

Biotechnology for methane mitigation

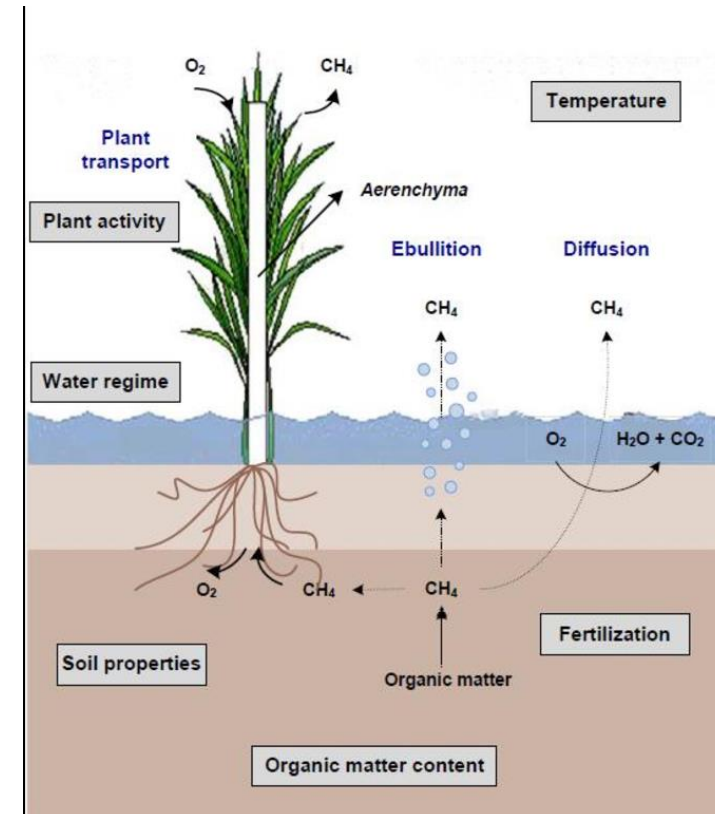
LETTER

doi:10.1038/nature14673

Expression of barley SUSIBA2 transcription factor yields high-starch low-methane rice

J. Su^{1,2*}, C. Hu^{1,2*}, X. Yan^{2*}, Y. Jin^{2,3}, Z. Chen¹, Q. Guan¹, Y. Wang¹, D. Zhong¹, C. Jansson⁴, F. Wang¹, A. Schnürer⁵ & C. Sun²

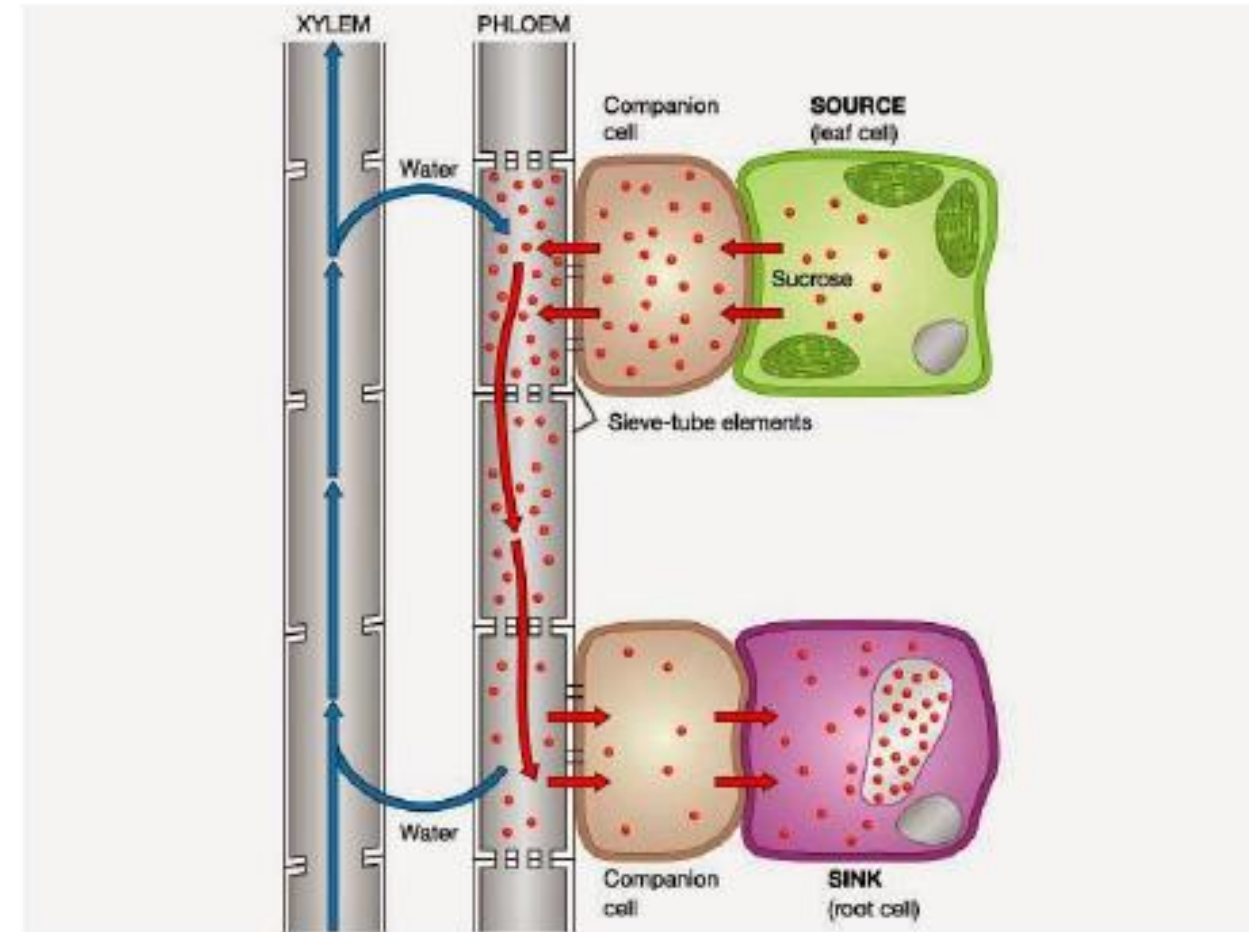
- First high-starch low-methane-emission rice reported.
- Generated via transcription factor technology.



What is SUSIBA2?



- Sugar signalling in barley 2 (SUSIBA2) is a plant-specific transcription factor
- SUSIBA2 regulates sugar-inducible gene expression mediating source–sink communication
- High expression of SUSIBA2 is associated with an increase in sink strength and starch biosynthesis

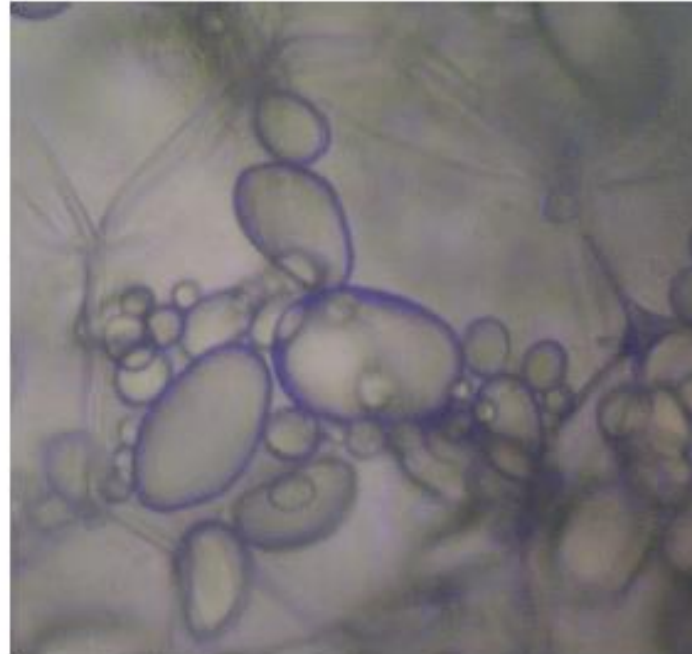
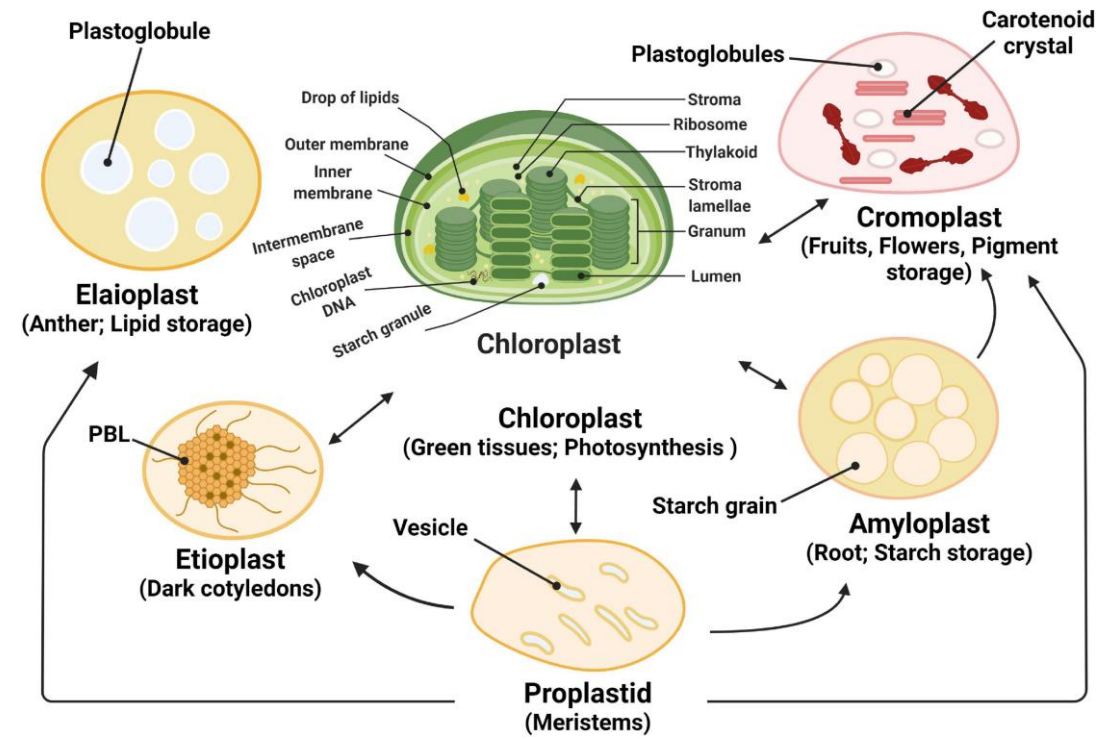


Starch

- Main form of carbon accumulated after carbon assimilation by Calvin-Benson cycle
- Primary starch, it forms granules in the chloroplasts of photosynthetic cells (transitory stock, degraded during the night)
- Secondary starch, import to amyloplasts (long term stock)

Amyloplasts

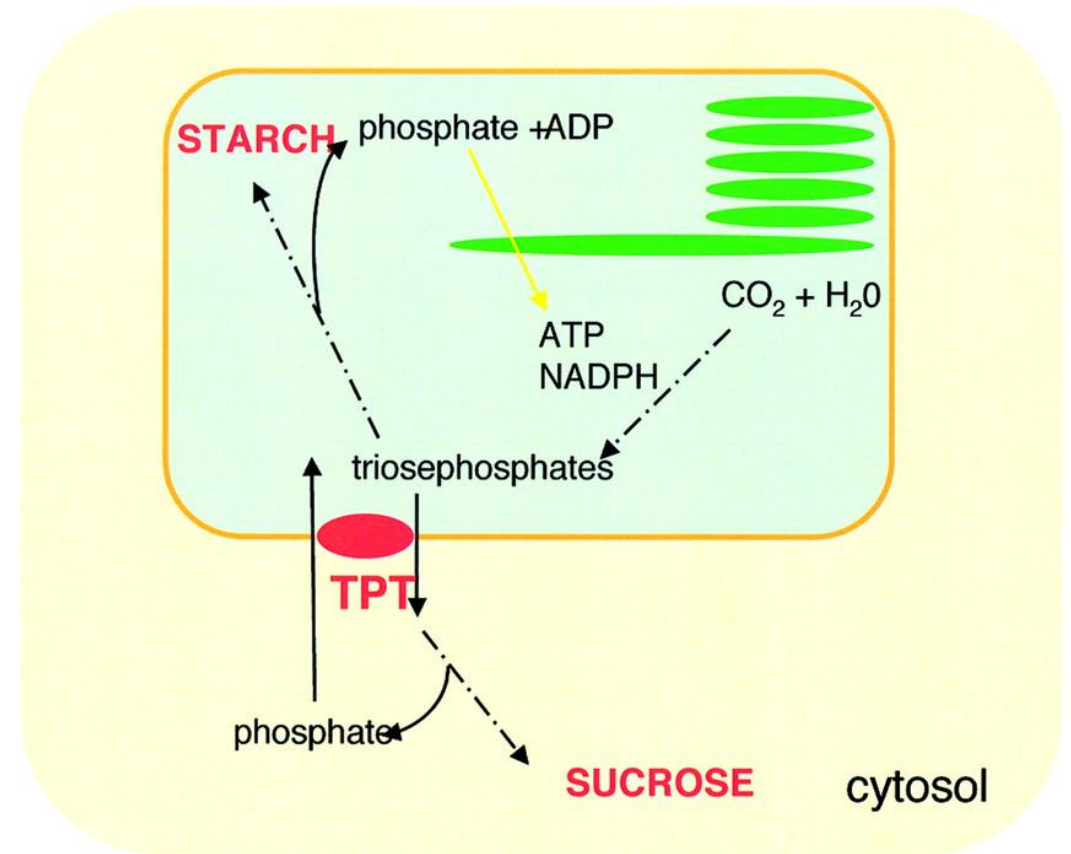
- Carbon stock in tubers
- Gravity perception, statolith positioning in seeds



Sucrose = glucose + fructose

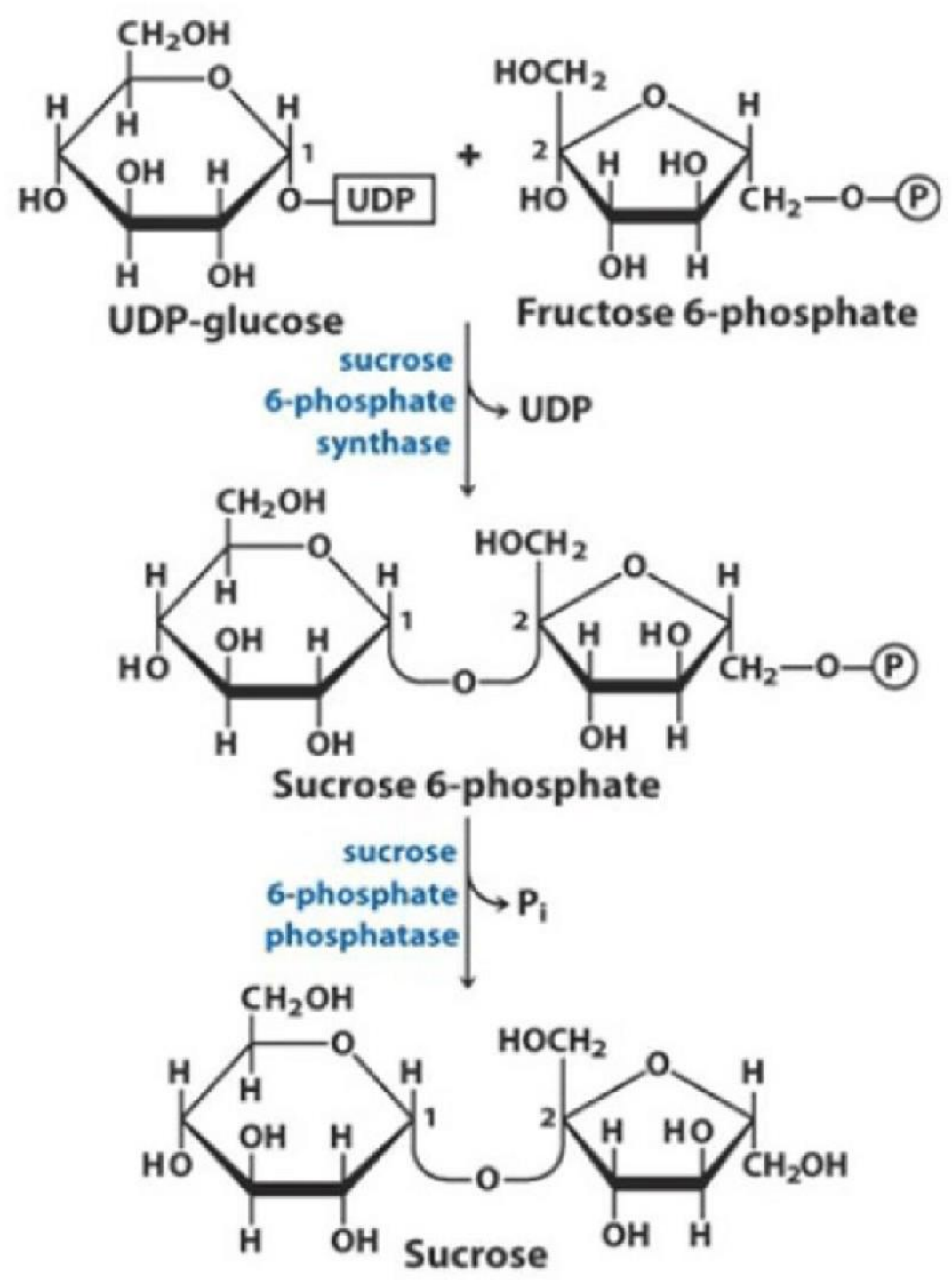
- Soluble
- Synthesized in the cytoplasm
- Transport carbon molecules
- Connected to starch metabolism

