Internship Presentation

Jessica Navarro

- BS Biology student
- IRRI Intern, Rice Breeding and Innovations

Acknowledgment

Supervisor: Dr. BP Mallikarjuna Swamy

Advisor: Dr. Alvey

Instructors/advisors: Ate Annie, Ate Eva, Dr. Bala, Ate Nirusha, Ate Muditha, Ate Raquel, Ma'am Mercy, Ate Kristel, Ate Elaine, Kuya Amery, Ate Cres, Ate Jean, Kuya Norman, Ma'am Joy, Ma'am Leeann

IRRI International Rice Research Institute

Table of Contents

- Acknowledgment
- Biofortification
- Molecular Analysis
- Heat Stress
- Submergence screening
- Golden Rice
- Carotenoid Degredation
- Carotenoid Extraction
- IRRI GeneBank and Seed Viability
- Genpotyping
- Hybridization Services
- Abiotic and Biotic Stress Evaluation Center
- Rapid Generation Advancement
- Seed Processing
- General Takeaways

Biofortification

- Improving nutrient content and resistance in rice
- The introgression of beta carotene (provitamin A), high iron/zinc, and resistance to abiotic/biotic stresses, into popular rice varieties
- MAGIC (Multi-parent advanced generation inter-cross) and mapping populations

Molecular Analysis

- Quantitative Trait Loci
  - Region of the genome that consists of multiple genes which influence a particular trait of interest
  - Identifying major/candidate genes within this region
- Markers
  - Genomic regions in the DNA for which all of us differ
  - Can be linked to important genes and analyzed to distinguish individuals
- Types of markers
  - Molecular markers: DNA v. Protein based
  - Site-specific restriction fragments (SSRs)
  - Single Nucleotide Polymorphism (SNPs)

Molecular Analysis

- Marker assisted selection
- Genotype and phenotype data
- R-squared values
- Linkage disequilibrium (LD) pruning
- LD decay plot

ICi Mapping

- removal of redunant markers
- identification of significant markers
  - LOD threshold: likelihood that at that position there is a QTL

- R and RStudio
- PLINK, GaPit, and Farmcpu
- RapDB

Hybridization Services

- Parentals from all divisions
- Selecting for the combination of desirable traits
- Cutting/removing panicles which have undergone selfing

Flowering and anther dehiscence

Hybridization Services

Emasculation

- Cutting each spikelet halfway
- Vacuming out pollen

Pollination

- Steady hand
- Attentiveness

Rapid Generation Advancement

- Generations F2, F3, F5, and F6
- F6 seeds are then planted in the field
- Advancing materials to make a fixed line
- Shortening time frame by 2 years

