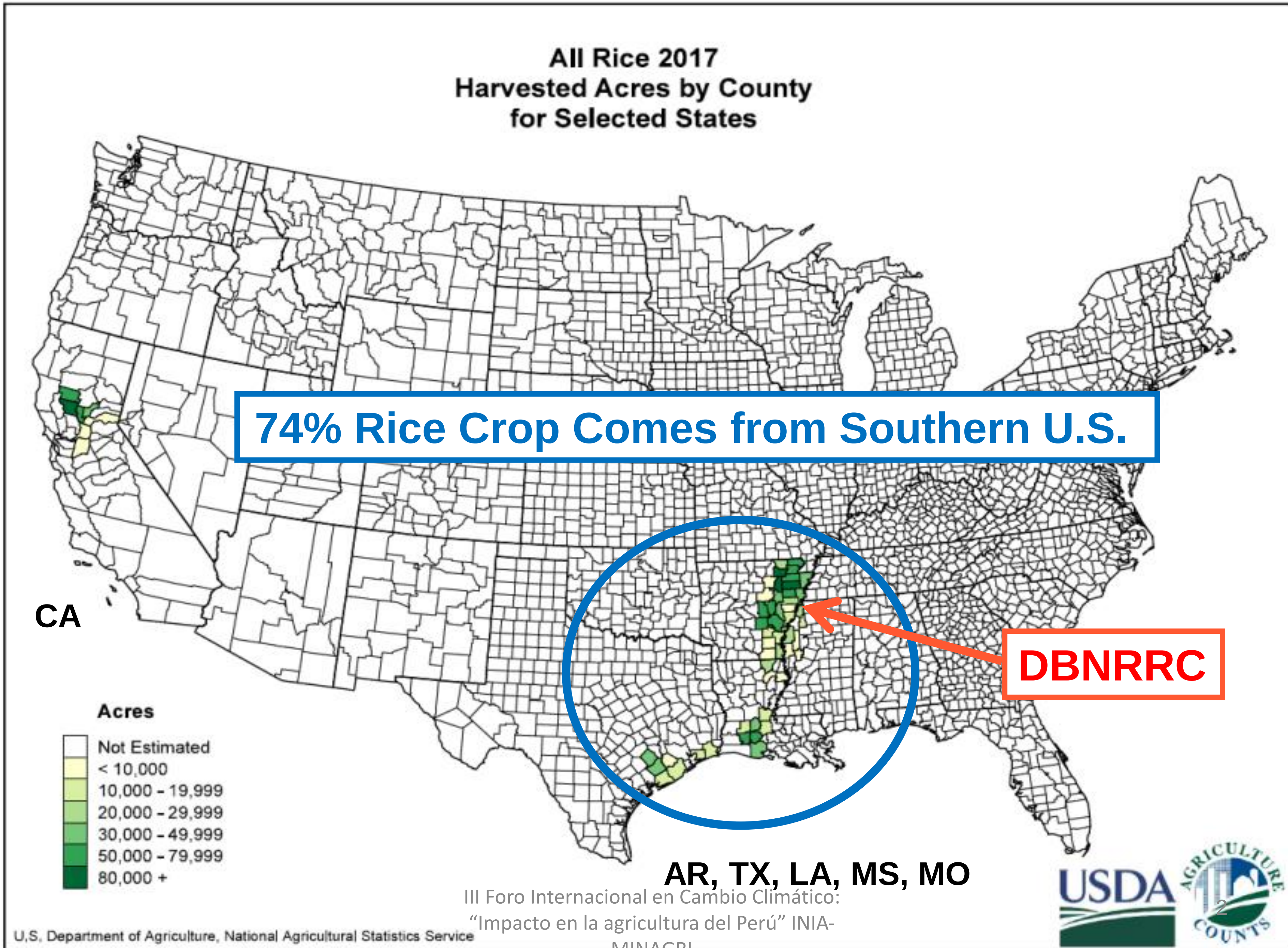
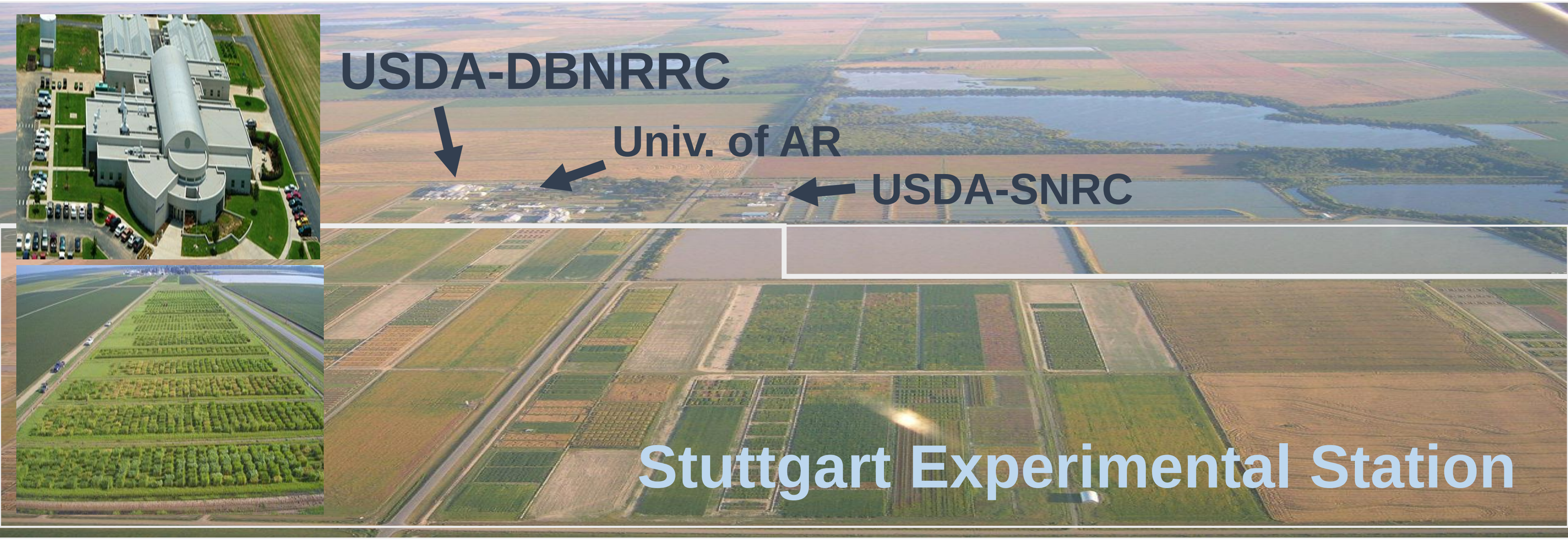
Impact of genotype and environment on yield and grain quality in rice

ARS-Dale Bumpers National Rice Research Center, Arkansas
*Affiliated with BARC-Adaptive Cropping Systems Lab, Maryland

Jinyoung Y. Barnaby, PhD

USDA


All Rice 2017
Harvested Acres by County
for Selected States
74% Rice Crop Comes from Southern U.S.
CA
DBNRRC
AR, TX, LA, MS, MO
Acres
Not Estimated
< 10,000
10,000 - 19,999
20,000 - 29,999
30,000 - 49,999
50,000 - 79,999
80,000 +
U.S. Department of Agriculture, National Agricultural Statistics Service



To Research Communities
(eg. GSOR (Genetic Stock Oryza), Genomics facility)

Service

Characterizing, Developing, and Utilizing genetic resources

Research

To Stakeholders
(eg. AR farmers, Rice companies-Riceland, Ricetech, etc)

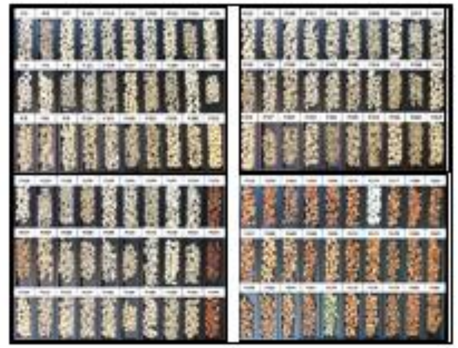
Outreach

USDA

Dale Bumpers National Rice Research Center

Genetic Stocks-ORyza (GSOR) Center

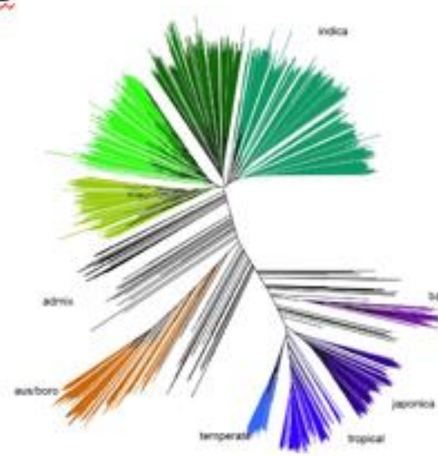
Genetic Diversity NSGC Collection Center - Genetic Stocks-ORyza (GSOR)



Genotyping Facility

Unique Gene Mapping Populations, diversity panel, etc

Genetic Bioinformatics



Gene Discovery (Ricebase)

Rice Field Evaluations

USDA

Barnaby's research issues important to USA rice production

- Changing management practices due to diminishing irrigation resources
- Adapting and mitigating to changing environments (heat, CO_2, and methane)
- Losing export markets due to poor quality (eg. chalk and arsenic)
- Utilizing omics-driven new technologies to breeding program

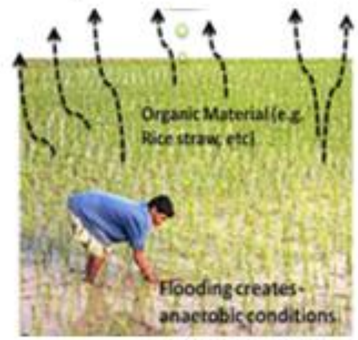
Lack of Irrigation

By static.ark.org

Yield Loss due to Heat Stress

By DBNRRC

CH_4 - Soil Microbe

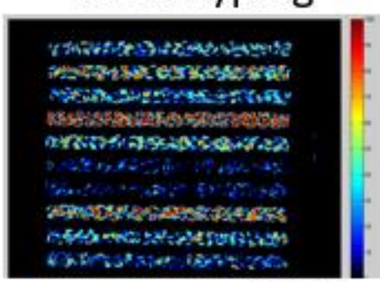


By Green Clean Guide

CO_2 – Plant response

By Ziska

High throughput Phenotyping

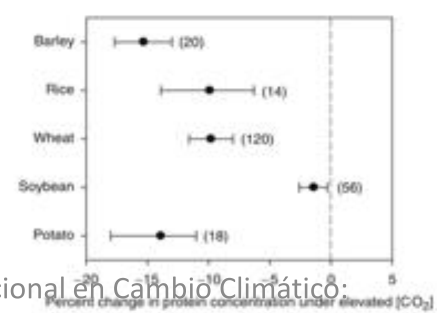


By Barnaby

Omics-driven Technologies

By Agilent

CO_2 - Protein



Taub et al (2008)

Grain Arsenic

By European Pharmaceutical Review

Grain Chalk

By DBNRRC

Partnerships on GHG & Grain Quality Studies

- USDA-Dale Bumpers Rice Research Center
- USDA-Beltsville Agricultural Research Center

Naturally existing genetic variation

DBNRRC
Collaborations

Soil Microbiome
SASL
Composition of soil microbiome affecting methane emissions and arsenic uptake

Mitigation

High throughput screening

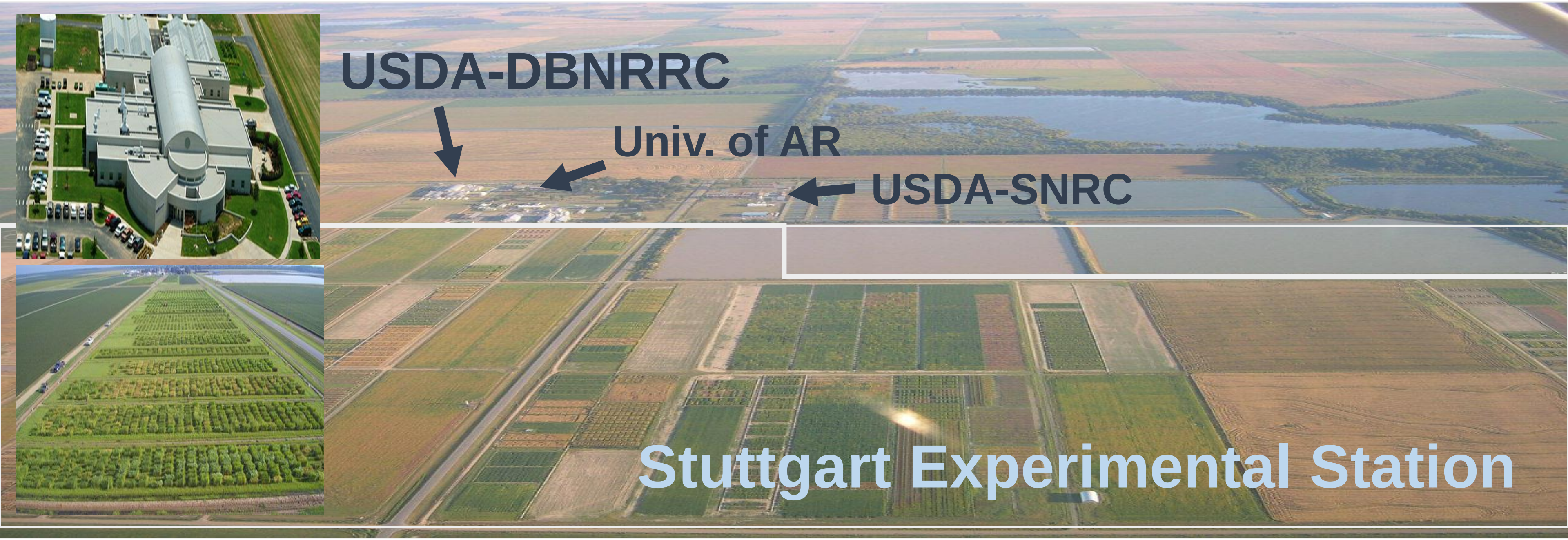
Hyperspectral Imaging System
EMFSL
High throughput phenotyping on grain physicochemical traits

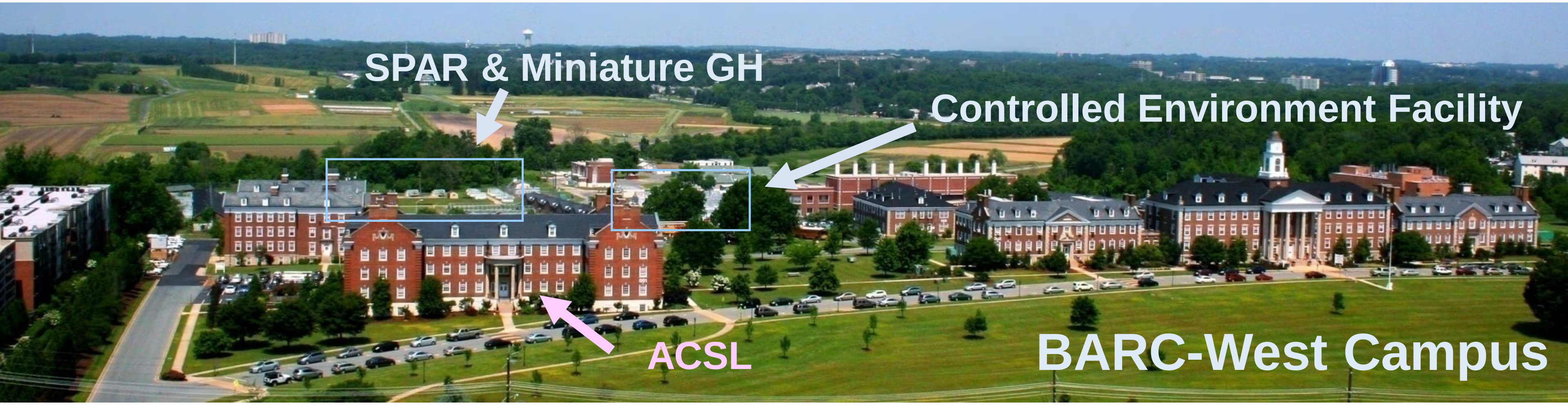
Climate Resilience

CO_2, heat, and heavy metals
Adaptive Cropping Systems Lab
Rice responses and grain quality (chalk and arsenic) to changing climates (heat stress, AWD), and developing a rice model for U.S. rice cultivars

