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A Proposal for IRRI to Establish a Grain Quality and Nutrition Research Center

Robin Graham

IRRI
INTERNATIONAL RICE RESEARCH INSTITUTE

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Mailing address: DAPO Box 7777, Metro Manila, Philippines
Phone: (63-2) 845-0563, 844-3351 to 53
Fax: (63-2) 891-1292, 845-0606
Email: irri@cgiar.org
Home page: www.cgiar.org.irri
Riceweb: www.riceweb.org
Riceworld: www.riceworld.org
Courier address: Suite 1009, Pacific Bank Building
6776 Ayala Avenue, Makati
Metro Manila, Philippines
Tel. (63-2) 891-1236, 891-1174, 891-1258, 891-1303

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Executive summary

IRRI has a major role to play in the new Global Challenge Program on Biofortification (CPB). If this program is fully funded, IRRI can expect to benefit from additional funding of up to US$1 million per year for 10 years. An increased emphasis on grain quality in the world and local rice trade is emerging concurrently with the increased emphasis on nutritional quality espoused by the CPB.

A Grain Quality and Nutrition Research Center (GQNRC) is proposed to capture the opportunities offered by the CPB, to give IRRI capability in micronutrient analysis of grain, to bring IRRI up-to-date in grain quality, and to give IRRI a research capability that will keep its breeders and agronomists abreast of developments in quality elsewhere, thus ensuring that IRRI will maintain its preeminent position in rice agronomy and breeding.

It is proposed that the GQNRC progressively appoint at least three internationally recruited staff (IRS) with expertise in grain quality, human nutrition, and cereal chemistry/biochemistry/analytical or micronutrient chemistry, and that one of them act as the head of the GQNRC. These appointments should be possible with sponsorship funding as proposed by Dr. Ren Wang, IRRI's deputy director general for research. Existing staff, particularly plant breeders and agronomists, should be encouraged to become joint members of the GQNRC and their division.

The GQNRC will require about 300 m^2 of space and up to US$350,000 in refurbishment and computing and new equipment, and will embody the upgraded grain quality laboratory and a new plant micronutrient analytical laboratory. A feature of the operation of these laboratory facilities will be computer control of all measurements, the instantaneous downloading of results into the International Rice Information System (IRIS), and established quality-assurance procedures.

The GQNRC will have both a service role for helping breeders incorporate quality traits into new germplasm and research and capacity-building roles to make this goal more efficiently achieved and the outputs more relevant both to NARES and to increasingly more sophisticated rice farmers and consumers.

Introduction

The year 2002 has seen the nomination of a human nutrition-oriented project focused on micronutrients in the food chain as a Global Challenge Program within the Consultative Group on International Agricultural Research (CGIAR), and IRRI has a major role to play in it. At the same time, the visit of Professor Robin Graham of The University of Adelaide and for nine years the scientific coordinator of the CGIAR Micronutrients Project, the forerunner of the CPB, has raised questions among IRRI staff as to how best to be prepared to handle the CPB and to capture fully at IRRI the opportunities offered by it. Added to this was the existence of a grain quality service laboratory that had served plant breeders outstandingly over many years but that did not have the time or a strong enough research arm to help it keep abreast of changes in perception and methodology of grain quality. Finally, the breeders involved in high-iron rice were sending their samples to Adelaide for analysis because trial analyses in the Analytical Service Laboratory (ASL) at IRRI were not acceptable. Dr. Ren Wang has proposed that Dr. Graham investigate the idea of a Grain Quality and Nutrition Research Center, funded by external sponsorship, that would house a revamped grain quality laboratory and a new plant micronutrients laboratory and also have a research capability, and produce a plan for debate among IRRI staff.

