Philippine Rice R&D Highlights 2013

RICE CHEMISTRY AND FOOD SCIENCE DIVISION

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Rice Chemistry and Food Science

Division Head: RCA Ramos

Executive Summary

The main task of the team is to conduct grain quality (GQ) evaluation of rice lines and varieties to assist breeders in identifying promising rice lines for entry into the National Cooperative Test and to advance the GQ assessment methods. The upgrading of methods will not only ensure better accuracy of data but also fast-track varietal screening. Current development in analytical assessment of quality characteristics of rice and the demand for reliable data by heightened breeding activities further necessitate an immediate updating of grain quality techniques. In addition, optimization of new analytical methods for measuring elemental composition and secondary metabolites important in Philippine rice is needed. This is to quantify micronutrients and non-nutrients important in health, nutrition, and food safety. In the process, the analytical capability is enhanced and methods are upgraded or developed.

For the project on grain quality screening, a total of 606 rice lines were received from the 2012 WS and 2013 DS of which 303 were completely processed and evaluated. In general, only 14 entries from 2012 WS (3 entries from aromatic, 4 entries from NFS, 5 entries from DS, 2 entries from hybrid), and 31 entries from 2013 DS (8 entries from DSR-ON, 20 entries from DSR PYT/GYT, 3 entries from PYT) passed the standard requirements for milling, physical, and eating qualities as set by the National Seed Industry Council.

Out of the 9 varieties subjected to consumer acceptability test of cooked, staled and reheated rice, IMS 2 remained highly acceptable (86.67 to 96.67%) and had the highest mean rating (3.47-3.57) even with ambient and refrigerated staling followed by NSIC Rc160 with 76.67 to 80.00% acceptability and 2.07 to 3.33 mean rating. Increase in Instron hardness was noted in intermediate AC-low/intermediate GT (1.65-3.32 kg/cm2) and high AC-low/intermediate GT (2.33-3.30 kg/cm2) rice varieties upon staling at ambient (28-30oC) and refrigerated (4-8oC) temperatures while waxy-low GT (0.75-0.78 kg/cm2) and low AC-low GT (1.58-2.02 kg/cm2) varieties remained soft.

For the second project, three studies were implemented in 2013. For the study on the improvement of an amylose field test kit and protocol composed of a spot plate, a coloring reagent, and a color stabilizer that can simultaneously differentiate AC class of seven samples for only 4-5 minutes was developed. Overall, AC class differentiation accuracy based on iodine colorimetry AC data on 25 selected varieties was 76% to 80%, while intermediate precision between analysis was 96%.

For the second study under Project 2, six PCR-based markers were tested for their association with each of the rice eating quality parameter and special trait, namely: amylose classification, gelatinization temperature, gel consistency, and three SNP marker for aroma or fragrance detection. Results show that molecular marker for gelatinization temperature was moderately associated (r= 0.60) with the alkali-spreading method while other markers for rice eating quality were weakly associated (r= 0.12-0.33) with the conventional methods. Results indicate that among the molecular markers validated, only the GC/TT SNPs marker for gelatinization temperature type, can complement the conventional alkali-spreading method.

The third study aims to establish a baseline data on the arsenic level of Philippine rices. In 2013, nine provinces were identified to be near volcanic and mining areas, rice and soil samples of which were prepared for physical, mineralogical, and chemical analyses and grain quality and arsenic analyses, respectively.

I. Grain Quality Assessment of Rice Lines

Project Leader: JBA Duldulao

Rice grain quality of both cooked and raw forms is an important key factor for plant breeding. A tool with exceptional accuracy and repeatability of results must be available to help breeders screen early generation rice lines prior to the next planting season. This would lessen the time and costs of production by selecting promising entries for the National Cooperative Testing (NCT).

A released variety is often served and consumed in freshly cooked form. However, not all rice are consumed as some remain stored in ambient condition prior to the next meal. Other times, it is stored or kept in the refrigerator and reheated the following day. The acceptability of this staled and reheated rice has not been measured using consumer panel. Identifying the types of rice that are still acceptable other than its freshly cooked form can help breeders identify the factors influencing the acceptability.

With all these perspectives, this project caters to two important components, namely: grain quality screening of early generation rice lines and measurement of acceptability of rice in other forms other than its usual freshly cooked form.

Grain quality evaluation of early generation rice lines

KB Bergonio, NC Ramos, CT Estonilo, JD Adriano, JBA Duldulao

Grain quality evaluation (GQE) plays an important role in the rice breeding program of PhilRice. Aside from yield, resistance to pests and diseases, and agro-morphologic qualities, grain quality, which includes milling recovery, physical attributes, and eating properties complete the traits of a variety. Early generation rice lines must, therefore, be evaluated and screened for grain quality to trim down the number of lines advanced for further trials, thus reducing cost and maximizing resources. Furthermore, early generation screening may also allow the identification of entries with properties suited for special purposes.

Highlights:

- A total of 606 rice lines were received from the 2012 wet season (WS) and 2013 dry season (DS). Grain quality properties of these inbred rice lines are presented in Table 1 for 2012 WS and Table 2 for 2013 DS. Milling recovery of the other 303 rice lines submitted on November 2013 is shown in Table 3.

