



Healthy grains and rice quality pre-breeding research at CSIRO
Healthy, safe, wholegrains for the food and beverage industries

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ZHONGYI LI, CSIRO AGRICULTURE AND FOOD, CANBERRA



CSIRO Agriculture & Food

Our advantage – Integrated Agricultural and Food Research

Genome

Phenome

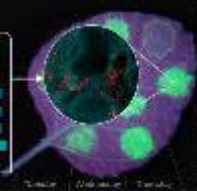
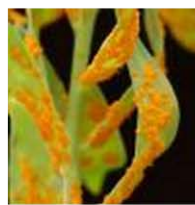
Breeding
& Selection

Farming
Systems

Landscapes &
Environment

Markets

Society &
Economy



CSIRO capability relevant to today's talk

CSIRO Agriculture and Food

- Cereal Quality Traits
- Innovative Food Centre

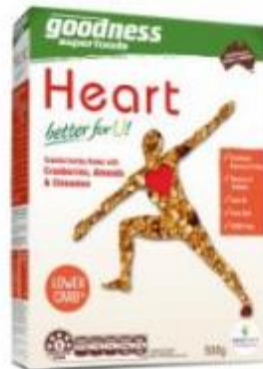
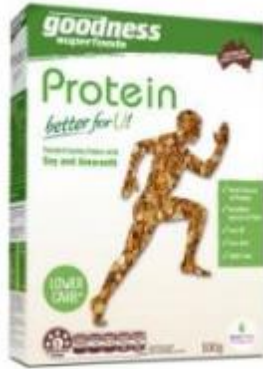


CSIRO Health and Biosecurity

- Nutritional and Health Substantiation



Some BARLEYmax products in Australia



BARLEYmax oat
soughdough



Spin-out

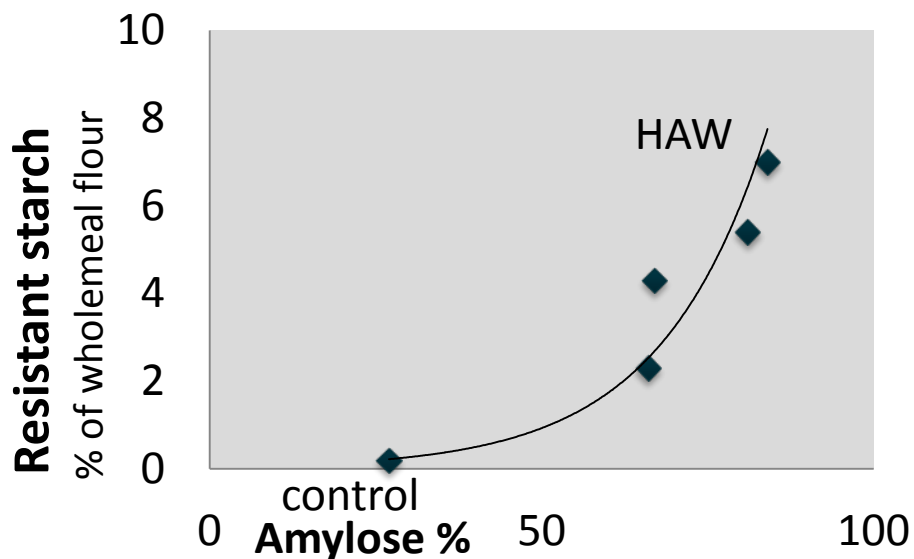
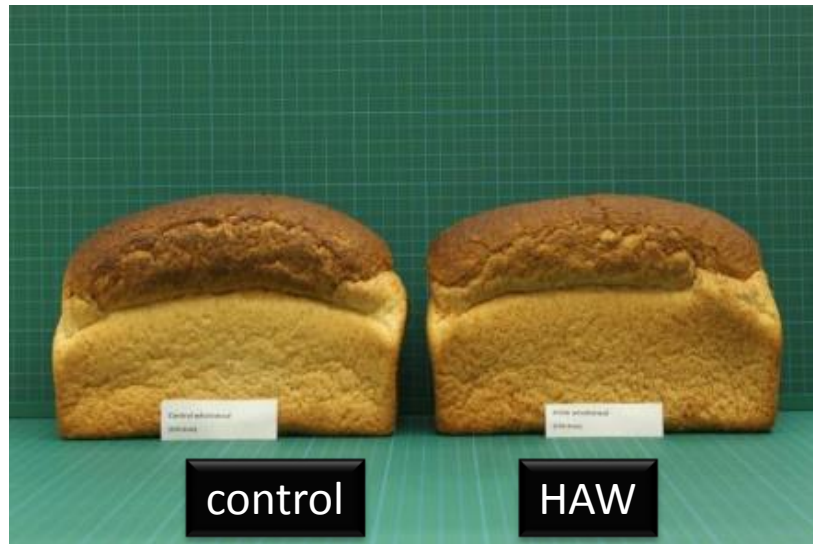


Japan first products launched August 2016
USA first products launched May 2017

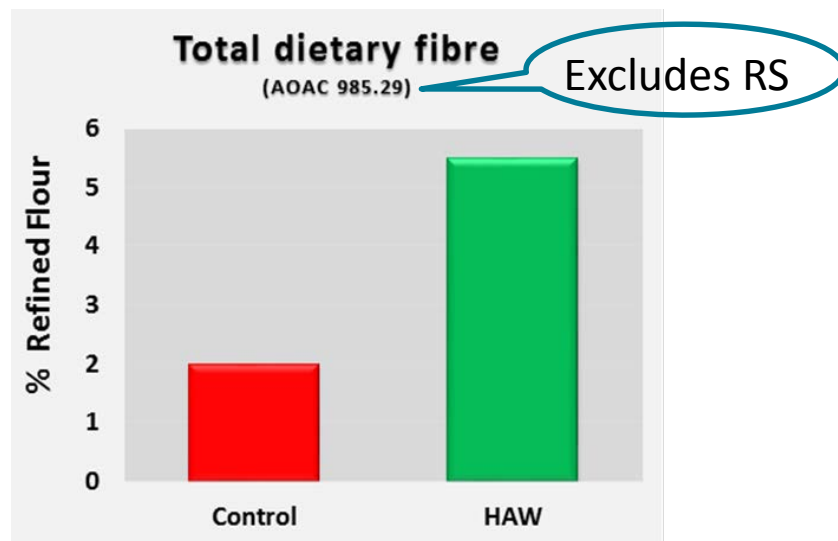


Marcus Newberry

Ahmed Regina



>>10 x resistant starch



3x TDF in refined flour
2x TDF in wholemeal

Hordein reduced >10,000x
56,000ppm to 3-5ppm

WHO Gluten-free target ≤ 20 ppm



First the beer

Commercial release April 2016



Crispin Howitt



Greg Tanner



Malcolm Blundell

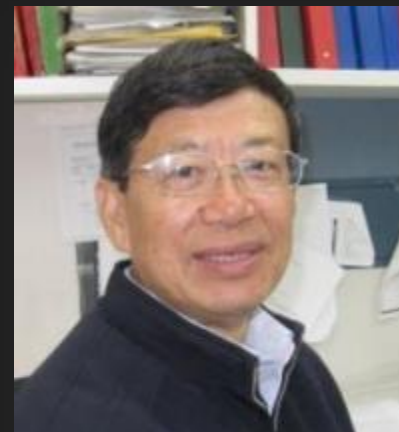
Today's presentation: Cereal Grain Traits

What we are doing:

- Resistant starch barley
- Resistant starch rice
- Micronutrient dense rice
- Long shelf life rice



Resistant starch barley BARLEYmax



Zhongyi Li

Why resistant starch? – a fermentable fibre

High amylose (>60%)

- Modifies starch granule structure to produce resistant starch

Resistant starch

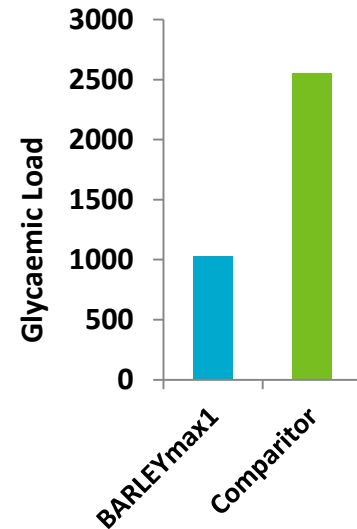
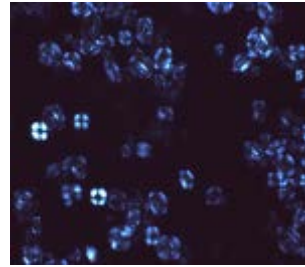
- Resists digestion in the small intestine
- Reduces glycemic index and glycemic load
- Improves satiety