The triose phosphate/phosphate translocator **TPT**. The main function of TPT is the export of recently fixed carbon in the form of triose phosphates (TP) from the chloroplast stroma to the cytosol. The transport occurs in strict counter-exchange with inorganic phosphate. The release of inorganic phosphate from TP during sucrose biosynthesis is thereby coupled to the export of TP. A reduction in cytosolic sucrose biosynthesis is thus communicated to the plastid stroma by a decrease in phosphate levels and a concomitant reduction of TP export. This decrease in stromal phosphate levels, together with an increase in 3-PGA levels, allosterically activates ADP-Glc pyrophosphorylase, thereby allocating recently fixed carbon dioxide into starch biosynthesis.



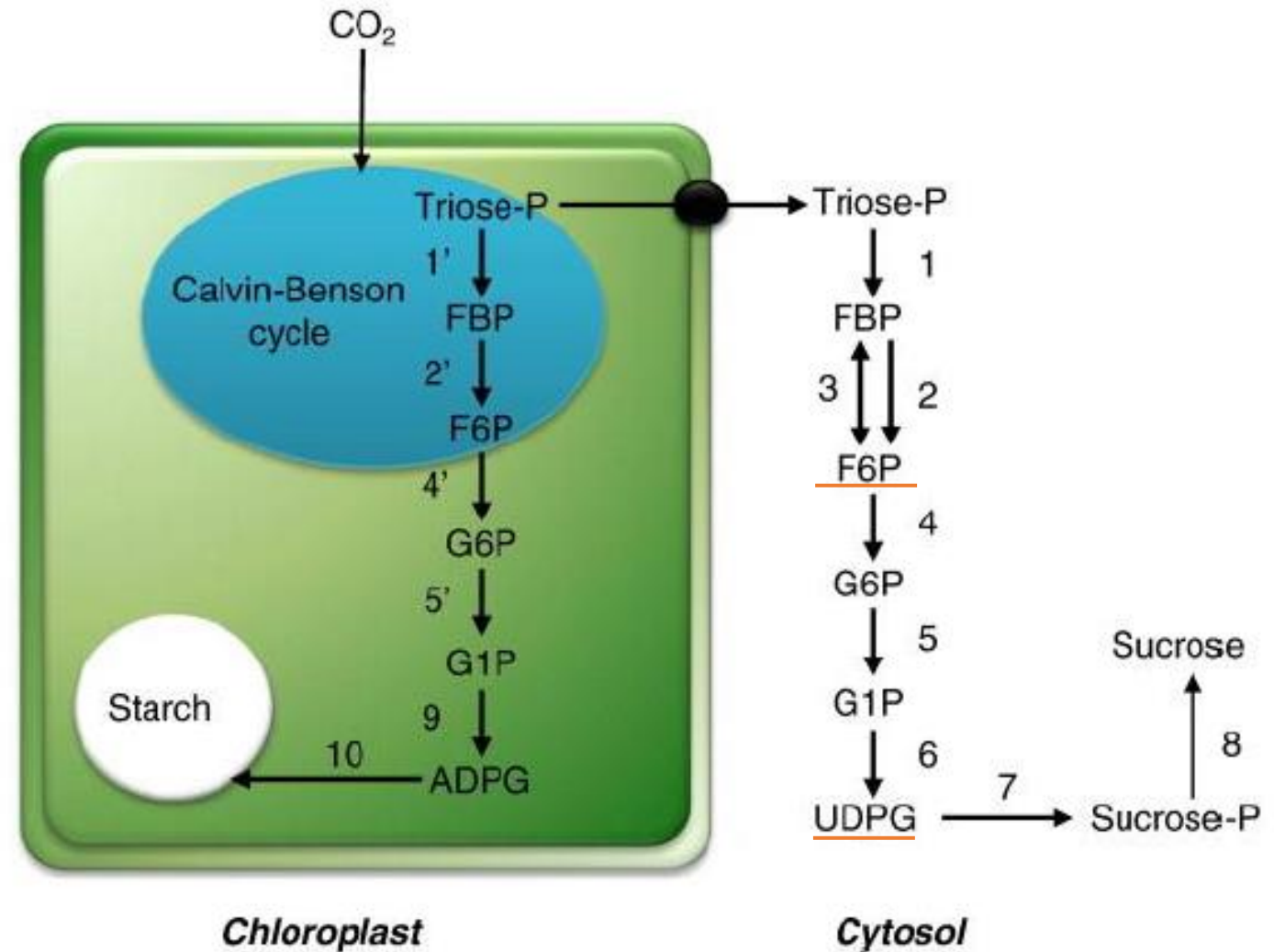
Mesophyll cells

Sucrose biosynthesis



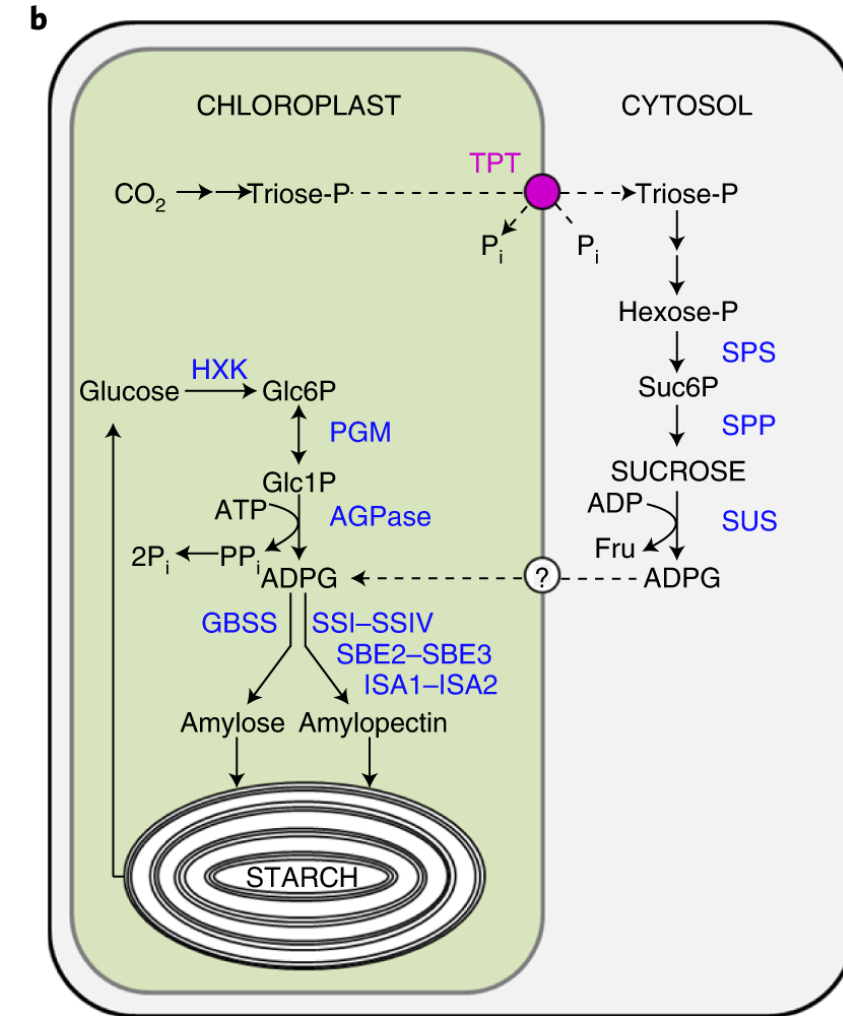
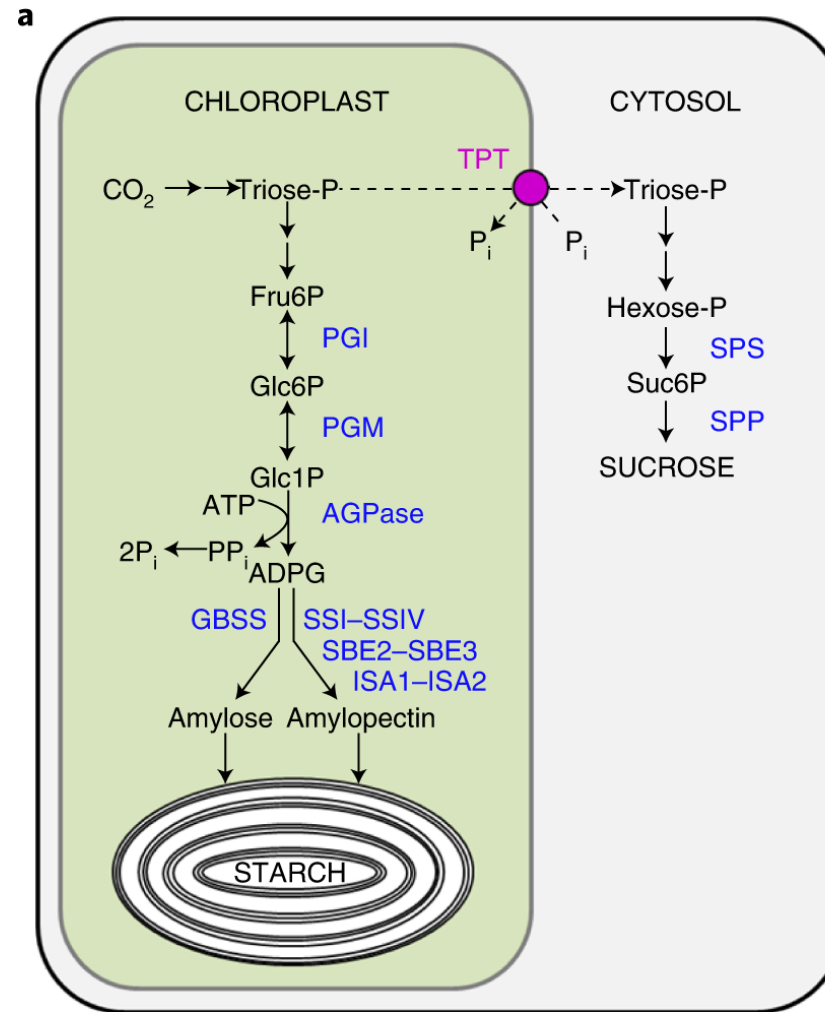
Sucrose biosynthesis

- TPT exchanger at the level of chloroplast membrane
- Gluco-neogenesis enzymes similar to those in the chloroplast (CCB)
- Reversible reactions
- F and G needed for sucrose
- UDP-glucosio pirofosforilasi



Starch biosynthesis

- Similarities with sucrose biosynthesis
- Fructose6P from CBB
- Glucose monomers
- 1-4 bonds



Amylose and amylopectin

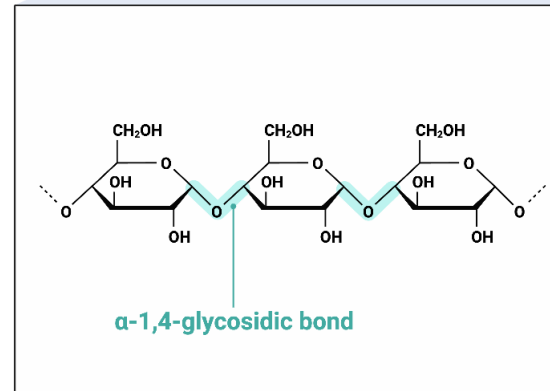
- Amylose linear chains $\alpha(1\rightarrow4)$ (<2000G)
- Amylopectins $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ (40000G)

iso1=isoamylase
Involved in the trimming of pre-amylopectin chains.

Types of Starch

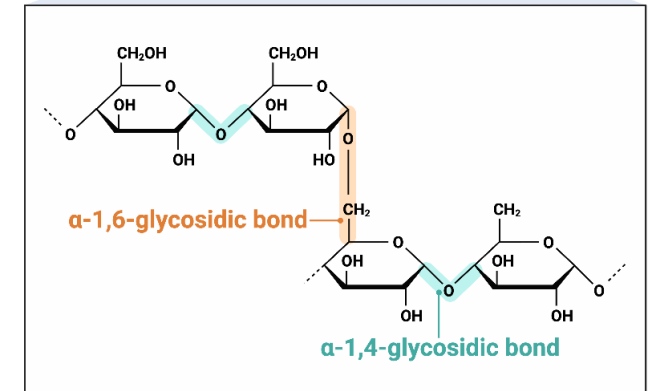
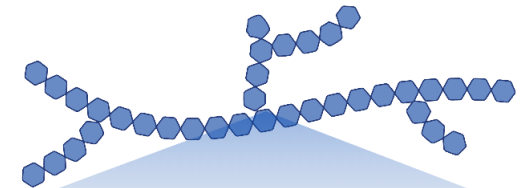
Structure of Amylose vs. Amylopectin

Amylose



Amylose is composed of glucose molecules connected by α -1,4-glycosidic bonds.

Amylopectin

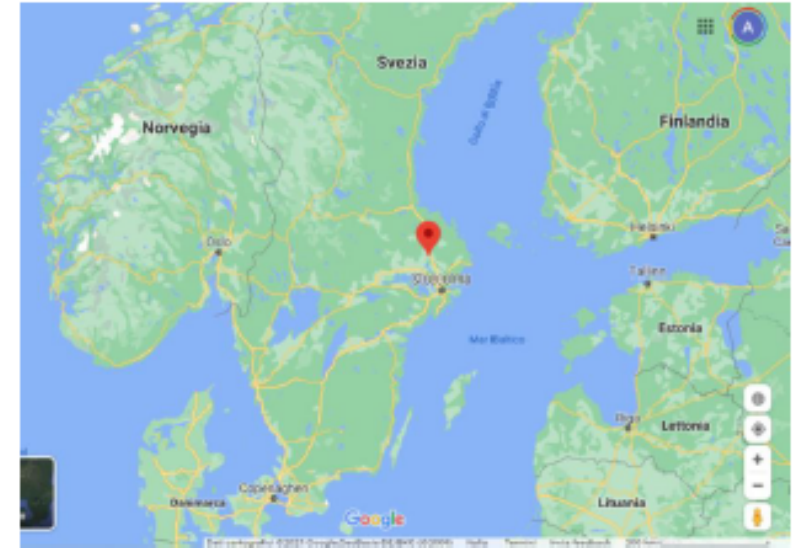


Straight-chain portions of amylopectin are connected by α -1,4-glycosidic bonds, whereas the branches are connected by α -1,6-glycosidic bonds.