Harvesting

Seed Processing of Biofortified and Transgenic Strains

Threshing

- grain separated from panicle

Milling

- husk removed from seed
- Prevention of contamination between strains

Seed sorting

- brown, long-grain, white, and GR2E strains of rice

Scooping

- Each brown bag contains the seeds of a single plant

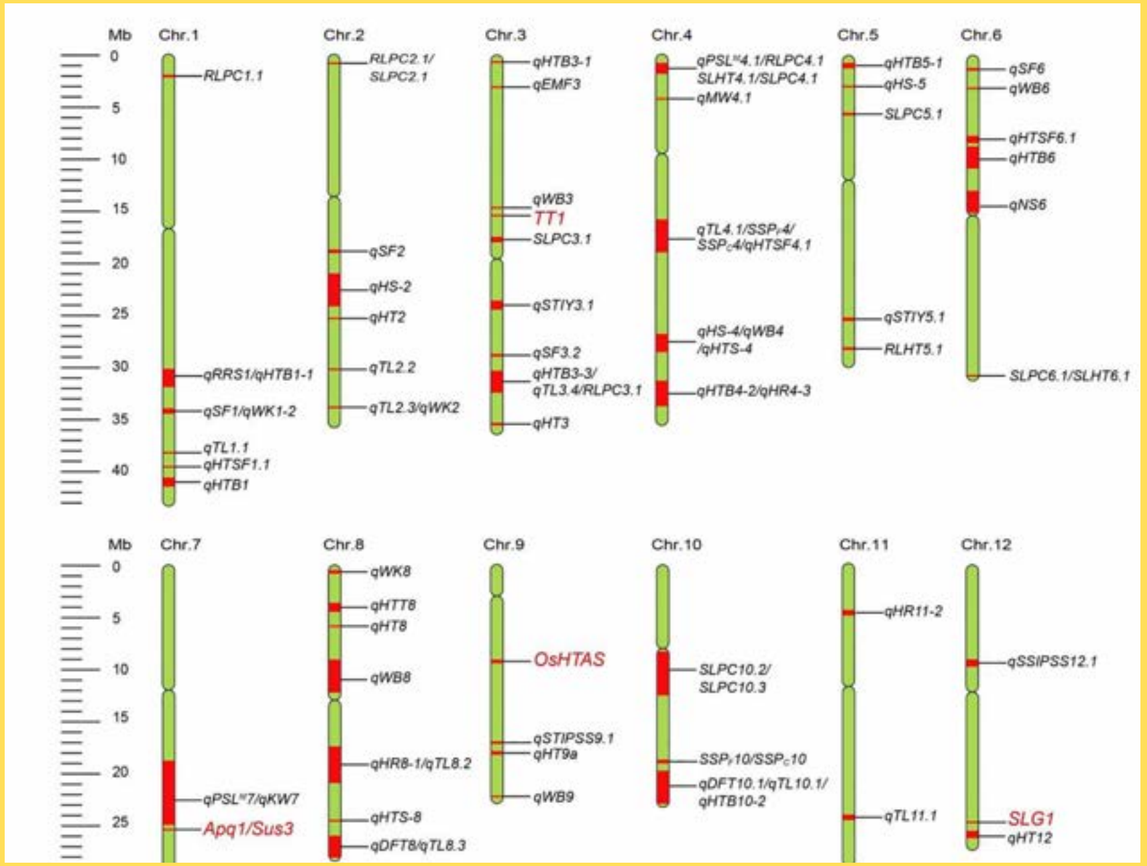
Abiotic and Biotic Stress Evaluation Center

Rice productivity limited by various abiotic factors such as drought, submergence, and high temperature

- Blast, green leafhopper (GLH), and brown planthopper (BPH) screening
- QTLs for BPH resistance on chromosomes 1, 3, 4, 6, 11 and 12
- Plant thinning and pruning
- Insects developing resistance

Heat Stress

- For each 1 °C increase in global temperature, yield decrease by 10%
- Pollen/spikelet sterility and anther indehiscence
- qHTSF1.1 and qHTSF4.1
- Indica v. japonica strains



Ye, C., Argayoso, M. A., Redoña, E. D., Sierra, S. N., Laza, M. A., Dilla, C. J., & Hernandez, J. E. (2012). Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. Plant Breeding, 131(1), 33-41.

Xu, Y., Chu, C., & Yao, S. (2021). The impact of high-temperature stress on rice: Challenges and solutions. The Crop Journal, 9(5), 963-976.