Background, opportunities, and challenges

A new paradigm for agriculture in the 21st century was proposed (Welch and Graham 1999, Graham et al

2001) that views agriculture as an instrument for public health and focuses attention on the role of agriculture in delivering nutrients to humans and animals in balanced amounts that can sustain maximal physical and mental activity of the humans who are at the same time the drivers of the food system and dependents on it. This is known as the productive, sustainable, and nutritious food systems paradigm for agriculture and human health. For papers relevant to the concepts and strategies within the food systems paradigm, see the references in Appendix D. Each food crop is seen for its contribution to balancing the diet of consumers in the context of WHO data that indicate that more than half of the 6 billion humans on Earth are deficient in micronutrients and another 15% suffer from protein-energy deficiency. The tried and proven approach to balanced nutrition of the past is to promote a highly varied diet to ensure that everyone receives all the nutrients required, whether they be known to science or not; but, such diets are relatively expensive and not available to the resource poor in developing countries of the South. The food systems approach uses several strategies to achieve a better balanced diet for all while ensuring sufficient production in a sustainable way. One strategy is the use of fertilizers to increase the density of certain micronutrients in staple foods; another is to breed staple food crops with a higher density of micronutrients.

The major micronutrient deficiencies in humans are those of iron, zinc, iodine, selenium, and vitamin A. The WHO Web site gives estimates of the numbers of people affected, except for zinc, for which there is no easy test, though zinc deficiency is considered to be possibly as extensive as iron deficiency (Gibson 1994). Even in the United States, the U.S. government estimates that nutrition-related chronic disease costs the economy US$300 billion annually. Plant breeding can exploit genetic variation in crop species to enhance their nutritional value, thereby helping to eliminate nutrient deficiencies, mainly of micronutrients, in both developing and developed countries as identified by WHO (1996). Exploiting the potential of plant breeding in this way has been shown to be more feasible and economically sound than the current medical interventionist approach (Bouis 1999).

A study of the physical work-rate of young women (Zhu and Haas 1998) has revealed that women even slightly deficient in iron (with adequate hemoglobin) cannot use their food energy efficiently, and so effectively waste 5% of their daily caloric intake. Iron supplementation allowed these women to complete the same work with 5% fewer calories (measured as oxygen consumed). An earlier study in China (Li et al 1994) showed similar results, namely, that iron-supplemented women in a cotton factory were able to do the same work with fewer calories. Humans deficient in other minerals and vitamins critical to metabolic efficiency are probably likewise unable to use all the calories they consume. In view of the WHO statistic that 4–5 billion people are iron-deficient (WHO Web site 2002), the incidence of people deficient in metabolic capacity because of one deficiency or another is probably 80% or more. This implies that many nutritionally compromised people eat more staple food than they can effectively use for energy and may do so only for the extra micronutrient they gain from consuming extra food. Consequently, it can be argued that the focus of the international agricultural research centers on breeding staple food crops for higher yield is unjustifiable if people largely cannot use the calories they are getting now. Greater health, work capacity, and overall productivity can be predicted from a new focus on raising the micronutrient density of staple foods to match the calories they contain. This is not an argument to decrease the calories now available but to package them with other metabolic components that will make for a more efficient use of the calories produced.

The CPB seeks to establish the food systems approach for research within the crop-based CGIAR centers responsible for staple crop improvement, including IRRI and its national agricultural research and extension systems (NARES) and advanced research institute (ARI) partners. In all, the CPB will support research and development of nutrient-dense cultivars of 17 crops, 6 Phase 1 crops and 11 Phase 2 crops. The six Phase 1 crops, rice, wheat, maize, beans, cassava, and sweet potato, have already completed an exploration of the germplasm and initial studies of the genetics and genotype by environment effects, and are therefore poised to take advantage of a major input of resources. Funding will support capacity building and farmer participatory research in NARES partners and will be allocated also for both CGIAR and partner research in biotechnology, studies of bioavailability, strategic initiatives, and economics and social marketing, along with administration and communications.

As milled rice has the lowest iron concentration of any modern staple crop yet is the major food source for nearly half of the world's population, it is essential that a major thrust of the CPB be on improving the nutritive value of rice. At the same

time, in all rice-growing areas there is an increased emphasis on cooking quality. IRRI is the obvious location for a critical mass of scientists in grain quality; micronutritional quality; nutrition science; organic, micronutrient, and analytical chemistry; biochemistry; and grain processing. This core of specialties will not only support the breeders and agronomists involved in improving the nutritional quality of rice, and investigating more efficient ways of doing so, but will also be the focus of capacity building in NARES and the conduit for the flow of knowledge from their colleagues in ARIs to other IRRI staff leading the effort in agronomy (in its broadest sense) and plant breeding. Finally, these researchers will be involved in developing new products that contribute to better nutrition. Research on milling efficiency will also be critical to the outcomes of this CPB.