A Novel WRKY Transcription Factor, SUSIBA2, Participates in Sugar Signaling in Barley by Binding to the Sugar-Responsive Elements of the *iso1* Promoter^W

Chuanxin Sun, Sara Palmqvist, Helena Olsson, Mats Borén, Staffan Ahlandsberg, and Christer Jansson¹

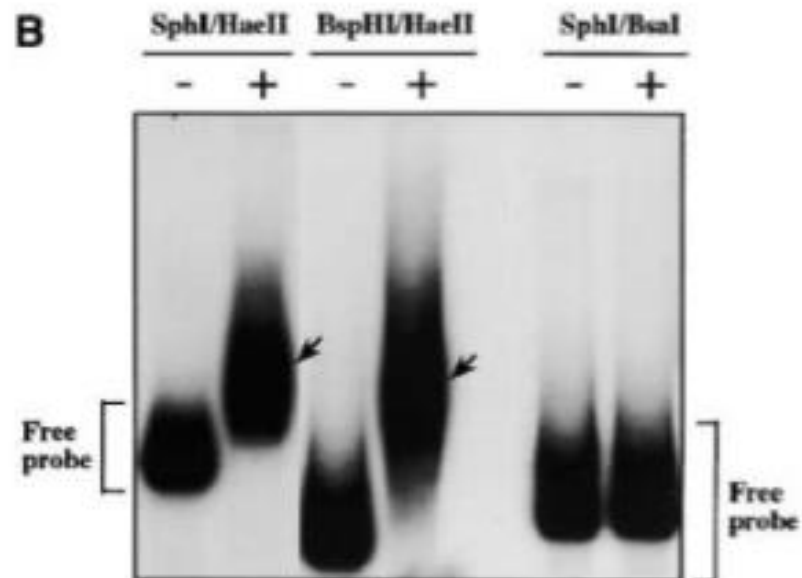
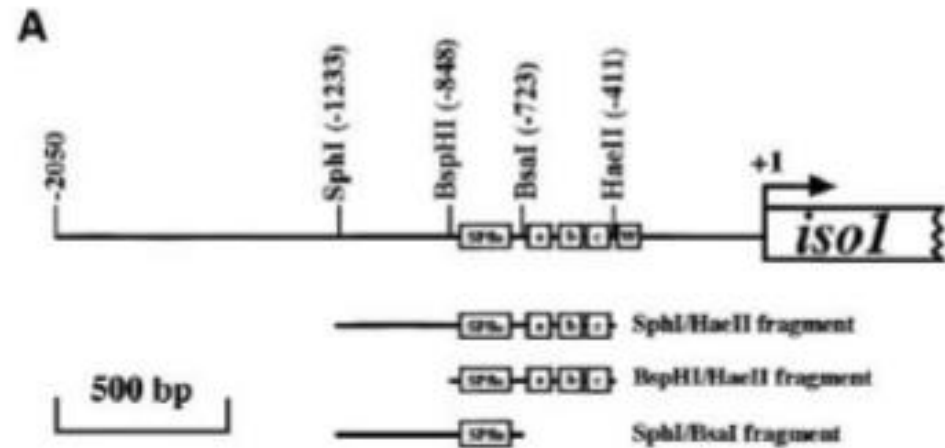
Department of Plant Biology and Forestry Genetics, The Swedish University of Agricultural Sciences, SE-75007 Uppsala, Sweden



- WRKY transcription factor, zinc finger motifs are highly conserved in SUSIBA2. Also, the N-terminal Ser- and Thr-rich regions that are present in many WRKY proteins

How did they confirm DNA binding capacities?

How did they confirm DNA binding capacities?

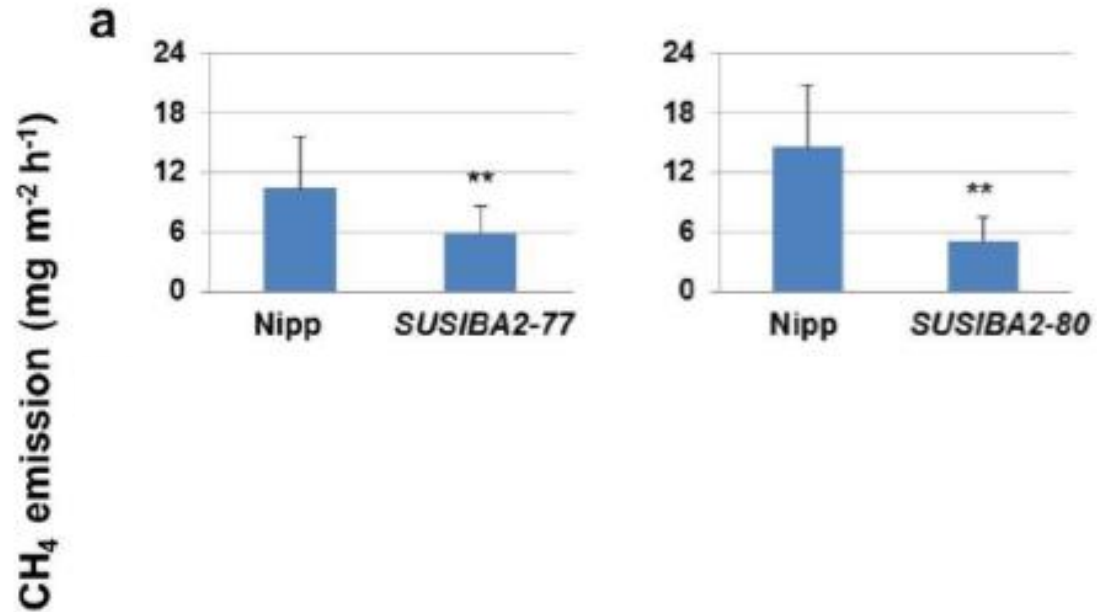


iso1=isoamylase
Involved in the trimming of pre-amylopectin chains. Accelerates the crystallization of nascent amylopectin molecules during starch synthesis. ISA1 and ISA2 work exclusively together as a multimeric holoenzyme. ISA1-ISA2 removes preferentially branches that are very close to other branches.

Phytotron experiments

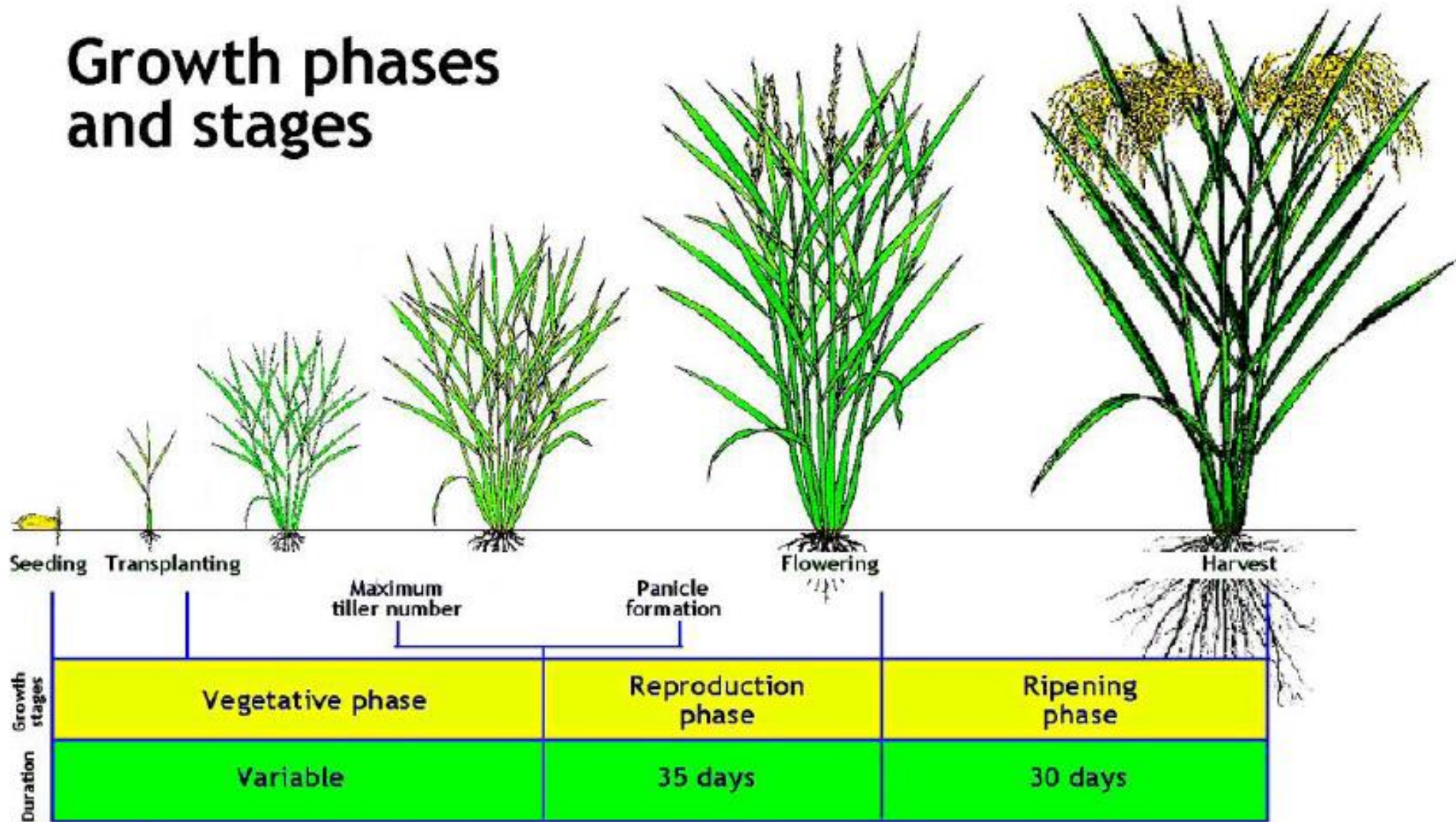
TWO LINES

- Methane emission of Nipp, SUSIBA2-77 and SUSIBA2-80 rice (15 daf).



What could be the next step?

Growth phases and stages

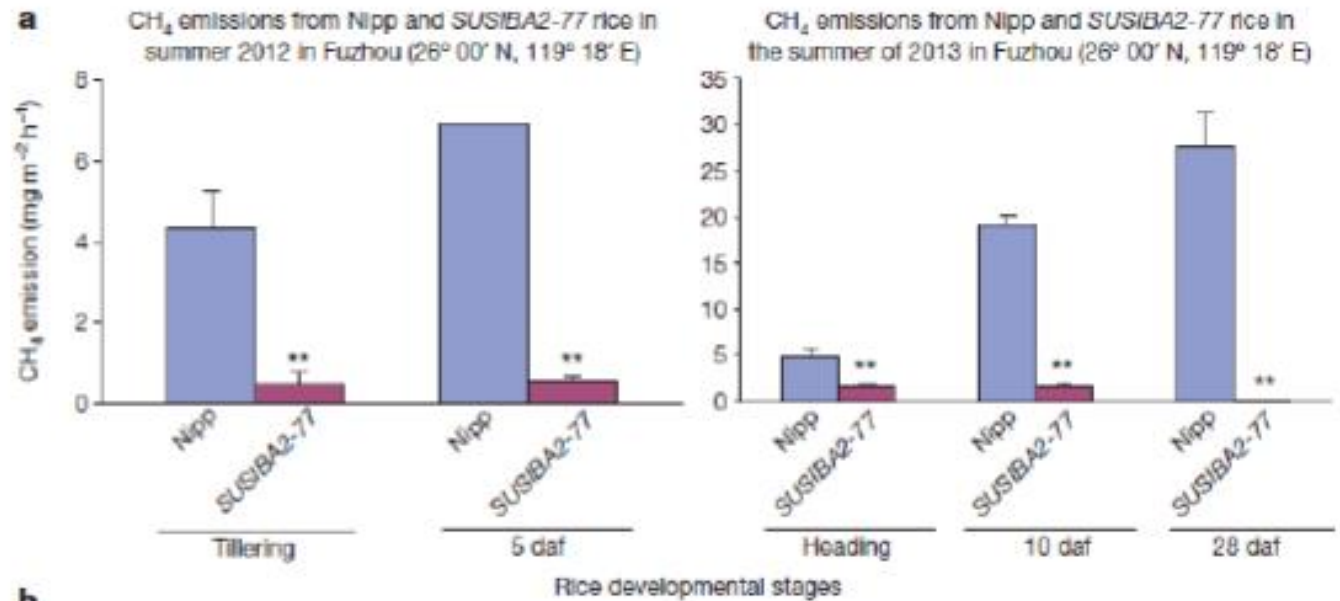


- The duration of the vegetative phase differs with variety.
- The reproductive and ripening phases are about the same for most varieties.

- Panicle formation to flowering takes about 35 days.
- Flowering to harvest takes about 30 days.
- Sowing to harvest ranges from 90 to 200 days or more.

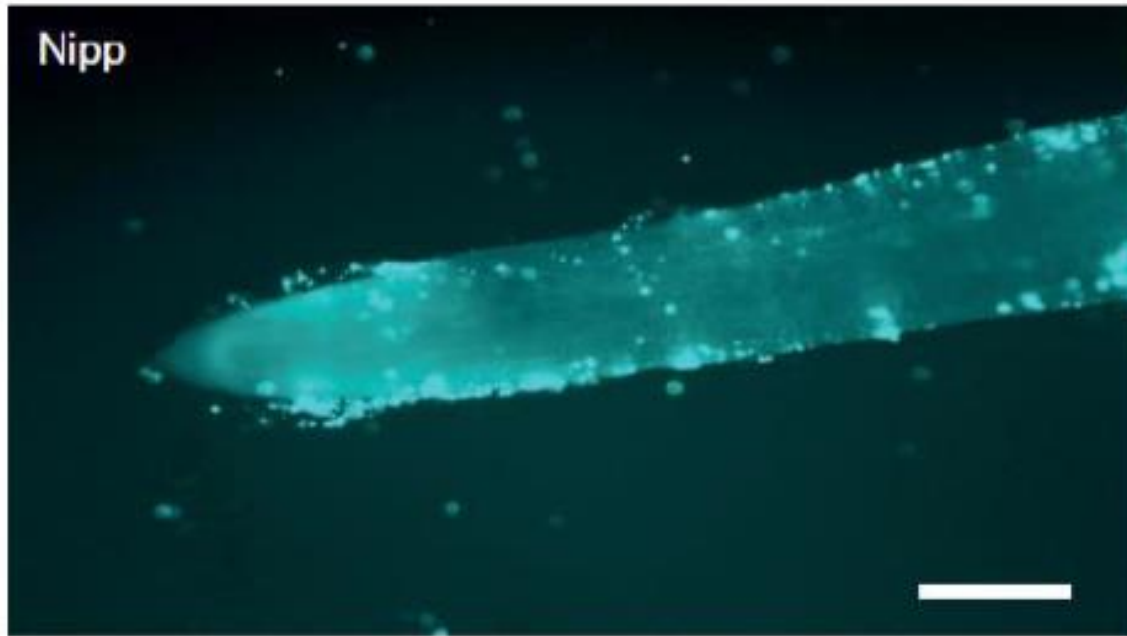
Field experiments

- Fuzhou (China) – 2012 - 2013
- Two stable rice lines (numbers 77 and 80) of homozygote transformants were selected in this study and defined as SUSIBA2 rice
- Results showed that cultivation of SUSIBA2-77 cut methane emissions to around 10% of control levels before flowering, and almost to zero (0.3% of the control level) at 28 days after flowering