Fermented to short chain fatty acids in large bowel

- Acetate, proprionate, butyrate – preferred energy for colonocytes
- Lower bowel pH - increases good bugs and reduces the pathogenic
- Enhanced Mg and Ca absorption

Healthier colonocytes

- Reduced rates of bowel cancer
- Reduced absorption of pathogens



Chronic disorders in Australia



Cardiovascular disease

18% of Australians
\$3.9 billion a year



Colorectal cancer

4,400 death pa,
cost \$2 billion a year



overweight or obese

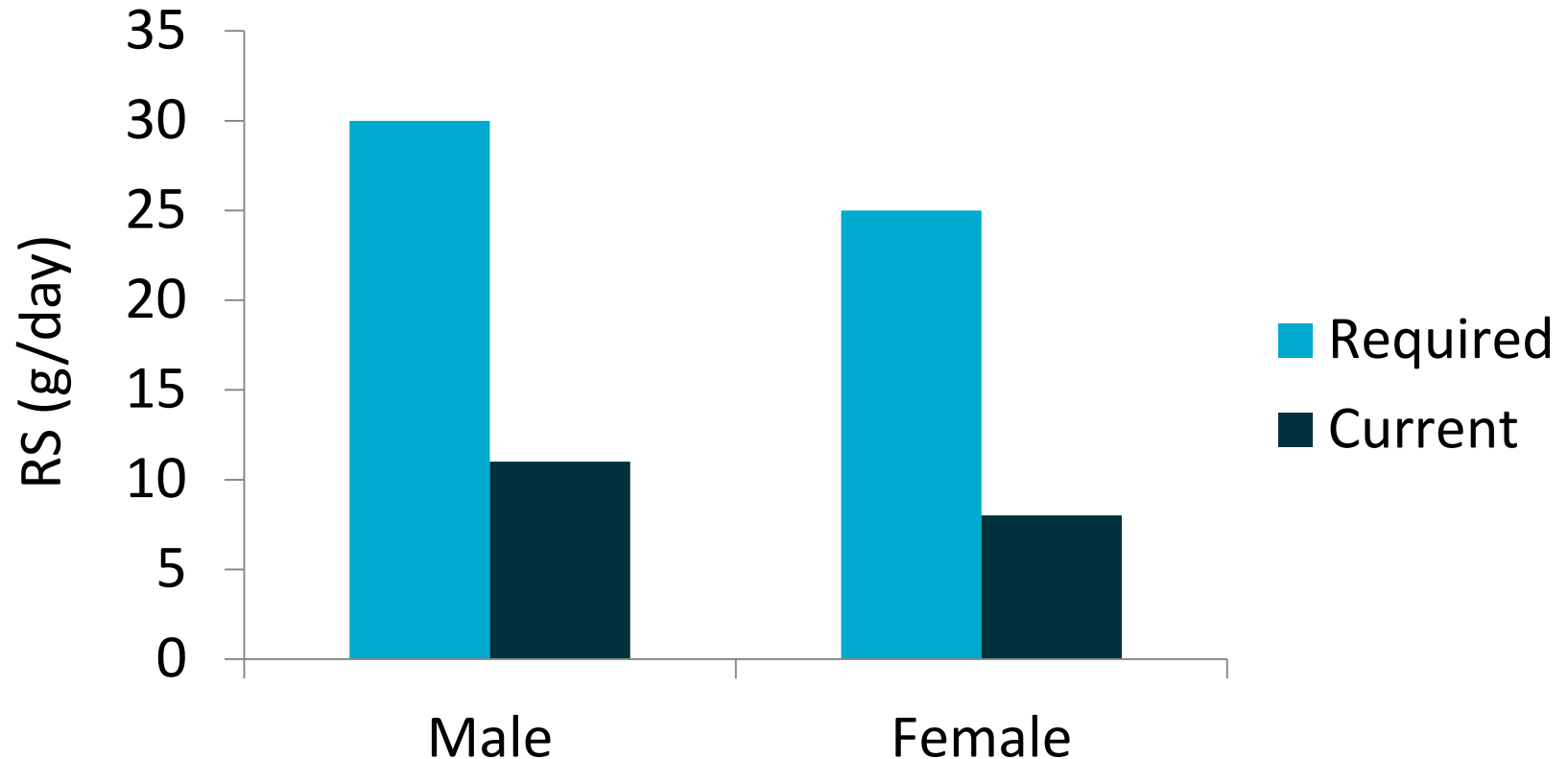
64% men,
47% women



Type II diabetes

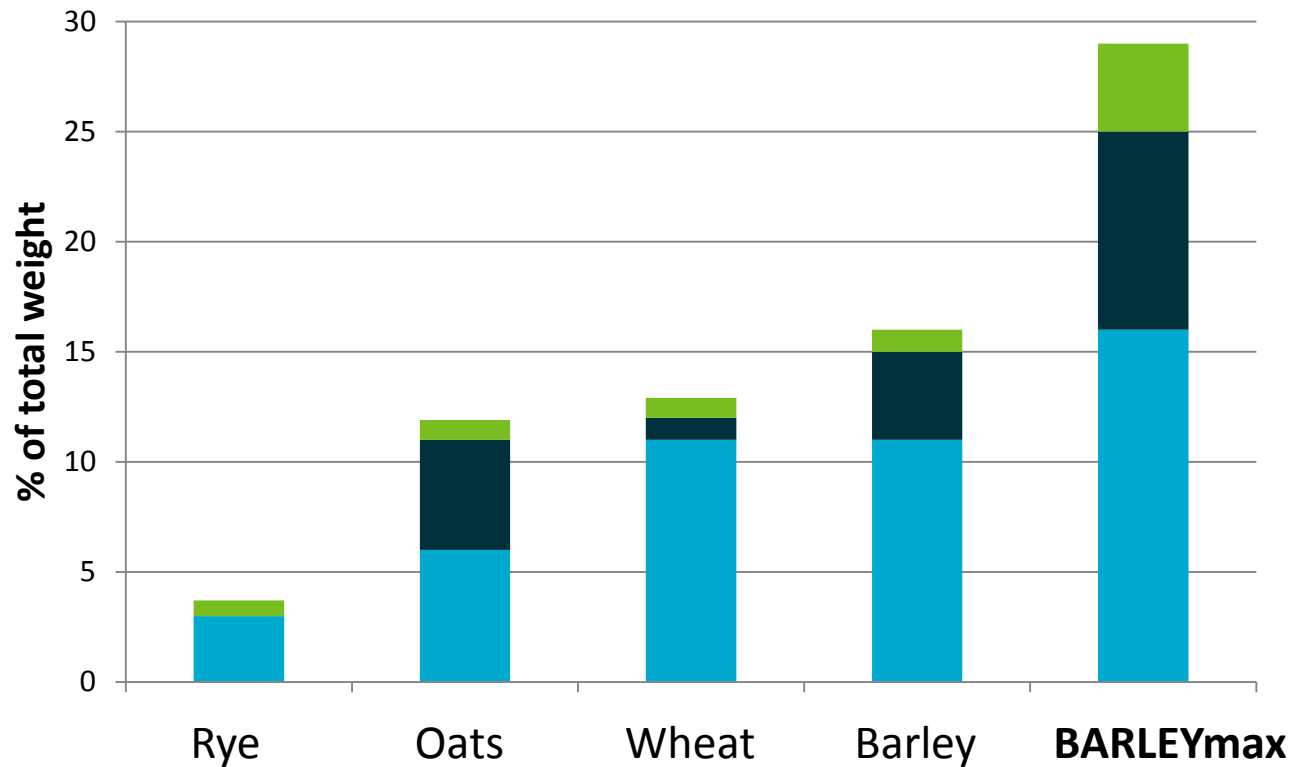
8% of Australians,
cost \$3 billion a year