Objectives

The purpose of the GQNRC is therefore

1. To provide a focus at IRRI for the effective use of the resources of the Global Challenge Program on Biofortification.
2. To provide IRRI plant science staff with expertise in human nutrition and grain quality both directly and through the GQNRC's nutrition contacts abroad.
3. To provide first-class laboratory support for all plant science activities at IRRI that require nutritional and cooking and eating quality assessments through grain analysis.
4. To promote collaborative efforts with agronomists and plant breeders to enhance the nutritional quality of rice to maintain IRRI's leading position in rice breeding and fulfill its mission.

GQNRC structure, links, and governance

The activities of the GQNRC are the responsibility of the GQNRC head, who reports to a senior IRRI position. The head is expected to create communication channels for all staff to contribute to the work and esprit de corps of the unit. No advisory committee is considered necessary as the Global Challenge Program will provide advice and review through its funding arrangements that are expected to provide a considerable proportion of the GQNRC's operating budget after establishment.

Space requirements

Criteria for the space requirements of the GQNRC containing an updated grain quality laboratory and a proposed plant micronutrient laboratory separate from the ASL are as follows:

1. An estimated 300 m^2 will be required. The plant micronutrient laboratory would need walled isolation from the grain quality laboratory and from all other IRRI activities to prevent contamination, especially by soil and dust, including milling dust, but also by copper, brass, galvanized materials, and, to a lesser extent, metals in general. At the same time, if one individual is to assume overall responsibility for both laboratories, ideally they ought to be contiguous or at least adjacent. It is my opinion that the reason the ASL has not produced satisfactory analyses for grain iron is that these activities have been co-located with the soil analysis activities that have been the major work of the ASL, and serious contamination is highly likely under such conditions. Nevertheless, the new plant micronutrient laboratory would need to use the inductively coupled plasma-atomic emission spectrometer (ICP-AES) located in the ASL and extra care would be needed when grain samples are being run on the ICP-AES located in the ASL.
2. The plant micronutrient laboratory will require a high-quality acid-resistant fume hood for digestion, with wash-down facility, and generally acid-resistant fittings all around—floors, benches, plumbing—everything. This obviously can be expensive. (A possible alternative to traditional digestion in nitric-perchloric acid is a new flow-through microwave digestion unit that uses hydrochloric acid, currently being "field"-tested under laboratory conditions at Cornell University. This does not need a fume hood and has only minimal plumbing requirements, so costs could be much less and locational constraints fewer. A decision on this new development must await the outcome of the Cornell tests.)
3. An excellent network of computers linking all instruments to the IRIS database is needed so that analytical data can be readily merged with breeding trial data.

4. The grain quality laboratory can possibly operate in its proposed reconfiguration within the amount of space it currently has, but additional space may be needed for research purposes.

Staff requirements

IRS: new position of head of the GQNRC

The GQNRC needs to be led by IRS-level appointments, and new positions are indicated both by the high workloads of current staff and by the skills required that are not available at IRRI now. An experienced person is indicated as the status of the GQNRC will depend on this more than anything else. This position could be filled from the fields of human nutrition, grain quality, cereal chemistry, organic or analytical or micronutrient chemistry, or biochemistry. At least two more appointments will be needed to cover this range of disciplines. Irrespective of training, a strong interest in biofortification and nutritional quality of grains is required.