Food Safety, Security

Food Quality





Dale Bumpers National Rice Research Center
Beltsville Agricultural Research Center



ACSL Controlled Greenhouses & Chambers



Controlling CO_2, temperature, light

ACSL Specialized Equipment

South Farm

6 Large open top chambers
12 Small open top chambers

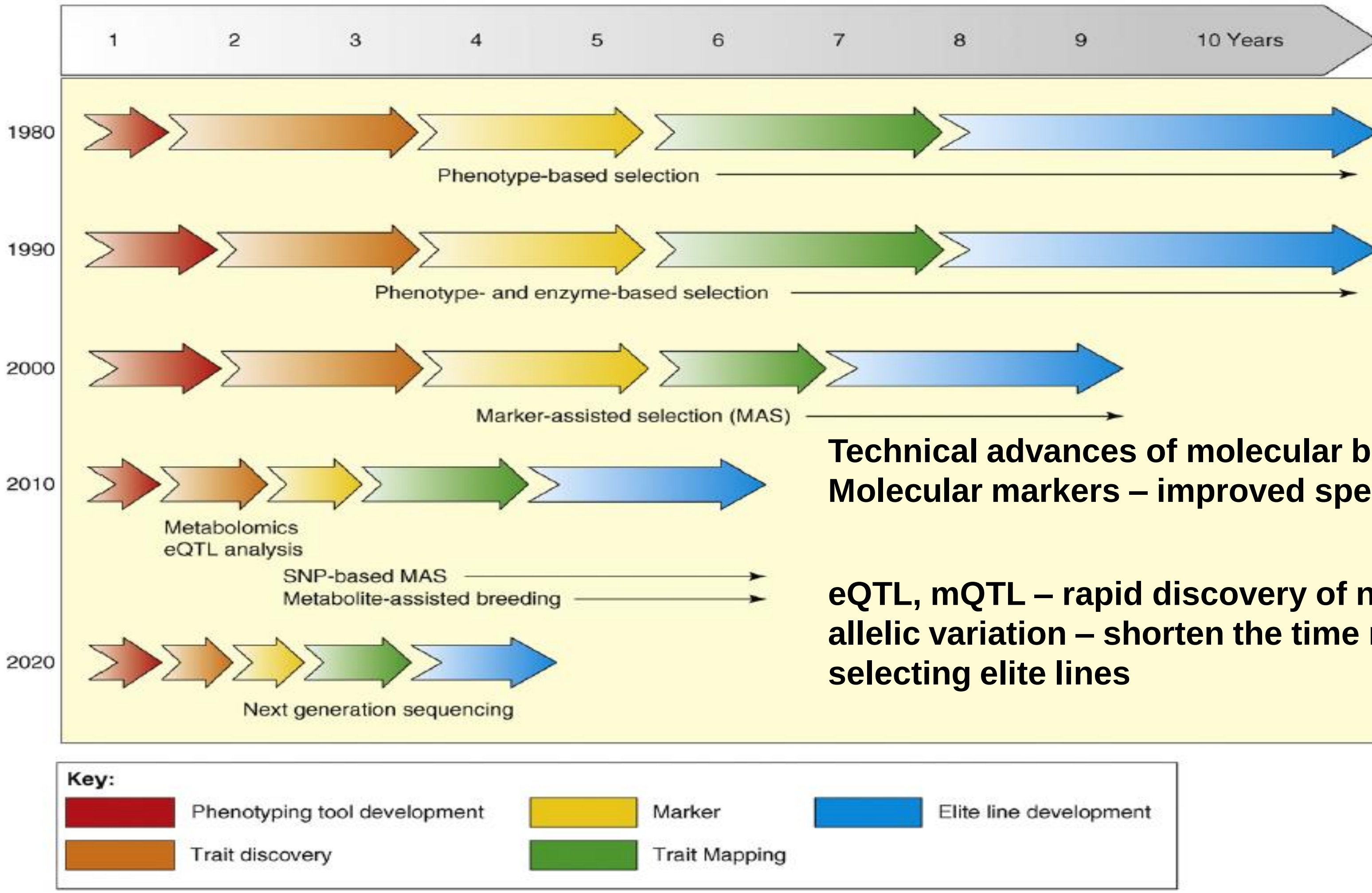
Controlled Environment Facility

Multiple Indoor Artificially Lit Growth Chambers

SPAR Unit

12 Soilbin Chambers
6 Chambers without soilbin

Breeding technology pipeline



Trait discovery

Phenotypic observation

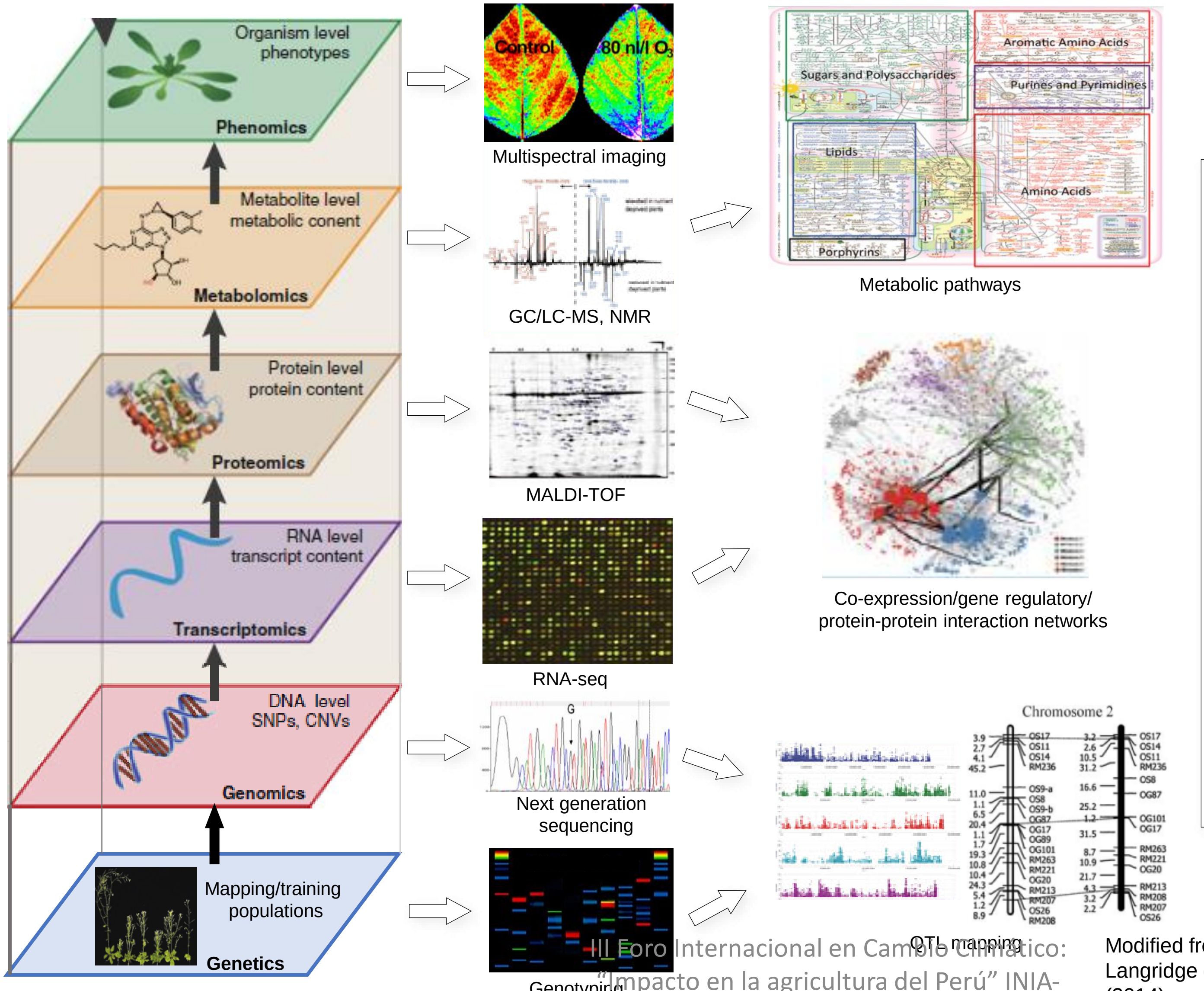
Restricted to Phenotypic, enzymatic or protein markers

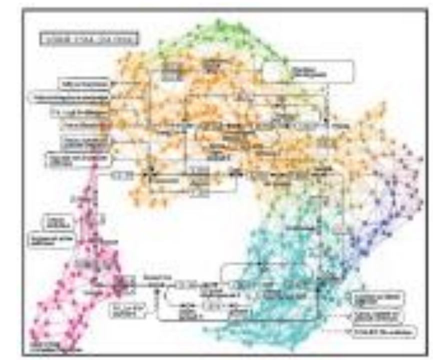
Technical advances of molecular biology
Molecular markers – improved speed of trait mapping

eQTL, mQTL – rapid discovery of new traits and allelic variation – shorten the time required for selecting elite lines

Fernie and Schauer (2008) *Trends in Genetics* 25 (1): 39-48

Omics: Multidisciplinary technology





Complete gene-phenotype networks
(G-P maps)

To understand mechanisms & identify genes for sustaining yield and quality

Modified from: Fernie (2008), Keurentjes (2009), Langridge (2011), Carreno-Quintero (2012), Chen (2014)

Tools to improve & accelerate the rice production in breeding

Genetic architecture of plant omics

Genetic mapping populations	Omics	Integration
Genetics Genomics	Transcriptomics Proteomics Metabolomics Phenomics	Bioinformatics

Application
Marker-assisted selection for production of climate resilient and high yielding rice cultivars in breeding

Content

- Assessment of crop responses to changing climates
- Adaptation process: Identification of climate resilient cultivars (e.g. yield)
- Adaptation process: Identification of QTLs for climate resilience (e.g. grain quality)
- Mitigation process: Identification of high-yielding with reduced GHG emissions

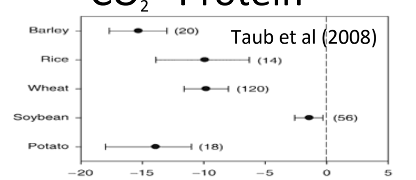
Yield Loss due to Heat Stress

By DBNRRC

Lack of Irrigation

By static.ak.org

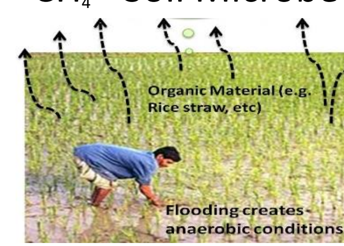
CO_2 - Protein



Grain Chalk

By DBNRRC

CH_4 - Soil Microbe



By Green Clean Guide

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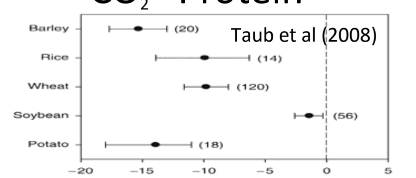

Grain Chalk

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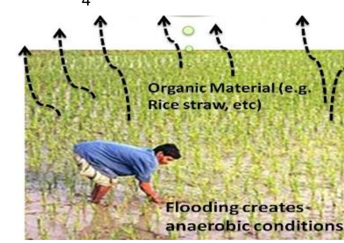
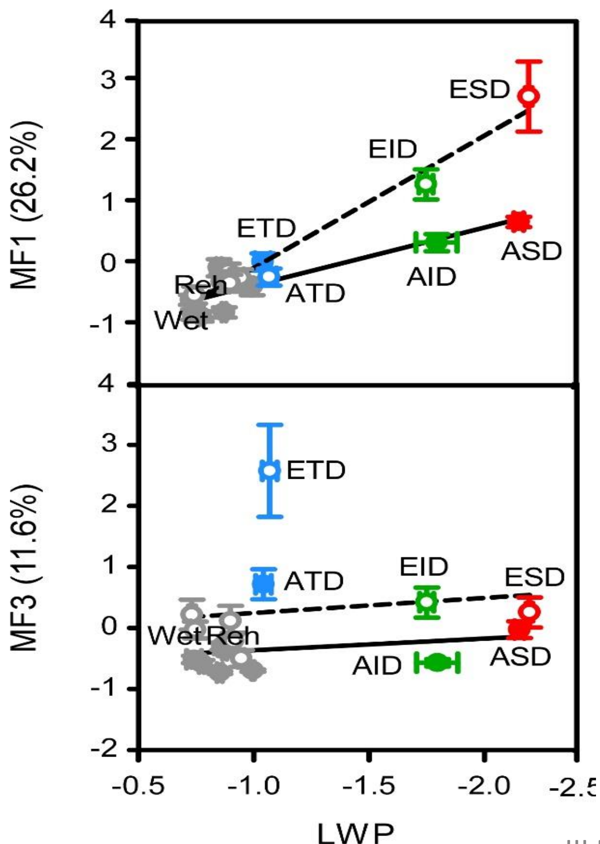
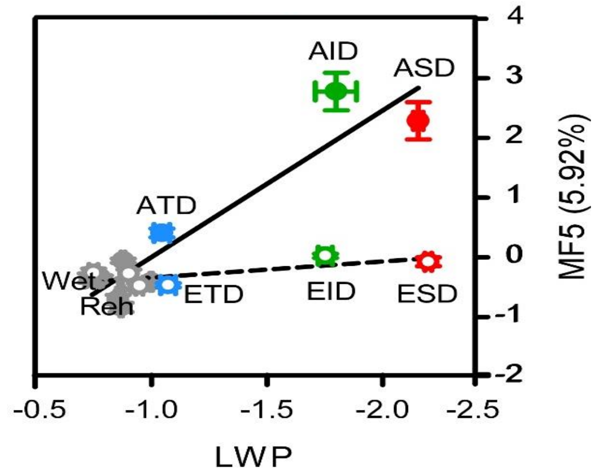


By Green Clean Guide

Maize responses to rising CO_2 + drought stress

- **Rising CO_2 level ameliorates drought responses**
- **Current stress markers may no longer be sufficient to screen superior germplasm under future climate conditions**





MF1: Leu, Val, Ile, Phe, Tyr
MF3: Glc, Fru, Mann
MF5: Pro, Raff

Barnaby *et al.* et al. *PLoS One* (2013), Yang *et al*, *Int. J Plant Biol.* (2014), Barnaby *et al*. submitted to *Int. J. Mol. Sci.* (2019)