Adequate RS intake in Australia



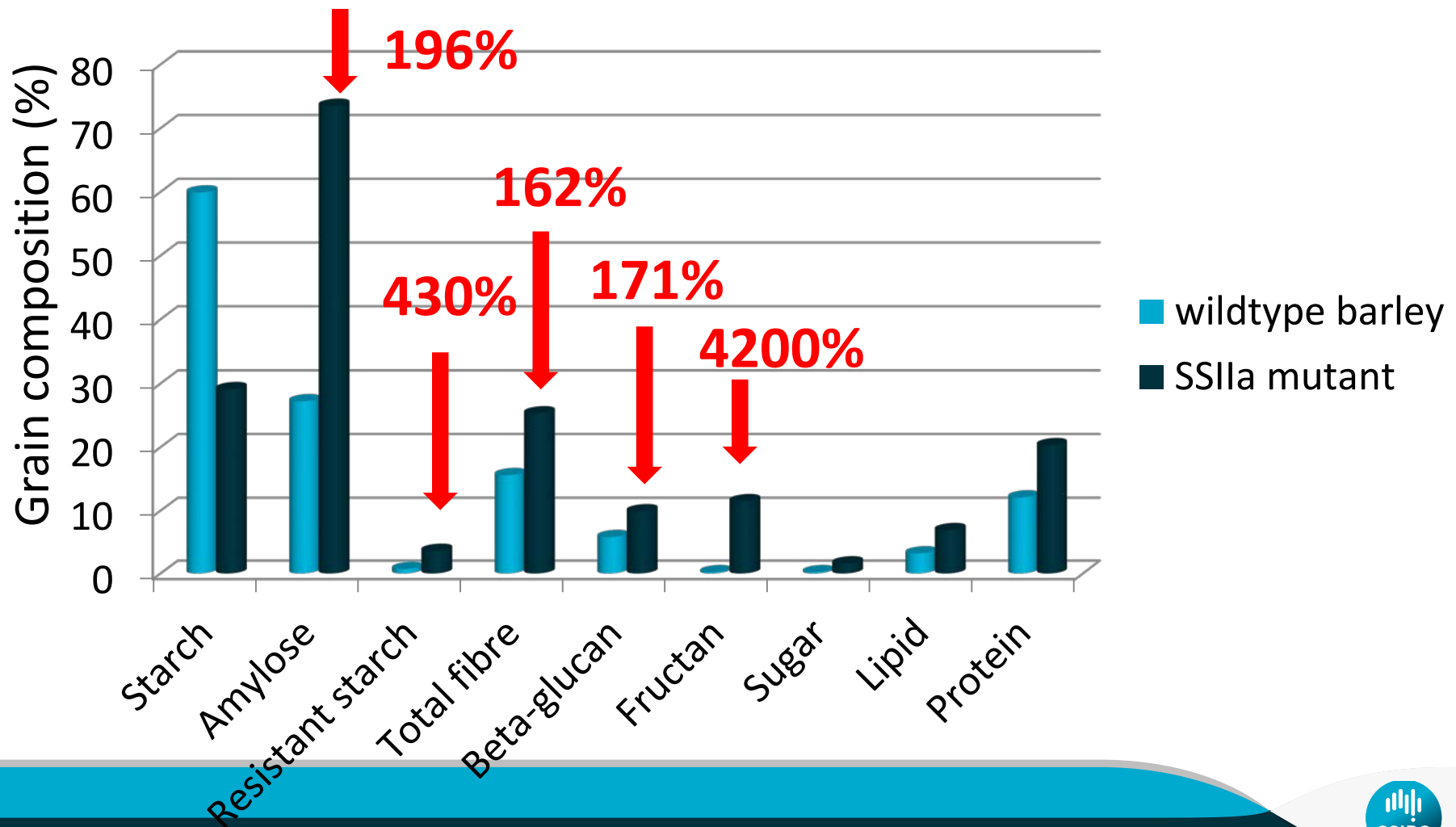
More fibre than any other cereal grain

High Resistant starch, fructan and fibre



- Resistant Starch
- Soluble Fibre
- Insoluble Fibre

SSIa mutants in barley increase resistant starch, β -glucan, fructan and total fibre



Clinical substantiation

- Reduced blood glucose concentration – lower GI (humans)
- Reduced plasma lipids (pig trial)
- Reduced pH in the large bowel (pig trial)
- Increased SCFA concentration in the large bowel (pig trial)