IRS/NRS: new position of analyst/laboratory manager

A senior graduate analyst is needed to oversee all day-to-day activities of both the micronutrient and grain quality laboratories. This position could be IRS or NRS, though IRRI will probably find it difficult to recruit such a person from within the Philippines owing to the high demand. Considerable experience and a commitment to quality assurance are essential requirements for this position. This person, in close collaboration with the GQNRC head, will be responsible for introducing new analytical capability as and when needed, choosing instrumentation, developing methods, introducing and maintaining quality assurance procedures, and closely supervising all laboratory staff. Essential skills are considerable experience in analytical chemistry and quality assurance, instrument maintenance, a high order of computing skills, including some programming skills, and a capacity to supervise junior laboratory staff to achieve the high standards of the laboratory. The seniority and critical importance of this position to the success of the GQNRC are emphasized. To underline this point, I note here that all four grain quality laboratories I visited overseas were run by quite senior research-oriented PhDs.

NRS:

It is expected that the GQNRC will have at least eight laboratory staff under the manager for the two laboratories. Some of the current staff of the grain quality laboratory would be required, and one or two staff of the ASL, depending on its new workload, should be considered for transfer as their skills would greatly enhance the new GQNRC.

Equipment requirements

Plant micronutrient laboratory (PML)

The laboratory requires a contamination-free environment, with a positive pressure ventilation system, and largely metal-free laboratory furniture, fittings, and facilities. For example, paint needs to be acrylic and carefully chosen for its low content of heavy metals.

Perchloric acid-resistant fume hood for digestion of grain and leaf samples: This is a major item and, hitherto, there has been no substitute. Such fume hoods exist in the ASL and one could be transferred to the PML, but they are coming to the end of their useful life and may require a major overhaul that may or may not be an economic option. However, recently, a flow-through microwave digester unit has been developed and the prototype is currently under test at Cornell University. If this unit passes the test, at its projected cost of US$50,000, it is an excellent alternative to the fume-hood digestion system.

Drying oven: stainless steel and forced draft.

Balances: electronic with breeze protection and computer interface for direct loading of weights into the autosampler file, capable of weighing accurately and rapidly 10–800-mg samples. Older computers as remote terminals of the network.

Nanopure water system: capable of 18 Mohm water with undetectable levels of heavy metals.

Autopipettors, vortex mixer, glassware and plasticware, standard solutions, and standard reference materials suitable for the purpose.

Access to the trace ICP-AES in the ASL.

Grain quality laboratory

This laboratory has the enviable reputation of doing 60,000–70,000 determinations annually (see Table 2 in Appendix A), half of which are gelatinization temperature (by alkali spreading) and another third are amylose content by autoanalyzer. The remaining sixth of the analyses includes aroma, grain elonga-

tion, gel consistency, and physical characteristics. This amazing workload has been achieved in recent years with only five core staff and two casuals. Each staff member can therefore be equated with 10,000 determinations per year, or more than 250 per week, or more than 50 every working day, including sample preparation and data reporting and management, laboratory and instrument maintenance, and other nonoutput but essential service activities. I believe that this level of service to the breeding programs could not be done cheaper anywhere by any means. It is most unfortunate that this laboratory has lost three of the five core people recently, including its leader. Nevertheless, even in the face of the past valued performance noted above, there remain opportunities and pathways to go forward, using the prospect of the new GQNRC to modernize and restaff the laboratory.

The three overseas grain quality laboratories I visited are more computer-controlled and database-oriented than the IRRI laboratory. The breeding database network is also less developed than in other centers abroad. This reflects the favorable position of IRRI in relation to the abundance of labor. Other centers have changed to computer control of breeding operations and associated laboratory operations through greater or earlier necessity. The opportunity is there for the IRRI breeding programs to make the current downsizing the time to change because the price of high-quality labor in the Philippines is going to continue to increase. The grain quality laboratory can take a lead by introducing objective, computer-based measurements progressively while database development can proceed in a way that it is easy for the laboratory computers to download each datum into its correct place in the database where its further use, for example, in ANOVA, is as simple as a keystroke. This requires standardized ID systems for every breeding program, trial, plot, and sample. IRIS could be the basis of the database and network.