Heat Stress Experiment Conducted by Ate Hsu

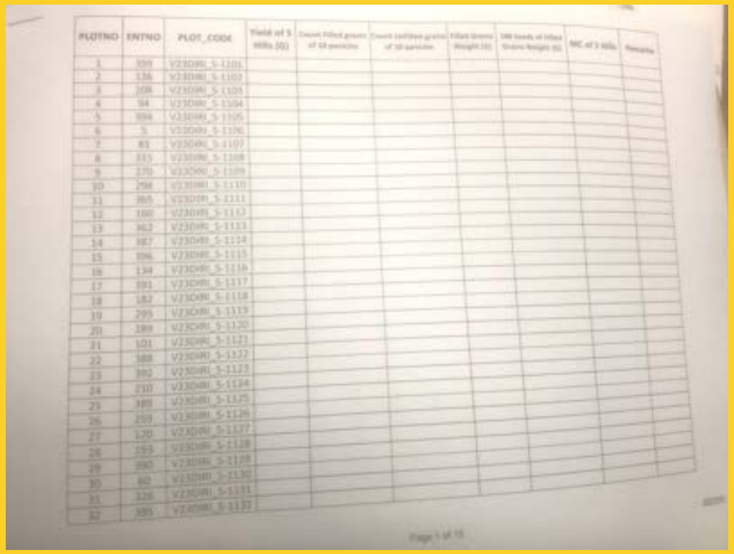
Seeding

- Swarna Sub-1

Transplanting

- flowering dates
- labeling
- fertilizer
- pulling
- strongest/healthiest in middle
- second plant near the side
- ensuring uniform growth

Filled and unfilled grains



Submergence screening

- Cihiram Sub-1 / GR2E
  - Tolerant to flooding and rich in vitamin A
- Submerged when 21 days old
- De-submerged after 14 days
- Strains that are resistant to flooding were seen to be less taller
  - Dormancy and delayed expansion
- Ultimately aiming to analyze if flooding has an effect on vitamin A content

Data collection of plant height and total number of plants

Leaf rolling and roots coming up from soil

Submergence screening

June 23

June 30

Golden Rice

- Vitamin A deficiency
- Beta-carotene is a precursor to vitamin A
- E.coli (pmi), maize (Zm-psy), and soil bacterium E. uredovora (crtI) genes
- Well-known varieties (BR29, RC82, IR64)
- Retainment of wild-type parent traits
- Phenotypic selection
- Harvest and character analysis

IR64 and IR64/GR2E

(prominent yellow hue, phenotypic indication that GR2E is likely present)

- Breaking dormancy: seeds dried for 3 days
- Once fixed (BC5F3), lines transferred to field
- Security measures
- IRRI seed health unit
- Multilocation trials
- Nutrition study

Golden Rice Fields

Carotenoid Degredation Analysis Conducted by Ate Raquel

- 6-8 week degredation is rapid
- After 8 weeks, plato
- Identification of candidate genes which increase the stability of carotenoid content in GR2E introgression lines
- Analyzing multiple agronomic traits
  - yield, seed weight, carotenoid content (at 2nd, 4th, and 6th months)
- Estimating stability
  - month 4 - month 2

Carotenoid Extraction

- Carotenoid content extracted and quantified every 2 months after harvest
- Polished seeds v. un-polished seeds
- Preparation and multi-tasking
- Setting up an ideal range for UV-VIS spectrophotmetry readings at 680 nm
  - 4 controls
  - aiming for an absorbance near 0.4 at 680 nm
  - readings/absorbance of samples averaged together
  - average x 80% = lower half of range
  - average x 109% = upper half of range
- Philrice samples: replicated 3x
- Indonesian lines: replicated 2x