Strong blue-green autofluorescence

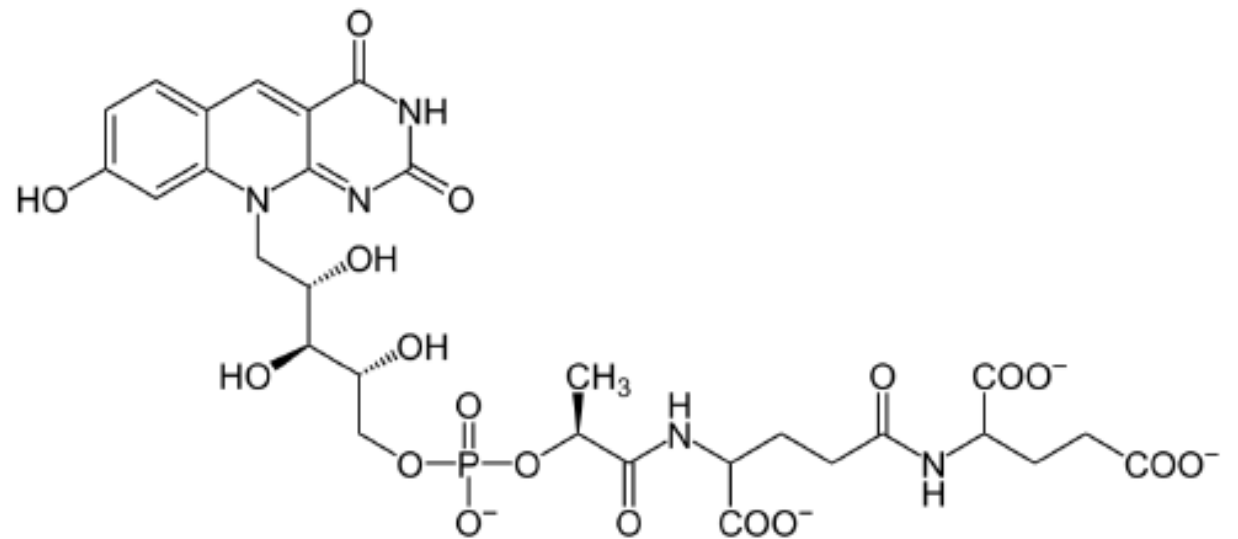
- When excited with light at 420nm wavelength is a characteristic of all methanogens due to the presence of the cofactor F420



To better understand, three-pronged approach

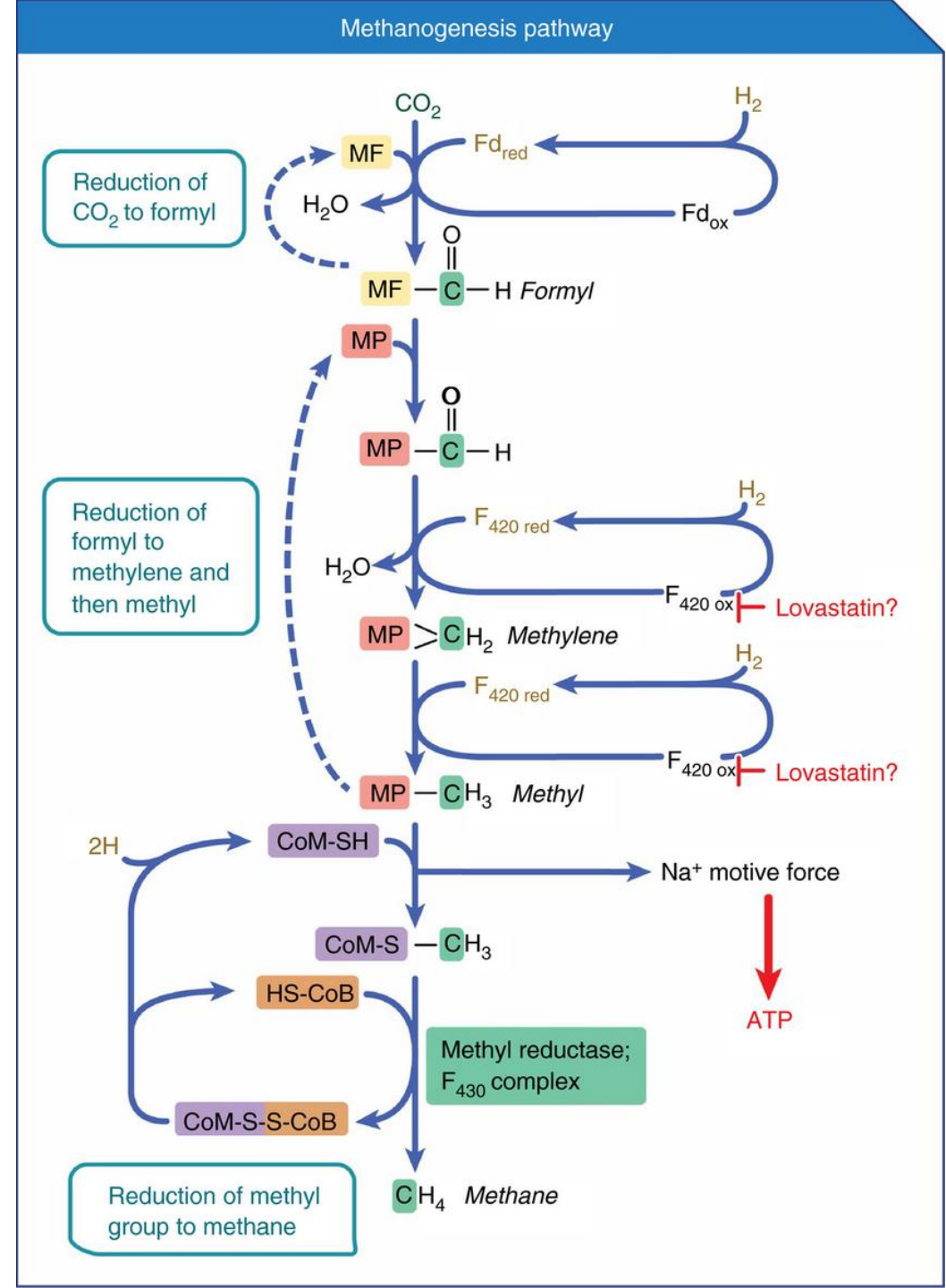
(1) quantifying rhizospheric methanogen communities

Coenzyme F420 is involved in redox reactions in methanogens



Coenzymes F430 and F420 play crucial roles in the methanogenesis pathway

The carbon atom reduced is highlighted in green. MF, methanofuran; MP, methanopterin; CoM, coenzyme M; Fd, ferredoxin; CoB, coenzyme B. This figure was adapted from reference.



To better understand, three-pronged approach

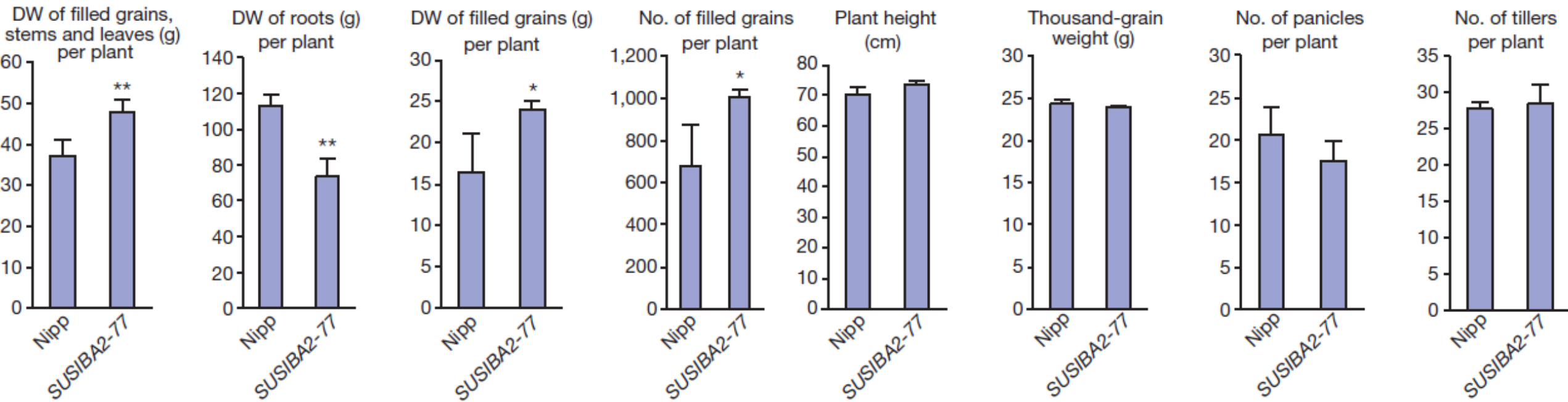
(2) measuring phenotypic traits of SUSIBA2 rice, to investigate if photosynthate partitioning in SUSIBA2 rice was altered



Compared with Nipp, SUSIBA2-77 has larger panicles with a higher proportion of filled grains, resulting in more drooping panicles (Fig. 2a, left and middle panels). In contrast, SUSIBA2-77 has a smaller root system than Nipp (Fig. 2a, right panel).

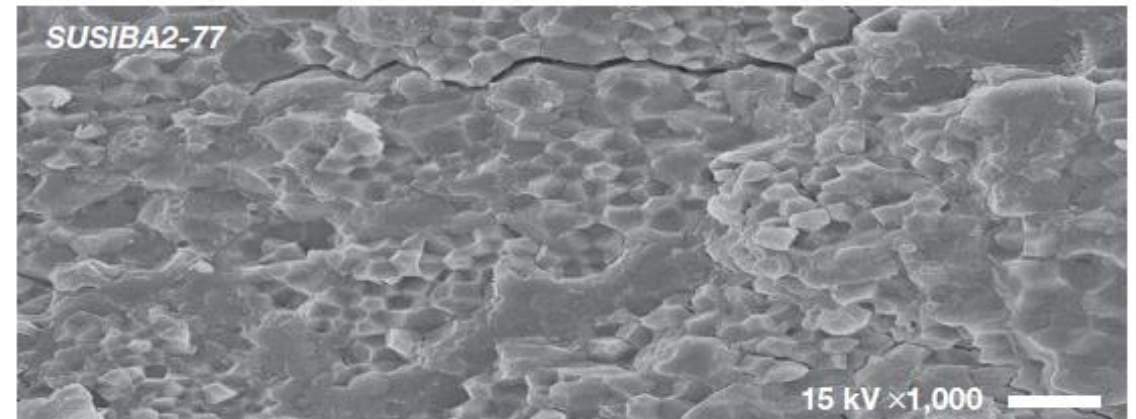
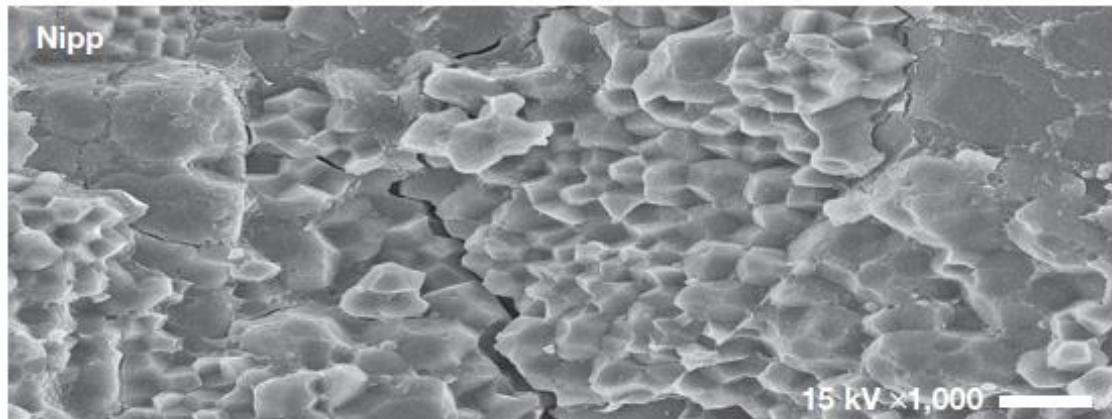
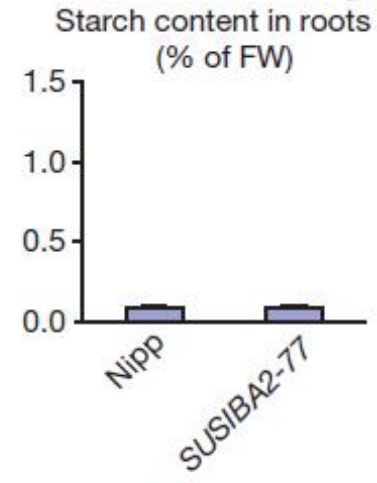
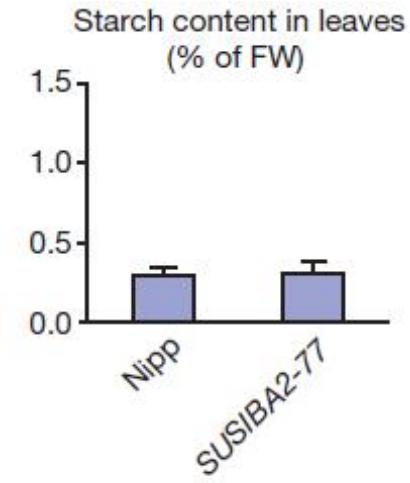
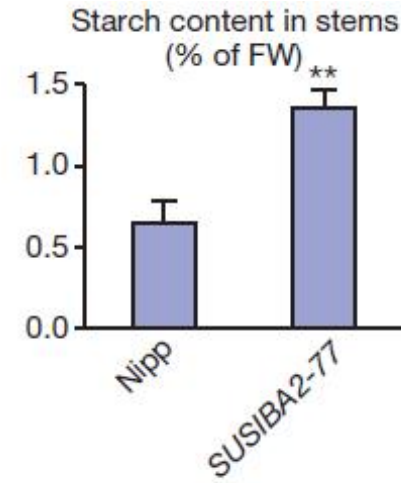
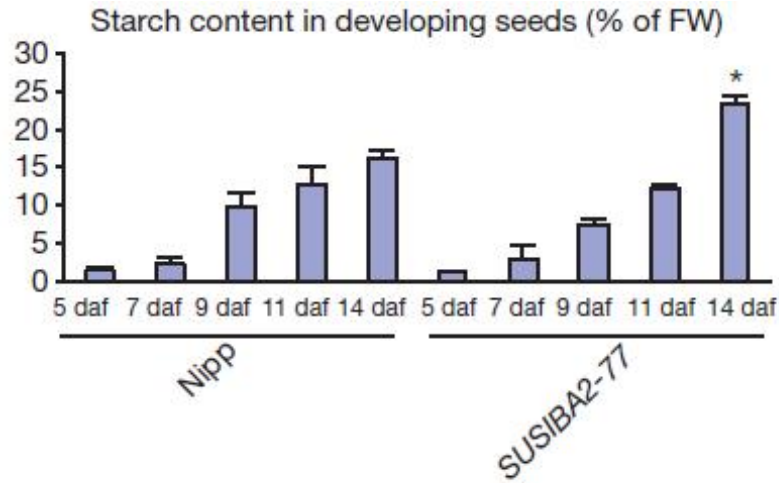
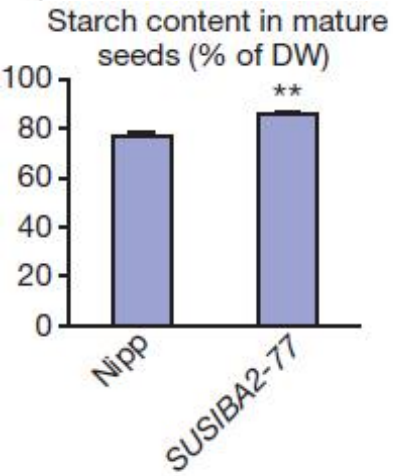
Quantitative data

- Plant height, thousand-grain weight (TGW), and numbers of panicles and tillers per plant were similar in SUSIBA2-77 and Nipp