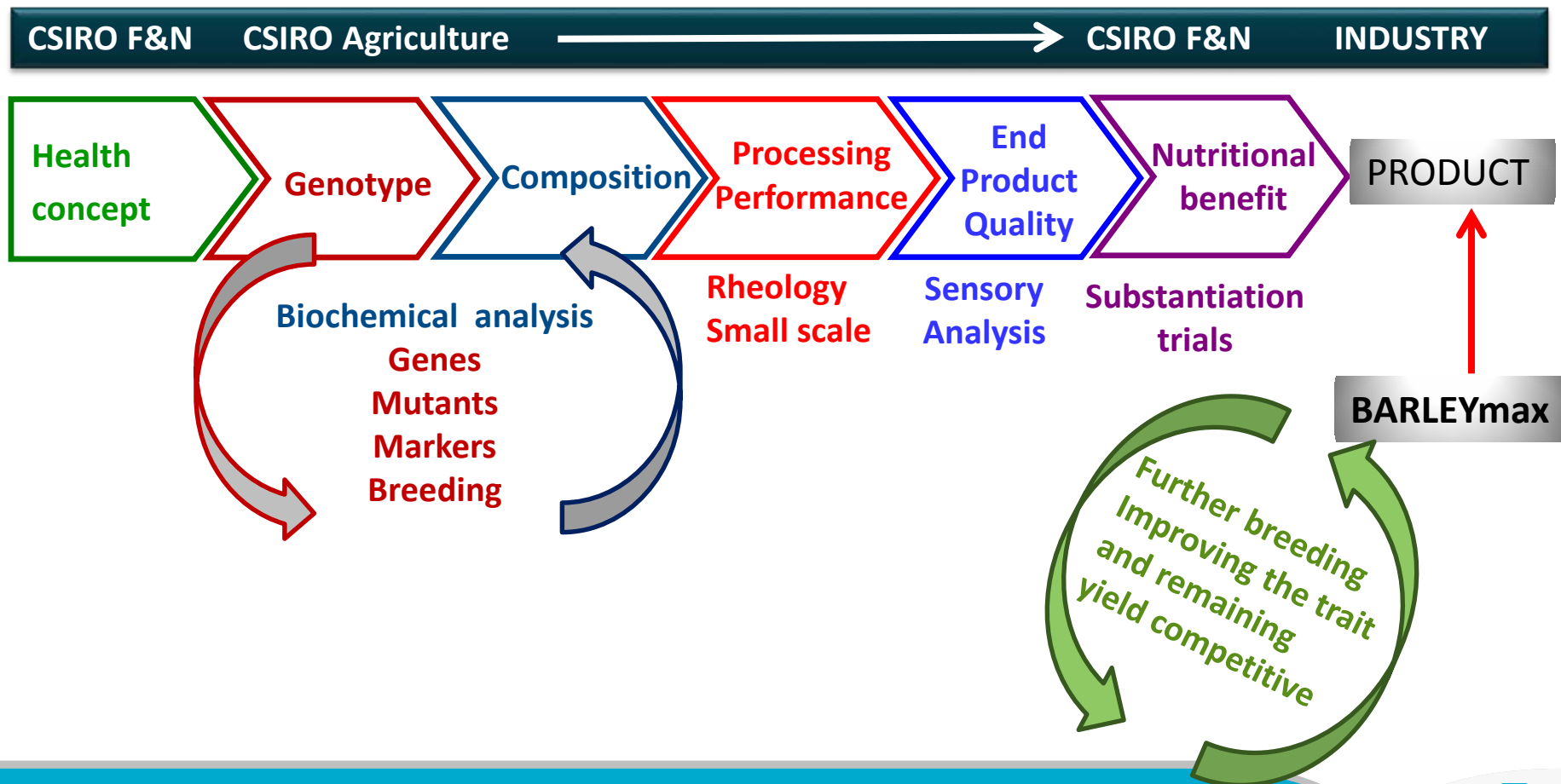
Tony Bird



David Topping

BARLEYmax – path to market and impact

The Healthy Grain



BARLEYmax 1, 2 and 3

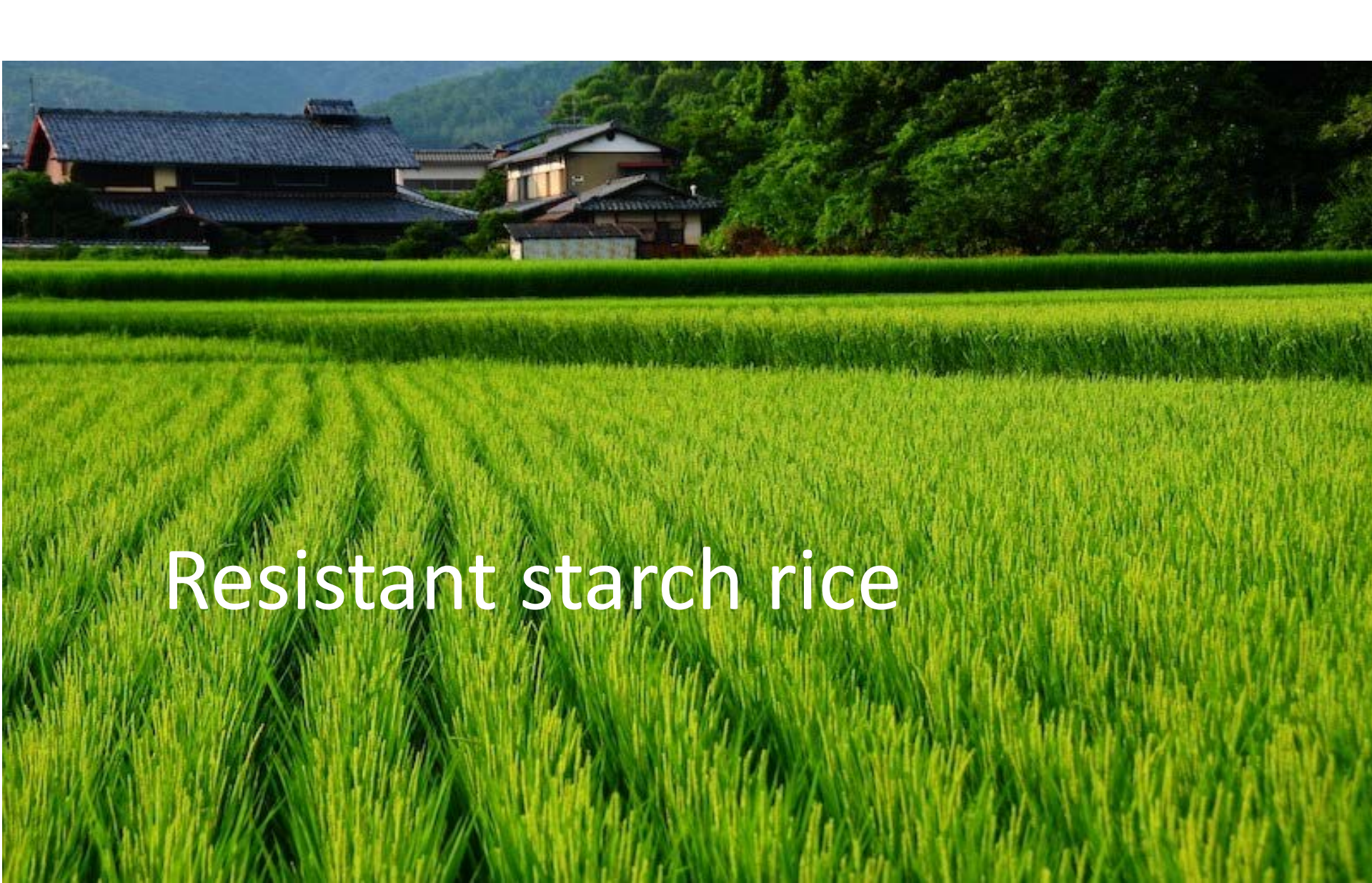
Commercial rights with *The Healthy Grain*

<http://www.thehealthygrain.com/>

CEO: Robert Burbury

Technical Business Manager: Dr Paul Sims





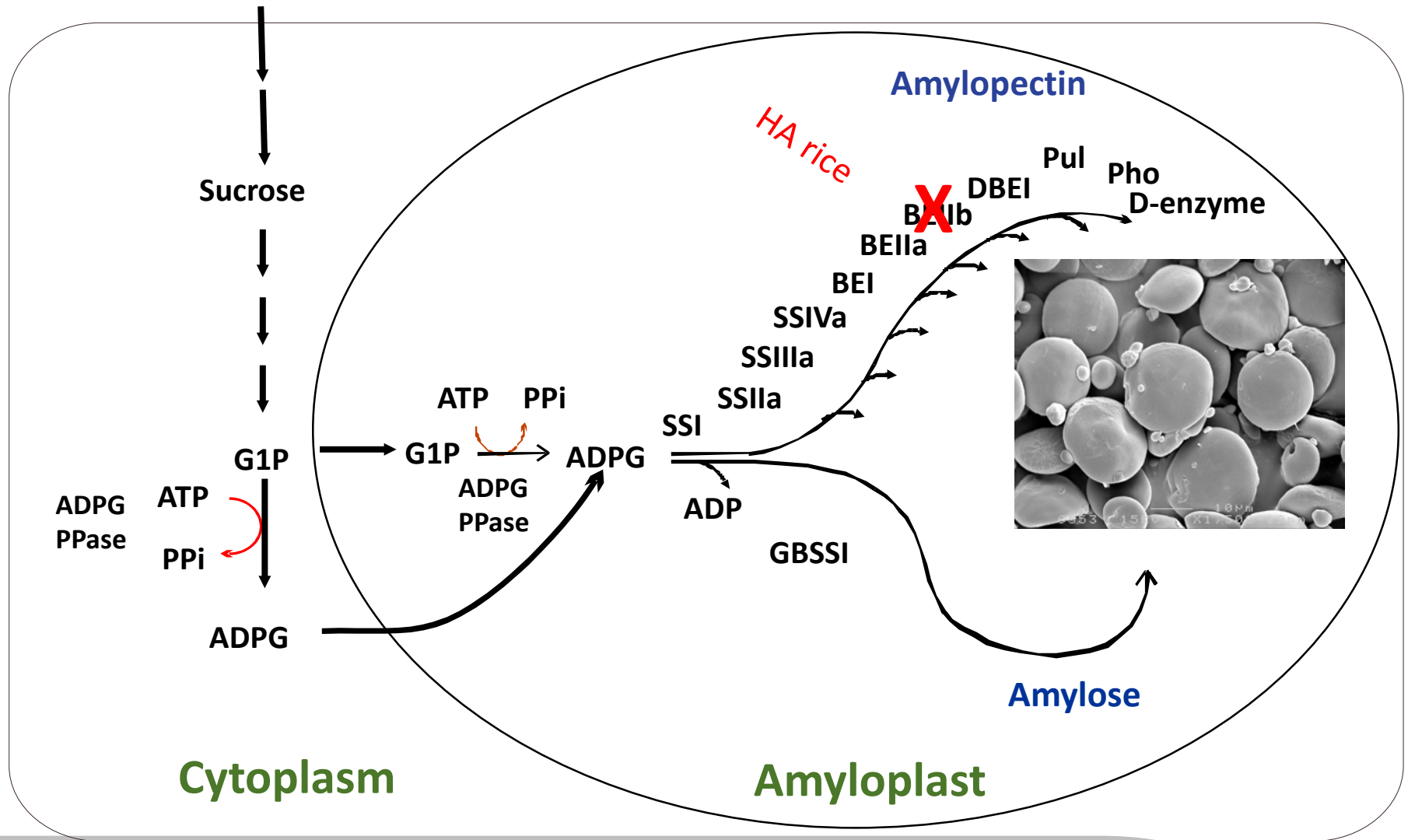
Resistant starch rice

Increasing amylose content in rice grain through down-regulating the expression of SBEIIb by RNAi and amiRNA approaches