Rice Responses to rising CO_2 + heat stress

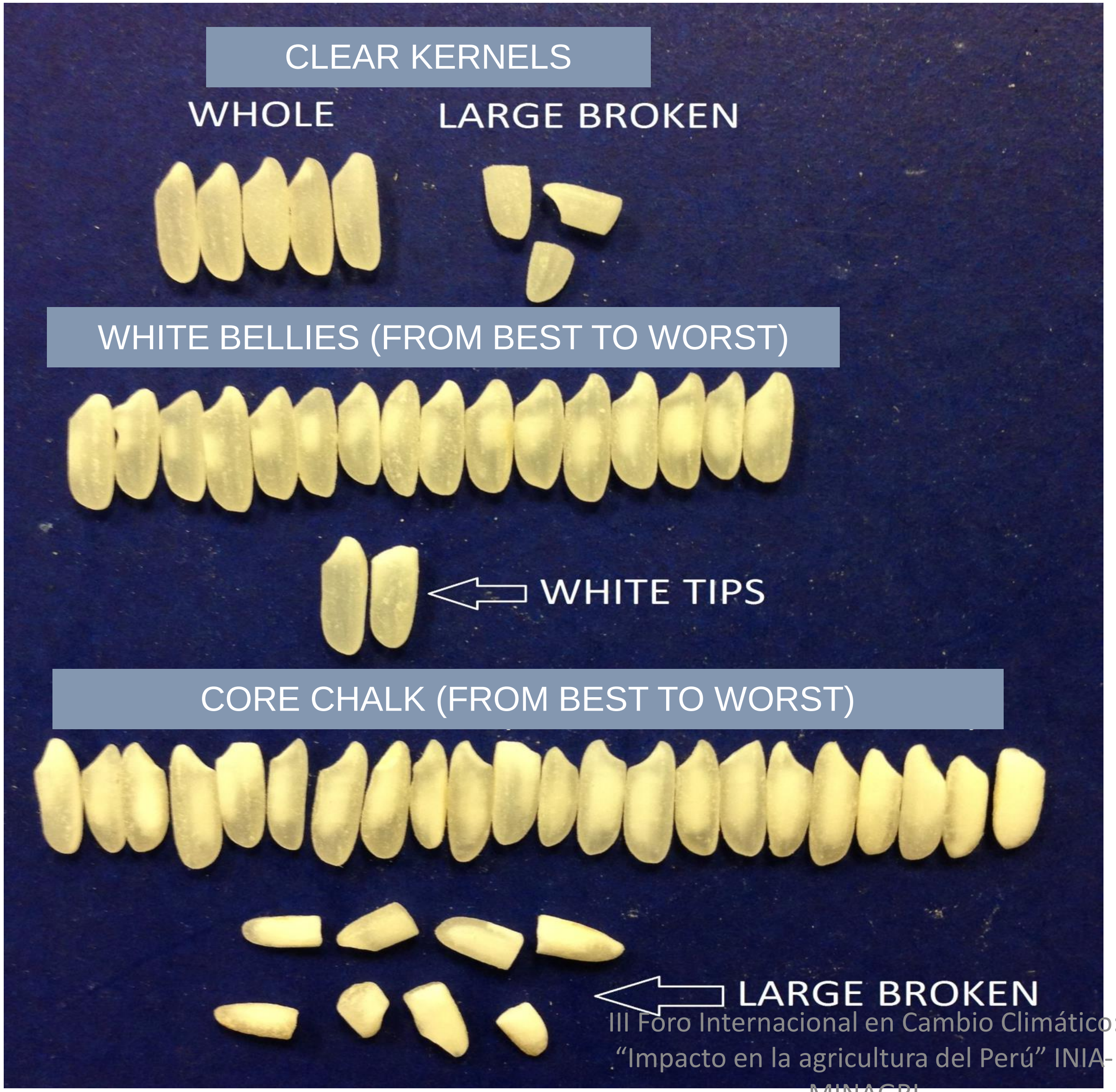
Rising CO_2 levels in the future may aggravate grain chalkiness

	400 ppm		600 ppm	
	Control	Heat	Control	Heat
KB				
ZH				

Bi-parental mapping population of KBNT and ZHE733 that is segregating for % chalk

Barnaby *et al*. Unpublished

Grain quality issue due to heat stress



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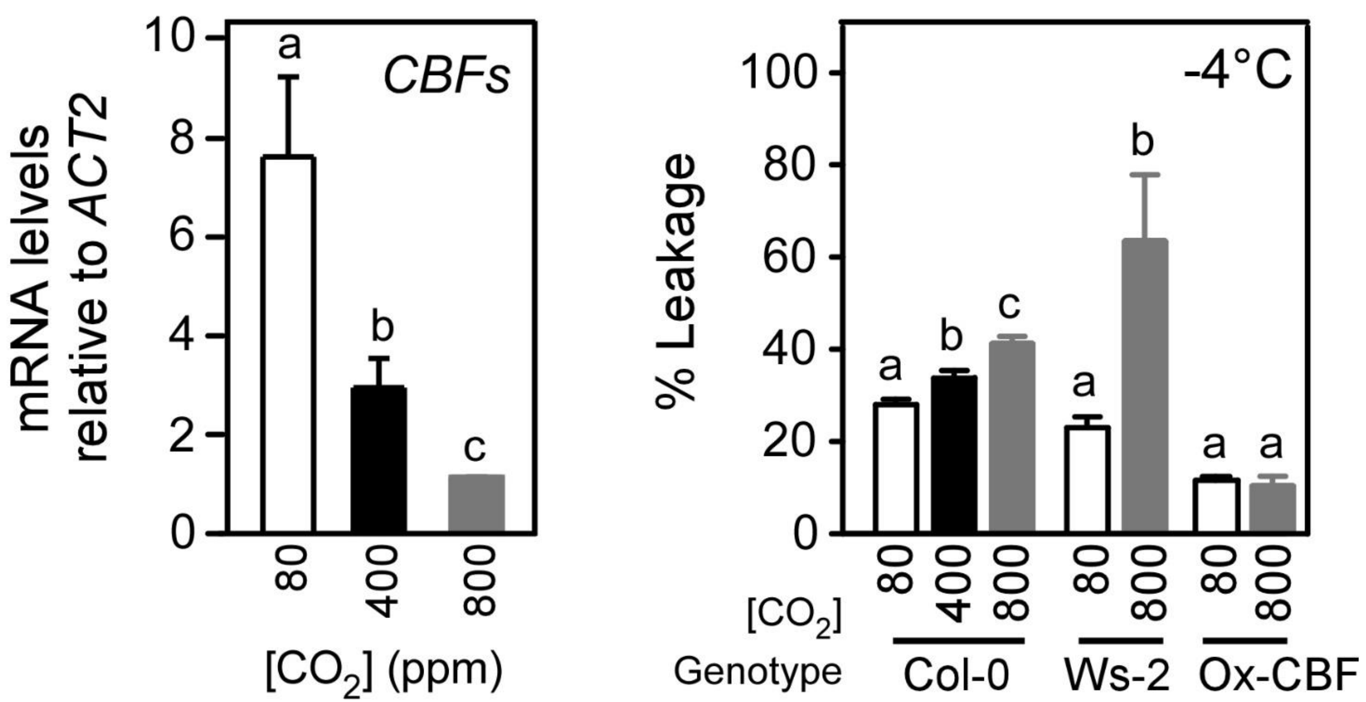
- Timing of high temperature stress during grainfill impacts type and amount of chalk
- High grain chalk causes grain breakage during milling and loss of crop value

Arabidopsis Responses to rising CO_2 + cold stress

Rising CO_2 levels in the future may suppress intact cold tolerance

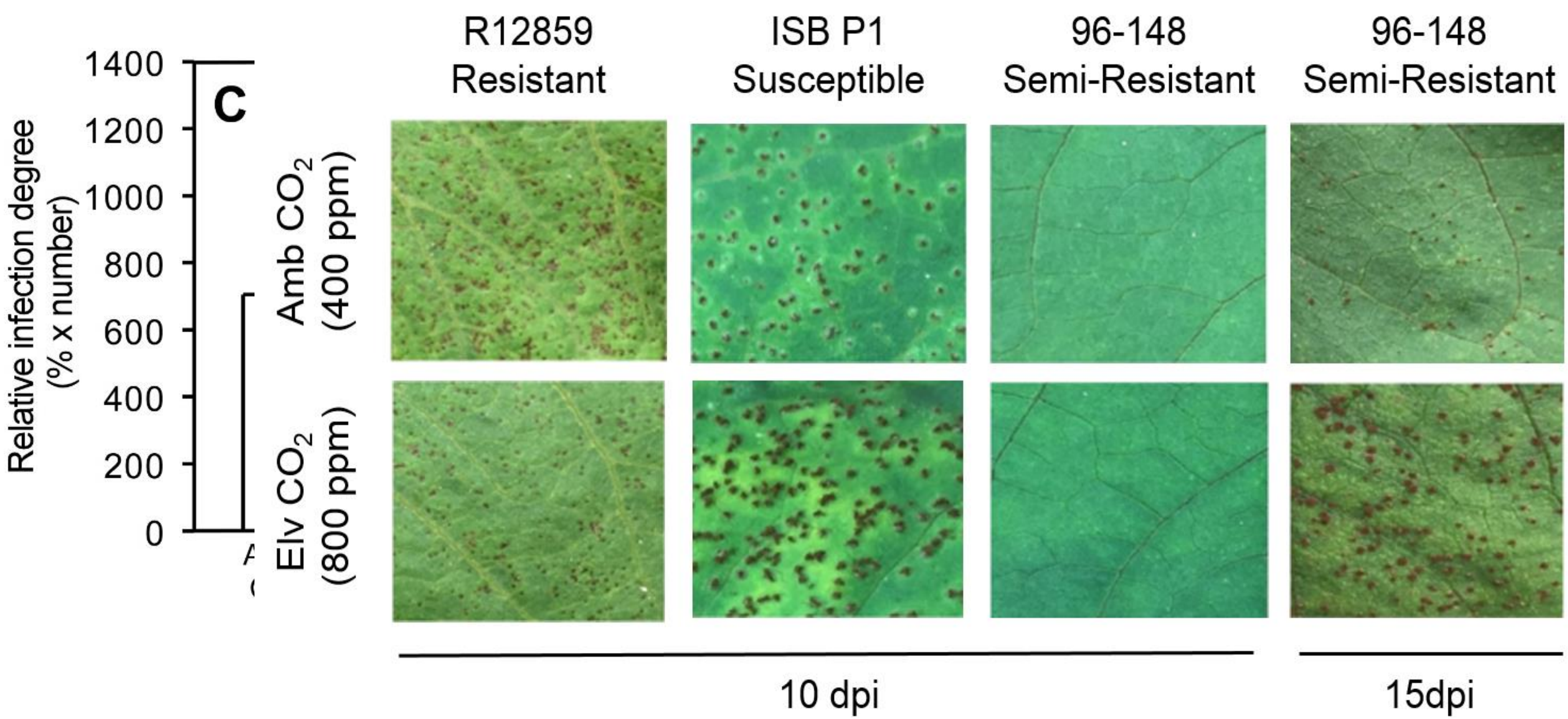


CBFs: C-repeat/dehydration responsive element binding Factor

Barnaby *et al*. submitted to *Int. J. Mol. Sci.*

Bean Responses to rising CO_2 + rust

Bean rust may become more aggressive under elevated CO_2 condition



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USDA
USDA-ARS-Dale Bumpers National Rice Research Center

Barnaby *et al*. Unpublished

Content

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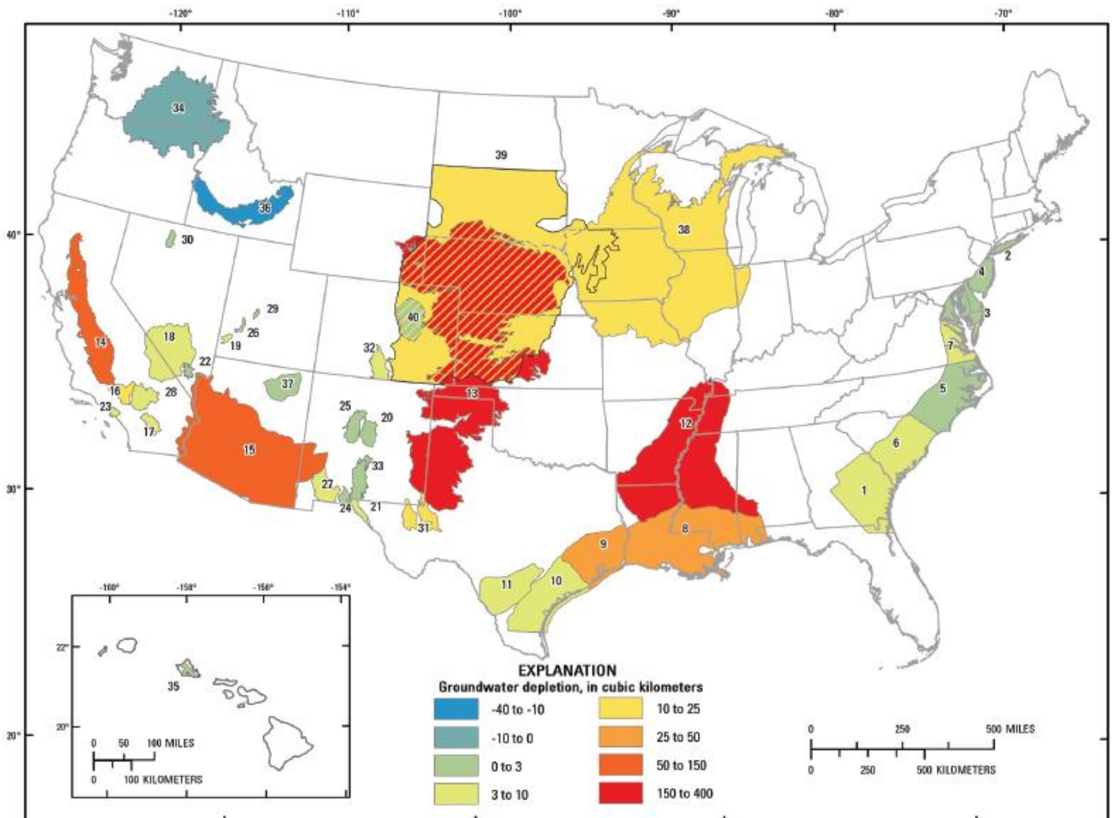
Lack of Irrigation

By static.ark.org

Diminishing irrigation resources
-> changing rice production practices
-> yield loss

Climate change is threatening water availability and access (Ground Water Depletion)



Cumulative ground water depletion, 1900 through 2008, in 40 assessed aquifer systems or subareas

Irrigating rice fields in Richvale, California.

ARS Grand Challenge by 2050

For transformation in U.S. agriculture that results in 20% more quality products, with a 20% reduction in environmental resources impacts

<u>(Genetics x Environment x Management) x p</u>

Water saving management practices (Alternate wetting and drying; AWD)

<u>Diminishing irrigation resources</u>

Changing management practices from flooded paddy to AWD can reduce water use up to 30%

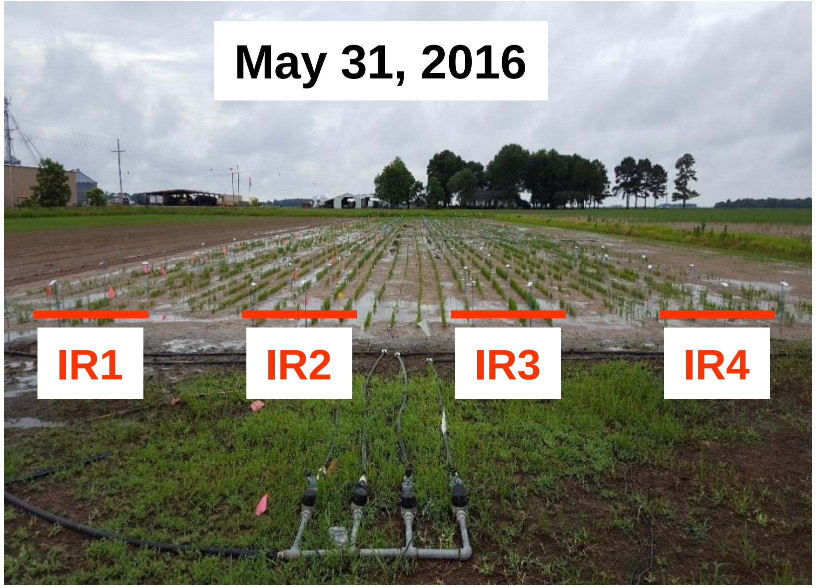
*** Yield reduction, rice blast, weed suppression, GHG emissions, nutrient uptake**

Research Objectives

- To identify stress response traits that can be used for breeding of cultivars without yield reduction upon water stress.
- To explore genetic variation in physiological and metabolic traits that could be used to evaluate yield variation under water deficit irrigation.