Starch

- Increased starch content
- Reduced starch granules size

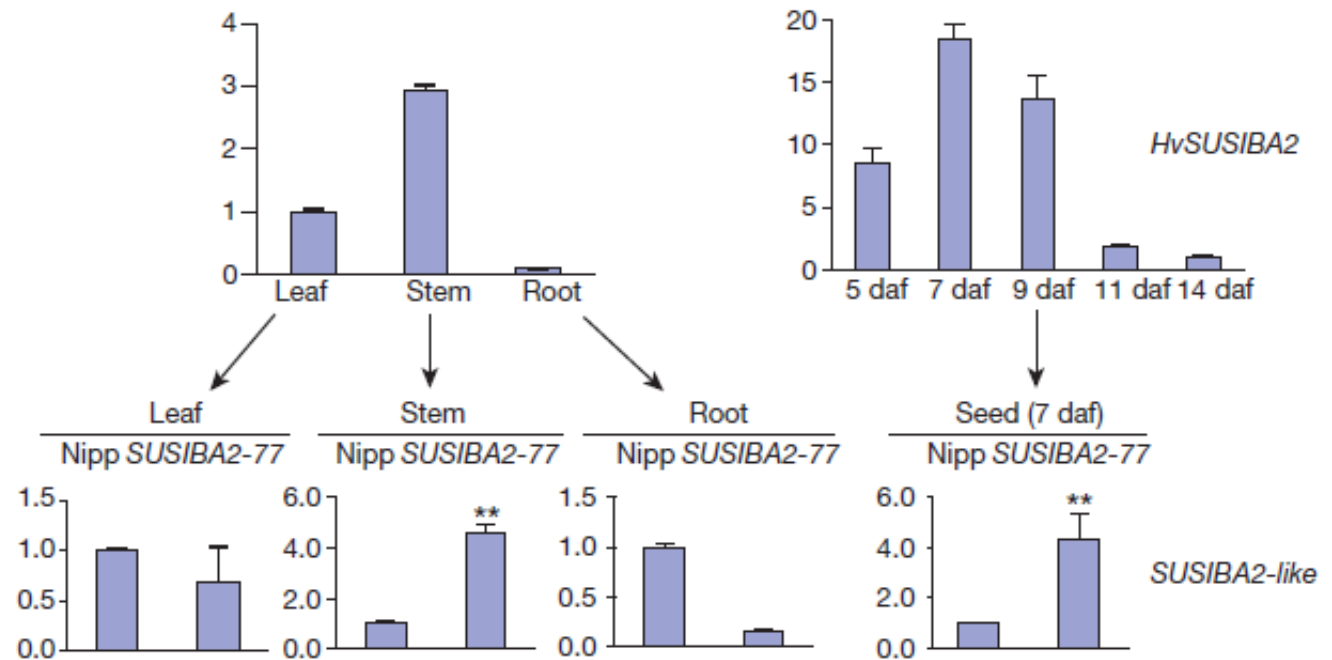


To better understand, three-pronged approach

(3) characterizing genotypic traits.

The presence of sugar-responsive elements (SUREs) in the promoters of genes targeted by SUSIBA2 is critical for executing the sugar-signalling cascade that controls starch biosynthesis

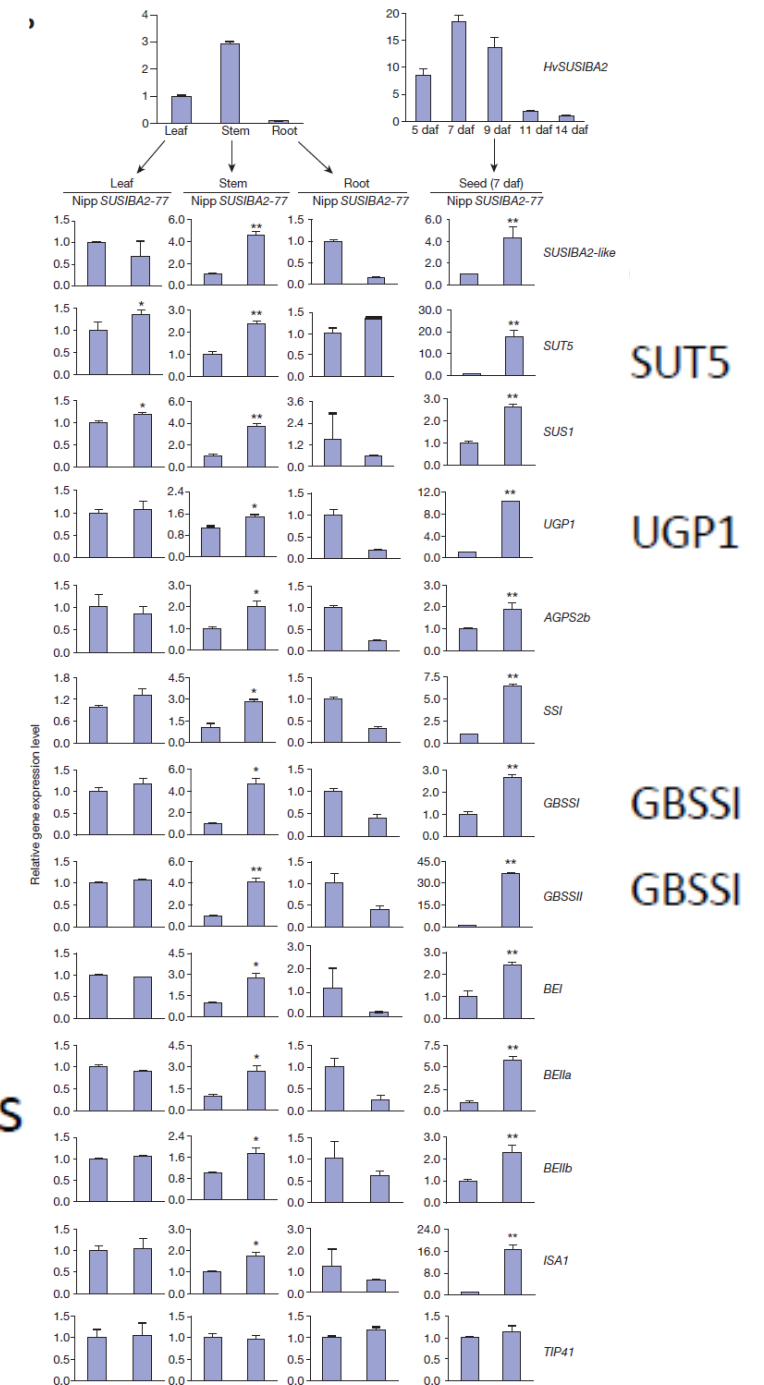
24 genes associated with sugar metabolism,
including HvSUSIBA2 and OsSUSIBA2-like.



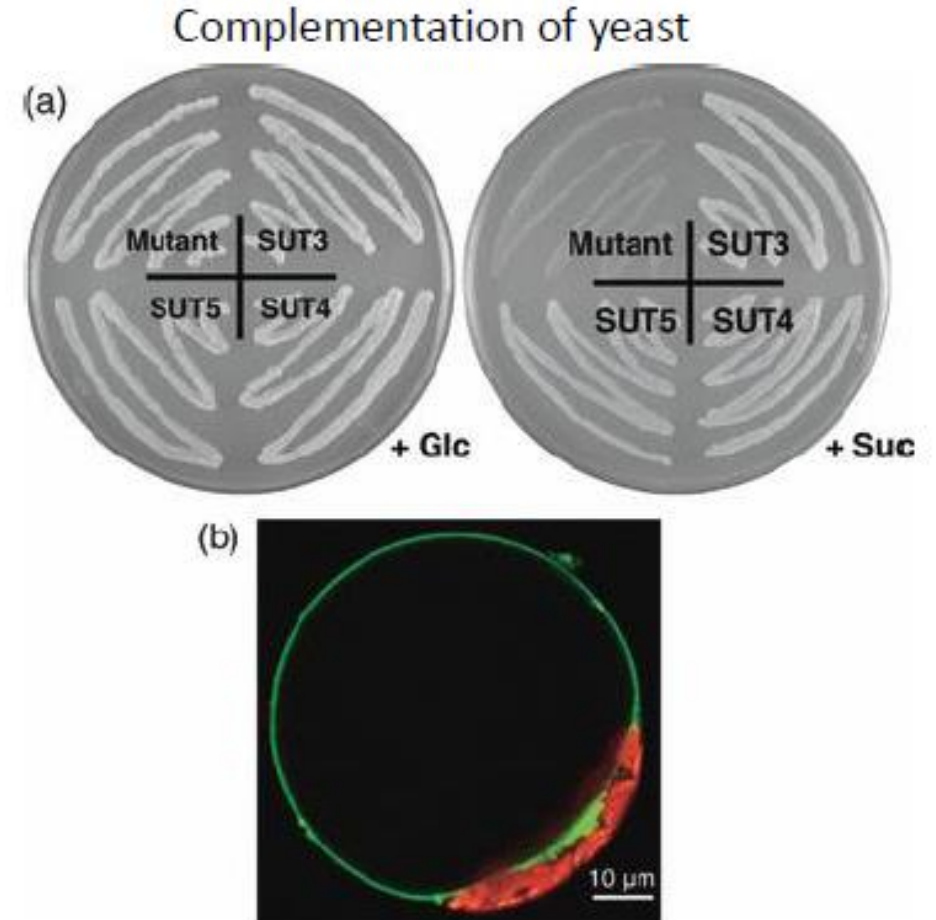
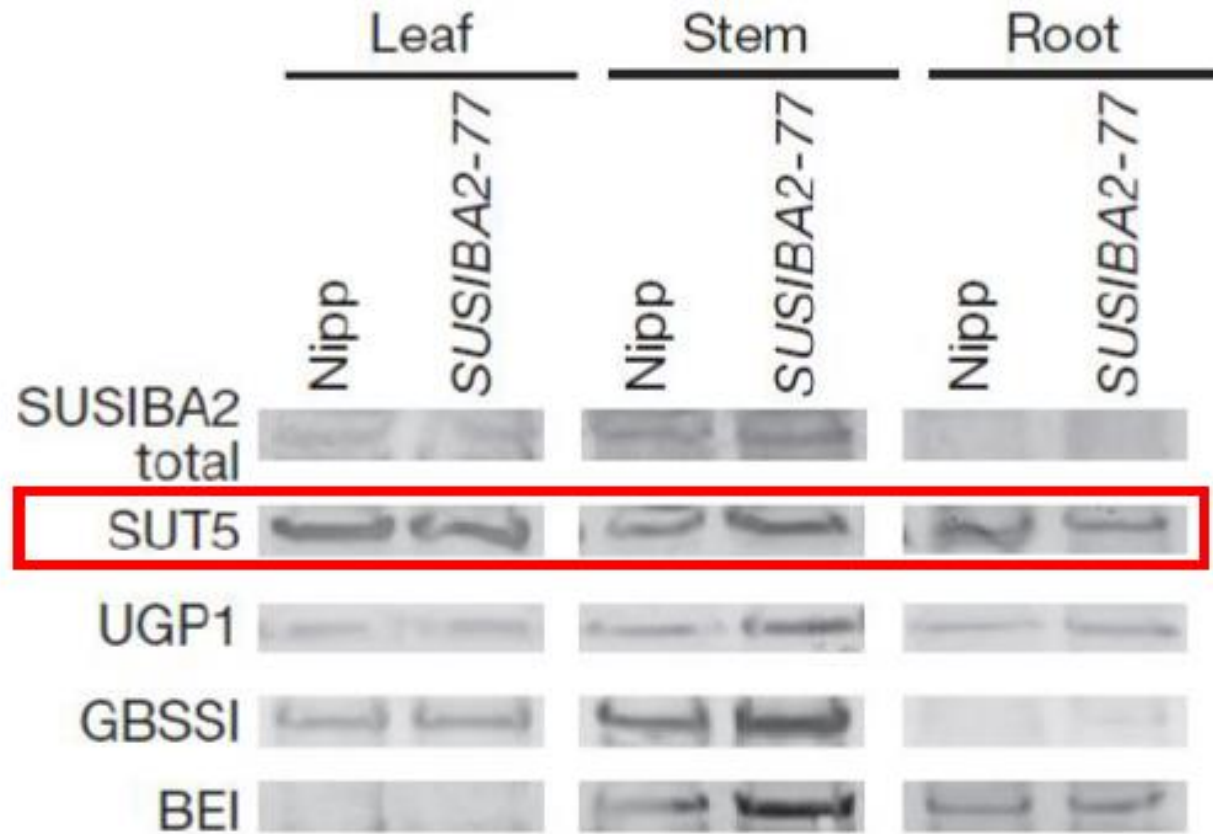
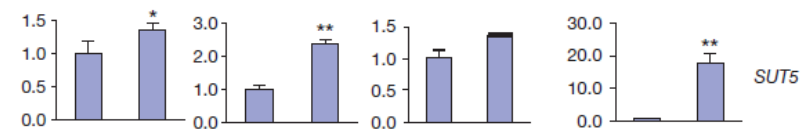
24 genes associated with sugar metabolism, including HvSUSIBA2 and OsSUSIBA2-like.

HvSUSIBA2 was highly expressed in early developing seeds and stems, and at very low levels in leaves, roots and late developing seeds.

The expression level of selected genes, apart from the control gene TIP41-like, followed the HvSUSIBA2 expression pattern with significant differences between SUSIBA2 rice and Nipp in seeds and in stems but not in leaves or roots.

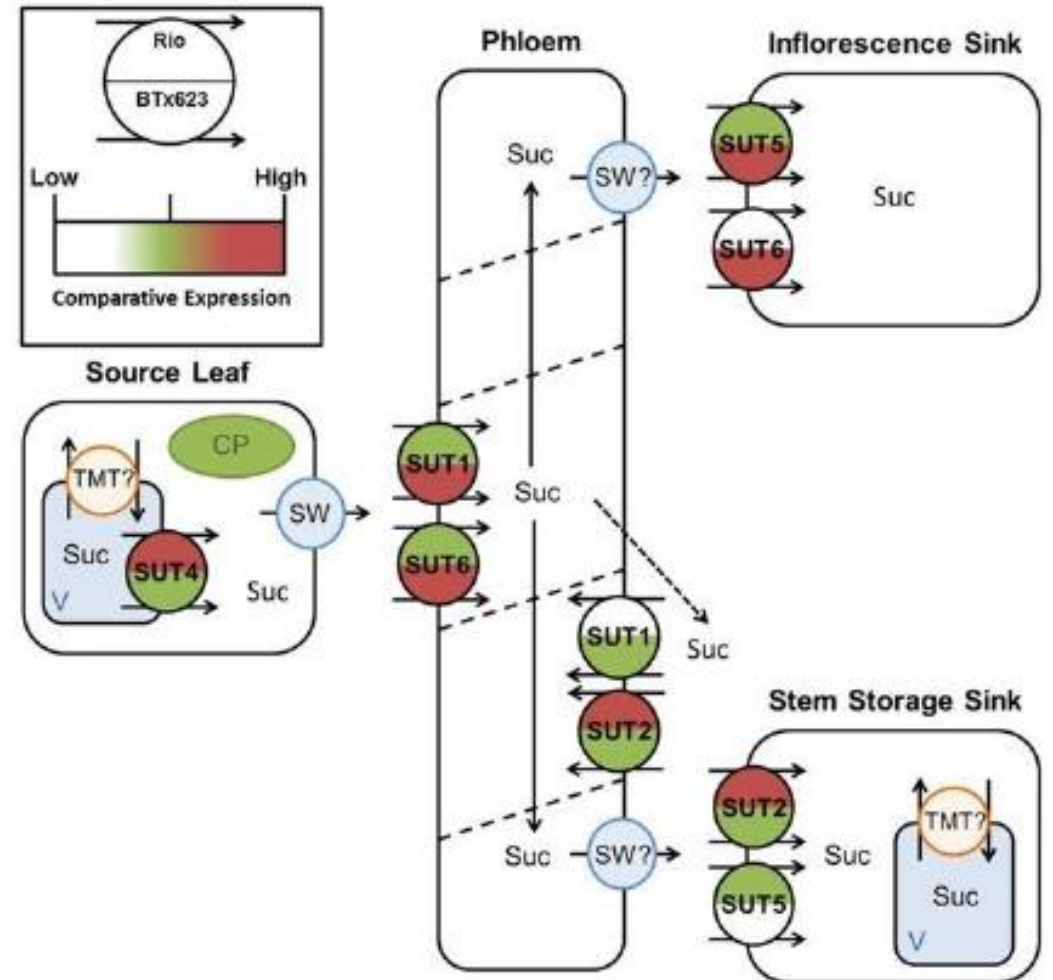


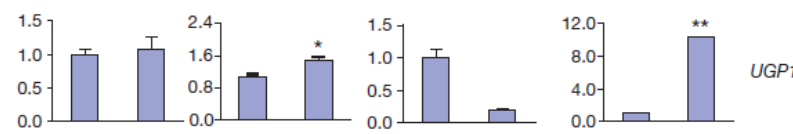
SUT5 = a membrane protein transporting sucrose