- A total of 432 samples (71%) met the standards for brown rice, 500 samples (83%) passed the total milled rice recovery and 180 samples (30%) for the head rice recovery. In general, only 18 samples from Aromatic, 8 samples from NFS, 24 samples from DS, 8 samples from Hybrid WS, 17 samples from DSR-ON, 36 samples from DSR PYT/GYT, 3 samples from PYT, 50 samples form UEM, and 1 sample from AON passed the standards for milling quality.

- Chalkiness was measured visually for all the samples. Five (5) samples from DSR PYT/GYT, 3 samples from DS, and 3 samples from Hybrid WS were classified as premium. About 6 samples from Aromatic, 1 sample from NFS, 9 samples from DS, 3 samples from Hybrid WS, 14 samples from DSR-ON, 39 samples from DSR PYT/GYT and 1 sample from PYT were scored grade 1. In general, there were 84 entries which passed the standards for chalkiness from all the samples.

- The preferred dimension for rice grain should be at least long grain length and slender shape. Majority of the entries from the 2012 WS were found to have at least long grain length and slender shape. The same was observed in 2013 DS where 28 samples from DRS-ON, 41 samples from DSR PYT/GYT, 32 samples from PYT, and 13 samples from Hybrid DS met the preferred dimension.

- Most of the entries from 2012 WS were found to be in the intermediate AC. There were 15 samples from Aromatic, 25 samples from DS, 12 samples from Hybrid WS. For samples in NFS, majority were low and waxy. On the other hand, most of the entries from 2013 DS especially on DSR PYT/GYT (47 samples), Hybrid DS (11 samples), and PYT (18 samples) were intermediate. It is only from DSR-ON that majority of the entries were low AC (60 samples).

- Majority of the 2012 WS entries (82 samples) were of low to intermediate GT type (<70oC – 74oC GT). Only 24 samples were high to intermediate GT. On the other hand, there were 169 samples from 2013 DS of low to intermediate GT. The remaining samples (23) were high to intermediate GT.

- Overall, 303 samples were processed completely for grain quality. In general, only 14 entries from 2012 WS (3 entries from Aromatic, 4 entries from NFS, 5 entries from DS, 2 entries from Hybrid), and 31 entries from 2013 DS (8 entries from DSR-ON, 20 entries from DSR PYT/GYT, 3 entries from PYT) passed the standard requirements for milling, physical, and eating qualities as set by the National Seed Industry Council.

Table 1. Grain quality properties of 2012 wet season inbred rice lines.

Grain Quality Property		Number of Entries			
Parameter/ Level/ Classification		AROMATIC 2013 WS (N=25)	NFS 2012 WS (N=9)	DS 2012 WS (N=45)	HYBRID 2012 WS (N=27)
Milling Recovery and Physical Attributes					
Brown Rice					
>80%	Good	-	-	1	1
75.1- 79.9%	Fair	14	-	44	23
<75%	Poor	11	9	-	3
Milled Rice					
>70.1%	Premium	9	-	16	7
65.1- 70.0%	Grade 1	14	7	29	18
60.1- 65.0%	Grade 2	1	2	-	2
55.5- 60.0%	Grade 3	1	-	-	-
<55.5%	Below Standards	-	-	-	-
Head Rice					
>57%	Premium	7	1	3	1
48.0- 56.9%	Grade 1	11	7	21	7
39.0- 47.9%	Grade 2	5	1	12	12
30.0- 38.9%	Grade 3	1	-	5	6
<30.0%	Below Standards	1	-	4	-
Chalkiness					
0.1- 2.0%	Premium	-	-	3	3
2.1- 5.0%	Grade 1	6	1	9	3
5.1- 10.0%	Grade 2	3	3	13	5
10.1- 15.0%	Grade 3	8	-	11	5
>15.0%	Below Standards	8	5	9	11

Table 2. Grain quality properties of 2013 dry season inbred rice lines.

Grain Quality Property		Number of Entries			
Parameter/ Level/ Classification		DSR-ON 2013 DS (N=72)	DSR PYT/GYT 2013 DS (N=56)	PYT 2013 DS (N=47)	HYBRID 2013 DS (N=22)
No Samples		3	2	-	-
Milling Recovery and Physical Attributes					
Brown Rice					
>80%	Good	-	-	2	-
75.1- 79.9%	Fair	69	45	44	22
<75%	Poor	-	9	1	-
Milled Rice					
>70.1%	Premium	2	2	-	3
65.1- 70.0%	Grade 1	66	49	33	19
60.1- 65.0%	Grade 2	1	3	14	-
55.5- 60.0%	Grade 3	-	-	-	-
<55.5%	Below Standards	-	-	-	-
Head Rice					
>57%	Premium	-	5	-	-
48.0- 56.9%	Grade 1	17	39	3	-
39.0- 47.9%	Grade 2	29	10	9	8
30.0- 38.9%	Grade 3	19	-	25	10
<30.0%	Below Standards	4	-	10	4
Chalkiness					
0.1- 2.0%	Premium	-	10	-	-
2.1- 5.0%	Grade 1	14	19	1	-
5.1- 10.0%	Grade 2	27	19	5	3
10.1- 15.0%	Grade 3	12	3	12	8
>15.0%	Below Standards	15	3	26	11

Table 3. Milling properties of IL-TP 2013 dry season samples.