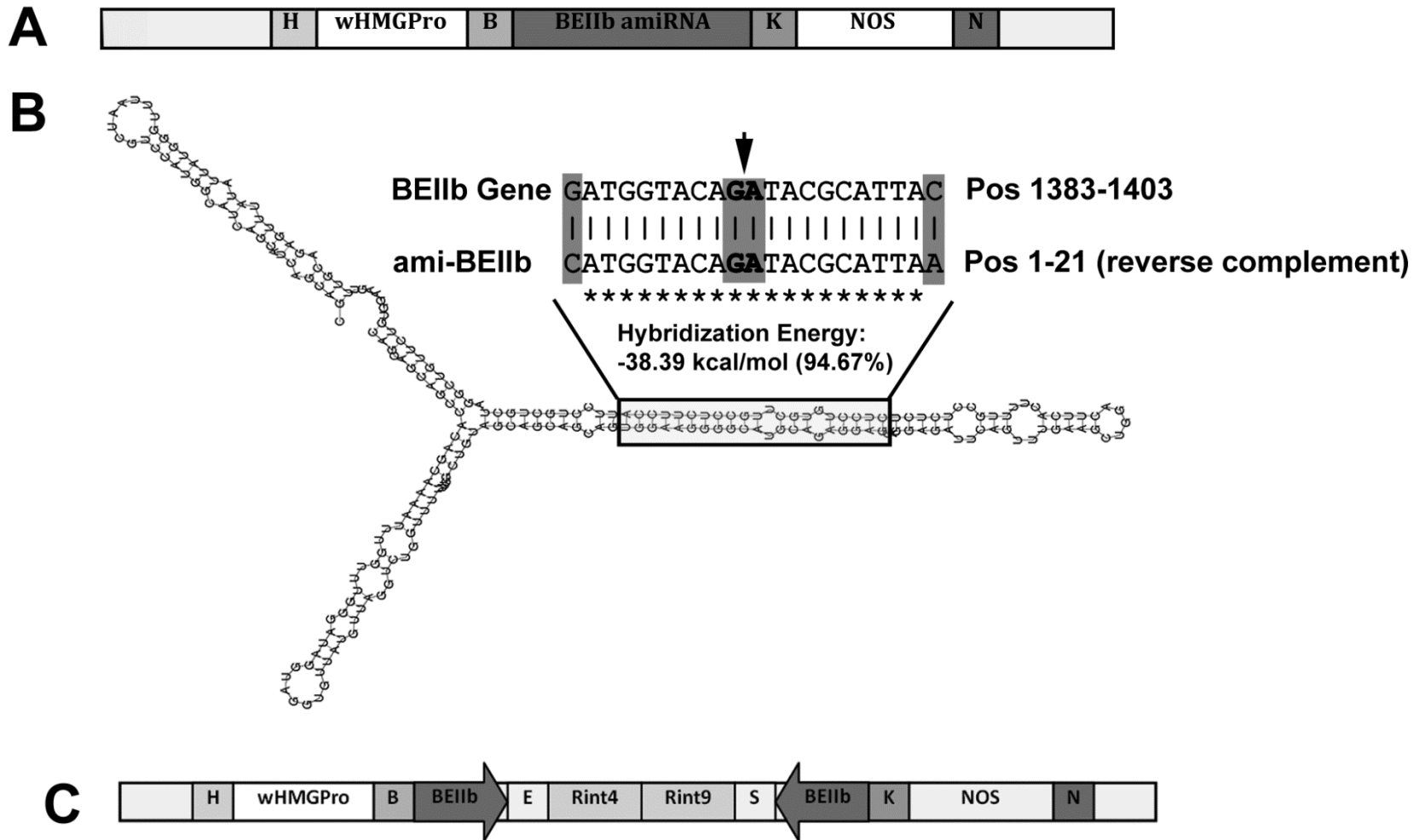
Vito Butardo



Increase amylose in rice



RNAi and amiRNA were used



Increasing amylose content in rice grain through combining different starch synthetic gene alleles

Jixun Luo



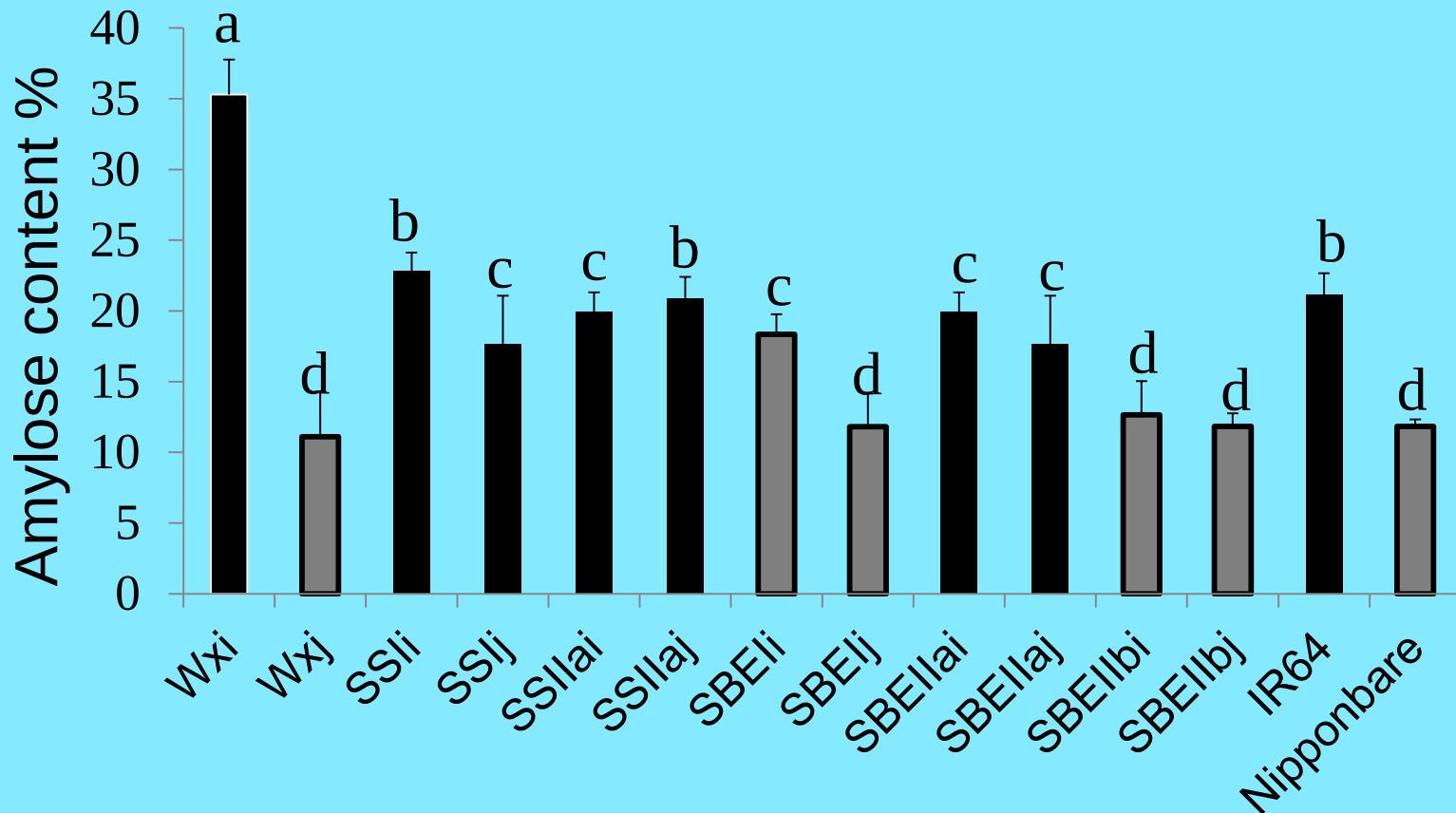
RILs following cross between IR64 (*indica*) and Nipponbare (*japonica*)

Genotypes of RILs of six starch biosynthetic enzyme groups

Geno- type	<i>Wxi</i>	<i>Wxj</i>	<i>SSIi</i>	<i>SSIj</i>	<i>SSIIai</i>	<i>SSIIaj</i>	<i>SBEIi</i>	<i>SBEIj</i>	<i>SBEIIai</i>	<i>SBEIIaj</i>	<i>SBEIIbi</i>	<i>SBEIIbj</i>
<i>Wx</i>	i	j	i		i		j		i		j	
<i>SSI</i>	i		i	j	j		i		j		i	
<i>SSIIa</i>	i		i		i	j	i		i		i	
<i>SBEI</i>	i		i		i		i	j	i		i	
<i>SBEIIa</i>	j		i		j		i		i	j	i	
<i>SBEIIb</i>	i		j		j		i		j		i	j

'i' and 'j' are abbreviations for *indica* (IR64) and *japonica* (Nipponbare) genotypes of each enzyme

Producing higher amylose rice grain through combining different starch synthetic gene alleles



Black columns indicate lines containing *Wxi* allele;
Grey columns indicate lines containing *Wxj* allele.

RILs following cross between A203 and 3-12

Genotypes of RILs of four starch biosynthetic enzyme groups

A203
SSIIa^{jap}SSIIa^{jap} / SBEIIb^{DR}SBEIIb^{DR}
 (opaque)

×

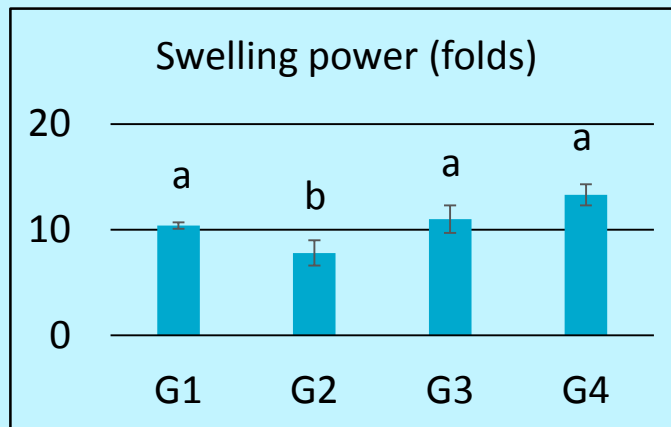
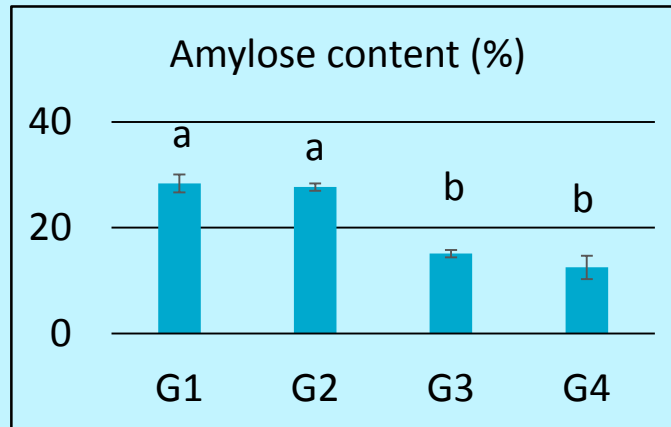
3-12
SSIIa^{ind}SSIIa^{jap} / SBEIIb^{WT}SBEIIb^{WT}
 (translucent)