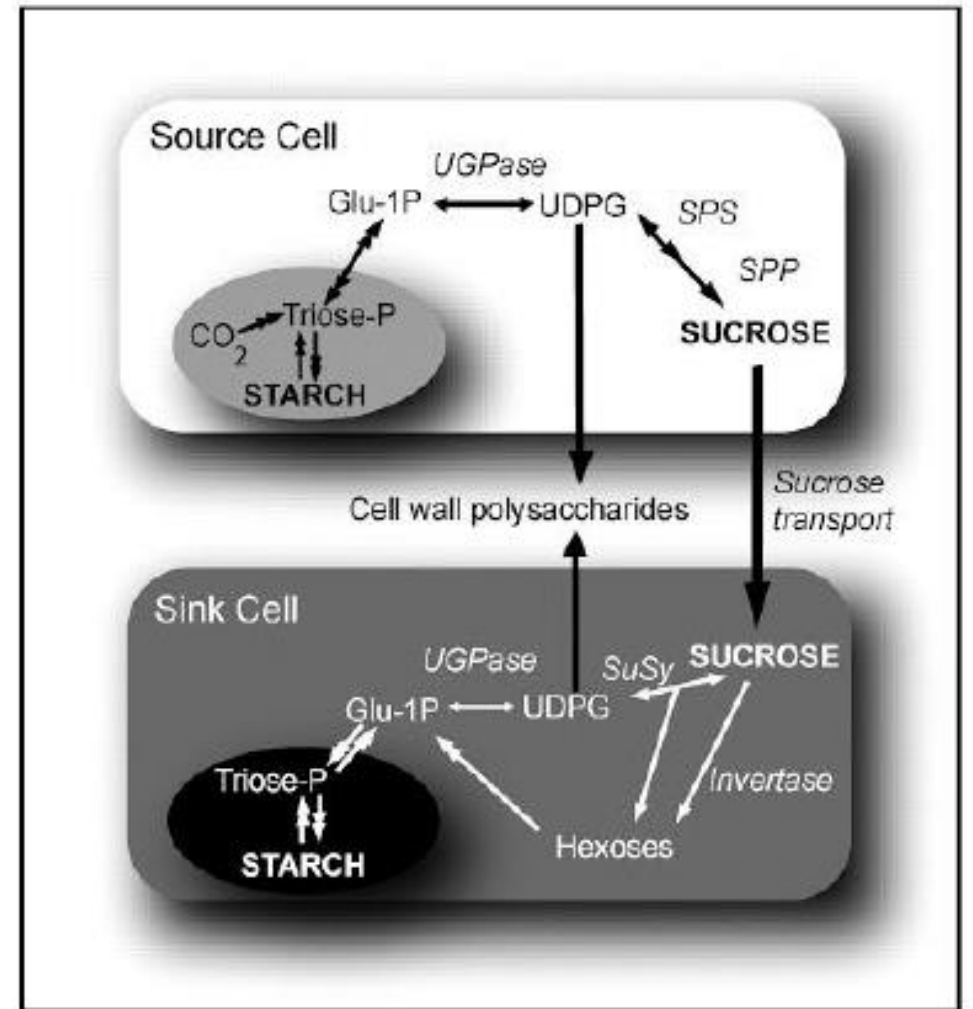
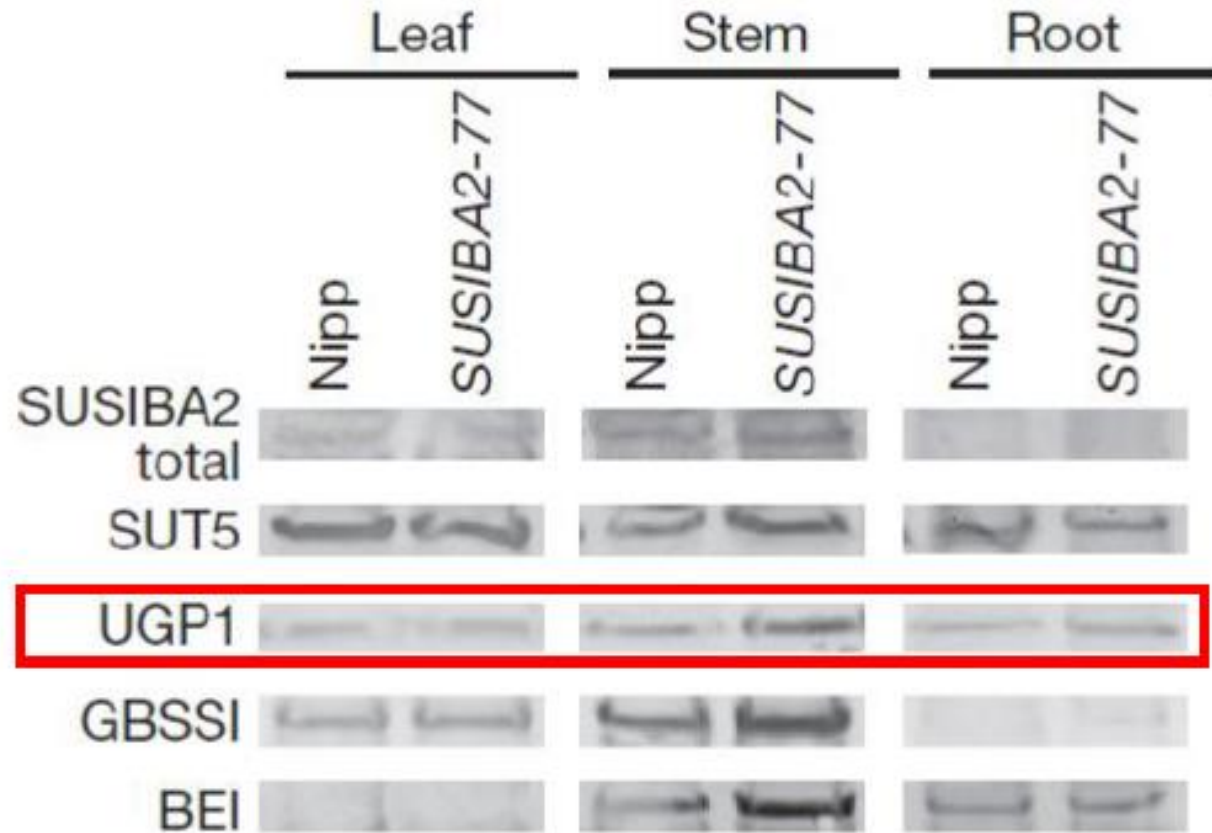
<https://doi.org/10.1111/j.1365-313X.2010.04463.x>

Source-transport-sink pathways

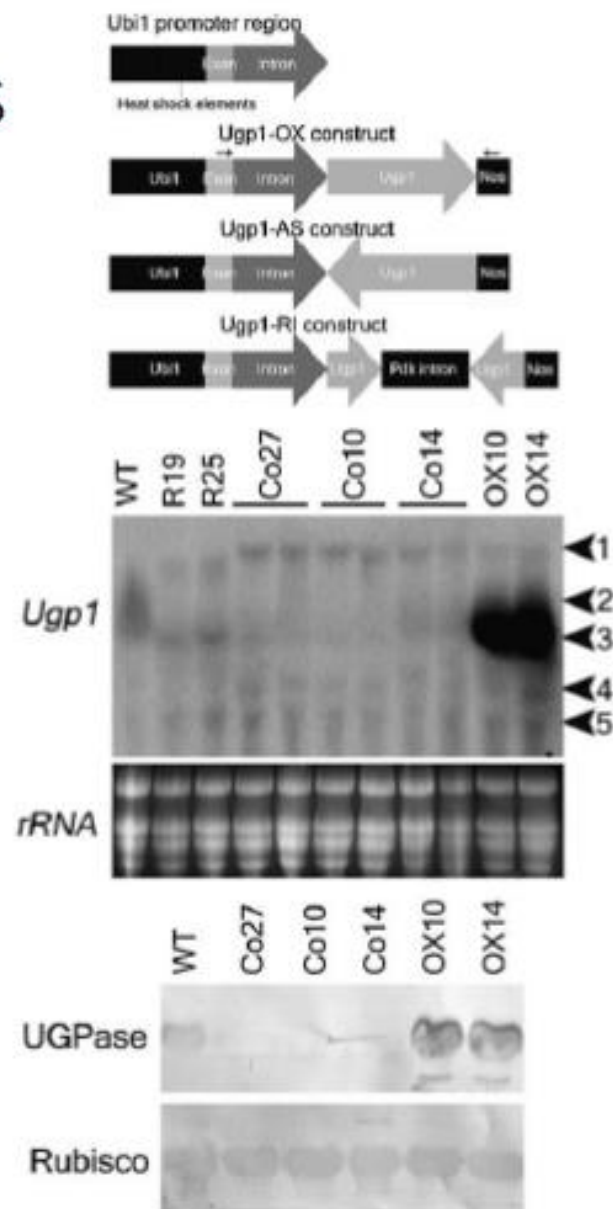
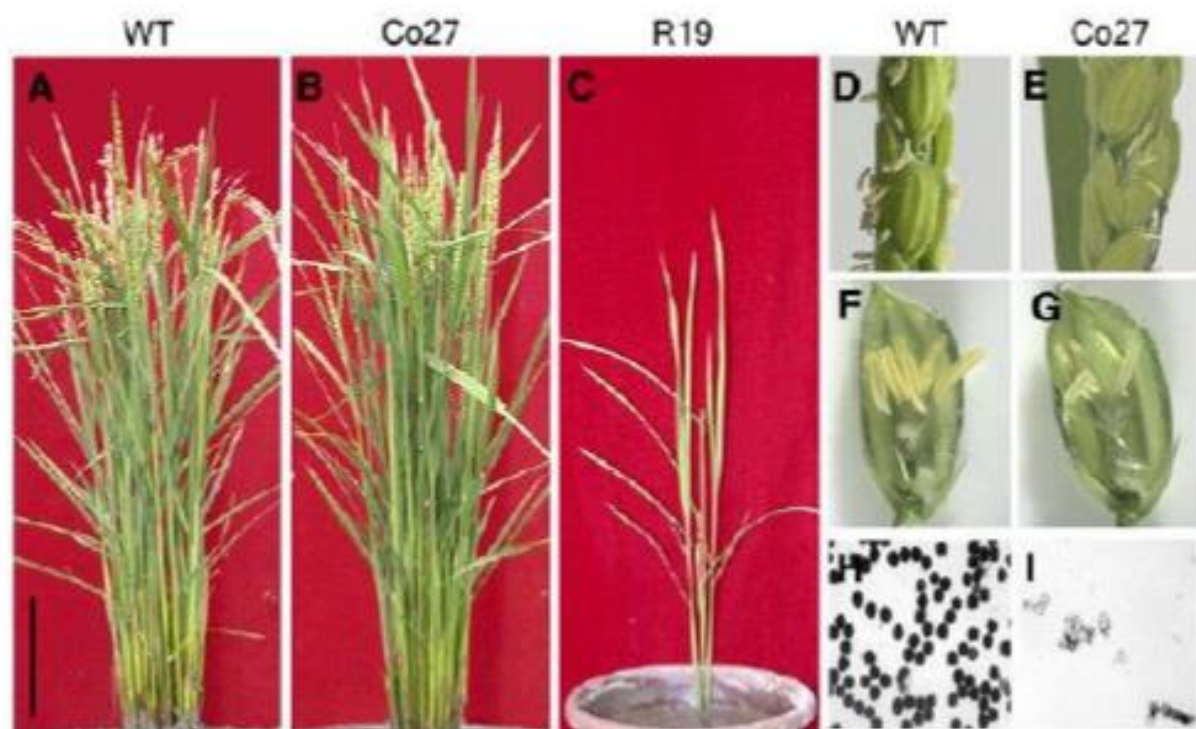




UGP1 = UDP-glucose pyrophosphorylase 1 (UGPase)



Rice UDP-Glucose Pyrophosphorylase1 Is Essential for productivity





Emerging strategies for precision microbiome management in diverse agroecosystems

Elizabeth French¹, Ian Kaplan¹, Anjali Iyer-Pascuzzi², Cindy H. Nakatsu³ and Laramy Enders¹✉

- Structural and functional diversity of soil
- Microorganisms as a source for agricultural sustainability
- Microbe-assisted crop production
- Microbiome management

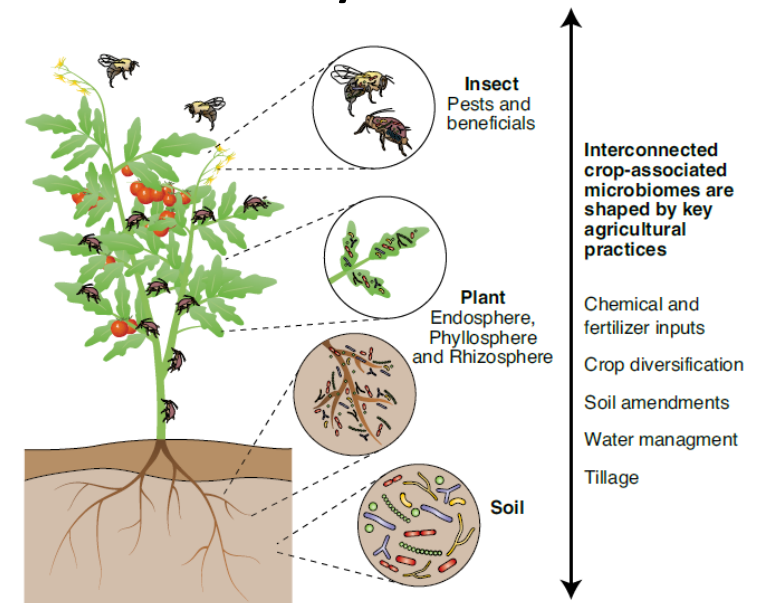
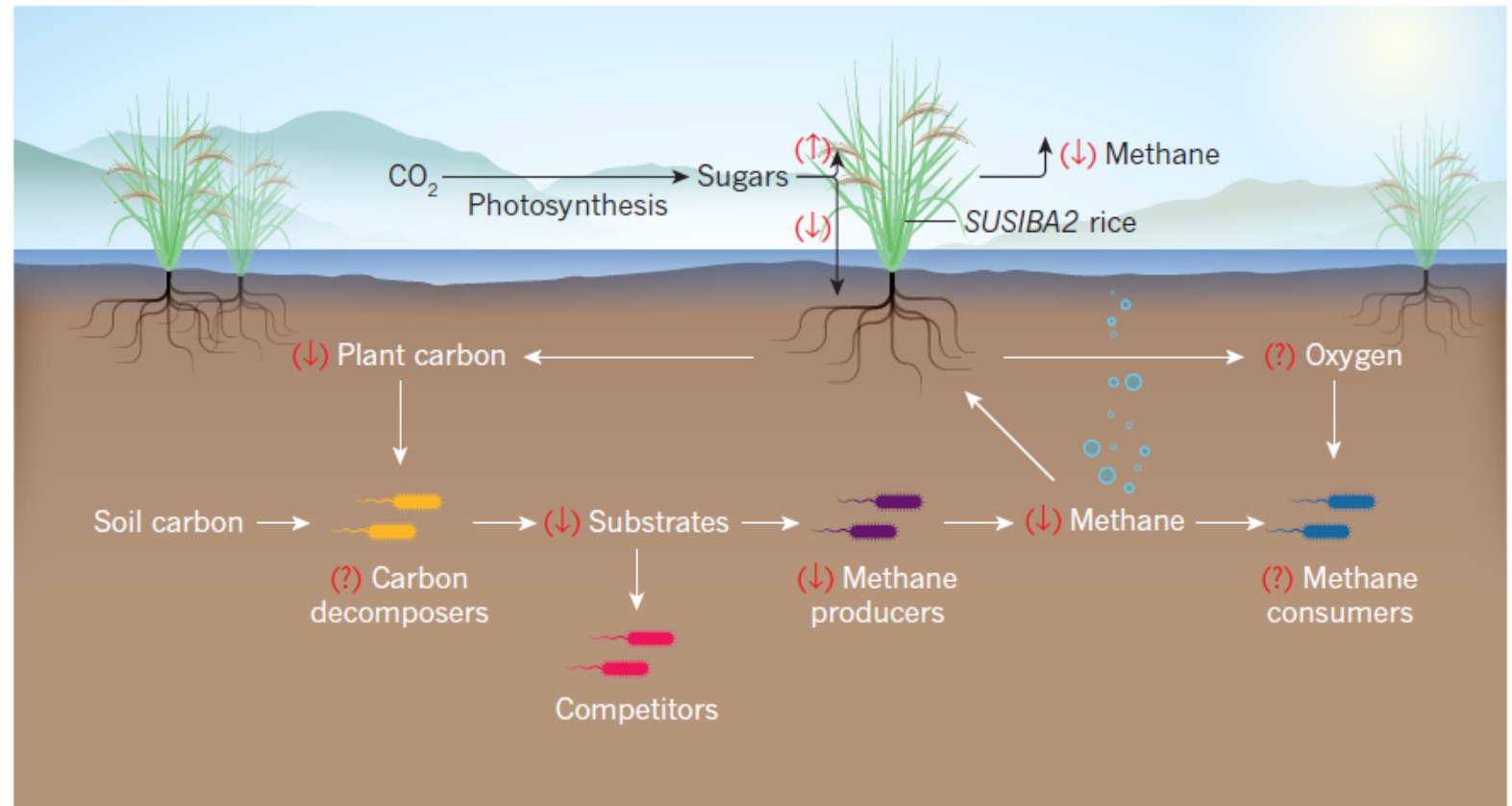