Grain Quality Property		Number of Entries			
Parameter/ Level/ Classification		UEM 2013 DS (N=92)	A2U 2013 DS (N=22)	AON 2013 DS (N=63)	M6 2013 DS (N=126)
Milling Recovery					
Brown Rice					
>80%	Good	16	1	-	15
75.1- 79.9%	Fair	64	11	19	41
<75%	Poor	12	10	44	70
Milled Rice					
>70.1%	Premium	54	1	6	25
65.1- 70.0%	Grade 1	34	14	39	53
60.1- 65.0%	Grade 2	4	5	16	29
55.5- 60.0%	Grade 3	-	2	2	19
<55.5%	Below Standards	-	-	-	-
Head Rice					
>57%	Premium	10	-	-	-
48.0- 56.9%	Grade 1	43	1	3	1
39.0- 47.9%	Grade 2	34	5	47	20
30.0- 38.9%	Grade 3	4	6	11	36
<30.0%	Below Standards	1	10	2	69

Consumer acceptability and quality of freshly-ooked, staled, and reheated rice

EH Bandonill, GG Corpuz, MJC Ablaza, PA Tibayan, and OC Soco

Rice is best served and consumed freshly cooked. However, there are times when leftover rice stored at ambient temperature is served for the next meal. It is also a common practice to refrigerate unconsumed rice then reheated and served the following day. Such practices result into staled rice with hard texture.

Texture of staled and reheated rice has been measured using an Instron meter by Juliano, et al (2007). Consumer scores of freshly cooked rice are regularly determined, but the acceptability of staled and reheated rice has not yet been scored by a consumer panel. This study thus aims to compare the acceptability of freshly-cooked, staled, and reheated rice and to determine what types of rice remain acceptable to consumers even in staled and reheated forms.

Instron hardness and sensory scores of 2011 dry season (DS) to 2012 wet season rice varieties were determined previously. Nine (9) varieties representing different amylose content (AC) and gelatinization temperature (GT) combinations were used. The same set of varieties were also used for the final season of analysis (2012 wet season). Freshly cooked, staled [at ambient (28-30oC) and refrigerated (4-8oC) temperatures], and reheated samples were evaluated using an Instron texture meter and by a consumer panel.

Highlights:

- Freshly cooked IMS2 (waxy-low GT) showed the lowest Instron hardness (0.75-0.78 kg/cm2) while NSIC Rc222 (intermediate AC-intermediate GT) staled at ambient temperature (4-8oC) and reheated showed the highest (2.40-3.32 kg/cm2) (Table 4).

- Increase in Instron hardness was noted in intermediate AC-low/intermediate GT (1.65-3.32 kg/cm2) and high AC-low/intermediate GT (2.33-3.30 kg/cm2) rice varieties upon staling at ambient and refrigerated temperatures while waxy-low GT (0.75-0.78 kg/cm2) and low AC-low GT (1.58-2.02 kg/cm2) varieties remained soft.

- IMS 2 remained highly acceptable (86.67 to 96.67%) and had the highest mean rating (3.47-3.57) even with ambient and refrigerated staling followed by NSIC Rc160 with 76.67 to 80.00% acceptability and 2.07 to 3.33 mean rating. They were consistently preferred over other samples evaluated. NSIC Rc222, which had hard texture and separated cooked grains, was least liked (6.67%) freshly-cooked or staled.

Table 4. Instron hardness and sensory scores of freshly-cooked and staled rice.

Rice Variety	AC-GT Type		Values								
			Instron Cooked Rice Hardness (kg/cm^2)			Acceptability (%)			Rating Score		
			Fresh	Staled		Fresh	Staled		Fresh	Staled	
				Ambient	Refrigerated		Ambient	Refrigerated		Ambient	Refrigerated
IMS2	Waxy	Low	0.78	0.78	0.75	86.67	86.67	96.67	3.47	3.50	3.57
NSIC Rc160	Low	Low	1.58	2.02	1.82	80.00	80.00	76.67	3.33	2.07	3.13
PSB Rc72H	Intermediate	Low	1.65	2.16	2.03	73.33	76.67	76.67	2.93	2.47	3.10
NSIC Rc122	Intermediate	Low	1.93	2.27	2.56	73.33	60.00	56.67	2.93	2.63	2.50
PSB Rc82	Intermediate	Intermediate	1.83	2.18	2.21	56.67	66.67	56.67	2.57	2.70	2.53
NSIC Rc222	Intermediate	Intermediate	2.40	3.32	3.08	6.67	6.67	6.67	1.73	1.77	1.53
IR64 (Control I)	Intermediate	Intermediate	1.99	2.77	2.57	25.00	20.00	35.00	2.00	2.53	2.15
IR64 (Control I)						52.50	37.50	32.50	2.58	2.23	2.10
NSIC Rc152	High	Low	2.38	3.30	2.91	33.33	30.00	23.33	2.27	2.27	1.93
PSB Rc68	High	Intermediate	2.33	3.26	3.09	66.67	36.67	20.00	2.87	2.33	2.00

II. Advancing Rice Grain Quality Assessment Methods

Project Leader: JBA Duldulao

Grain quality improvement is one of the most important goals in a rice breeding program. It dictates the market value and consumer acceptability. Rice consumers discriminates rice quality relevant to color, shape, foreign matter content, milling recovery or head rice, and even amylose content. Rice quality characteristics should be specified precisely to consider commodity-demand relationship. The development of advance and rapid methods for grain quality evaluation is a priority of the chemists and food scientists in the division.