Carotenoid Extraction

Overview

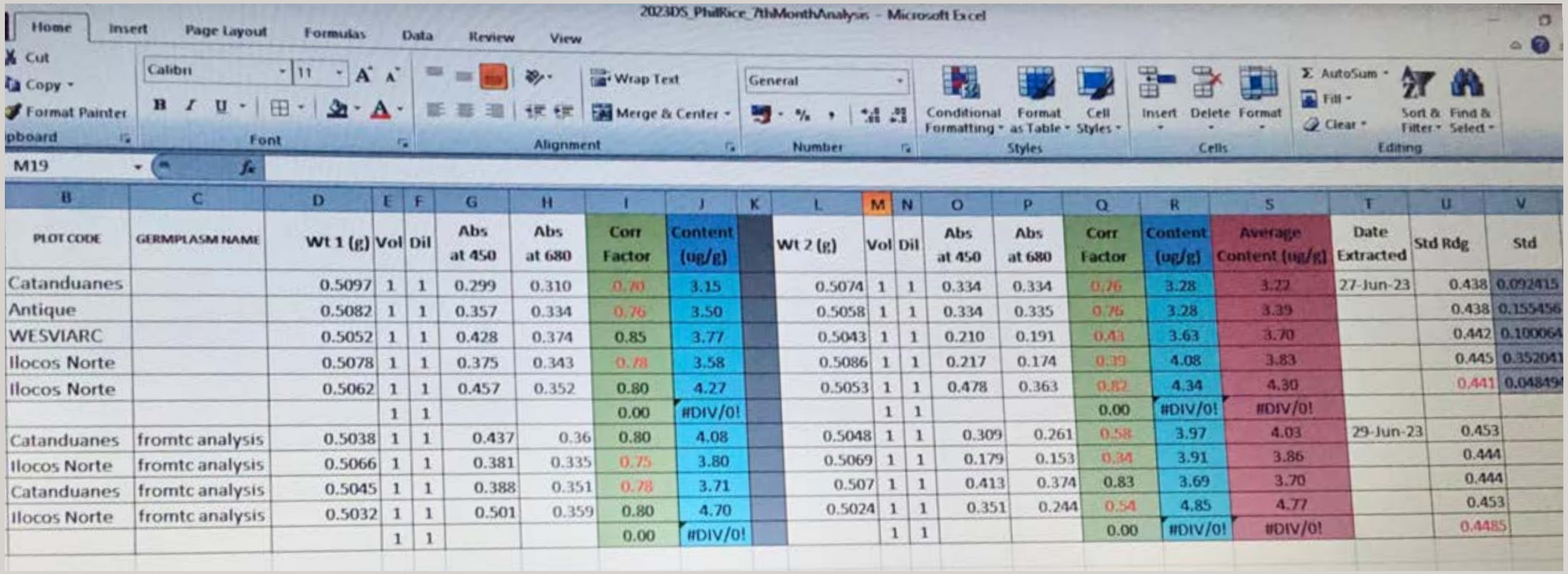
- 7-month samples
- Obstacles
  - seperation of water and supernatent phases