Fig. 1 | The major sectors of crop-associated microbial communities (soil, plant and insect) are shaped by key agricultural practices. Arrows highlight how soil, plant and insect microbiomes interact to influence crop health, and show how each is affected by the listed management practices. See Table 1 for summary and descriptions of management practices.

Bypassing the methane cycle

A genetically modified rice with more starch in its grains also provides fewer nutrients for methane-producing soil microbes. This dual benefit might help to meet the urgent need for globally sustainable food production. [SEE LETTER P.602](#)

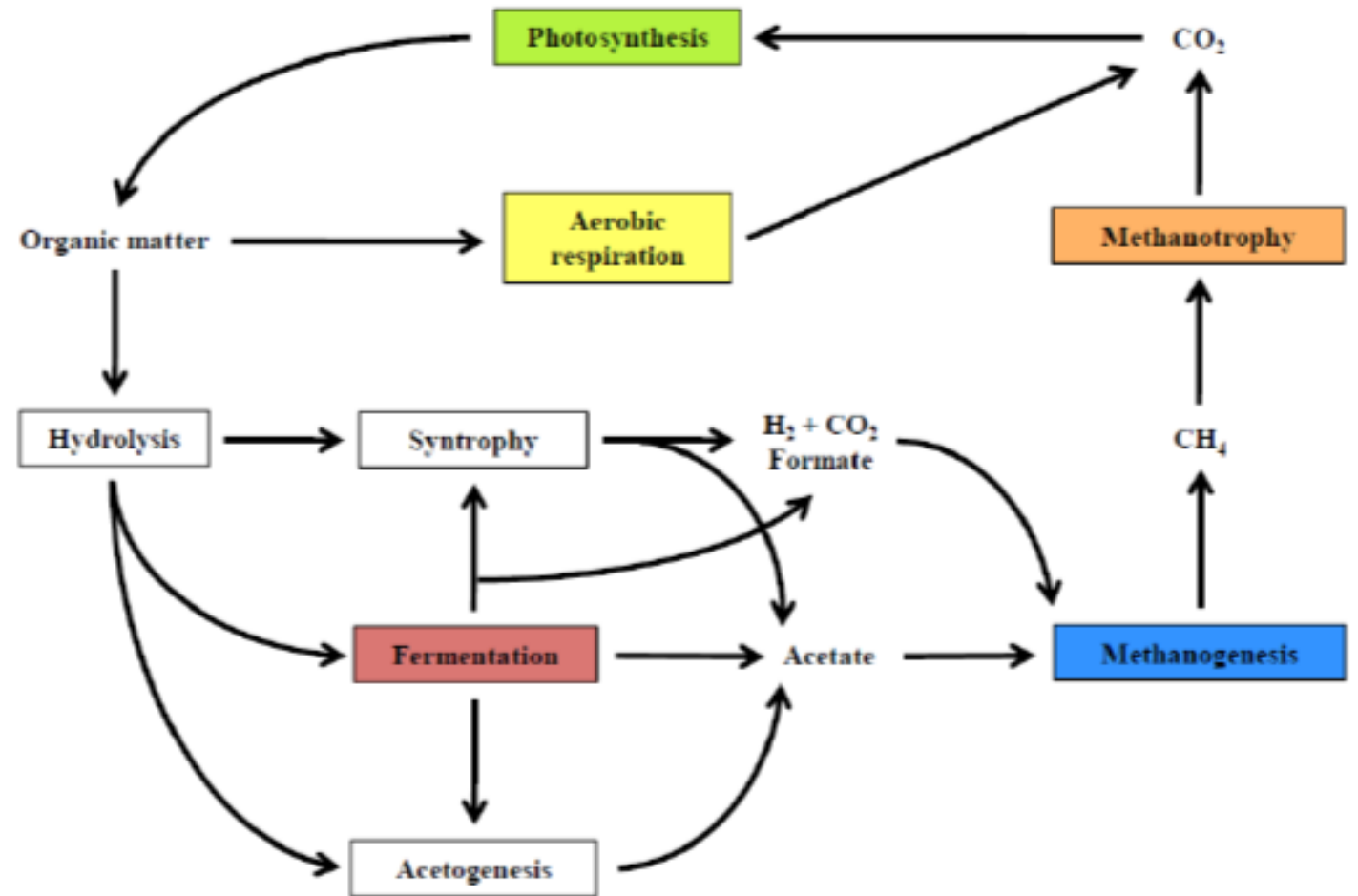
PAUL L. E. BODELIER



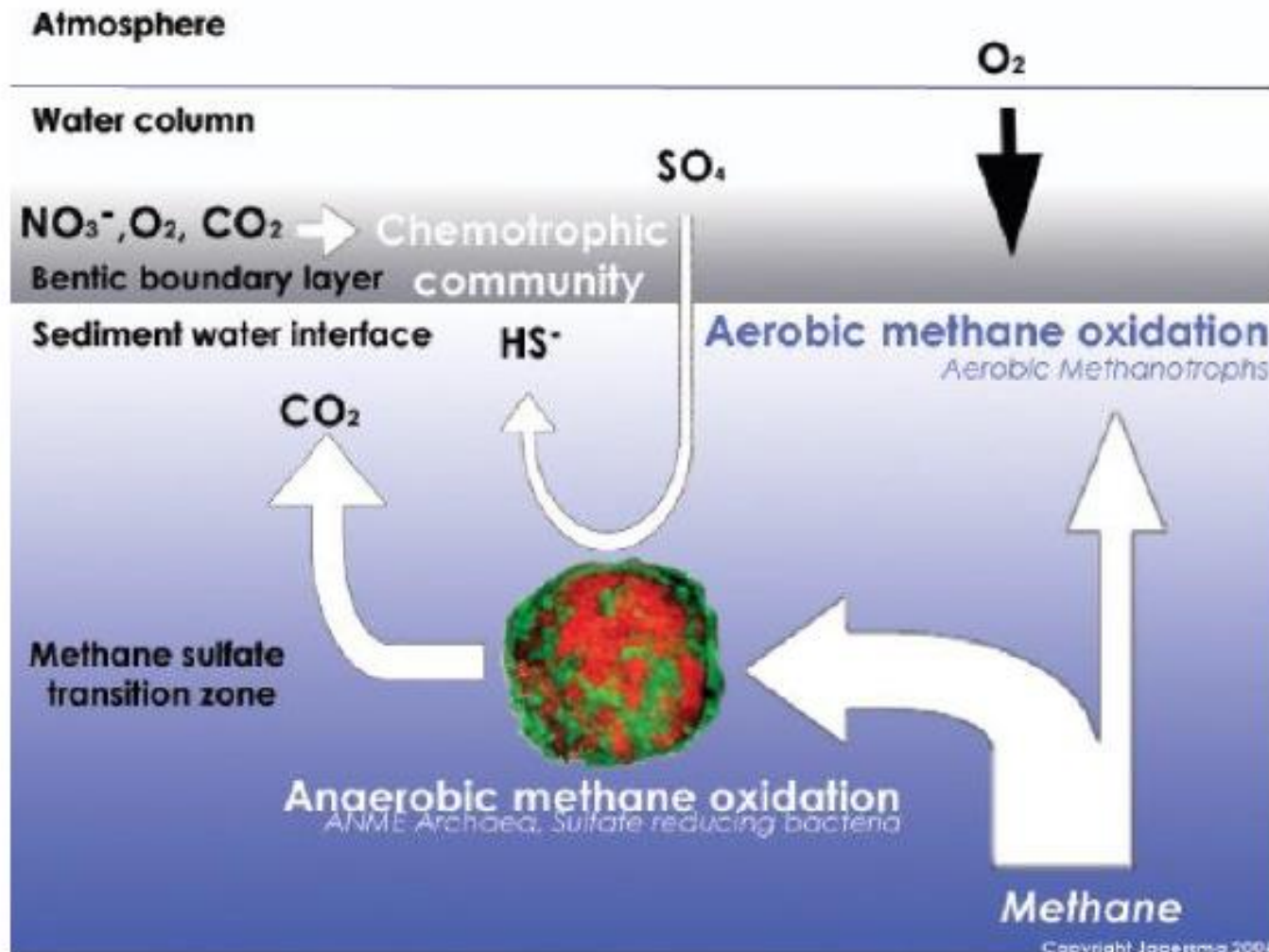
Minireview

Methane, microbes and models: fundamental understanding of the soil methane cycle for future predictions

- Global methane cycle in nature and its linkage with carbon cycle.
- The five main compartments of C processing are indicated by the colored boxes. Arrows indicate the substrates either produced by a process or for which other process intermediates are produced.



Anaerobic oxidation of methane



- Methanotrophs are bacteria capable of using CH_4 as their sole carbon source (Strong et al., 2015).

- Three major functional types of methanotrophs are known to consume CH_4 :

- 1) **anaerobic methane-oxidising archaea, also called anaerobic methanotrophs (ANME);**

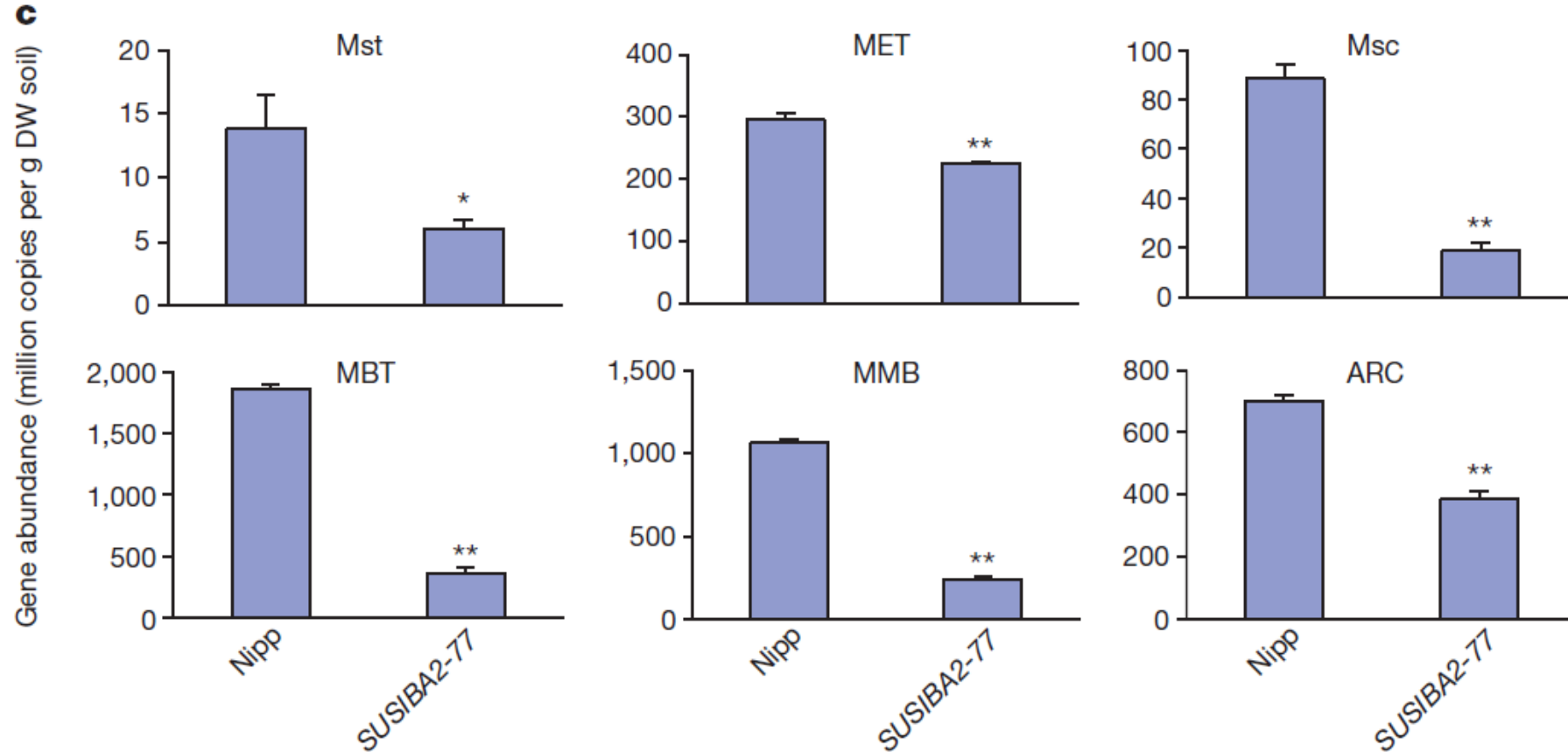
- 2) anaerobic methane-oxidising bacteria (NC10 phylum)

- 3) **aerobic methane-oxidising bacteria (Proteobacteria and Verrucomicrobia).**

These conduct different types of methanotrophy (Kalyuzhnaya et al., 2018)

Quantification of the methanogenic communities

- total archaea (ARC) and methanogens (MET), and the orders Methanobacteriales (MBT), Methanomicrobiales (MMB) and Methanocellales and two families, Methanosaetaceae (Mst) and Methanosarcinaceae (Msc).