Grain quality is one of the major selection criteria in rice breeding programs. In the marketplace, how the grains appear dictates the price of rice. Millers prefer rice with high milling recovery, while consumers favor rice which have little chalk and brokens.

The task of grain quality evaluation is in the hands of a few people at the Rice Chemistry and Food Science Division (RCFS) of PhilRice. They assist breeders in the identification of promising rice lines which then enter the National Cooperative Test.

Although the tools for grain quality evaluation tools are already established, they are not fool proof. Some are tedious, cumbersome, and outdated. For instance, the determination of amylose content for the past few years saw some significant variations among the same check varieties. Determination of chalkiness involved manual separation of chalky grain from translucent grains taking so much time and eye strain. Gel temperature is determined by the degree of disintegration of rice grains in alkali as scored by the analyst. Different analysts often give different scores to a sample and even the same sample gives a wide range of dispersion per grain. These experiences stress the need to come up with more improved tools or the adoption of new tools that only need validation in the PhilRice laboratories. Such upgrading of methods will not only ensure that accurate data are generated but also the efficiency of the rice screening is fast-tracked.

The developments in analytical assessments of quality characteristics of rice, as well as the demand for reliable data by the increasing breeding activities, therefore, necessitate the updating of grain quality techniques at PhilRice.

Improving amylose determination method for rice: Development of an amylose field test kit

JBA Duldulao, KB Bergonio, CT Estonilo, NC Ramos

Amylose content (AC), the most important factor affecting cooking and eating quality and processing behavior of rice, plays a critical role in a rice breeding program. AC of rice has been conventionally measured by the amylose-iodine complex method of Juliano (1971). This method is relatively reproducible but suffers interference from amylopectin and lipids, leading to variability of measures, even with check varieties. Recently, Duldulao and Bergonio (2011) reported an ammonium-based amylose determination method with distinct and stable blue color, minimal interference from amylopectin and lipids, and with an improved accuracy across amylose classes (from waxy to high amylose). In spite of this development, the method is still limiting when used for rapid analysis of breeding materials numbering to thousands. There is, therefore, a need to develop a rapid amylose test based on the improved method.

Kongseree et al. (2002) developed an amylose field,test by iodine staining method using milled rice sample (3 g) and defatting (70% isopropanol or ethanol rubbing alcohol) and staining reagent (0.01% I2 in 0.1% KI) in 0.05N sodium acetate buffer (pH 8) to detect contamination of low AC Khao Dawk Mali 105 rice with high-AC rice. This was also adapted and modified by Juliano et al. (2009) in the Philippines using the same iodine staining reagent but different buffer (sodium phosphate, pH 8) to differentiate milled rice into waxy, low AC, intermediate AC, and high AC. The kit takes 30-35 min to differentiate 4-6 rice samples simultaneously and 25 minutes for a single sample.

We previously reported a moderately accurate (85%) one-step amylose staining field test using only 5 milled rice grains and mixed reagent (borate buffer and I2 in NH4I solution) without defatting.. Colors are immediately scored (within 5-7 minutes) as staining tends to fade upon drying. This test takes 3-5 min to differentiate 5-10 samples simultaneously. Validation of the rapid field-test conducted by two analysts of RCFS PhilRice Los Baños using selected NCT 2009 DS and WS entries revealed scoring accuracy of 70% and 80%, respectively, and repeatability of 80%.

This study aims to re-evaluate and optimize our rapid amylose test using the improved colorimetric method as reference.

Highlights:

- A rapid amylose test kit and protocol composed of a spot plate, a coloring reagent, and a color stabilizer that can differentiate AC class of 7 samples simultaneously for only 4-5 minutes were developed.

- The rapid amylose test follows this protocol: (1) Place seven whole milled rice grains on a spot plate and stain with 0.5 mL of coloring reagent mix by swirling for about 10-15 sec. (2) Aspirate off the coloring reagent solution using Pasteur pipette. (3) Add 0.5 mL of color stabilizer and swirl for about 10 sec. (4) Aspirate off the color stabilizer solution using Pasteur pipet (optional). (5) Classify the AC class of the samples based on the color produced. The resulting colors are white (no stain) to light violet for waxy to very low-AC, light violet to light gray for low-AC, violet to gray for intermediate-AC, and dark violet to black for high-AC (Figure 1).

Figure 1. Iodine staining for (L to R) waxy, low-AC, intermediate-AC and high-AC varieties.

- Overall AC class differentiation accuracy based on iodine colorimetry AC data by two analysts on 25 selected varieties was 76% and 80%, while intermediate precision between analysts was 96% (Table 5). For 150 test samples (NCT 2011WS entries), AC class differentiation accuracy was 73%. Differentiation accuracy for each AC class was 100% for waxy, 75-80% for low-AC, 80-87% for intermediate-AC and 42-100% to high-AC rice samples.