Upper phase indicative of carotenoid presence

Supernatent solution

Carotenoid Extraction

Results



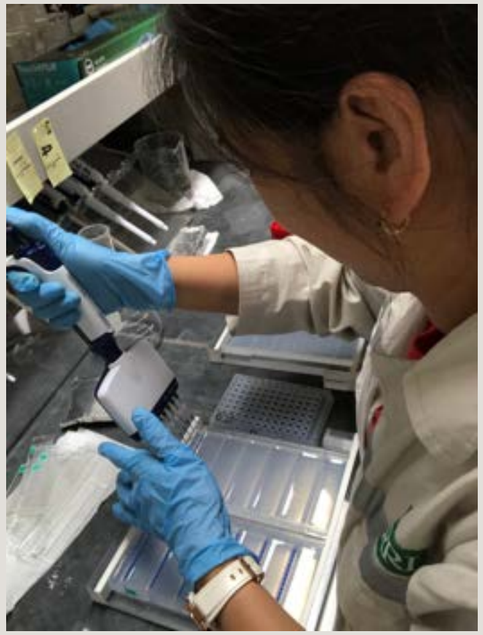
PLOT CODE	GERMPLASM NAME	Wt 1 (g)	Vol	Dil	Abs at 450	Abs at 680	Corr Factor	Content (ug/g)		Wt 2 (g)	Vol	Dil	Abs at 450	Abs at 680	Corr Factor	Content (ug/g)	Average Content (ug/g)	Date Extracted	Std Rdg	Std
Catanduanes		0.5097	1	1	0.299	0.310	0.80	3.15		0.5074	1	1	0.334	0.334	0.76	3.28	3.22	27-Jun-23	0.438	0.092415
Antique		0.5082	1	1	0.357	0.334	0.76	3.50		0.5058	1	1	0.334	0.335	0.76	3.28	3.39		0.438	0.155456
WESVIARC		0.5052	1	1	0.428	0.374	0.85	3.77		0.5043	1	1	0.210	0.191	0.43	3.63	3.70		0.442	0.100064
Ilocos Norte		0.5078	1	1	0.375	0.343	0.78	3.58		0.5086	1	1	0.217	0.174	0.39	4.08	3.83		0.445	0.352041
Ilocos Norte		0.5062	1	1	0.457	0.352	0.80	4.27		0.5053	1	1	0.478	0.363	0.82	4.34	4.30		0.441	0.048496
			1	1			0.00	#DIV/0!			1	1			0.00	#DIV/0!	#DIV/0!			
Catanduanes	fromtc analysis	0.5038	1	1	0.437	0.36	0.80	4.08		0.5048	1	1	0.309	0.261	0.58	3.97	4.03	29-Jun-23	0.453	
Ilocos Norte	fromtc analysis	0.5066	1	1	0.381	0.335	0.75	3.80		0.5069	1	1	0.179	0.153	0.34	3.91	3.86		0.444	
Catanduanes	fromtc analysis	0.5045	1	1	0.388	0.351	0.78	3.71		0.507	1	1	0.413	0.374	0.83	3.69	3.70		0.444	
Ilocos Norte	fromtc analysis	0.5032	1	1	0.501	0.359	0.80	4.70		0.5024	1	1	0.351	0.244	0.54	4.85	4.77		0.453	
			1	1			0.00	#DIV/0!			1	1			0.00	#DIV/0!	#DIV/0!		0.4485	

- Ideal correction factor: 80% or above
- Ideal range for absorbance at 680 nm
  - 0.352 to 0.480 (experiment 1)
  - 0.358 to 450 (experiment 2)
- Unsuccessful samples: correction factor is highlighted red
- Aiming for little deviation between content (ug/g) of replicates
- Average Content