- Archase (ARC)
- Methanomicrobials (MMB)
- Methanobacterials (MBT)
- Methanosarcinales (two families: Methanosarcinaceas, Msc; Methanosaeaceae, Mst)
- Methanogens (MET)
- Methytobacter/Methylosarcina (MBAC; TYPE I)
- Methylosinus (TYPE II)



Notably, SUSIBA2 showed a more pronounced effect in mitigating CH₄ emissions than in improving yield traits

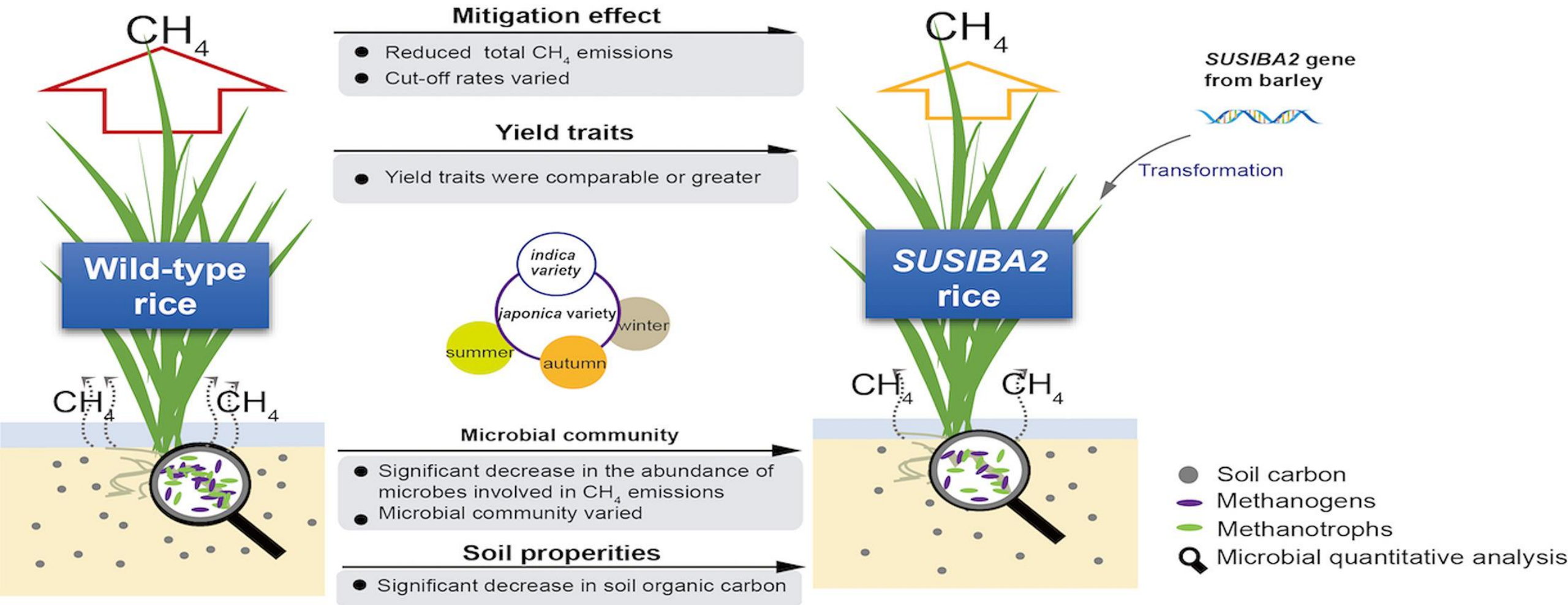
low methane emissions from SUSIBA2 rice were due to
a decrease in the rhizospheric abundance of
methanogens



Comprehensive analysis of *SUSIBA2* rice: The low-methane trait and associated changes in soil carbon and microbial communities

Lin Du, Yunfei Wang, Zhen Shan, Xueliang Shen, Feng Wang*, Jun Su*

Fujian Provincial Key Laboratory of Genetic Engineering for Agriculture, Institute of Biotechnology, Fujian Academy of Agricultural Sciences, Fuzhou 350003, China





Comprehensive analysis of *SUSIBA2* rice: The low-methane trait and associated changes in soil carbon and microbial communities

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Fujian Provincial Key Laboratory of Genetic Engineering for Agriculture, Institute of Biotechnology, Fujian Academy of Agricultural Sciences, Fuzhou 350003, China



- The total CH₄ emissions varied with growing season and rice variety. late rice > early rice > winter rice
- Two or three crops of rice can be grown in a year

Table 1

The CH₄ emissions of *SUSIBA2* rice and the wild-type rice in paddies under natural growing seasons.

The CH₄ emissions and reduction rate of MH86 and 86R-10 in this research were calculated based on the same data source as those in Su's previous report (Su et al., 2018).

| Cultivars | Growth season | Lines | Tested days | Total CH ₄ emission (mg/m ²) | Reduction rate |
|-----------------|-----------------|--------|-------------|---|----------------|
| <i>japonica</i> | Summer | Nip | 78 | 10,288.16 | |
| | | SRB77 | | 5043.6 | −50.98% |
| | Autumn | Nip | 83 | 15,830.16 | |
| | | SRB77 | | 7761.12 | −50.97% |
| | Winter | Nip | 57 | 5928.02 | |
| | | SRB77 | | 4608.60 | −22.26% |
| <i>indica</i> | April–September | MH86 | 73 | 7249.68 | |
| | | 86R-10 | | 5057.16 | −30.8% |

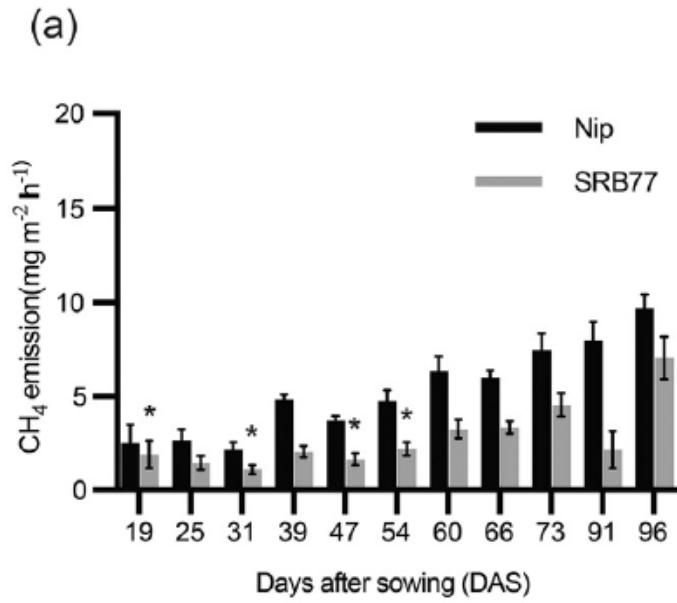
(Su et al., 2018)



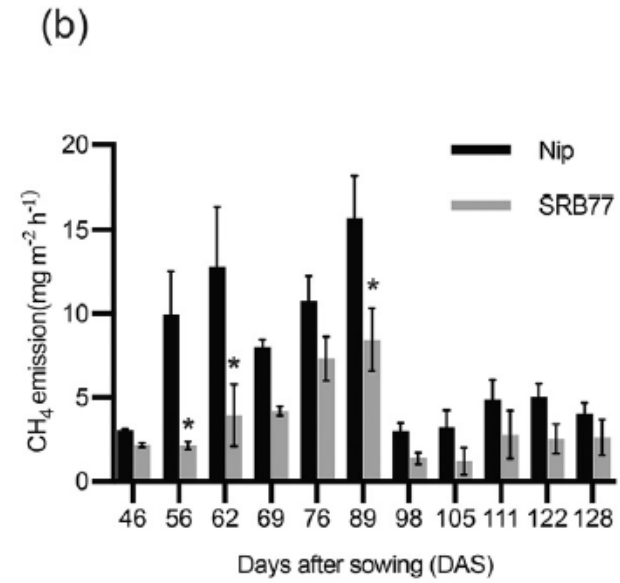
- *Oryza sativa* subsp. *Japonica* (Japan / Korea)
0 – 20 % amylose content

- *Oryza sativa* subsp. *indica* (India)
> 20 % amylose content

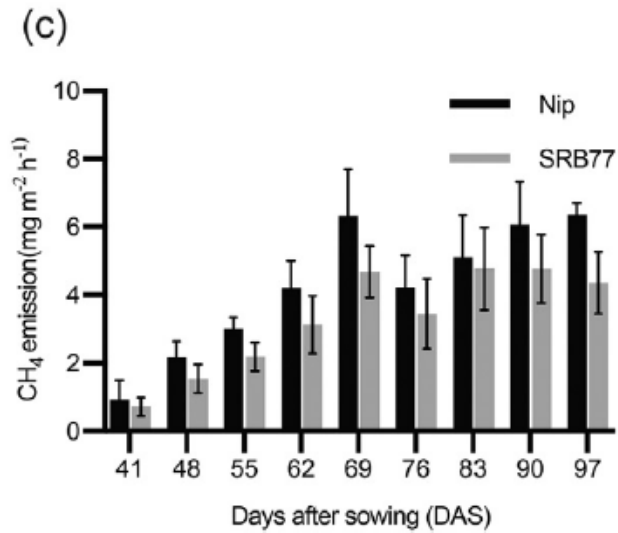
Japonica
early



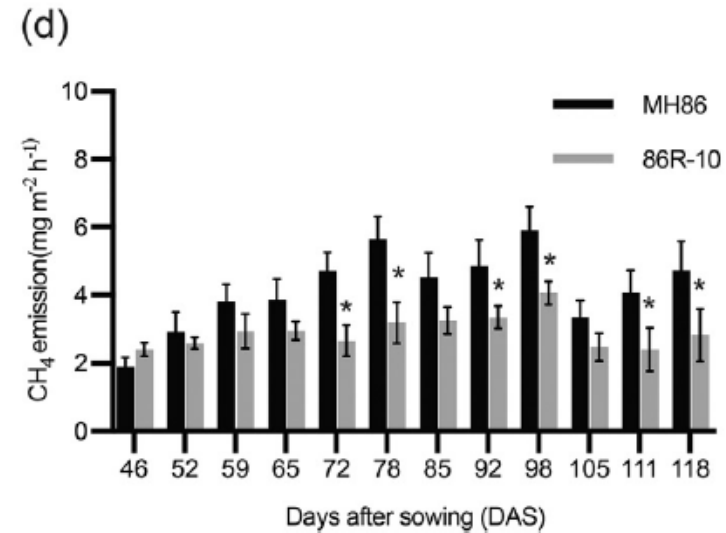
Japonica
late



Japonica
winter

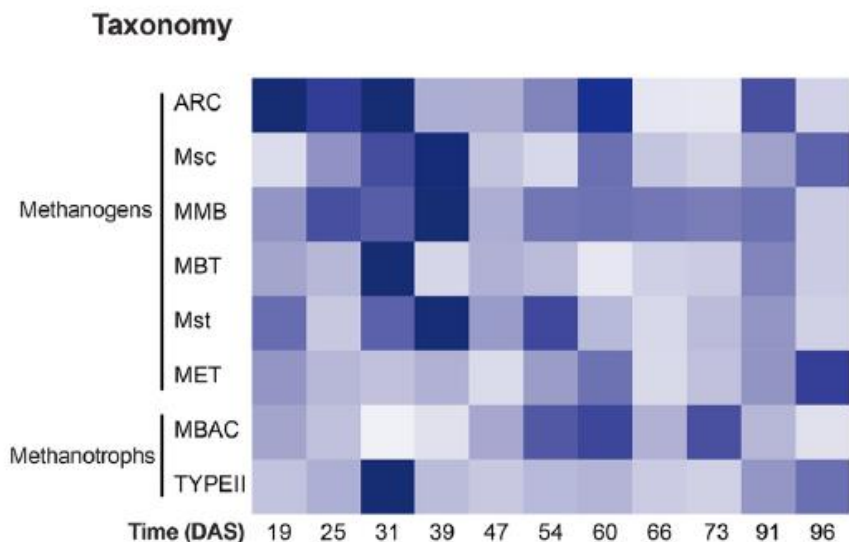


Indica

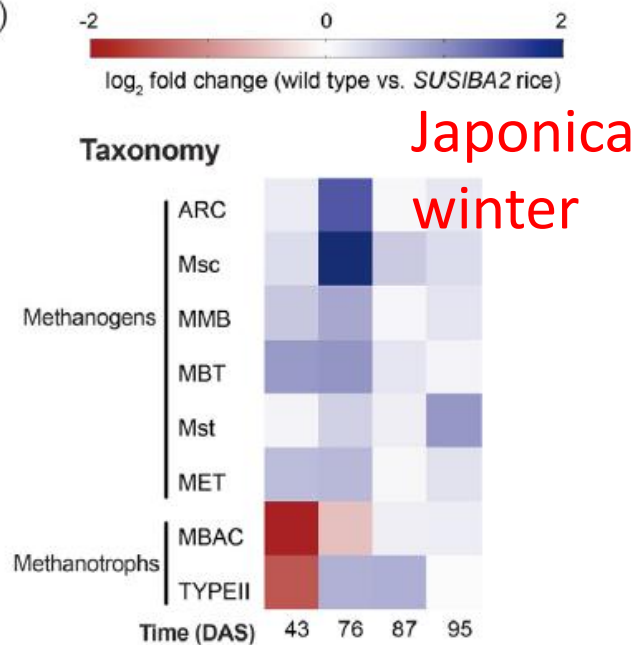


Japonica

(a) early



(c)

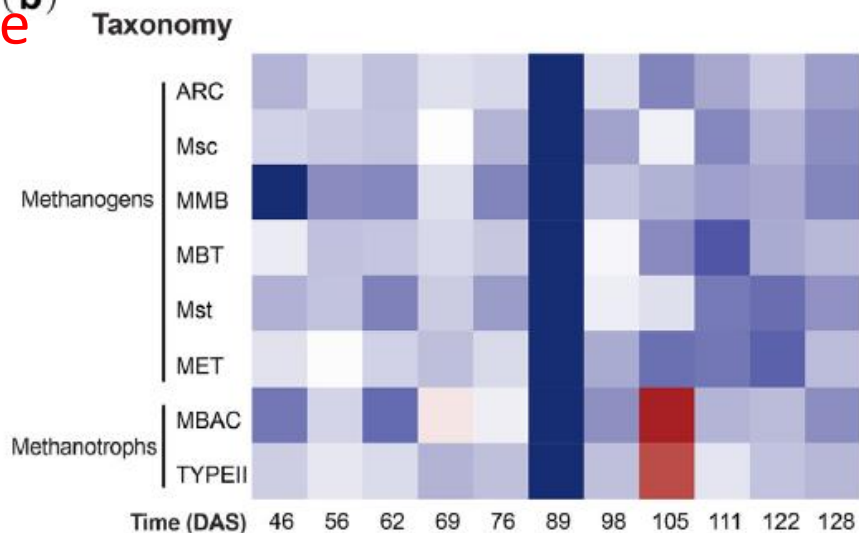


- Effects of rice varieties on the abundance of CH₄ production-associated microbes.

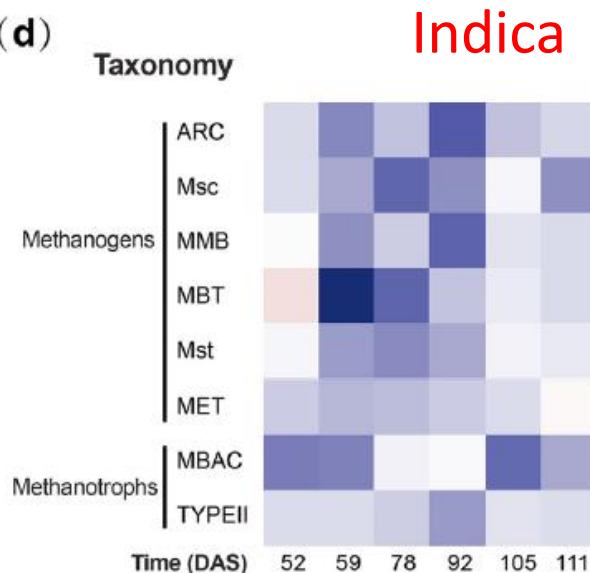
- Heatmap presenting the log₂ fold change in the abundance of methanogens and methanotrophs in

Japonica late

(b)



(d)



- the wild-type Nip paddy soils versus the SRB77 paddy soils at different sampling times during the early rice-growing season

SUSIBA2 model

- Sink remodelling