Materials and Methods

A sub-surface drip irrigation (SDI) system was implemented to deliver soil moisture conditions that ranged **from saturated soil to near drought conditions** in order to determine plant stress response.



- Irrigation (IR) 1 (30% VWC): Field capacity=29%, 1% above
- Irrigation (IR) 2 (24% VWC): 25% water deficit
- Irrigation (IR) 3 (20% VWC): 50% water deficit
- Irrigation (IR) 4 (14% VWC): 80% water deficit (just above wilting point)

Barnaby et al, (2019) *Int. J. Mol. Sci.* 20(8): 1846

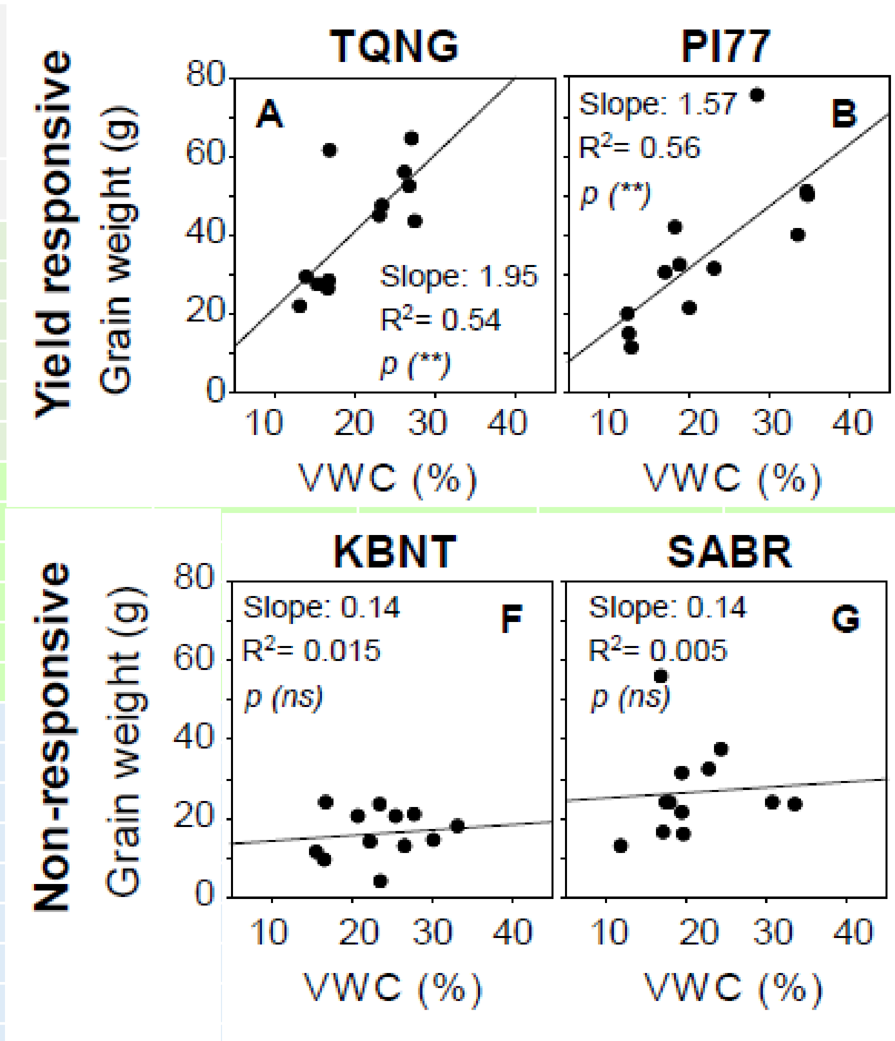
The physiological, biochemical, and agronomic status of 7 out of 15 cultivars to reduced soil water availability were evaluated

Variety	Sub-population	Grain Type	Selected
Zhe 733	Indica	Long	
Rondo	Indica	Long	
Teqing	Indica	Medium	*
PI312777	Indica	Medium	*
Cybonnet	Tropical japonica	Long	
Lemont	Tropical japonica	Long	*
RoyJ	Tropical japonica	Long	
Lagrue	Tropical japonica	Long	*
CL151	Tropical japonica	Long	
Kaybonnet	Tropical japonica	Long	*
Katy	Tropical japonica	Long	
Francis	Tropical japonica	Long	*
Saber	Tropical japonica	Long	*
Mars	Tropical japonica	Medium	
Jupiter	Tropical japonica	Medium	

Barnaby et al, (2019) *Int. J. Mol. Sci.* 20(8): 1846

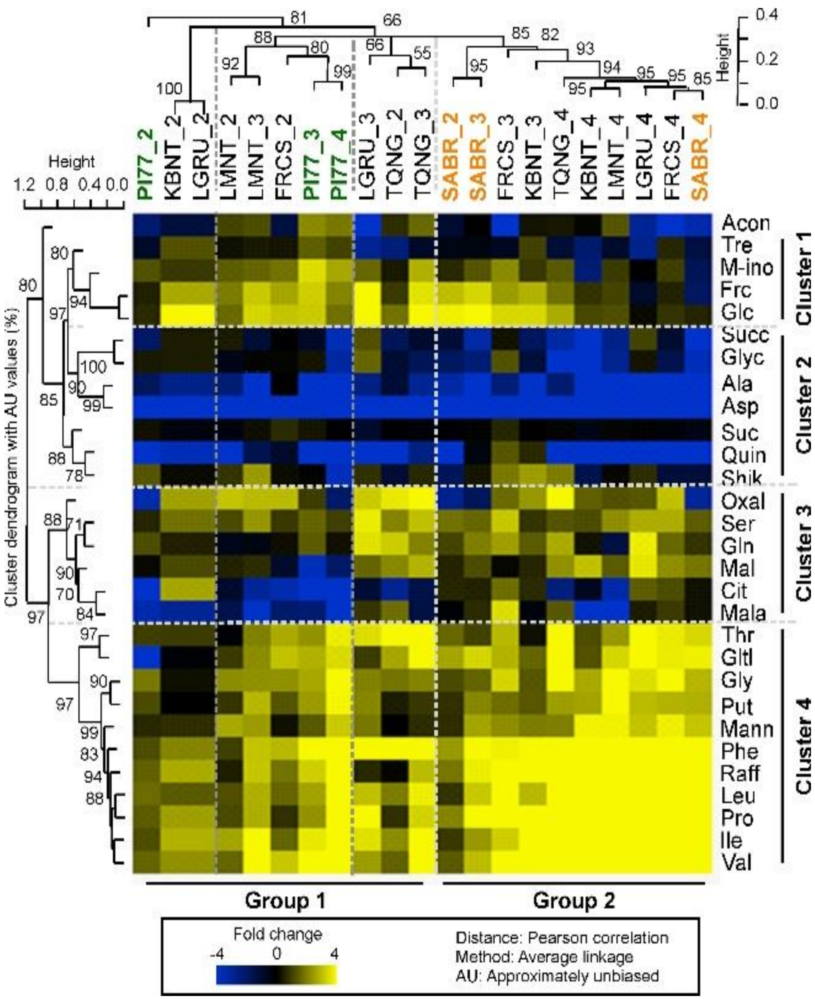
Seven rice cultivars (2014-2016) were selected based on yield response (g/plant) to reduced water availability

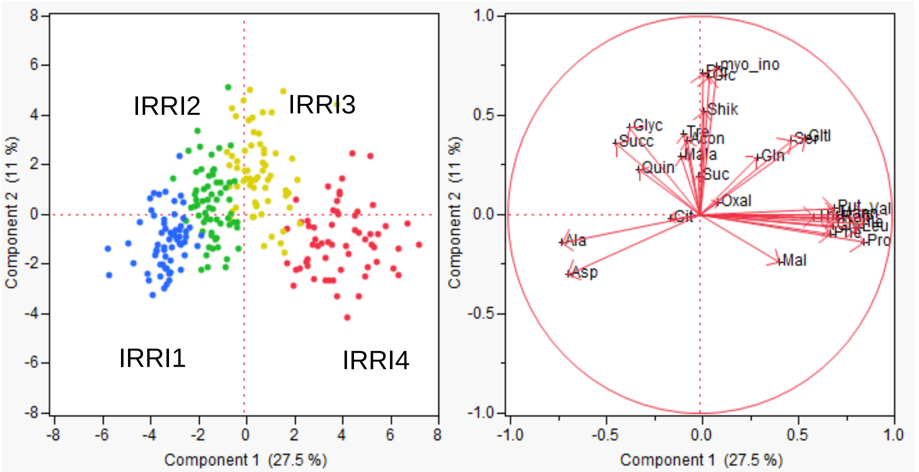
Cultivar	Year	Yield performance under water stress
TQNG	2014	High responsive (High yielding varieties)
	2015	
	2016	
PI77	2014	
	2015	
	2016	
LGRU	2014	Intermediate responsive
	2015	
	2016	
LMNT	2014	
	2015	
	2016	
FRCS	2014	Non- or low responsive
	2015	
	2016	
KBNT	2014	
	2015	
	2016	
SABR	2014	
	2015	
	2016	



Barnaby et al, (2019) *Int. J. Mol. Sci.* 20(8): 1846

Metabolic changes among cultivars that differ in yield response to water stress





<u>29/40 metabolites (significant impact by water stress)</u>

- Cluster 1: soluble sugars (osmotic adjustment?)
- Cluster 2 and C3: Organic acids
- Cluster 4: amino acids (stress responsive markers)

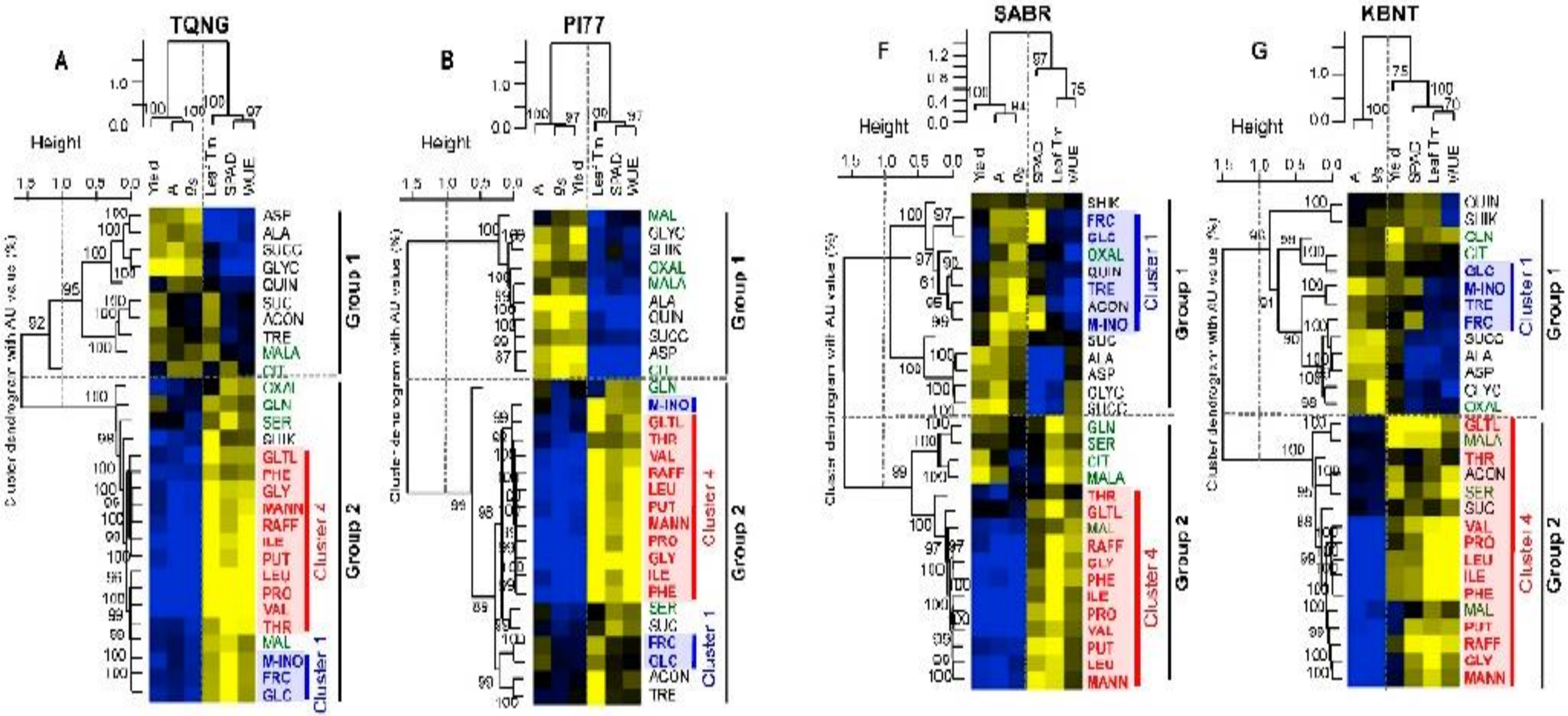
There exists genetic variation in accumulating metabolites upon water stress

Barnaby et al, (2019) *Int. J. Mol. Sci.* 20(8): 1846

Contrasting patterns of metabolic change between high vs int. and low responsive cultivars

High yield responsive cultivars

Low yield responsive cultivars



Barnaby et al, (2019) *Int. J. Mol. Sci.* 20(8): 1846

Summary

- Adopting water saving management practices in rice production will significantly **conserve water resources** as well as **reduce input** (e.g. pumping underground water) costs. However, farmers will **not adopt such practices** if there is a significant risk of economic loss (i.e. reduced yield and/or grain quality).

- There exists **cultivar variation** in response to different stress levels indicating a **genetic balancing** between survival (stress tolerance) and grain production.

- **Different breeding strategies** may be needed to develop new cultivars for deficit irrigation production systems.
  - **Under mild stress (mild water saving)**, **high yielding** cultivars with a **high transpiration rate** would likely sustain higher yield than lower yielding with lower stomatal conductance.
  - Under sever stress (when **aggressive water savings** is attempted): cultivars having **lower transpiration rates** and also accumulate **soluble sugars** would be a better option.