Table 5. AC class differentiation accuracy of rapid amylose test kit on check varieties. (N=25) and NCT 2011WS entries (N=150)

AC Class by Iodine Colorimetry	Rapid Amylose Test Kit Accuracy		
	Check Varieties (N=25)		NCT 2011WS Entries (N=150)
	Analyst 1	Analyst 2	
Amylose Classification			
Waxy-Very low (0-10.0%)	100%	100%	100%
Low (10.1-18.0%)	25%	25%	31%
Intermediate (18.1-25.0%)	80%	87%	84%
High (>25.0%)	100%	100%	42%
Overall Accuracy (%)	76%	80%	73%
Intermediate precision between 2 analysts	96%		Not applicable

Validation of molecular markers for rice eating quality

KB Bergonio, DJS Timbol, CT Estonilo, JBA Duldulao, and NC Ramos

Accurate screening and selection of parent germplasm and early generation lines with good eating quality is critical in rice breeding. Physicochemical and sensory analyses are the methods of choice, but these have limitations. Sensory methods require large quantities of samples and only a few samples can be analyzed per day. Moreover, results are sometimes inconsistent even for the same sample, presumably due to the physical and emotional condition of evaluation panel or subtle differences in sample preparation (Lestari et al., 2009). On the other hand, methods to determine physicochemical properties of rice, such as amylose content, gelatinization temperature, gel consistency, and fragrance, are relatively accurate and reproducible, but they also require large quantities of samples and are tedious, expensive, and time-consuming. Consequently, these factors slow down the screening process as breeders need to immediately know the physicochemical and sensory properties of progeny across multiple years and locations.

In other countries, molecular techniques based on the polymerase chain reaction (PCR) have been employed in rice eating quality assessment as physicochemical properties, such as amylose content, gelatinization temperature, and gel consistency, and are controlled by one to three major genes with one or more modifiers (Lestari et al., 2009). Molecular assessment by PCR is simple, efficient, cost-effective, and allows for early growth stage evaluation unlike the conventional methods. Hence, it can complement or completely replace conventional methods for physicochemical and sensory assessment. However, prior to local adoption, methods of generating molecular descriptors must be optimized and validated.

This study aimed to validate previously reported PCR-based markers for rice eating quality against conventional assessment methods, to subsequently formulate a marker-based assessment and prediction method of eating quality of rice, and to generate molecular descriptors of the accessions of the PhilRice Genebank.

Highlights:

- Six PCR-based markers [3 single nucleotide polymorphisms (SNP) and 1 simple sequence repeats (SSR)] were tested for their association with each of the rice eating quality parameter and special trait, namely: (CT)n microsatellite (or SSR) in the Waxy (Wx) gene for amylose class (Ayres et al., 1997), GC/TT SNP in the starch synthase IIa (SSIIa) gene for gelatinization temperature type (Jin et al., 2010), C/T polymorphism in Ex10 of the Wx gene for gel consistency (Tran et al., 2011), and 8-bp del and 3 SNP in Ex7 of the gene encoding the putative betaine aldehyde dehydrogenase 2 (badh2) for fragrance detection (Bradbury et al., 2005). PCR conditions for each of the DNA markers were optimized prior to use.

- Eighty-four validation samples, composed of selected varieties representing all eating quality types, and 215 test samples from the PhilRice Genebank, were used. These were processed and prepared for genotypic analyses to determine the accuracy and repeatability of each molecular marker vs. conventional sensory and chemical analyses. Correspondence between each molecular marker and conventional methods were also evaluated by Pearson regression and correlation analysis. 62 out of 84 validation samples and 213 out of 215 test samples successfully germinated and grown under conventional practices at the PBBD screen house. DNA from 2-week old leaf tissue samples was collected by the CTAB method. DNA quality and quantity were checked using agarose gel electrophoresis and Nanodrop® spectroscopy.

- Amylose classification by the (CT)n microsatellite marker showed an average overall prediction accuracy of 77% using the validation set. Prediction accuracy for high amylose content samples (N=14) was 42.9%, while 78.9% for waxy-low-intermediate amylose content (N=38). Repeatability between two separate analyses of the same validation samples was 100%. For test samples, overall prediction accuracy was 70.0%. Genotypic amylose classification for waxy-low-intermediate samples (N=167) obtained 79.0% accuracy, while 40.0% accuracy for high-amylose content samples (N=46) (Table 1).

- Gelatinization temperature type by the GC/TT SNP marker showed an average overall prediction accuracy of 91.3% with the validation set. Prediction accuracy for high intermediate gelatinization temperature samples (N=30) was 93.3%, while 88.6% for low gelatinization temperature samples (N=22). Repeatability between two separate analyses of the same validation samples was 98.1%. On the other hand, overall prediction accuracy obtained with test set was 89.2. High intermediate gelatinization temperature type (N=142) obtained 93.7% prediction accuracy, whereas, the low gelatinization temperature type (N=71) obtained 80.3% (Table 5).