Physical attributes, including milling properties and grain elongation. After observing operations in the three other quality laboratories and then discussions with Mrs. Normita dela Cruz, I recommended that objective measurement of the physical characteristics be installed first. This is probably the area where computers with image analysis software will bring the most benefit to a labor-deficient laboratory and this area of operations can be used to develop the database integration. These measurements are among the fewest currently requested so the savings will be modest. However, it is possible that, as the reproducibility of the measurements increases, their usefulness may also increase. Required are two or three Sony Video Pro32 cameras, a flatbed scanner, and several old PCs to act as remote terminals for downloading data to the database. Software is the Color Image Analysis System from Leading Edge P/L.

Gel consistency. A Relative Viscosity Analyzer (RVA) was purchased some time ago but it is compatible with 386 PCs only and has hardly been used. A 386 PC can be found for it or it can be upgraded or replaced, but this measurement could be used to replace the gel consistency (GC) technique after some cross-calibration studies on the amount of variance in GC that is accounted for by the RVA measurement. Another simple instrument is available that is considered to add extra interpretative value to the measurement of this trait. Genetic studies with these instruments could possibly resolve this complex trait to several quantitative trait loci (QTLs) once measurements are quantitated.

Amylose. I strongly recommended that the two markers associated with this trait be made available to breeders initially at least for research purposes, including genetic studies, and as a matter of some urgency. However, it is not expected at this stage that the autoanalyzer method for amylose will be replaced by either markers or near-infrared spectrometry (NIR). Because the IRRI laboratory services large and genetically diverse rice breeding programs, it seems clear that the autoanalyzer should be retained for routine assessment of amylose and be upgraded with a larger autosampler and with software suitable for downloading the electronic data to IRIS after inspection of the daily standardization procedures by the analyst. Concerns about the stability of this instrument must be examined at the same time (on this point, the Beaumont, Texas, laboratory strongly recommended the Series II instrument as being far more stable and reliable than the Series III instrument in use at IRRI).

An excellent NIR Systems 6500 exists in the ASL. It is therefore possible for IRRI to investigate the potential of the NIR either for its own purposes or as a cheap way for NARES partners to measure quality parameters in their breeding programs. This should be viewed only as a research project, and not as a development for the grain quality laboratory itself, though that could possibly come from extensive and successful research results.

Gelatinization temperature. This critical quality measurement that is a function of the enthalpy of gelatinization and the temperature of the phase transition is approximated in the laboratory by the slaking of the white rice grains in the presence of aqueous alkali. This curious correlation allows a very

rapid estimate of this parameter, and its importance is reflected in the fact that more than 30,000 estimates are requested by breeders annually. A sophisticated *differential scanning calorimeter (DSC)* is now available to measure this parameter directly as temperature and enthalpy of the phase transition. Expendables alone are US$0.60 per sample and the technique probably takes three times as long as the current method. However, this instrument could be used to investigate the irreversibility of the hydration and dehydration of amylose on heating and cooling (hysteresis), which might lead to a better understanding of the trait, its genetics, its dependence on grain maturation temperature, and its efficient manipulation in breeding programs. IRRI should purchase a DSC and begin to investigate how efficient it will be in the routine quality assessment portfolio of the grain quality laboratory. The instrument should be used first as a research instrument and then for advanced lines and chosen populations, leading to its wider use when justified.

Aroma. Other laboratories, perhaps without the advantage of an experienced staff in the matter of aroma, have gone to gas chromatography for aroma assessment, measuring only the primary component, 2-acetyl 1-pyrroline (2AP) (Buttery et al 1982). Apparently, the difference between Basmati and Jasmine fragrance is that Jasmine contains other components as well as 2AP. These extra components are unknown, being one or more of about 300 compounds appearing in the GC-MS (gas chromatograph-mass spectrometer). In the GC-MS, aroma is also digitized and made suitable for downloading into the database. The advantage of this needs to be considered now that Mrs. dela Cruz may not be available with her incomparable skill in this area. Certainly, as with other major quality traits and their increasing importance to world trade in rice, a research capability in aroma would undoubtedly pay dividends if IRRI is to maintain its preeminence in rice research. A gas chromatograph should be used to develop the 2AP method for quantitative assessment of aroma. A little-used but suitable gas chromatograph may already be available at IRRI, and, if so, it should be moved to the grain quality laboratory. It would not be practicable to operate it efficiently in another building.