Parents

	<i>SSIIa</i>	<i>SBEIIb</i>	<i>Wx</i>	Seed colour
G1:	<i>ind</i>	- ve	<i>jap</i>	opaque
G2:	<i>jap</i>	- ve	<i>jap</i>	opaque
G3:	<i>ind</i>	+ ve	<i>jap</i>	translucent
G4:	<i>jap</i>	+ ve	<i>jap</i>	translucent

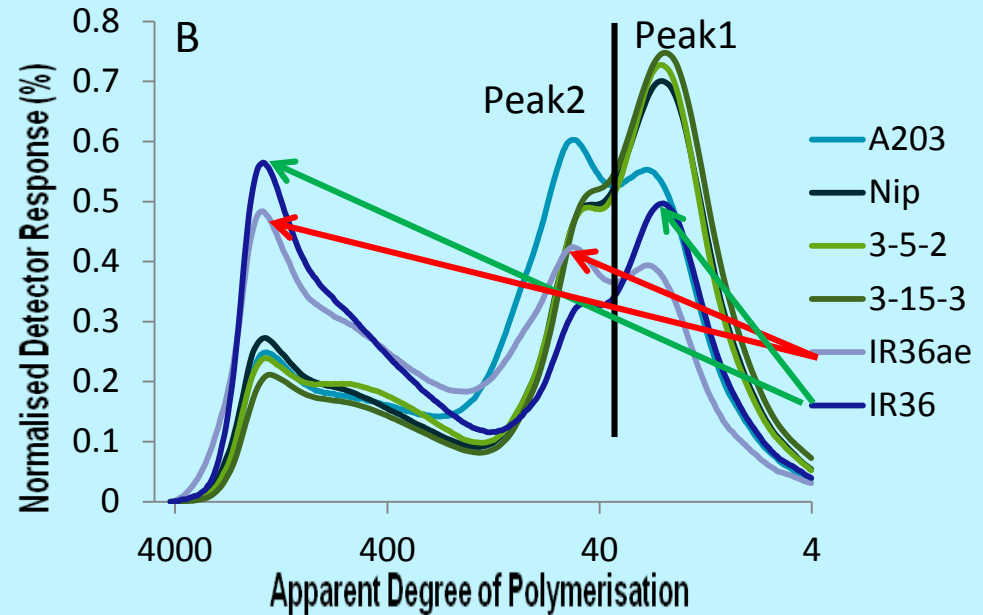
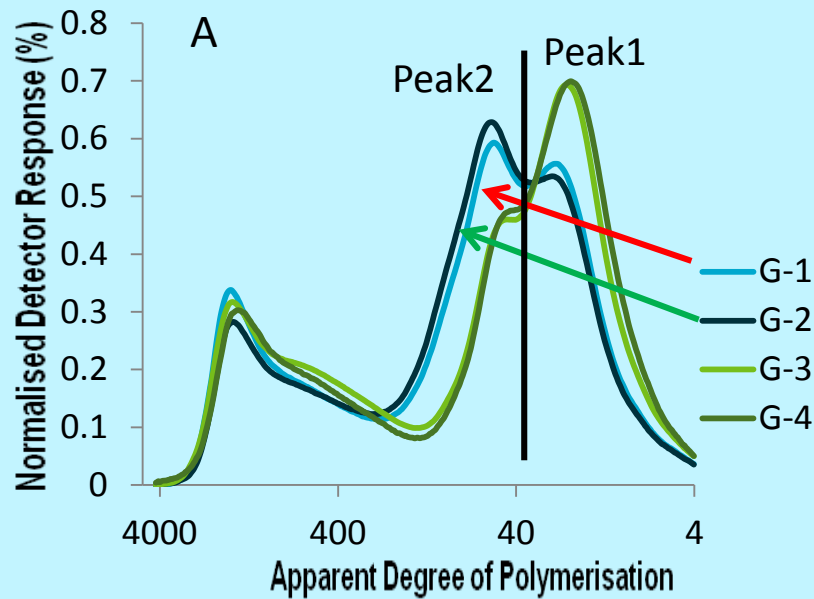
F3

The impact of the *indica* *SSIIa* allele on the starch properties of high amylose starch from rice grains



	<i>SSIIa</i>	<i>SBEIIb</i>
G1:	<i>ind</i>	- ve
G2:	<i>jap</i>	- ve
G3:	<i>ind</i>	+ ve
G4:	<i>jap</i>	+ ve

Two different mechanisms for increasing amylose content in rice grains

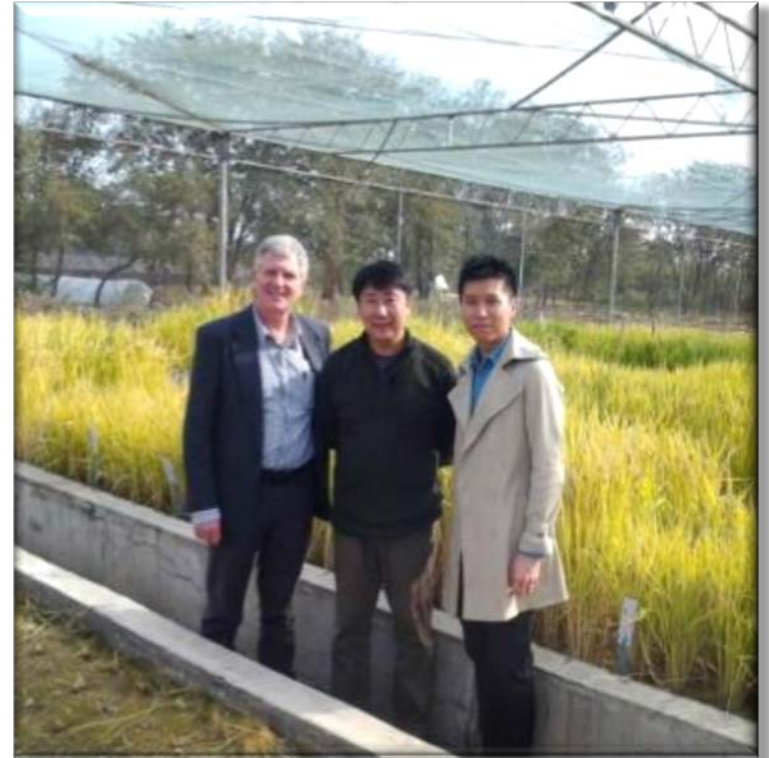


	<i>SSIIa</i>	<i>SBEIIb</i>	<i>Wx</i>
G1:	<i>ind</i>	-ve	<i>jap</i>
G2:	<i>jap</i>	-ve	<i>jap</i>
G3:	<i>ind</i>	+ve	<i>jap</i>
G4:	<i>jap</i>	+ve	<i>jap</i>

	<i>SSIIa^{jap}</i>	<i>SBEIIb</i>	<i>Wx^{jap}</i>
A203:	<i>jap</i>	-ve	<i>jap</i>
Nip:	<i>jap</i>	+ve	<i>jap</i>
3-15-3:	<i>jap</i>	+ve	<i>jap</i>
3-5-2:	<i>ind</i>	+ve	<i>jap</i>
IR36ae:	<i>ind</i>	-ve	<i>ind</i>
IR36:	<i>ind</i>	+ve	<i>ind</i>

Summary for manipulating starch in rice grain

- Both suppression of SBE IIb expression and gene recombination can increase amylose content.
- High amylose trait can be achieved through two different mechanisms.
- The *indica* SSIIa can improve starch properties for high amylose starch from rice grain through down-regulation of SBEIIb.