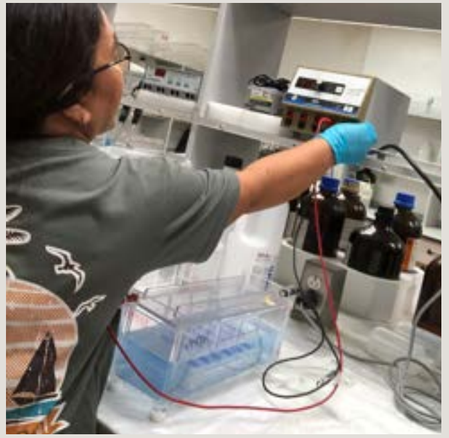
Genpotyping

DNA Extraction

PCR



Gel electrophoresis and imaging



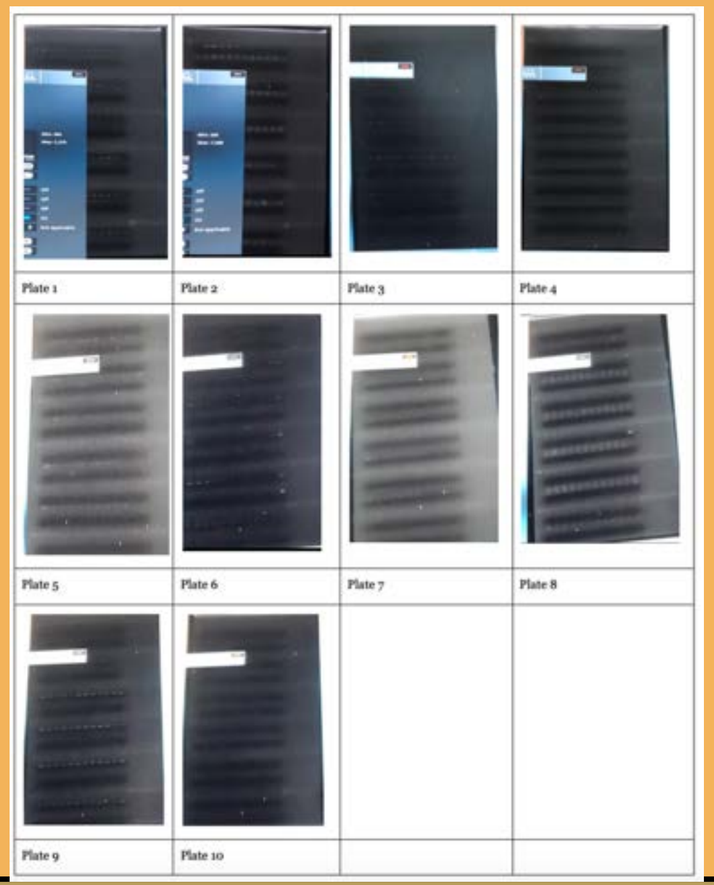
Genpotyping - Experiment 1

Overview

- Zinc and yield are negatively correlated traits
- QTL 6.2
- Crossing of 2 parents (trait of high yield) with 4 donor parents (trait of high zinc),
- Confirming the true heterozygotes

Results

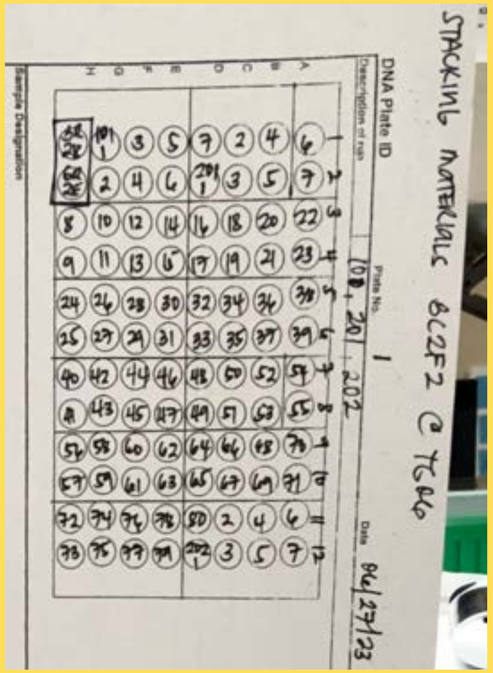
- Some samples did not amplify
- Single band results indicative of need for further backcrossing



Genpotyping - Experiment 2

Overview

- Wild Type Parents: BR28, BR29, NC238, GR2E
- Donor Parent: GR2E
- Generation progenies: BC2F2
- Plate ID