- Gel consistency by the C/T SNP marker showed an average overall prediction accuracy of 76.9% with the validation set. Prediction accuracy for medium-hard gel consistency samples (N=11) was 54.5%; 87.8% for soft gel consistency samples (N=41). Repeatability between two separate analyses of the same validation samples was 92.3%. For the test set, overall prediction accuracy was 81.7%. Soft gel types samples (N= 167) obtained 91.0% prediction accuracy, while medium-hard gel types samples (N= 46) obtained 47.8% prediction accuracy (Table 5).

- Fragrance or aroma detection by the 8 bp del and 3 SNP marker showed an average overall prediction accuracy of 82.7% with the validation set. Prediction accuracy for non-aromatic samples (N=44) was 95.5%, while 12.5% for aromatic samples (N=8). Repeatability between two separate analyses of the same validation samples was 100%. On the other hand, test samples (N= 213) showed 88.3% overall aroma prediction accuracy. Prediction accuracy obtained for non-aromatic (N=159) and aromatic (N=42) of test samples were 98.7% and 57.4%, respectively (Table 6).

- Average overall accuracies of amylose, gelatinization temperature, gel consistency, and fragrance markers using validation and test samples were 76.9% and 70.0%, 91.4% and 89.2%, 76.9% and 81.7%, and 82.7% and 88.3%; respectively, in reference to the conventional organoleptic and chemical methods. Intermediate precision between two separate genotypic analyses conducted by different analysts using the same validation samples were 100% for both amylose and fragrance; while 98.1% and 92.3% were obtained for gelatinization temperature and gel consistency, respectively.

- Regression and correlation analyses found that molecular marker for gelatinization temperature was moderately associated (r= 0.60) with the alkali-spreading method. Other markers for rice eating quality were weakly associated (r= 0.12-0.33) with the conventional methods. These indicated that among the molecular markers validated, only the GC/TT SNPs marker for gelatinization temperature type can complement the conventional alkali-spreading method.

Table 6. Accuracy and repeatability of the markers for rice eating quality using validation (N=52) and test (N=213) samples.

Eating Quality Parameter/ Classification	Validation Samples (N=52)			Test Samples (N=213)	
	No. of Sample	Accuracy (%)		No. of Sample	Accuracy (%)
		Analyst 1	Analyst 2		
Amylose Content					
Waxy-Low-Intermediate	38	78.9	78.9	167	79.0
High	14	42.9	42.9	46	40.0
Overall accuracy		76.9	76.9		70.0
Repeatability between 2 analysts using validation samples=100%					
Gelatinization Temperature					
High-Intermediate	30	93.3	93.3	142	93.7
Low	22	90.9	86.4	71	80.3
Overall accuracy		92.3	90.4		89.2
Repeatability between 2 analysts using validation samples=98.1%					
Gel Consistency					
Medium-Hard	11	63.6	45.5	46	47.8
Soft	41	85.4	90.2	167	91.0
Overall accuracy		78.8	75.0		81.7
Repeatability between 2 analysts using validation samples=92.3%					
Aroma Detection					
Non-aromatic	44	95.5	95.5	159	98.7
Aromatic	8	12.5	12.5	54	57.4
Overall accuracy		82.7	82.7		88.3
Repeatability between 2 analysts using validation samples=100%					

Arsenic content in Philippine rices
JBA Duldulao and CT Estonilo

Grain quality is not only measured in terms of physicochemical and sensory properties but also on safety. More often safety is not easily apparent to the naked eye. Microbial contaminants must be first grown in culture media to be detected. Microbial and fungal contaminants and their by-products, like aflatoxins, can be detected by polymerase chair reaction (PCR) based methods or instrumental methods like high performance liquid chromatography (HPLC).

Another major safety concern in food is heavy metal contamination. The most recent concern on rice is arsenic (As) contamination. Arsenic in rice from the US and Europe (0.198 mg/kg) was significantly higher than rice from Asia (0.07 mg/kg) (Zavala and Duxbury, 2008). The global normal range is 0.08-0.20 mg/kg based on data from literature values and those of Zabala ang Duxbury (2008). Several countries currently use a 1 ppm limit for As in food and this is often cited as a "safe" level for rice.

There are only a few countries that have set maximum levels of As in their rice and rice products. China has set its standard to 0.15 mg/kg inorganic As; Argentina, Brazil, Paraguay and Uruguay at 0.3 mg/kg total As; and Australia and New Zealand at 1 mg/kg total As for all cereals (Codex Committee on Contaminants in Food March 2012 Report).

The Philippines, so far, has not conducted studies on the As levels of rices locally grown or those imported. Likewise, it has not set maximum limit of As in rice. Since arsenic in a known carcinogen, and chronic exposure even at low levels has been linked to increased risks of bladder, lung and skin cancer as well as Type 2 diabetes and cardiovascular diseases, the evaluation of locally grown and imported rices for As levels is necessary. Accordingly, maximum level of As must be imposed by regulatory authorities so that Filipinos who subsist daily on rice are not put in danger.

On these premises, this study shall therefore establish a baseline data on the As level of Philippine rices. Subsequently, in-house capability to analyze As shall be developed so that more rices especially from potentially As-contaminated areas and rices exported into the country can be analyzed for their safety for human consumption.