Oryzinol and bran lipids. These, along with tocopherols and carotenoids, are easily if not rapidly measured by high-pressure liquid chromatography and methods for these should be investigated and made available to breeders. As a first service, the screening of all existing commercial varieties and advanced lines should be aimed for.

Laboratory rice mill. The laboratory visits abroad emphasized the importance of milling and created the conviction that the quality and value of all the measurements mentioned above are immediately dependent on the quality of the laboratory mill and the extent to which it is able to simulate the performance of a good commercial mill in the hands of a competent miller. Without that, all the above measurements are of questionable value, and a major effort should be put into the question of the mill design and its maintenance, using the good offices and experience of the head of the Agricultural Engineering Unit. It will become increasingly important that absolute values for quality parameters be used and be interchangeable with those of quality laboratories and their published works everywhere. The Beaumont laboratory used a laboratory Udy cyclone mill, and they are happy with it, though it may not be useful for micronutrient work because of the poor condition of the metal surfaces. The RiceTec laboratory also used a Udy mill but with in-house modifications for dust control and throughput efficiency.

Funding possibilities

The CPB may fund some of these developments but the preferred strategy is corporate sponsorship from any of the major companies of Asia.

Rice grain quality evaluation procedures

Normita M. Dela Cruz[1]

Grain quality in rice is very difficult to define with precision as preferences for quality vary from country to country. Few people realize its complexity and the various quality components involved. The concept of quality varies according to the preparations for which grains are to be used. Although some of the quality characteristics desired by growers, millers, and consumers may be the same, each may place different emphasis on various quality characteristics. For instance, the miller's basis of quality is dependent upon total recovery and the proportion of head rice and brokens on milling. Consumers base their concept of quality on grain appearance, grain size and shape, behavior upon cooking, and the taste, tenderness, and flavor of cooked rice.

Cooking quality preferences vary in the different countries of the world (Azeez and Shafi 1966). Rice is one cereal that is consumed mainly as whole milled and boiled grain. The desired properties may vary from one ethnic group or geographical region to another and may vary from country to country (Juliano et al 1964). Quality in rice may therefore be considered from the viewpoint of milling quality; grain size, shape, and appearance; and cooking characteristics.

Milling quality

Milling yield is one of the most important criteria of rice quality, especially from a marketing standpoint. A variety should possess a high turnout of whole-grain (head) rice and total milled rice (Webb 1985).

Milling yield of rough rice is the estimate of the quantity of head rice and total milled rice that can be produced from a unit of rough rice. It is generally expressed as a percentage (Khush et al 1979). Thus, the milling quality of rice may be defined as the ability of rice grain to stand milling and polishing without undue breakage so as to yield the greatest amount of total recovery and the highest proportion of head rice to brokens.

The milling process generally consists of five fundamental operations:

1. Cleaning the rough rice to remove leaves, rice stems, and other foreign matter.
2. Shelling or dehulling the cleaned rice to remove the hulls.
3. Cleaning the brown rice to remove the hulls not totally removed by dehulling.
4. Milling or polishing the brown rice.
5. Separating whole grains from broken kernels.

Milling yield determination

Duplicate 125-g rough rice samples are used for milling determinations. Moisture content for these samples should be in the range of 12–14%. A Motomco or Steinlite moisture meter usually determines the moisture content.

Rough rice samples are dehulled with a Satake laboratory sheller. The sample is poured into the hopper. Samples with many partially filled grains of reduced thickness usually require two passes. The resulting brown rice is weighed to obtain the percentage of hulls.

The brown rice is milled in a McGill mill number 2 (Adair 1952) for 30 seconds with the prescribed added weight (680 g) on the pressure cover, followed by a second milling for another 30 seconds without the weight. The fraction removed may be considered bran in the first milling and that after the second milling polish. The milled rice sample is collected in a jar or thick paper bag and sealed immediately. The rice is allowed to cool before weighing. This procedure minimizes grain cracking during cooling. The weight of the total milled rice is recorded.