Content

- Assessment of crop responses to changing climates
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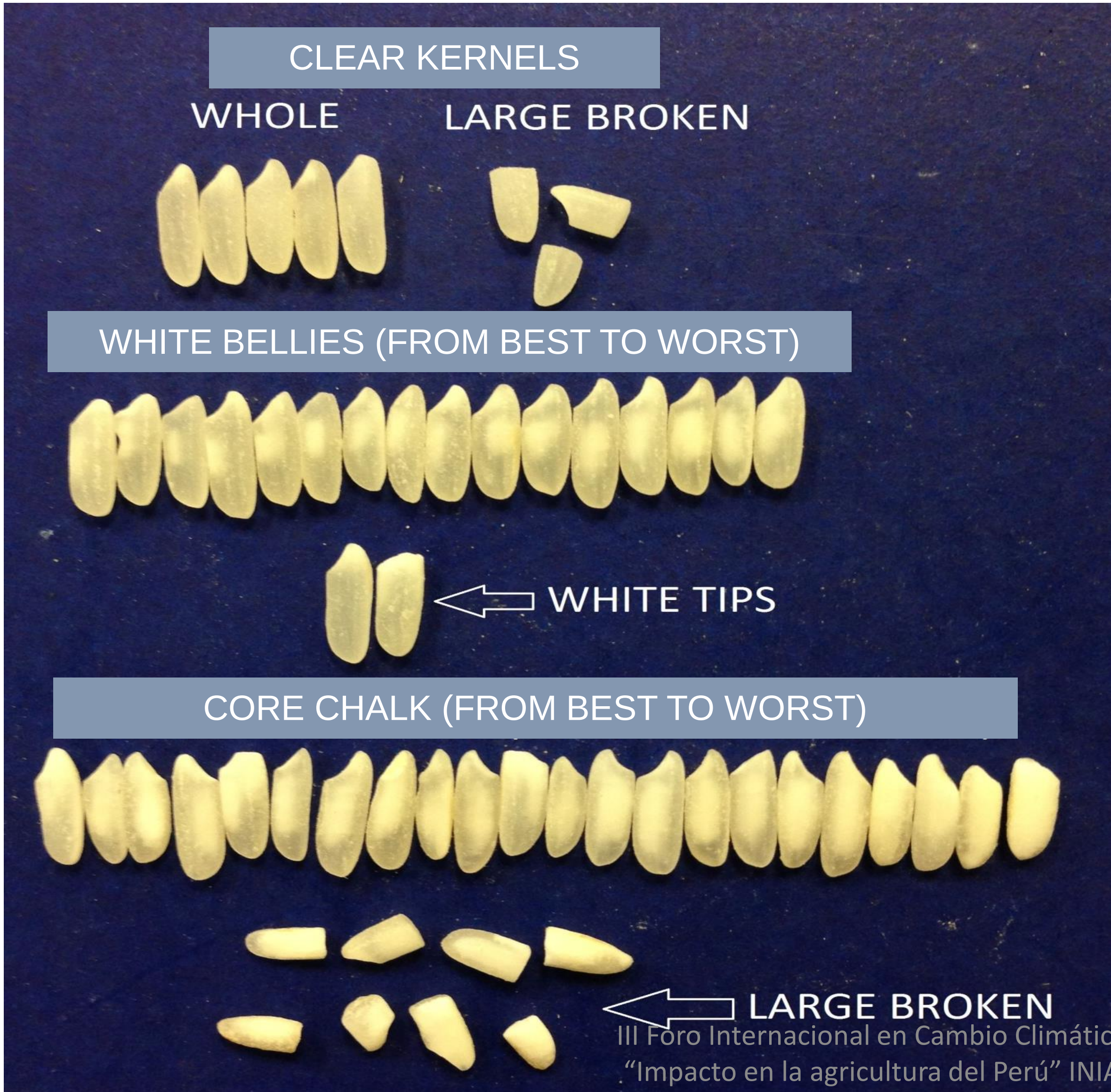
Grain quality issue due to heat stress



- Timing of high temperature stress during grainfill impacts type and amount of chalk
- High grain chalk causes grain breakage during milling and loss of crop value

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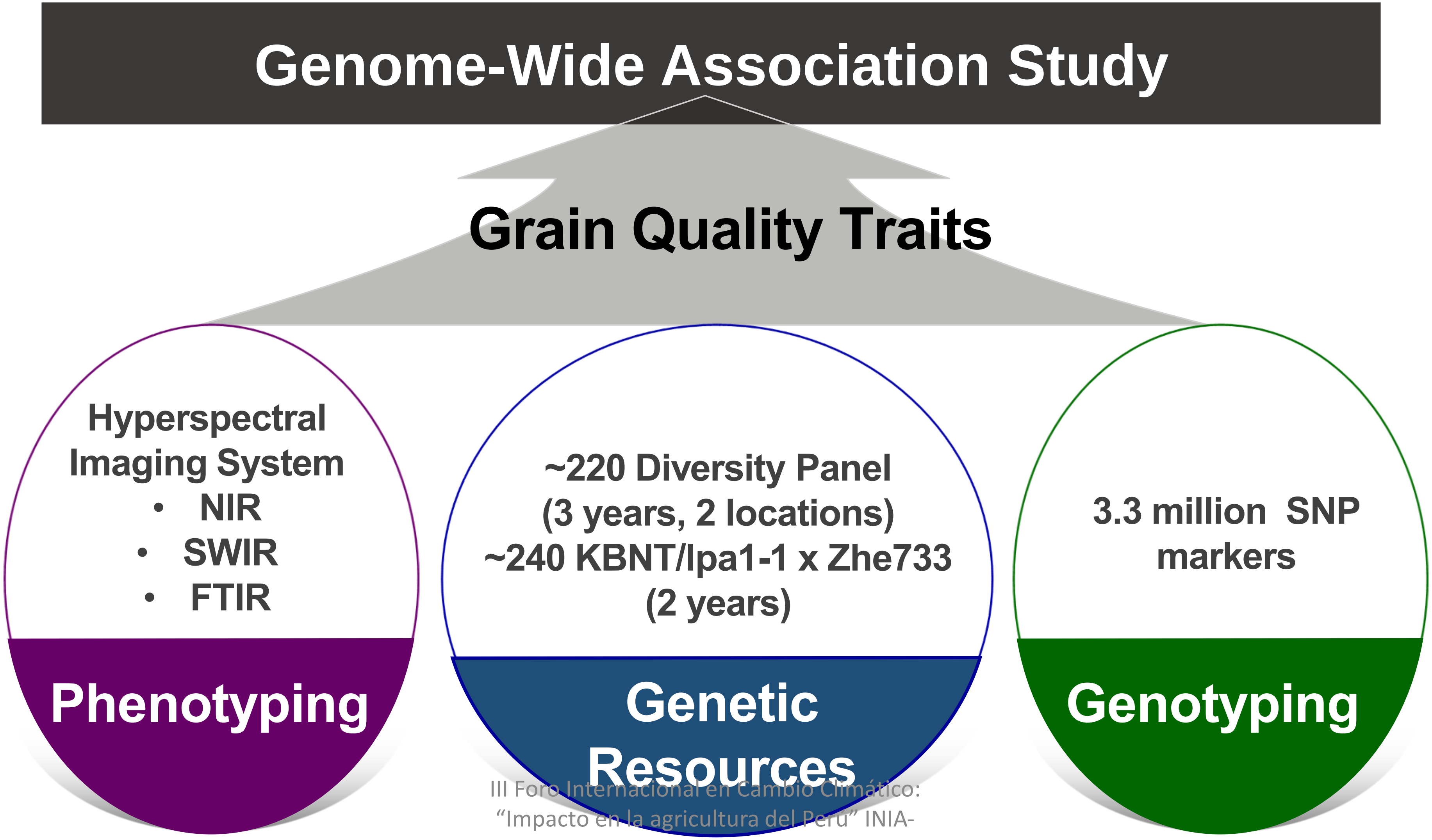
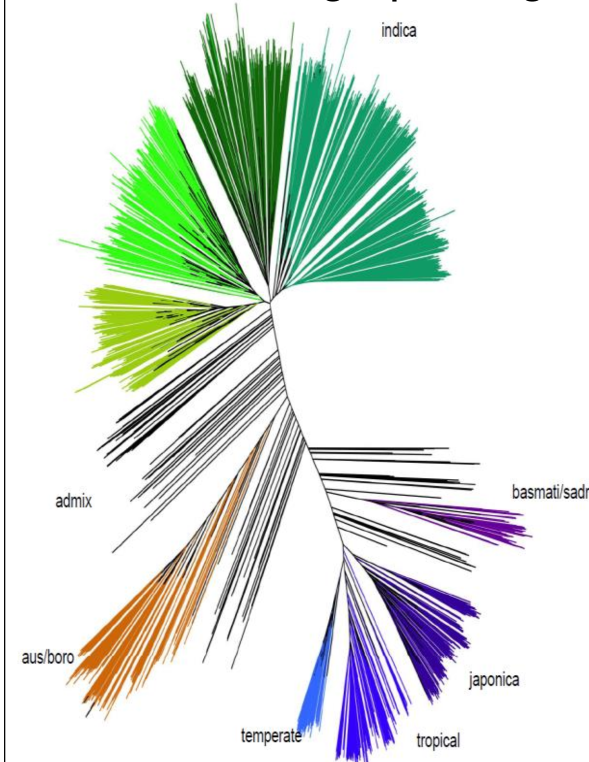
Exploring genomic regions associated with grain quality traits



USDA minicore rice germplasm collection

Genetic Fingerprinting

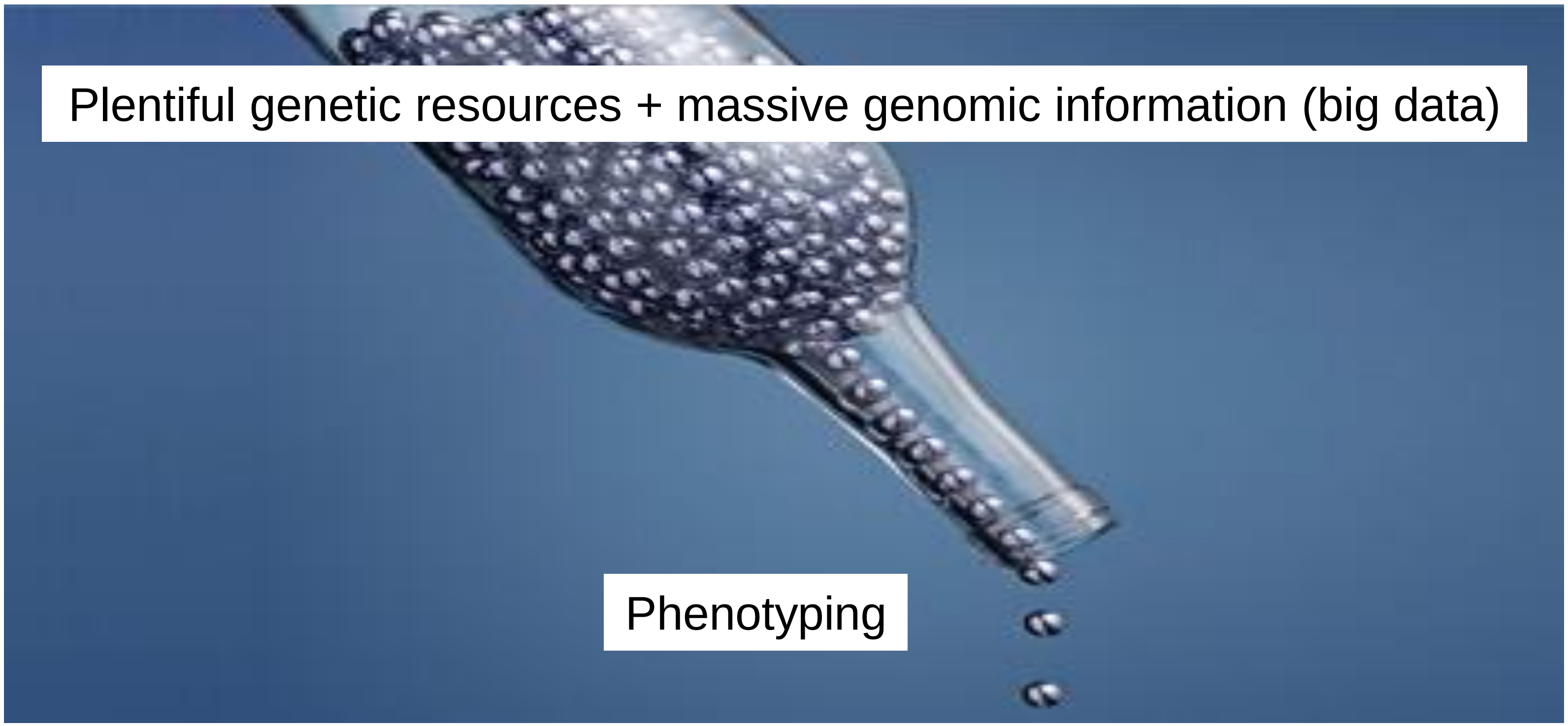


Alexandrov et al. doi:10.1093/nar/gku1039

- Represents 1% of worldwide rice collection (~220 varieties out of 19,000)
- Includes 5 subpopulation of *O.sativa* (AUS, IND, TRJ, TEJ, ARO)
- Has genomic dataset of 3.3 million SNP markers

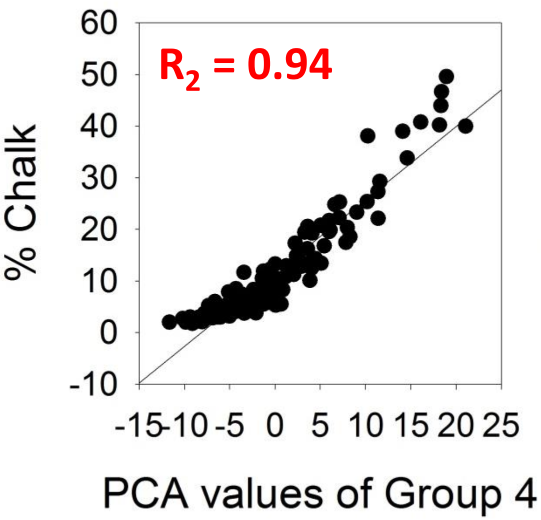
Research Rationale

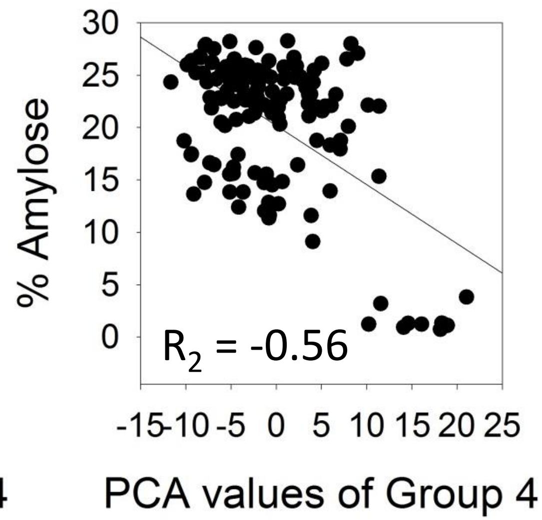
Having means to rapidly and accurately evaluate thousands of genotypes is needed to fully utilize genomics data that is currently available

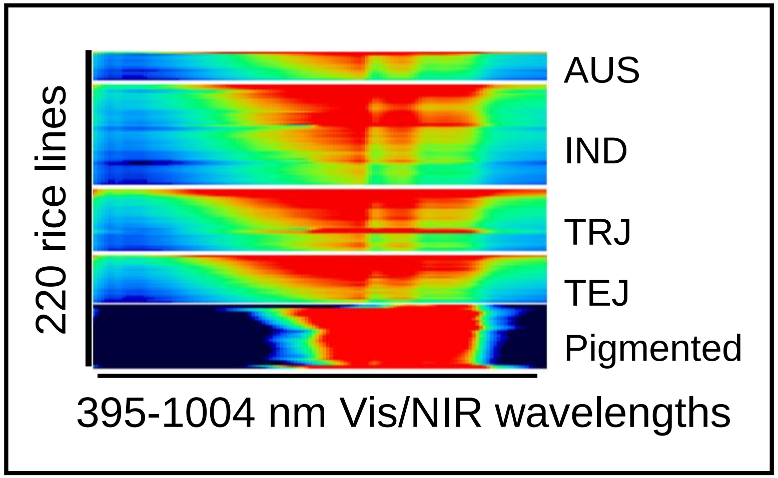


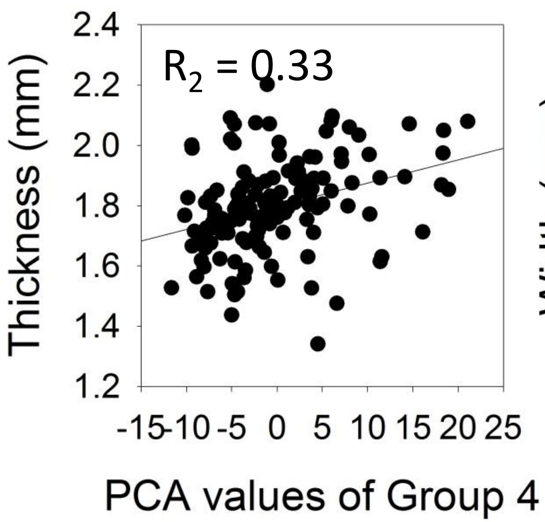
Hyperspectral imaging system as a mean of high-throughput phenotyping of grain physicochemical traits