Highlights:

- There are nine (9) provinces in the country that were identified near volcanic and mining areas. The provinces near volcanic activities are Pampanga, Albay, and Sorsogon. Whereas provinces near mining activities are La Union, Camarines Norte, Masbate, Compostela Valley, Agusan Del Sur, and

Surigao Del Norte. There are three to four municipalities in each province that identified some farmers willing to provide soil and rice samples.

- A total of 40 farmers have provided rice and soil samples. There are 38 soil samples collected and properly prepared for physical, mineralogical and chemical analyses. There are 40 rice samples properly stored for grain quality and arsenic analyses.

Table 7. Collected rice samples for elemental analyses.

PROVINCE	MUNICIPALITY	BARANGAY	FARMER	VARIETY	AFFECTED BY
Pampanga	Bacolor	Sta Barbara	Ferdinand Pineda	PSB Rc18	Mt. Pinatubo
	Porac	Along	Eugenio Serrano	NSIC Rc226	Mt. Pinatubo
	Mabalacat	Cacutud	Orlando Garcia	NSIC Rc224	Mt. Pinatubo
		Atlu Bola	Rizal Carbungco	NSIC Rc216	Mt. Pinatubo
La Union	Rosario	Nangcamotian	Bernardo Collad	NSIC Rc160	Benguet Mining Industries
		Udiao	Marcelino Packing	Diamong X	Benguet Mining Industries
		Tay-ac	Romy Llaneras	NSIC Rc158	Benguet Mining Industries
		Puzon	Pablo Costales	Diamond X	Benguet Mining Industries
		Tabtabungao	Rolando Rocapor	NSIC Rc156	Benguet Mining Industries
Camarines Norte	Labo	Dalas	Arnel Sayco		Small Scale Mining
			Nicanor Manaog		Small Scale Mining
	Jose Panganiban	Sta. Rosa Norte	Fernando Calsada	PSB Rc18	Elnar's Gold Processing
		Sta. Rosa Sur	Roberto Vasquez		Escober Gold Processing
Albay	Guinobatan	Masarawag	Alfredo Opinia	NSIC Rc240	Mt. Mayon
		Doña Tomasa	Jaime Mataba	PSC Rc82	Mt. Mayon
	Camalig	Quirangay	Jeanly Millares	Inbred	Mt. Mayon
		Sua	Rodolfo Moyo	Bulao (tradvar)	Mt. Mayon
		Tumpa	Joseph Obligacion	Bulao (tradvar)	Mt. Mayon
	Legaspi	Bagong Abre	Christopher Nuñez	NSIC Rc222	Mt. Mayon
Sorsogon	Irosin	Carriedo	Cris Basa	NSIC Rc224	Mt. Bulusan
		San Pedro	Antonio Ete	NSIC Rc240	Mt. Bulusan
	Juban	Catanusan	Martin Fulay	IR60	Mt. Bulusan
		Buraburan	Leonilo Militante	NSIC Rc240	Mt. Bulusan
	Casiguran	Escuala	Amador Masarate	NSIC Rc222	Mt. Bulusan – no soil, #38
		San Juan	Nelia Derla	Binat-Ang (tradvar)	Mt. Bulusan – no soil, #39

Abbreviations and acronymns

ABA – Abscicic acid
Ac – anther culture
AC – amylose content
AESA – Agro-ecosystems Analysis
AEW – agricultural extension workers
AG – anaerobic germination
AIS – Agricultural Information System
ANOVA – analysis of variance
AON – advance observation nursery
AT – agricultural technologist
AYT – advanced yield trial
BCA – biological control agent
BLB – bacterial leaf blight
BLS – bacterial leaf streak
BPH – brown planthopper
Bo - boron
BR – brown rice
BSWM – Bureau of Soils and Water Management
Ca - Calcium
CARP – Comprehensive Agrarian Reform Program
cav – cavan, usually 50 kg
CBFM – community-based forestry management
CLSU – Central Luzon State University
cm – centimeter
CMS – cystoplasmic male sterile
CP – protein content
CRH – carbonized rice hull
CTRHC – continuous-type rice hull carbonizer
CT – conventional tillage
Cu – copper
DA – Department of Agriculture
DA-RFU – Department of Agriculture-Regional Field Units
DAE – days after emergence
DAS – days after seeding
DAT – days after transplanting
DBMS – database management system
DDTK – disease diagnostic tool kit
DENR – Department of Environment and Natural Resources
DH L– double haploid lines
DRR – drought recovery rate
DS – dry season
DSA - diversity and stress adaptation
DSR – direct seeded rice
DUST – distinctness, uniformity and stability trial
DWSR – direct wet-seeded rice
EGS – early generation screening
EH – early heading
EMBI – effective microorganism-based inoculant
EPI – early panicle initiation
ET – early tillering
FAO – Food and Agriculture Organization
Fe – Iron
FFA – free fatty acid
FFP – farmer's fertilizer practice
FFS – farmers' field school
FGD – focus group discussion
FI – farmer innovator
FSSP – Food Staples Self-sufficiency Plan
g – gram
GAS – golden apple snail
GC – gel consistency
GIS – geographic information system
GHG – greenhouse gas
GLH – green leafhopper
GPS – global positioning system
GQ – grain quality
GUI – graphical user interface
GWS – genomwide selection
GYT – general yield trial
h – hour
ha – hectare
HIP - high inorganic phosphate
HPL – hybrid parental line
I - intermediate
ICIS – International Crop Information System
ICT – information and communication technology
IMO – indigenous microorganism
IF – inorganic fertilizer
INGER - International Network for Genetic Evaluation of Rice
IP – insect pest
IPDTK – insect pest diagnostic tool kit
IPM – Integrated Pest Management
IRRI – International Rice Research Institute
IVC – in vitro culture
IVM – in vitro mutagenesis
IWM – integrated weed management
JICA – Japan International Cooperation Agency
K – potassium
kg – kilogram
KP – knowledge product
KSL – knowledge sharing and learning
LCC – leaf color chart
LDIS – low-cost drip irrigation system
LeD – leaf drying
LeR – leaf rolling
lpa – low phytic acid
LGU – local government unit

