved ethanol conversion
- Photoperiod-sensitive
  - Longer growing season-maximize biomass

Model system

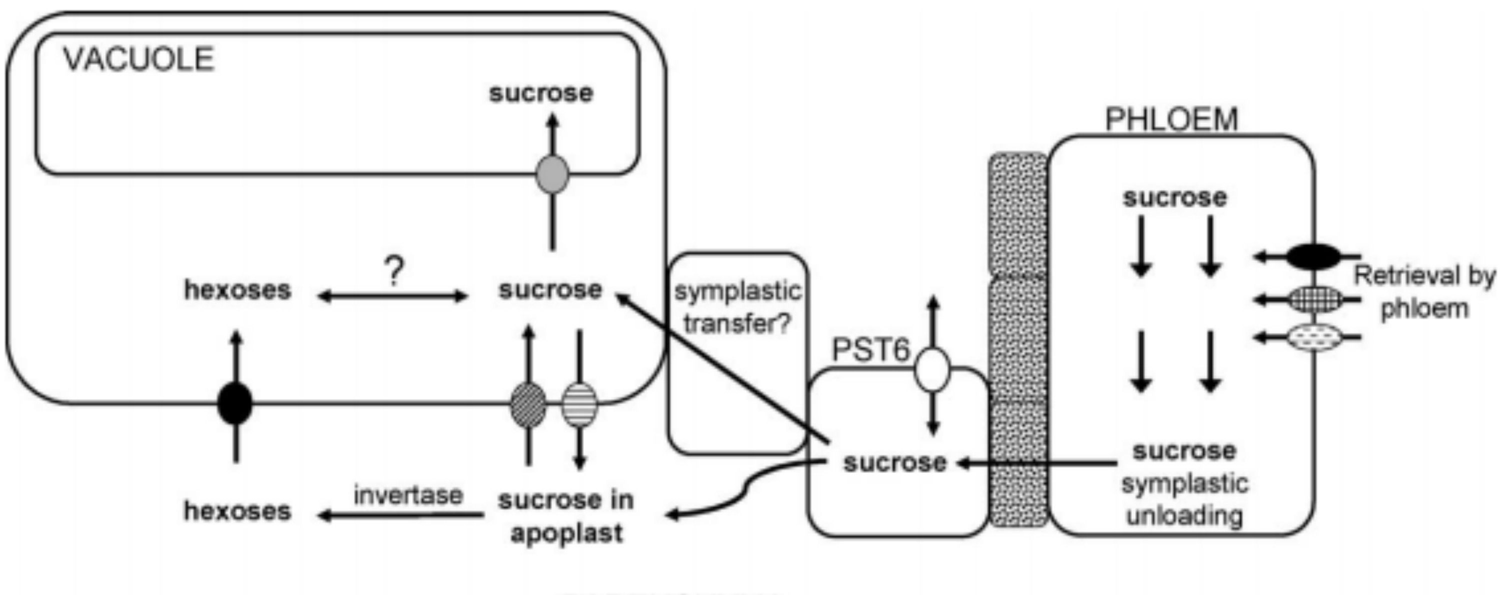
- Smaller genome
- Sequenced
- Grain and sweet exist
  - Can look for potential changes that lead to sugar storage
- Good genetic and physiological model for sugarcane

Genes linked to higher sugar content

- Microarray analysis of sweet vs. grain sorghum
- Same species so it is much easier to do this
- 103 down-regulated
  - Cellulose and lignocellulose related genes
  - Cell wall formation genes
- 51 up-regulated
  - Starch degradation
  - Heat shock proteins
  - Abiotic stress proteins
- Application
  - Better understand sorghum
  - Improve other biofuel crops
  - "sweet sorghum-like transgenic corn"

Sugar accumulation

- Sucrose transport not fully understood
- Sugarcane and sorghum similar
- Apoplastic vs. symplastic movement
- Intermediate hexoses



Comparison to other potential crops

- Efficient (low cost; high output)
- 2/3 ethanol production compared to sugarcane
- Higher quality sugar
- Stalks can be used as bioenergy or to feed animals
- Grain can instead be used as food or fodder
- Cheaper to produce ($75/L) than sugarcane ($112/L) and corn ($89/L)
- No fuel-food competition

Barriers to Biofuels

- Second generation biofuels, more costly, but necessary
- Difficult to break down secondary cell wall
- Lignin composition
  - Inhibits degradation, fermentation
  - Reduces conversion efficiency
- Cellulose, difficult and costly to break down

Possible Improvements for Sorghum

- Traditional breeding with available germplasm
- Hybrids
- High-energy sorghum (grain+sweet)
- Transformants!
  - Pest and disease resistance
  - Cellulase producing
  - Altered lignin composition
  - Altered cell-wall composition
  - Improved sugar storage

Genetic Transformation of Sweet Sorghum

- Anshu Raghuwanshi, Robert Birch
- Motivation: sorghum as a biofuel!
- Sweet sorghum has never before been successfully transformed

Overview

- Screened for varieties amenable to tissue culture
- Optimized tissue culture and regeneration conditions
- Established transformation and selection protocol

Initial Screening

- Screened 32 Sweet sorghum varieties
  - Callus initiation (3-4 months in dark)
- 5 different media
- Results:
  - R19188, Ramada, Wray
  - Modified M11 (mM11)

Regeneration and Hormones

- Ramada showed the highest regenerability after tissue culture (greater than 85%)
- Hormones were optimized for Ramada: vary through callus induction, selection, and multistep regeneration

Protocol

- Callus Induction
  - 4 days on mM11
- Selection
  - 3 weeks on selection media
    - mM11 + 40mg/L hygromycin
  - 8-12 weeks on callus proliferation media
    - mM11 + 0.5mg/L kinetin + 40mg/L hygromycin
- Regeneration w 40mg/L hygromycin
  - 2 weeks on regeneration medium
    - mM11 - 2,4-D, + 3mg/L BAP + 1mg/L TDZ
  - 2-3 weeks on shoot elongation medium
    - MS salts, sucrose, casein hydrolysate, CuSO4, 2mg/L BAP, NAA, phytagel
- Rooting
  - 5-8 weeks on rooting media
    - MS salts, sucrose, casein hydrolysate, NAA, phytagel

Transformation, Selection, Regeneration

- Bombardment
- Co-transformation
  - Hygromycin phosphotransferase
  - Luciferase
- Selection
  - Hygromycin
- Regeneration
  - 0.09% efficiency
  - 16 lines
  - 2 albino, 1 only roots
  - 9 planted in greenhouse, 3 stunted and sterile
  - 17,000 embryos!!

My Project

- Transform Ramada using the protocol from this paper
- Use Agrobacterium rather than bombardment
- Use PMI-GFP rather than hpt
- Gentler transformation and selection
- Super cool because no one has done it before!!

References

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mM11

- 0.004 µM BAP
- No micronutrients
- MES buffer
- Copper Sulfate
- Pyridoxal-5-phospate (not HCl)
- Agar
- pH 5.8