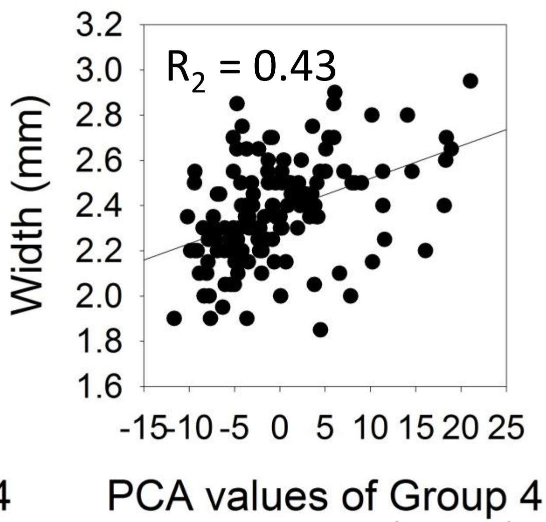
707-922 nm of NIR spectral region is correlated with chalk

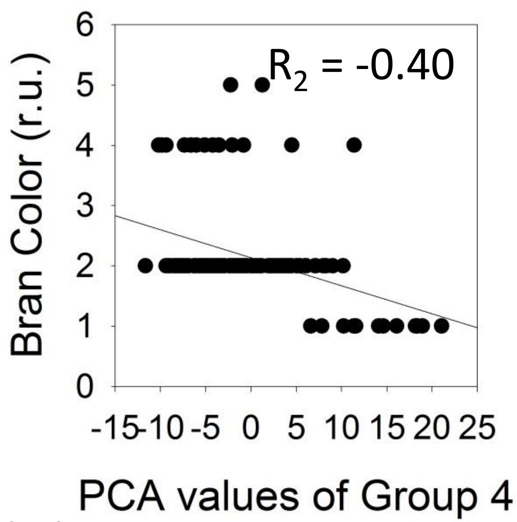












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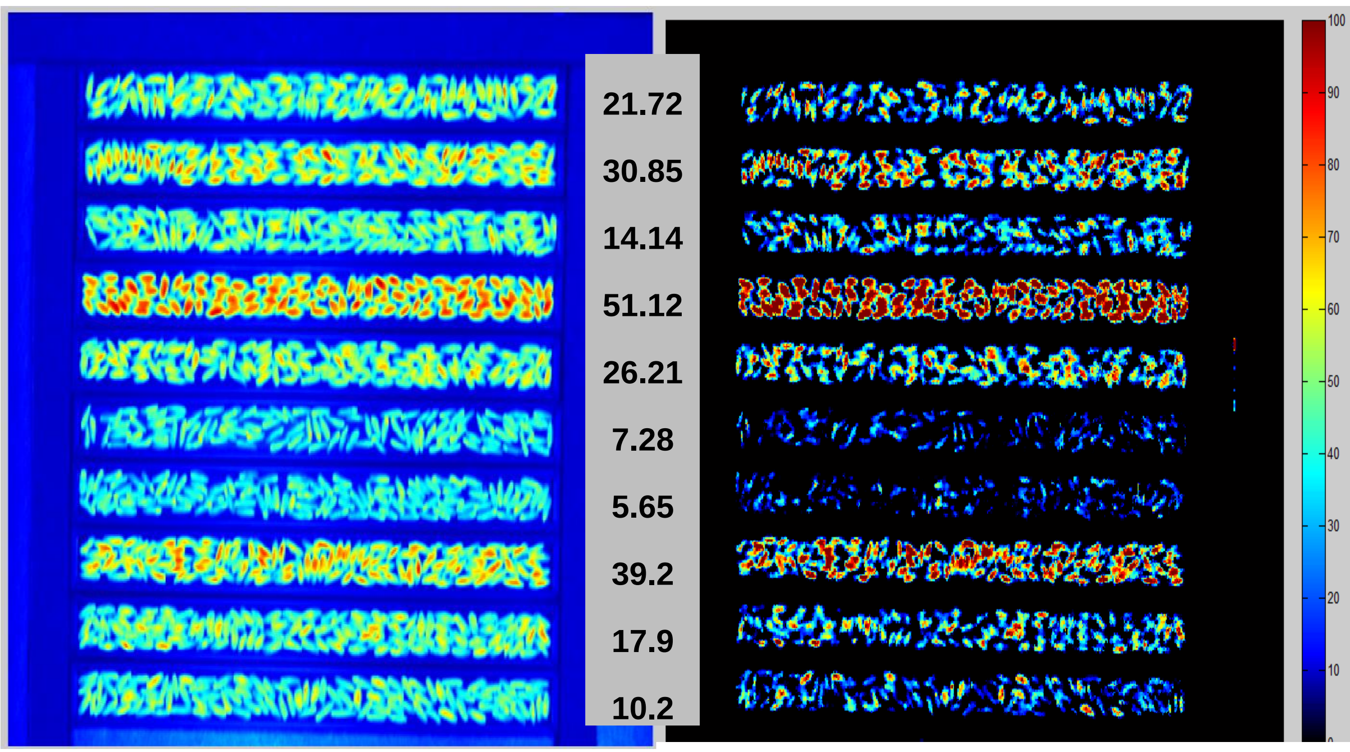
Barnaby *et al*, submitted to *Scientific Reports* (2019)

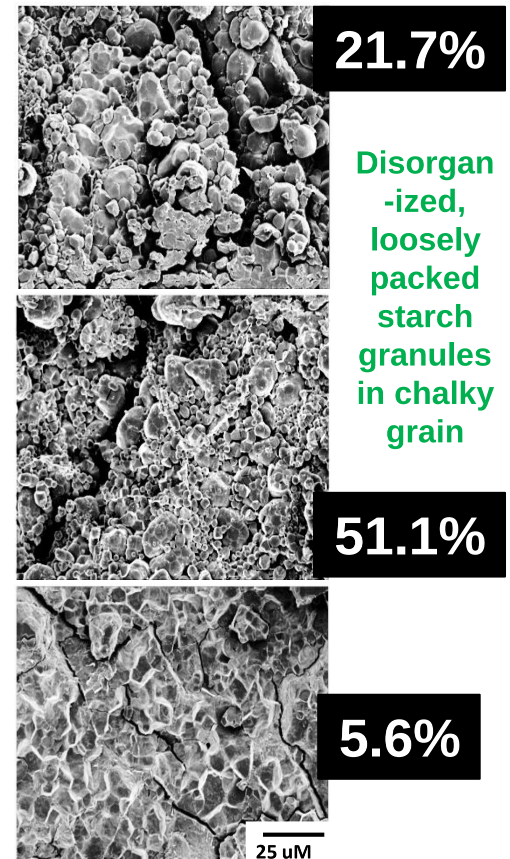
Validation of spectral regions for predicting chalkiness by bi-parental mapping population segregating for % chalk

~240 progenies from KBNT x ZHE mapping population (2 yrs) with % chalk data were used to identify the specific spectral regions



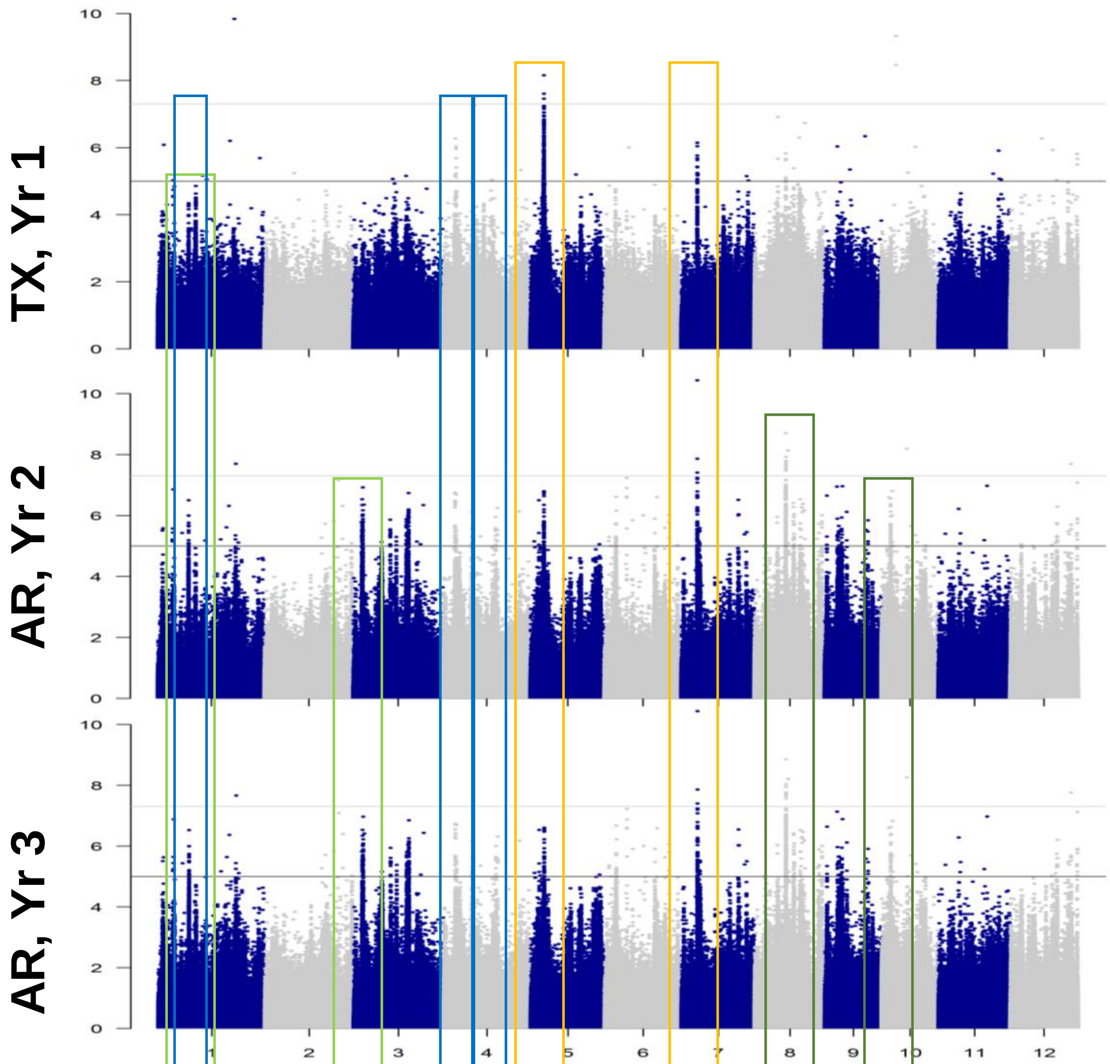
Spectral regions in Group 4

Partial Least Square image result



Barnaby *et al*, submitted to *Scientific Reports* (2019)

Some QTLs are robust across all locations, and are genotype driven, others expressed in just AR



Ch1(Phosphatase protein), Ch3 (Ser/Thr kinase): Expressed in the Rice Chalky Grains Produced Under High-temperature Conditions (Kaneko et al (2016) Rice)

Ch5 (Phospholipase): Expressed during grainfill stage - chalkiness formation (Liu et al (2010) BMC Genomics)

Ch7 (Glycosyl hydrolases family 16 protein0, Ch8 (Hypothetical protein), Ch10 (Protein kinase domain containing protein)

Major Chalk genes identified (Edwards et al (2016) Field Crops Research

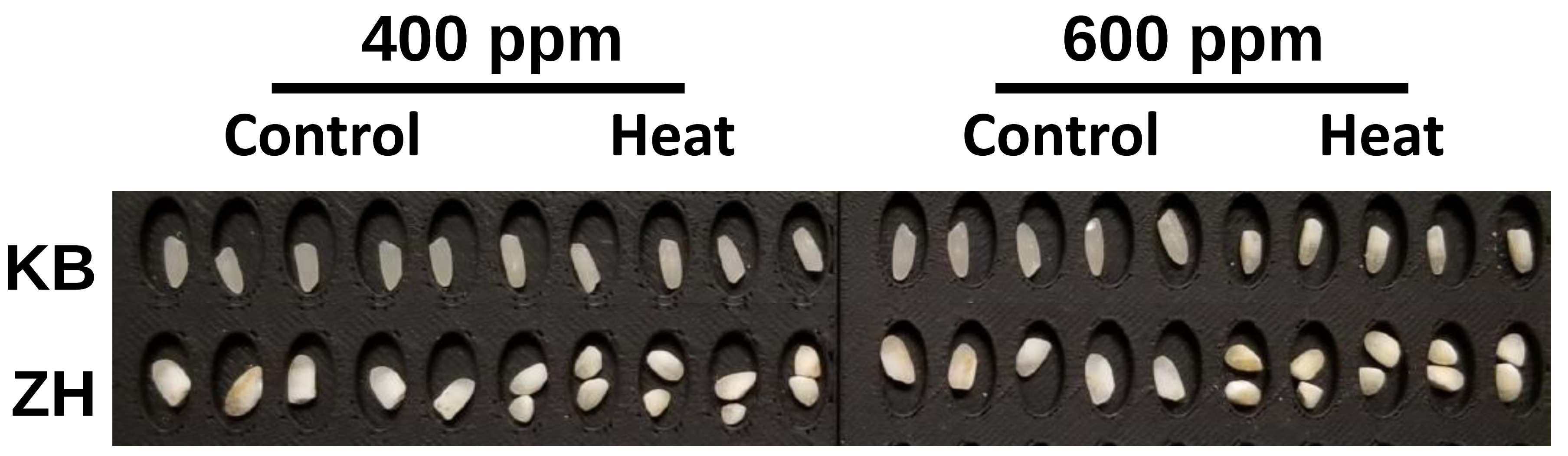
Barnaby *et al*, submitted to *Scientific Reports* (2019)

Summary

- **The genetic architecture of a globally diverse rice panel using NIR hyperspectral profiles in rice grains was demonstrated.**
- **Vis/NIR spectroscopy can detect phenotypic differences in rice physiocochemical properties that are associated with genetic sub-population and environmental responses.**
- **Specifically, the 702-900 nm range of the NIR spectrum is highly associated with the grain chalk phenotype, a major quality characteristic that causes grain breakage during milling and loss of crop value.**
- **GWAS revealed the genome regions associated with variation in grain chalk.**

Rice Responses to rising CO_2 + heat stress

Rising CO_2 levels in the future may aggravate grain chalkiness



Bi-parental mapping population of KBNT and ZHE733 that is segregating for % chalk

Barnaby *et al*. Unpublished

Content

- Assessment of crop responses to changing climates
- Adaptation process: Identification of climate resilient cultivars (e.g. yield)
- Adaptation process: Identification of QTLs for climate resilience (e.g. grain quality)
- **Mitigation process: Identification of high-yielding with reduced GHG emissions**