Results

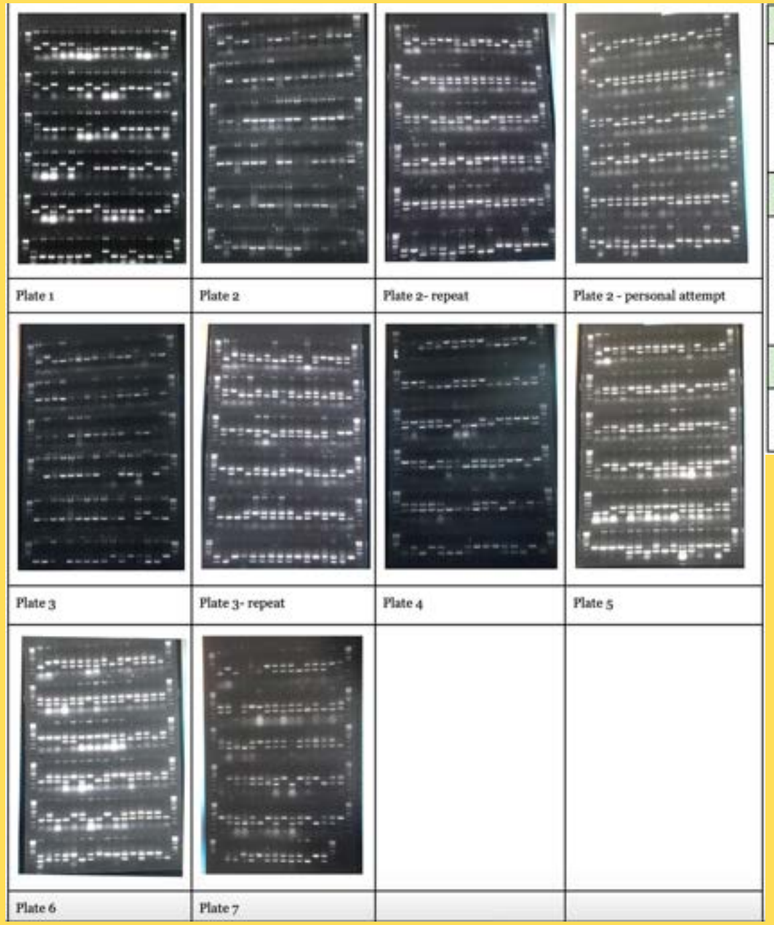


Plate 1	Plate 2	Plate 2- repeat	Plate 2 - personal attempt
Mainly homozygous results for wild type parent.	First attempt demonstrated that many samples were not successfully amplified.	Second attempt indicative of amplification, mainly heterozygous results.	When comparing the second attempt and my personal attempt, results are consistent and coincide with one another.
Plate 3	**Plate 3- repeat**	**Plate 4**	**Plate 5**
First attempt demonstrated that many samples were not successfully amplified.	Second attempt indicative of amplification, mainly heterozygous results.	Somewhat equal distribution of heterozygous results and homozygous results for wild type or donor parent.	Mainly heterozygous results.
Plate 6	**Plate 7**		
Mainly heterozygous results.	Mainly heterozygous results.		

Genpotyping - Experiment 2

Scoring

Genpotyping - Experiment 3

Overview

- Wild-type parents: NSCRc 400, 436, 420, 442, 480
- Donor parent: GR2E
- Generation: BC5F3
- Plate ID

Results

Plate 1	Plate 2	Plate 3	Plate 4
Mainly homozygous for GR2E results.	Mainly homozygous for GR2E results.	Mainly homozygous results for GR2E and heterozygous results. Some samples in the first row did not amplify.	Mainly homozygous for GR2E results.
Plate 5	Plate 5- repeat	Plate 6	Plate 6- repeat
Many samples did not amplify.	Mainly heterozygous and homozygous for GR2E results.	Many samples did not amplify.	Mainly heterozygous and homozygous for GR2E results.
Plate 7	Plate 8	Plate 9	Plate 9- repeat
Mainly homozygous for GR2E results.	Mainly heterozygous and homozygous for GR2E results.	Many samples did not amplify.	Mainly homozygous for GR2E results.

IRRI GeneBank and Seed Viability

- Viability of short-term storage seeds is monitored every 5 years
- Viability testing of rice strains *Sativa* (from Asia) and *Glaberrima* (from Africa)
- Raised for 21 days in seed beds, pulled, transplanted the following day
- Plants cut after transplanting
- 5 rows per strain
- Post-harvest stage characterization
  - Amylose, gel consistency, and gelatinization
- Photosensitivity affecting harvest days

IRRI GeneBank seed beds

IRRI GeneBank transplanting field

Short term gene bank

General Takeaways

- Production, testing, and analysis of biofortified rice is a process that involves many roles and perspectives; requires teamwork and collaboration
- Being able to visit different divisions has allowed me to better understand rice production
- Met strong and passionate people

Salamat!