LSTD – location specific technology development
m – meter
MAS – marker-assisted selection
MAT – Multi-Adaption Trial
MC – moisture content
MDDST – modified dry direct seeding technique
MET – multi-environment trial
MFE – male fertile environment
MLM – mixed-effects linear model
Mg – magnesium
Mn – Manganese
MDDST – Modified Dry Direct Seeding Technique
MOET – minus one element technique
MR – moderately resistant
MRT – Mobile Rice TeknoKlinik
MSE – male-sterile environment
MT – minimum tillage
$mtha^{-1}$ - metric ton per hectare
MYT – multi-location yield trials
N – nitrogen
NAFC – National Agricultural and Fishery Council
NBS – narrow brown spot
NCT – National Cooperative Testing
NFA – National Food Authority
NGO – non-government organization
NE – natural enemies
NIL – near isogenic line
NM – Nutrient Manager
NOPT – Nutrient Omission Plot Technique
NR – new reagent
NSIC – National Seed Industry Council
NSQCS – National Seed Quality Control Services
OF – organic fertilizer
OFT – on-farm trial
OM – organic matter
ON – observational nursery
OPAg – Office of Provincial Agriculturist
OpAPA – Open Academy for Philippine Agriculture
P – phosphorus
PA – phytic acid
PCR – Polymerase chain reaction
PDW – plant dry weight
PF – participating farmer
PFS – PalayCheck field school
PhilRice – Philippine Rice Research Institute
PhilSCAT – Philippine-Sino Center for Agricultural Technology
PHilMech – Philippine Center for Postharvest Development and Mechanization
PCA – principal component analysis
PI – panicle initiation
PN – pedigree nursery
PRKB – Pinoy Rice Knowledge Bank
PTD – participatory technology development
PYT – preliminary yield trial
QTL – quantitative trait loci
R - resistant
RBB – rice black bug
RCBD – randomized complete block design
RDI – regulated deficit irrigation
RF – rainfed
RP – resource person
RPM – revolution per minute
RQCS – Rice Quality Classification Software
RS4D – Rice Science for Development
RSO – rice sufficiency officer
RFL – Rainfed lowland
RTV – rice tungro virus
RTWG – Rice Technical Working Group
S – sulfur
SACLOB – Sealed Storage Enclosure for Rice Seeds
SALT – Sloping Agricultural Land Technology
SB – sheath blight
SFR – small farm reservoir
SME – small-medium enterprise
SMS – short message service
SN – source nursery
SSNM – site-specific nutrient management
SSR – simple sequence repeat
STK – soil test kit
STR – sequence tandem repeat
SV – seedling vigor
t – ton
TCN – testcross nursery
TCP – technical cooperation project
TGMS – thermo-sensitive genetic male sterile
TN – testcross nursery
TOT – training of trainers
TPR – transplanted rice
TRV – traditional variety
TSS – total soluble solid
UEM – ultra-early maturing
UPLB – University of the Philippines Los Baños
VSU – Visayas State University
WBPH – white-backed planthopper
WEPP – water erosion prediction project
WHC – water holding capacity
WHO – World Health Organization
WS – wet season
WT – weed tolerance
YA – yield advantage
Zn – zinc
ZT – zero tillage

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Department of Agriculture

PHILRICE

PHILIPPINE RICE RESEARCH INSTITUTE

We are a chartered government corporate entity under the Department of Agriculture. We were created through Executive Order 1061 on 5 November 1985 (as amended) to help develop high-yielding, cost-reducing, and environment-friendly technologoies so farmers can produce enough rice for all Filipinos.

We accomplish this mission through research and development work in our central and seven branch stations, coordinating with a network that comprises 57 agencies and 70 seed centers strategically located nationwide.

To help farmers achieve holistic development, we will pursue the following goals in 2010-2020: attaining and sustaining rice self-suffiency; reducing poverty and malnutrition; and achieving competitiveness through agricultural science and technology.

We have the following certifications: ISO 9001:2008 (Quality Management), ISO 14001:2004 (Environment Management), and OHSAS 18001:2007 (Occupational Health and Safety Assessment Series).

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