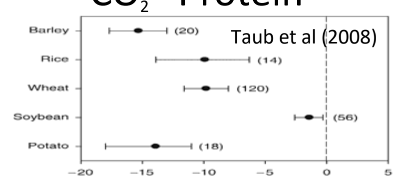
Yield Loss
due to Heat Stress

By DBNRRC

Lack of Irrigation

By static.ark.org

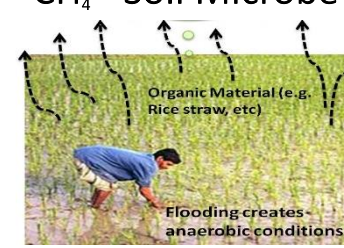
CO_2 - Protein



Grain Chalk

By DBNRRC

CH_4 - Soil Microbe

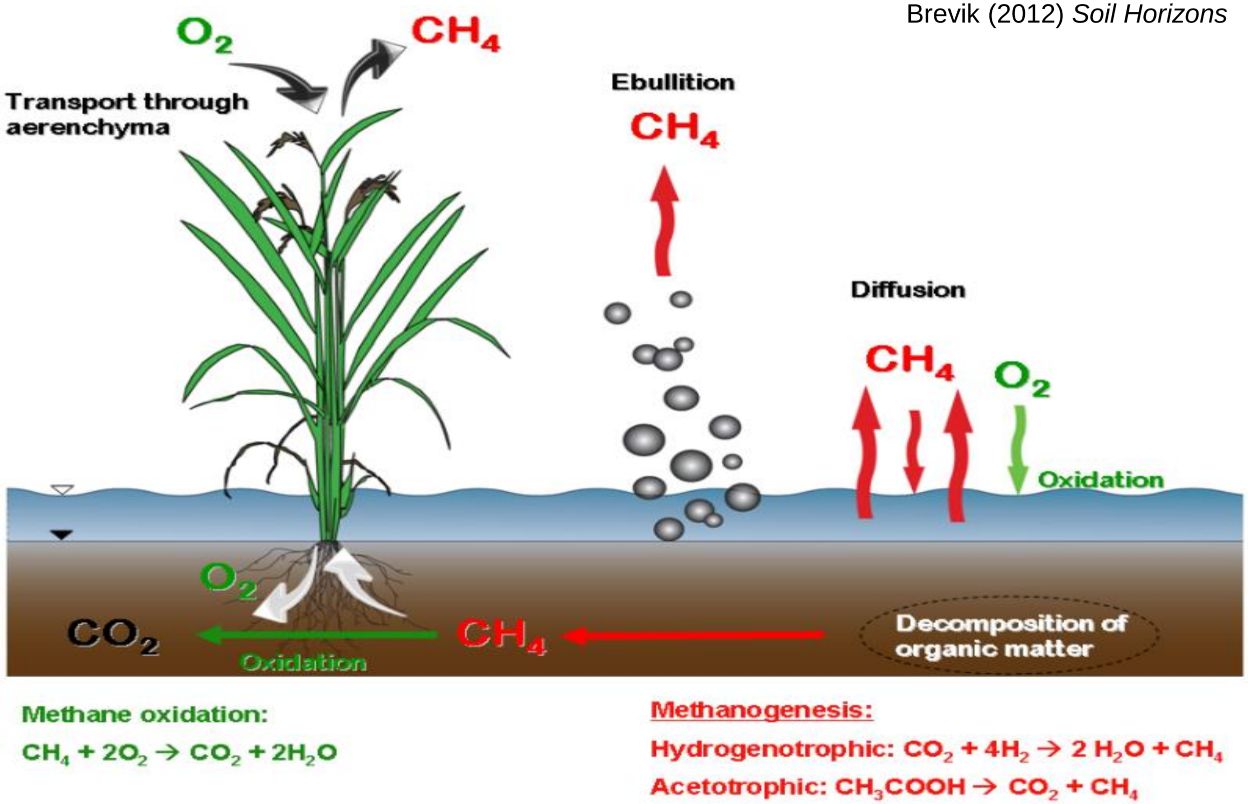


By Green Clean Guide

Rice paddy field:
An ideal condition for methanogenesis

Generation and Emission of Methane from Wet Soils

Brevik (2012) *Soil Horizons*



The warm, water logged soil of rice paddies

- The methanogens in the soil utilize organic matters from rice root exudates as well as fertilizers in the soil, and produce methane.

How can we reduce methane emissions?

Ongoing efforts:

- Management practices (AWD)
- Screening for cultivars producing reduced emission in methane

⬇

Characterizing cultivars that differ in CH_4 emission for phenoloigical, anatomical & agronomic traits

⬇

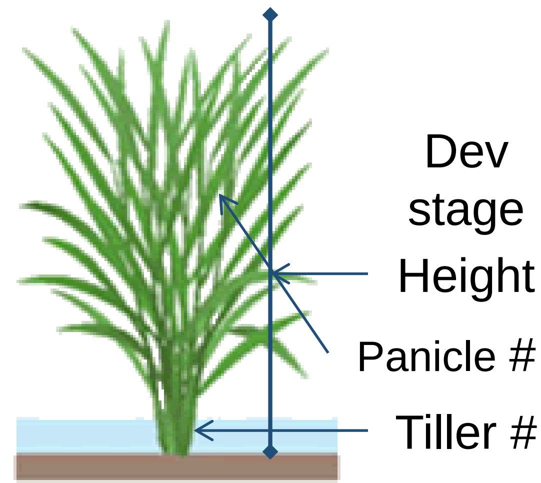
Understanding the physiological and molecular mechanisms of rice-microorganism interactions that produce methane (methanotrophic bacteria)

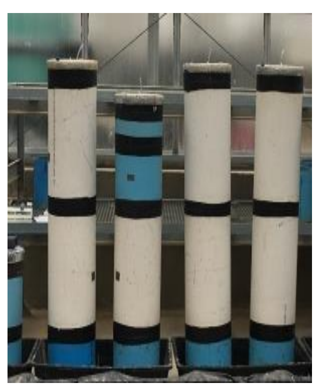
Methane emissions: relationship with agronomical and anatomical traits

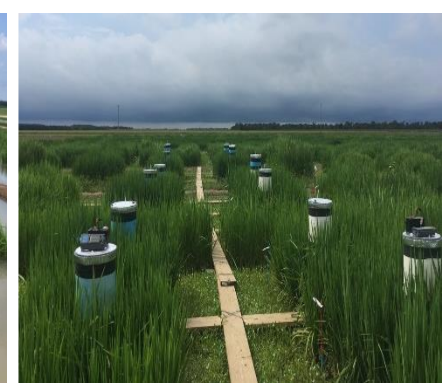
Seasonal CH_4 profile

~ Association

- Tiller number
- Height
- Panicle number
- Developmental stage
- Aerenchyma tissue
- Root Biomass
- Shoot Biomass
- Yield

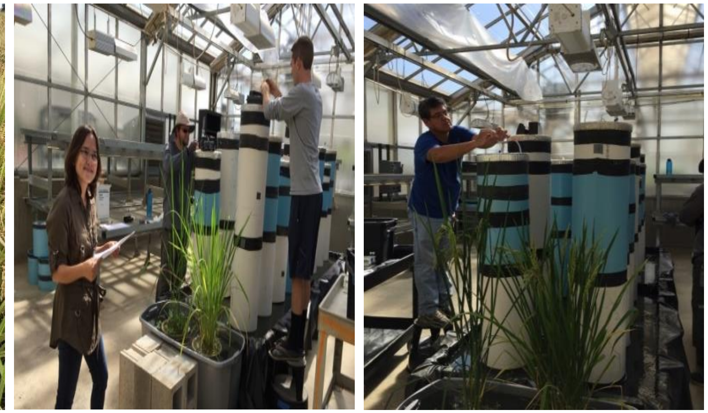




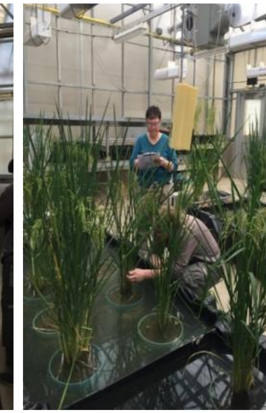


Assessment of agronomic and anatomical traits linked with methane emissions

Methane emission studies in a commercial field
Collecting CH_4 samples and root samples in the field



Capturing CH_4 release by the plant and harvesting tillers and roots

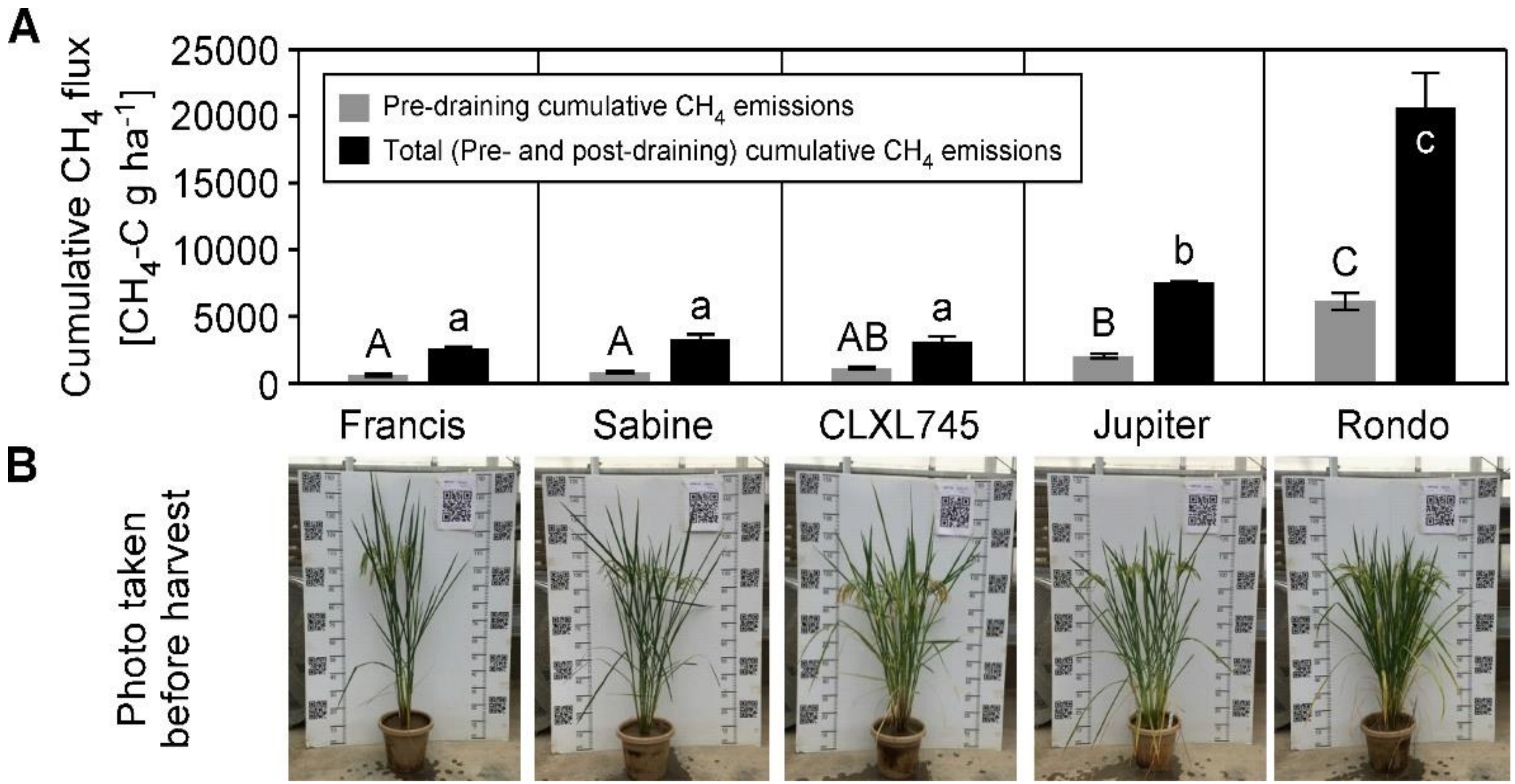


Determining growth stage

Capturing CH_4 samples in a field

Assessing shoot/root architecture traits, and collecting leaf sample for DNA marker analysis

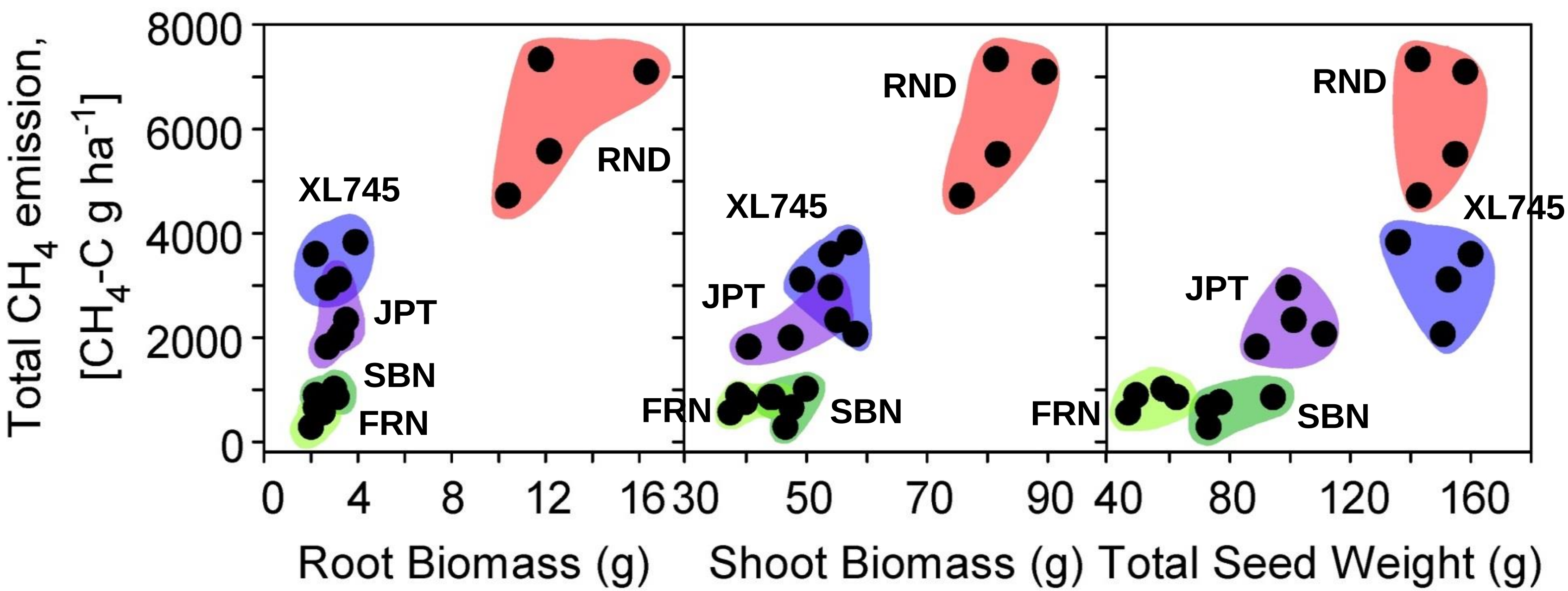
Methane emissions and plant traits differ among the five varieties