Micronutrient dense rice

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中国科学院植物研究所
INSTITUTE OF BOTANY, THE CHINESE ACADEMY OF SCIENCES



New type of rice - thick aleurone mutants

Rice dominant diets:

Zn and Fe deficient
5th and 6th most important
contributors to disease in
developing countries
(WHO, 2002)

Dietary fibre

Proteins, enzymes

Phenolics, lignans

Vitamin E

B group vitamins

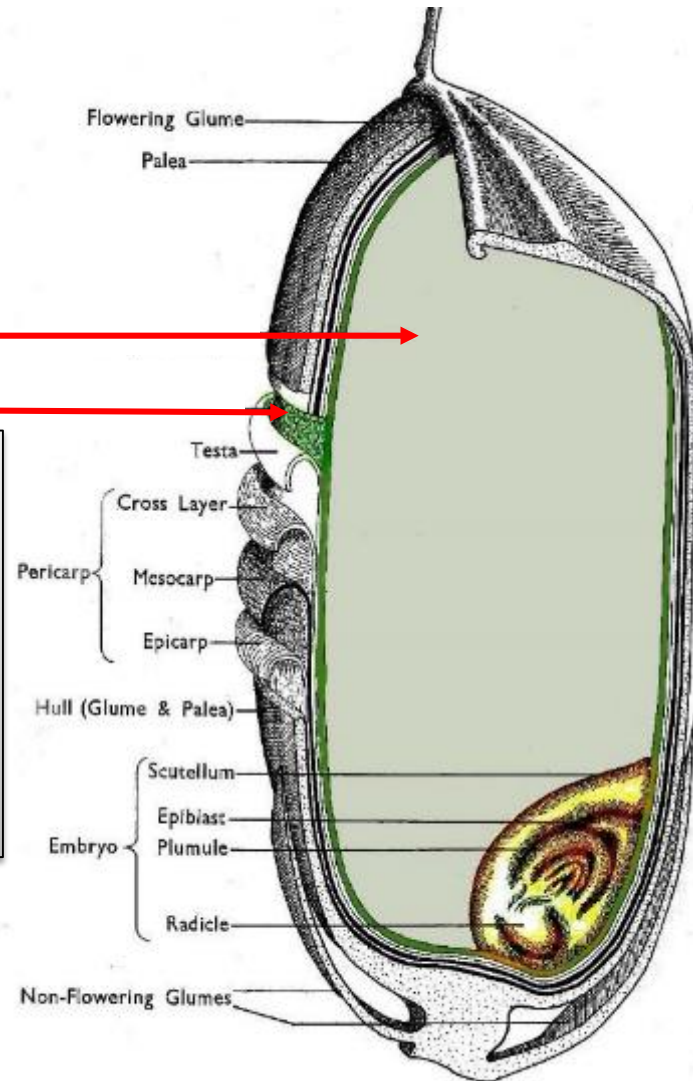
Minerals and phytic acid

Lipids, plant sterols

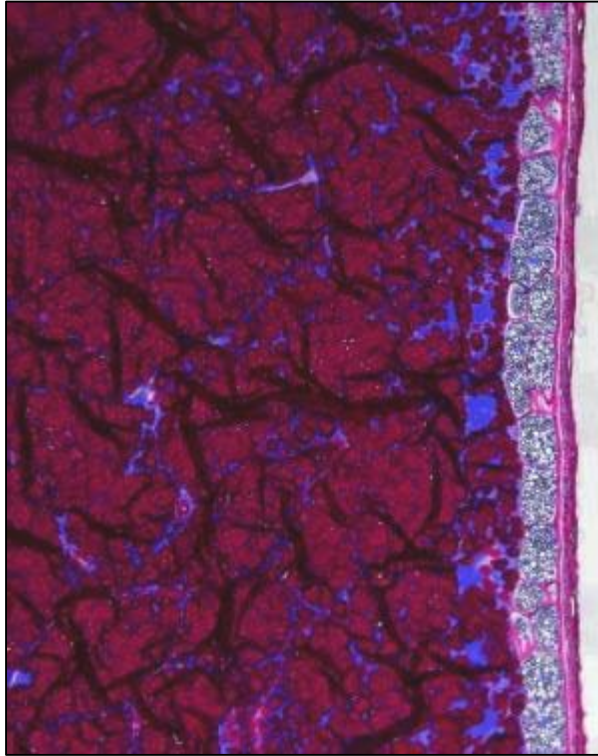
Aleurone important sink for
micronutrients

Starchy endosperm

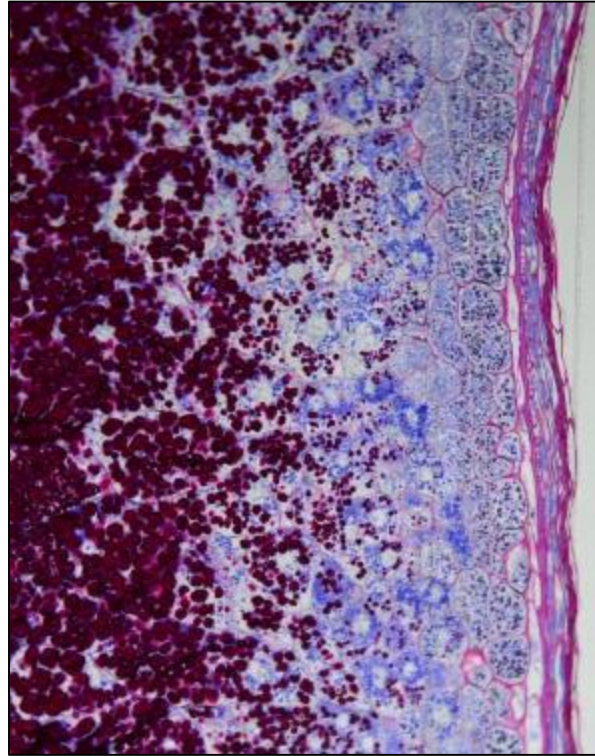
Aleurone layer



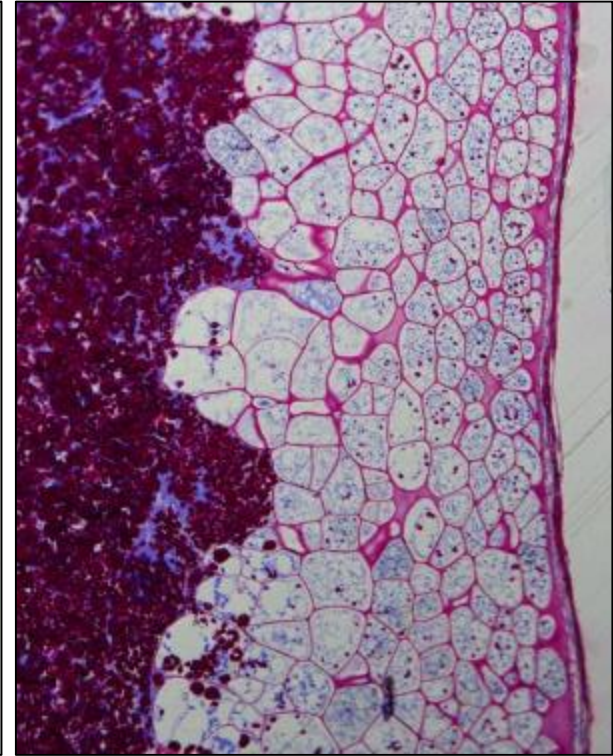
ta-1 and *ta-2* showed increased aleurone layers



ZH11



ta1



ta2

27 days after pollination

Nutritional analyses of *ta2* mutant wholemeal flour compared to normal wholemeal flour

50% increase in lipid

≥20% increase in
Zn, Fe, Mg, K, P, S

80% increase
antioxidant capacity

60% increase in
Vitamin B6 &
B9



30% increase in fibre

20% increase in
Vitamin B3

Summary for micronutrient dense rice

- Micronutrient dense rice has been generated through increasing aleurone sink for micronutrients in rice grain.
- Thick aleurone rice grain also contains more dietary fibre and vitamins.
- Patent application is in place.



Long shelf life rice

Oil oxidation

Oxidation stages

Oleic acid
Linoleic acid
Linolenic acid

Autoxidation
Stage 1

Autoxidation
Stage 2

Good Oil

Oxidation process

Bad Oil



Hydroperoxides

Aldehydes
Carboxylic
Alcohol
hydrocarbons



Oxidized flavor

Products

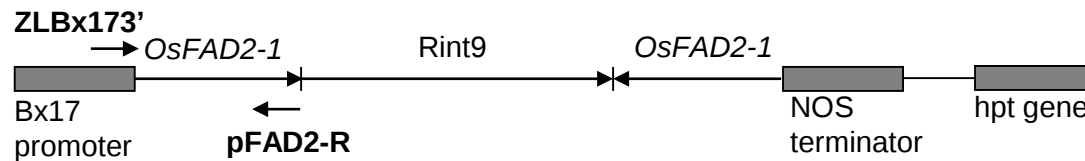
eg. For Linoleic acid
9-Hydroperoxide
13-Hydroperoxide

eg. For Linoleic acid
Pentanal
Hexanal
2,4-decadienal

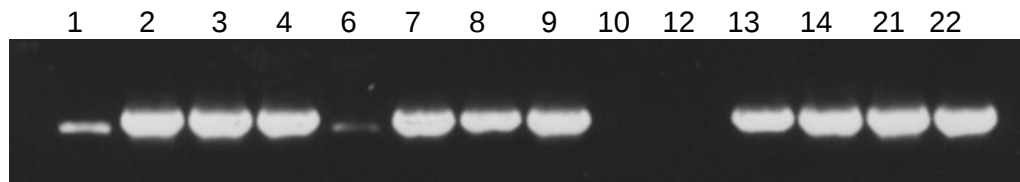
eg. soybean and corn oil
contain 23.5% Hexanal
in volatile compounds

