GENOMICS-INFORMED GENETIC ENGINEERING AND GENE EDITING IN SORGHUM

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Improving productivity and quality
It’s great to talk to an audience who know sorghum.
“If we had a way to accurately tally the world’s vast and complex drinking practices, sorghum is clearly one of the world’s most imbibed plants.”

(Stewart, 2013)
The Matrix — breaking down the starch:protein matrix

Lower digestibility
Higher gelatinization temp
Altered processing
Different ways to manipulate sorghum grain quality

Breeding aka

Engineering approaches

Or you can just shoot it

The Australian leadership group has arrived. 😂
Whole-genome sequencing reveals untapped genetic potential in Africa’s indigenous cereal crop sorghum.

Emma S. Mace¹,*, Shuaishuai Tai²,*, Edward K. Gilding³, Yanhong Li², Peter J. Prentis⁴, Lianle Bian², Bradley C. Campbell², Wushu Hu², David J. Innes⁵, Xuelian Han², Alan Cruickshank¹, Changming Dai², Céline Frère³, Haikuan Zhang⁷, Colleen H. Hunt¹, Xianyuan Wang², Tracey Shatte¹, Miao Wang², Zhe Su², Jun Li², Xiaozhen Lin², Ian D. Godwin³, David R. Jordan⁶ & Jun Wang²,⁷,⁸
Starch debranching – changes starch structure

Allelic variation at a single gene increases food value in a drought-tolerant staple cereal

Edward K. Gilding¹,*, Celine H. Frere²,*, Alan Cruickshank³, Anna K. Rada⁴, Peter J. Prentis⁵, Agnieszka M. Mudge¹, Emma S. Mace³, David R. Jordan⁶ & Ian D. Godwin¹
Pullulanase activity changes starch structure

- SbPUL-RA 70% starch, SbPUL-GD 65% starch
- Differences in amylopectin and amylose structure and size
Pullulanase sorghum lines

- NILs grown in field trials under bird-netting
- Replicated poultry feeding trials in progress at Sydney University
Genomics can inform Transgenic and Editing approaches

Guoquan Liu
20-25% efficiency
ALTERING EXPRESSION OF KAFIRIN AND STARCH BIOSYNTHESIS GENES

Gene Expression

Whole grain

Embryo-specific

Endosperm-specific

Altering the Protein: Starch Matrix
Natural $\beta$-kafirin null mutants

$\beta$-kafirin is not essential

That makes it a target to play with!

- Higher protein
- Higher digestibility
- Ogi porridge in West Africa
**Transgenics and genome editing**

Approaches to improve grain quality

BIGGER GRAIN and MORE DIGESTIBLE PROTEIN

1. Overexpress a synthetic β-kafirin
2. Change the way proteins fold (RNAi-foldase)
3. Manipulate G-proteins associated with grain size
Transgenic synβkaf lines

More Protein + Higher Digestibility

Protein content %

Independent T1 transgenics  Tx430

Digestibility %

5-1 6-1 8-1 9-1 9-2 Tx430

Digestibility by pepsin

Digestibility by chymotrypsin
G protein gamma-subunits

1. Overexpression
2. RNAi silencing
3. Overexpression of truncated genes

- RNAi silencing (~50-70% downregulation) from independent transgenic events
  - GGC1 (GS3) – mean 7% increase in seed size
    - Same as the QTL in NAM
  - GGC2 – mean 7% increase in seed size
  - GGC3 (DEP1) – mean 9% increase in seed size
Grain size characteristics

10 seeds

RNAi GGC1 (GS3)
No reduction in grain number
9-12% increase in TKW
“Foldase” RNAi lines – more protein, bigger grain

Significant change in the protein-rich peripheral endoperox

Tx430
Wild-type

Foldase RNAi line 12-8-1
Also – grain is slightly larger volume and heavier

Field trials summer 2018
• Inbred lines
In grain fill in SE Queensland
Current status of GM sorghums

- Best GM sorghum lines with:
  - 12-14% larger grain
  - No reduction in seed number – YIELD
  - Up to 30% higher protein content
  - 10-15% more digestible

- Better Food
- Better Feed
- Better returns for farmers and intensive animal industries
Gatton campus and Research Farm 80 km from Brisbane
27 transgenic lines – 5 genes manipulated

UQ + NuSeed + Qld Dept of Agriculture and Fisheries collaboration
Gene editing in sorghum – our progress

Brown Midrib sorghum – lignin biosynthesis (rice U6 promoter)

Sorghum albinism
(rice v sorghum U6/U3 promoters)
Most edits were seen in selfed progenies

- Brown midrib (rice U6 promoter) – knockout of lignin biosynthesis
  - 5 of 20 transgenics were homozygous in regenerated plants
  - Additional 7 of 20 showed BMR phenotype in selfed progenies
  - Edits varied from 8 bp deletion to 1 bp insertion

(Peng et al, 2017, *Plant J.* under revision)

- Now our focus is tissue culture-free editing
PIN gene expression and plant architecture

1. PIN genes
2. Plasticity
3. Pleiotropy

Small canopy expression

Large canopy expression

See Andy Borrell’s talk coming up soon

Andy Borrell and Guoquan Liu
PIN genes confer ‘nodal root number’ plasticity in sorghum
Genomics + GM/editing + physiology ➔ better sorghum
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