Barnaby et al, submitted

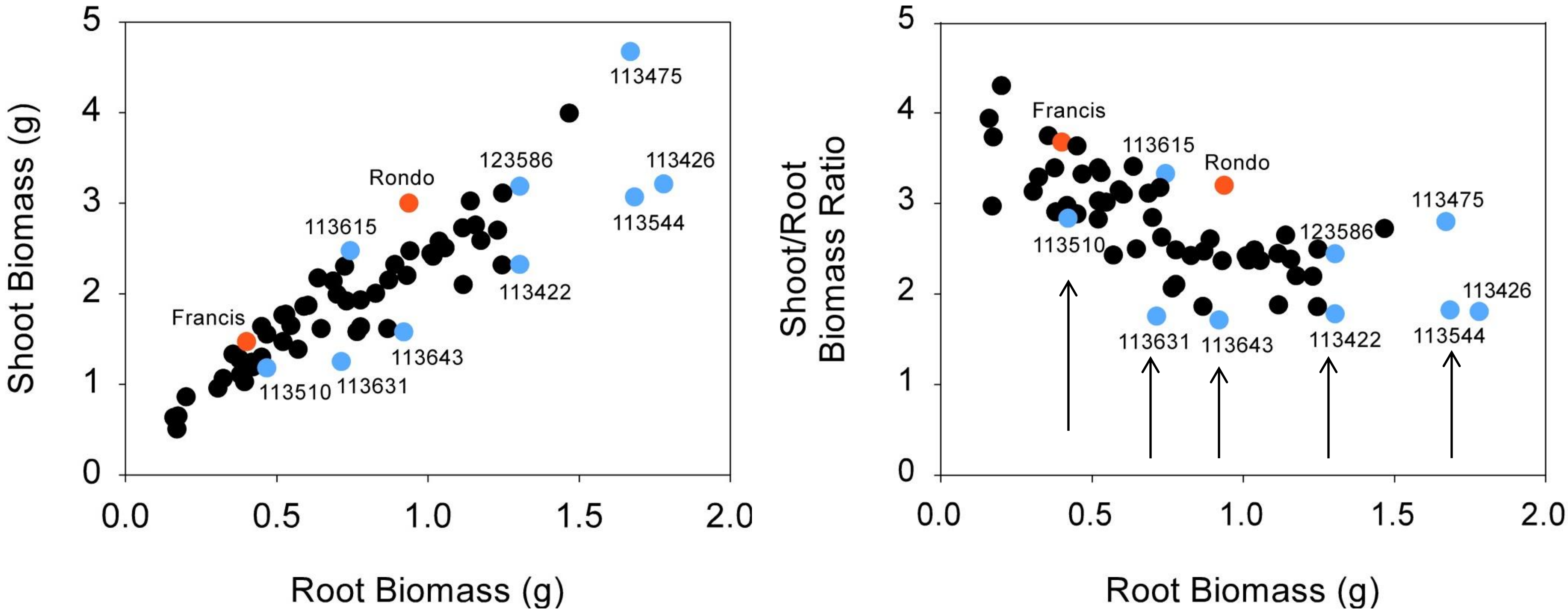
Anatomical & physiological traits affecting CH_4 emissions

Root/shoot biomass: positively correlated with total CH_4 emissions



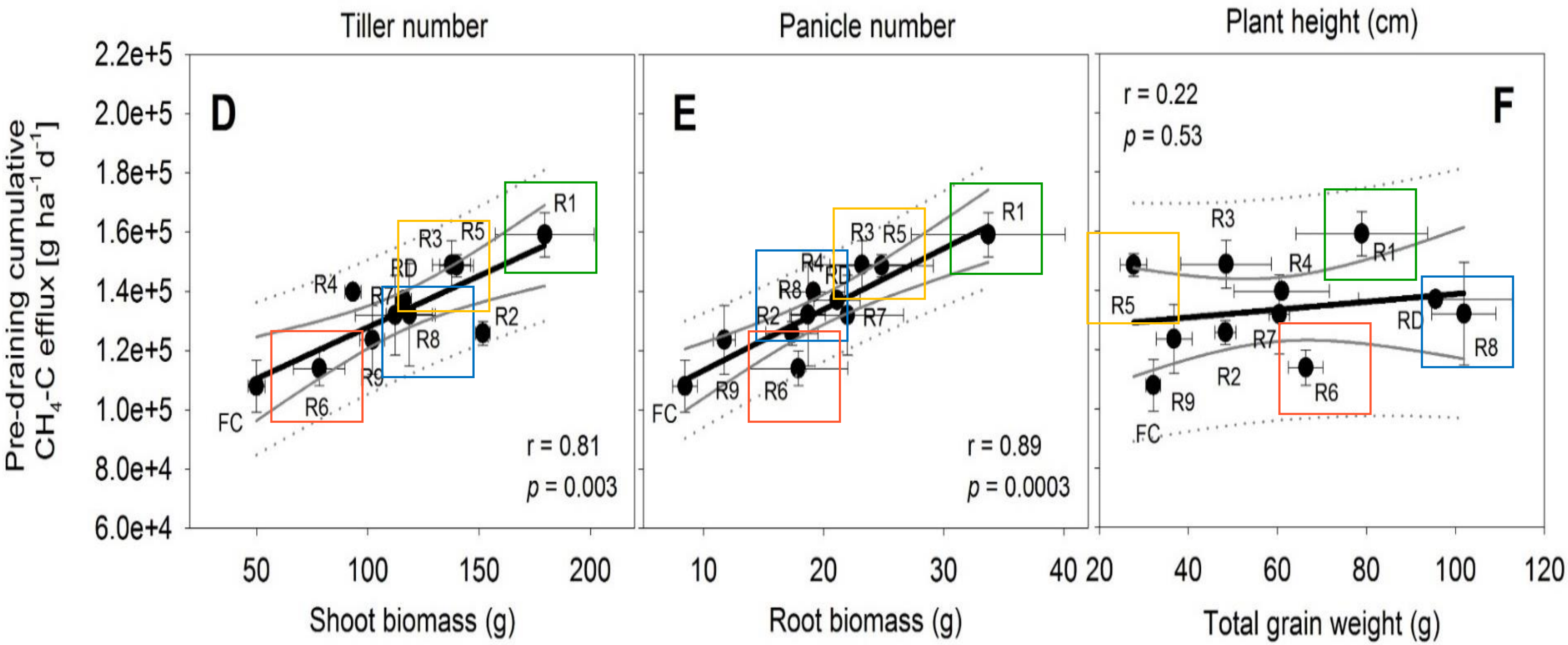
Aerenchyma Tissue Observation

62 FRN x RND Mapping Progenies: Based On Shoot And Root Phenotypes



: Rondo, Francis, and 9 RILs were selected, and has conducted GHG study In the greenhouse and field in 2017

Relationships between agricultural traits and CH_4 emissions using 9 FR-RILs plus the parents



- RIL6 & RIL8: high yielding, low RB, low vs int. SB, and low CH_4 emissions
- RIL5: low yielding, high SB and RB, and high CH_4 emissions

Barnaby et al, submitted

Summary

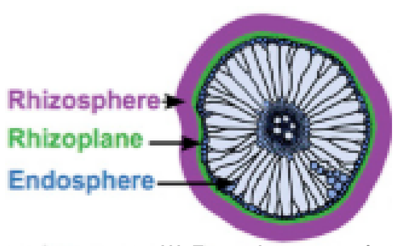
- There exists **genetic variation** in CH_4 emissions
- **Rondo** was a **high CH_4** emitter and **Francis** was a **low CH_4** emitter
- Among physiological and agronomic traits observed, **root and shoot biomass** showed positive correlation with CH_4 emissions
- **Root biomass** as a major driver affecting methane emissions was confirmed by a subsequent experiment using **FR-RILs** segregating for root biomass
- Identified **RIL6 & RIL8** with high yielding, low RB, low vs int. SB, and low CH_4 emissions

Understanding root-soil-microbe interactions in methane emissions

- Temporal and spatial profile of soil microbial composition using 8 RILs and 2 parents (Francis and Rondo) will be assessed (using 16S rDNA sequencing)

- Four developmental stages:
  I. Heading (HD) – 3 weeks
  II. HD (CH_4 emissions start to increase)
  III. HD + 3 weeks (at grainfill stage: peak)
  IV. Harvest (start to decrease)

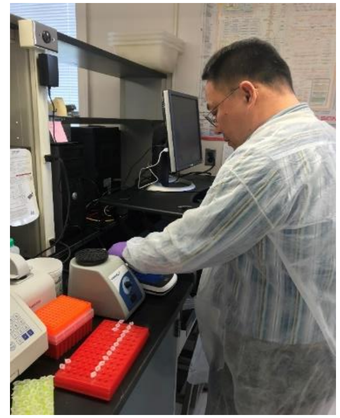
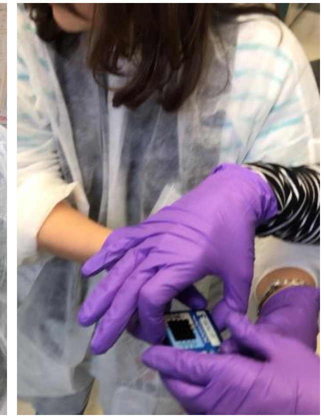
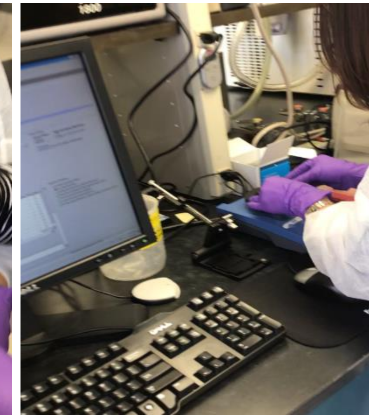
- Three root compartments:
  I. Rhizosphere
  II. Rhizoplane
  III. Endosphere



Top: 10 FR RILs and two parents , Rondo and Francis, growing in a greenhouse; **Bottom**: Collecting soil microbiome samples

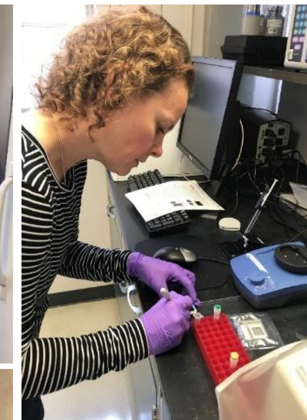
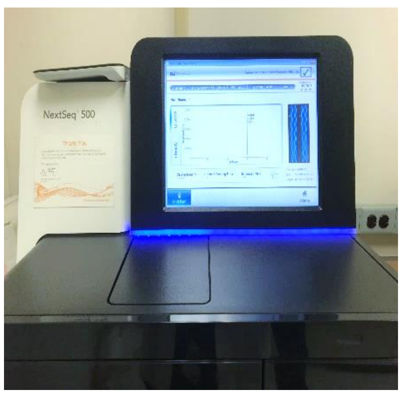


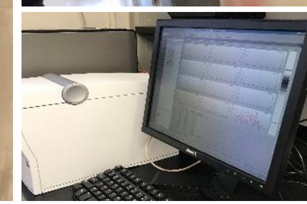
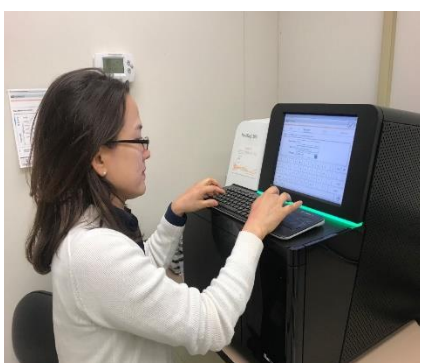


Soil and microbiome study

- Soil C & N measurement
- Soil microbial DNA extraction
- Library construction
- Tagment gDNA
- Amplify tagmented DNA
- Clean up amplified DNA
- Normalize libraries
- Pool libraries
- Run on the NextSeq

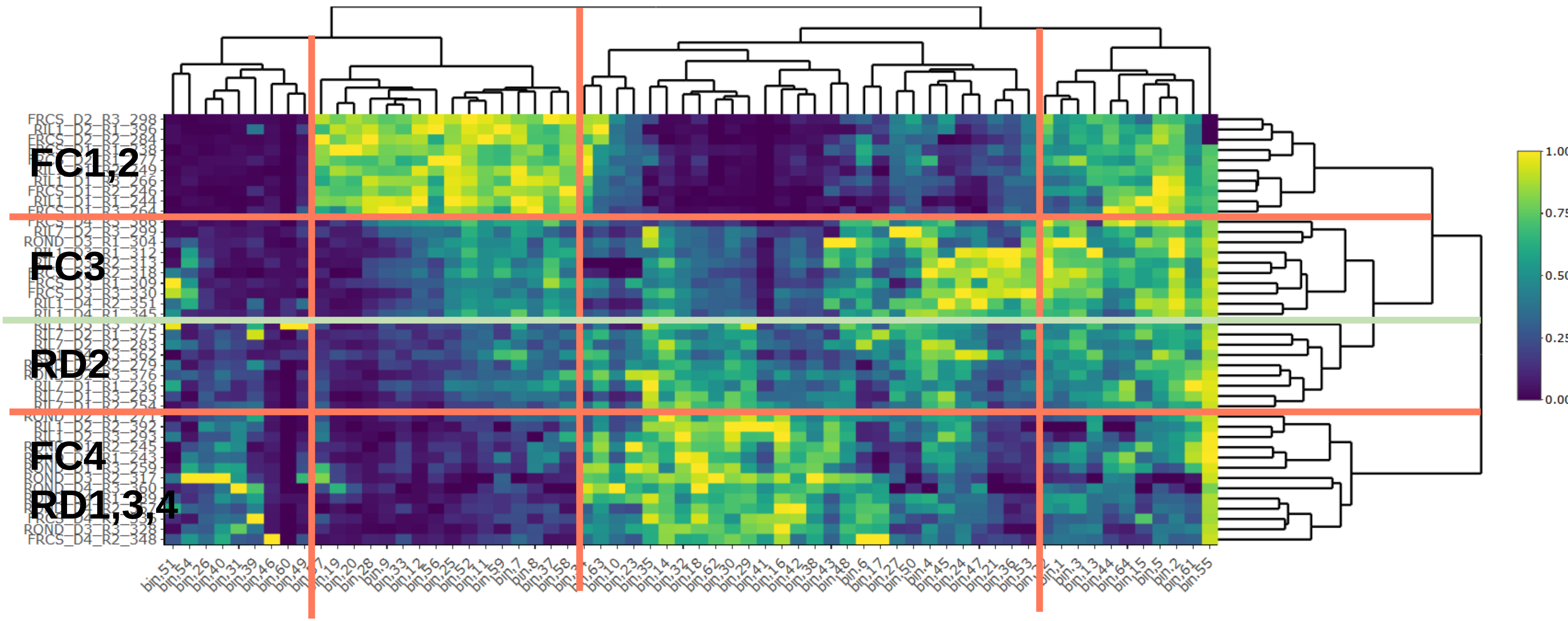









Heatmap of bin abundance



- FC and RD show different microbial profile
- Microbial profile drastically changes in FC based on developmental stage

Fernandez-Baca *et al.* Submitted to *Applied Environ. Microbiol.* (2019)

Summary

- **The lowest and highest CH_4 emitters, Francis and Rondo, showed contrasting soil microbial profiles.**
- **Heading stage showed the most diverse soil microbial composition among 4 developmental stages known to differ in CH_4 emissions**
- **Genetic variation differing soil microbial communities were largely found in D1 (booting stage) and D2 (heading stage)**

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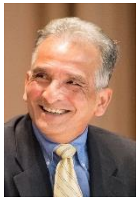

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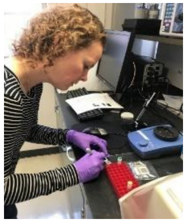

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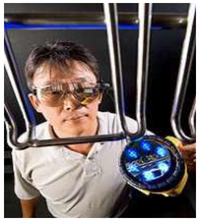

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