PCR screening of *OsFAD2-1* RNAi T₀ plants (Nipponbare)

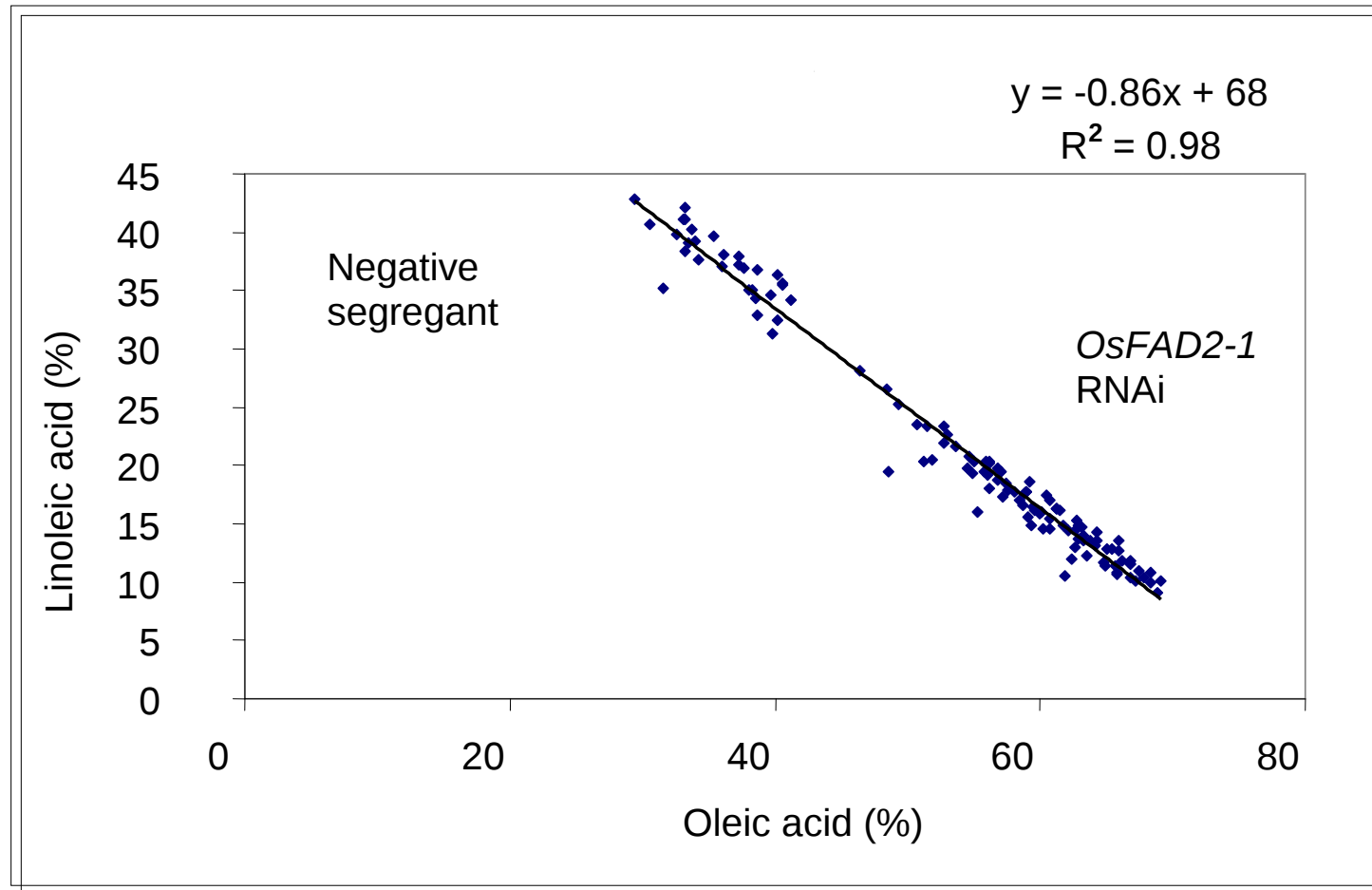
A



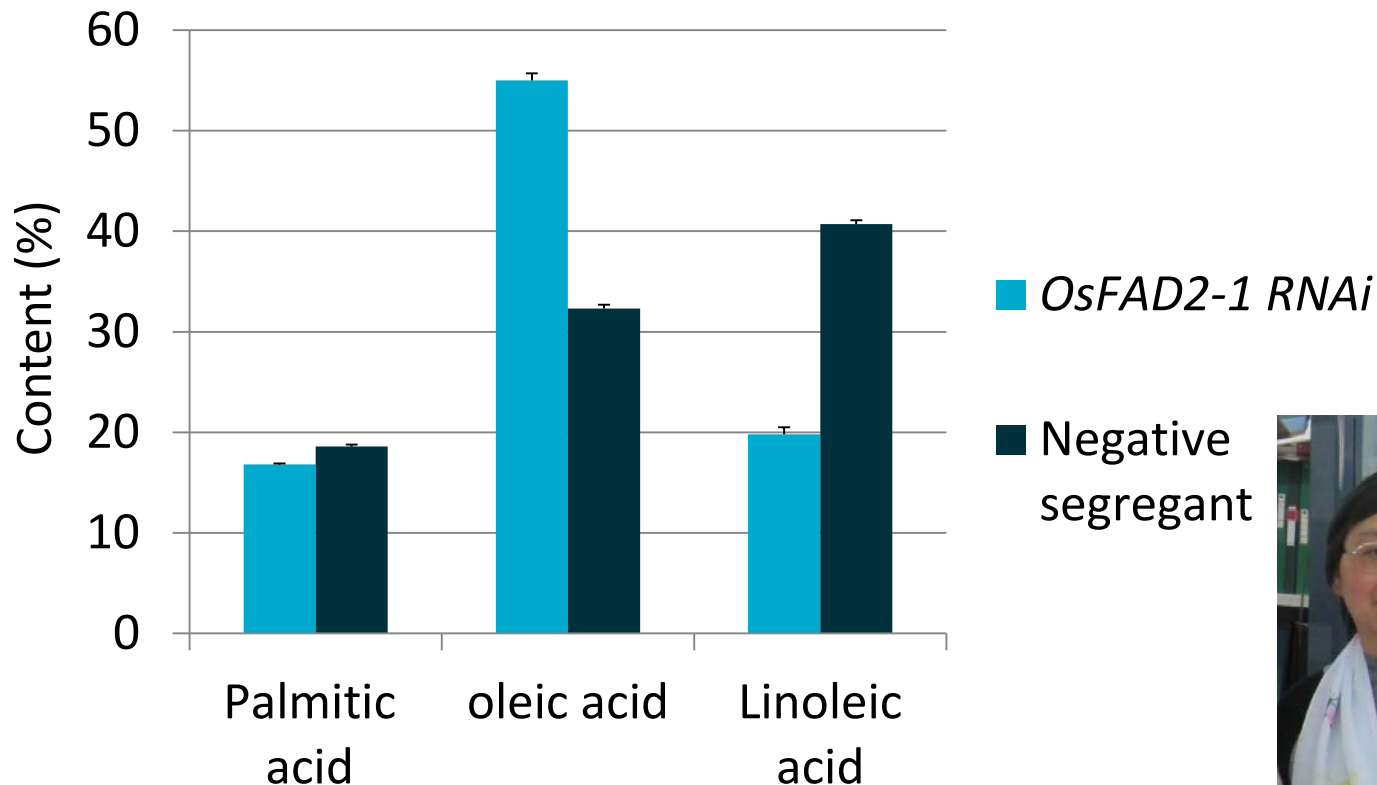
B



Linear relationship between linoleic acid and oleic acid content for *OsFAD2-1* RNAi and negative segregant T_1 grains



Increased oleic acid and reduced linoleic acid contents of T₄ grains for *OsFAD2-1* RNAi lines



Hong Wang Ming Huang

Summary



Producing BARLEYmax, HA wheat and Kebari grains containing novel traits that are commercially available.

Developed rice grain with increased RS and low GI.

Developed micronutrient dense rice.

Producing long shelf life rice grain.

Looking for the opportunities to work with other partners for improving rice grain quality.

Acknowledgement

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Limagrain (ARISTA)

Rob Defeyter

Lindsay Adler

Paul Sims

CSIRO Plant Industry

CAISO Food Future Flagship

CSIRO Agriculture Flagship

Thank you

CSIRO Agriculture Flagship

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Presenter Title: Manipulating
starch in

cereal grains

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