Whole grains (head rice) are separated from the total milled rice with a rice-sizing device. The

[1]Senior associate scientist, International Rice Research Institute, Los Baños, Laguna, Philippines.

indentation size of the device depends on the grain size. Two plates of the same size are used for each run. The resulting head rice is weighed. Samples should be at least 3 to 4 months old after harvest to obtain reliable head-rice yields.

Calculations

The percentage of hulls of rough rice is calculated as follows:

$$\text{Brown rice (\%)} = \frac{\text{Weight of brown rice}}{\text{Weight of rough rice}} \times 100$$

$$\text{Hull (\%)} = \frac{\text{Weight of hulls}}{\text{Weight of rough rice}} \times 100$$

$$\text{Total milled rice (\%)} = \frac{\text{Weight of total milled rice}}{\text{Weight of rough rice}} \times 100$$

$$\text{Head rice (\%)} = \frac{\text{Weight of head rice}}{\text{Weight of rough rice}} \times 100$$

$$\text{Degree of milling (\%)} = \frac{\text{Weight of total milled rice}}{\text{Weight of brown rice}} \times 100$$

The proportions of the various components vary according to the method of milling used and the variety of rice. Generally, the hulls form 20% to 22% of the rough rice, although variation of 18% to 26% has been recorded. Bran and embryos constitute another 8% to 10%. Thus, from a given sample of rough rice, about 70% milled rice is obtained. The proportion of whole grains is known as head rice recovery and is expressed as percentage of rough rice. Thus, if from a sample of 100 g of rough rice, 70 g of milled rice is obtained and 20 g of this is broken, head rice recovery is 50%. The head-rice recovery may vary from as low as 25% to as high as 65% (Khush et al 1979).

Grain size, shape, and appearance

The appearance of milled rice is important to consumers. Thus, grain size and shape are among the first criteria of rice quality that breeders consider in developing new varieties for release for commercial production (Adair et al 1966). A length:breadth ratio (L/B) from 2.5 to 3.0 has been considered widely acceptable as long as the length is more than 6 mm (Kaul 1970). Consumers prefer rice with a translucent endosperm and pay a premium price for it, even though opacity disappears during cooking and does not alter eating quality.

Preferences for grain size and shape vary from one group of consumers to another. Some ethnic groups prefer short bold grains, some prefer medium-long grains, and others highly prize long slender grains. In general, long grains are preferred in the Indian subcontinent, but, in Southeast Asia, the demand is for medium to medium-long rice. In temperate areas, short-grain varieties are prevalent. There is a strong demand for long-grain rice on the international market.

Grain appearance depends upon the size and shape of the kernel and translucency and chalkiness of the grain. The physical dimensions of rice kernels are of vital interest to those engaged in the many facets of the rice industry. Rice varieties may be objectively classified into grain-type categories based upon two physical parameters: length and shape. Length is a measure of the rice kernel in its greatest dimension. While grain size and shape can be visually classified, more exact measurements are needed for a more critical comparison of varieties.

Standards for evaluating grain length and shape of breeding materials vary among countries and marketing areas. Below is a useful classification for a routine breeding evaluation.

Size classification:

Scale	Size category	Length in mm
1	Very long	More than 7.50
3	Long	6.61 to 7.50
5	Medium or intermediate	5.51 to 6.60
7	Short	Less than 5.50

Shape classification:

Scale	Shape	Length/width ratio
1	Slender	More than 3.0
5	Medium	2.1 to 3.0
9	Bold	2.0 or less than 2.0

Grain appearance is also largely determined by endosperm opacity, the amount of chalkiness on the dorsal side of the grain (white belly), on the ventral side (white back), or in the center (white center), and the condition of the “eye.” In some varieties, the grain tends to break more frequently at the “eye” or pit left by the embryo when it is milled. Rice samples with damaged eyes have a poor appearance and low market value. Similarly, the greater the chalkiness, the lower the market acceptability. The starch granules in the chalky areas are less densely packed vis-à-vis translucent areas. Therefore, the chalky areas are not as hard as the translucent areas and the grains with chalkiness are more prone to breakage during milling.

Milled grains are visually scored for the presence or absence of white belly, white back, white center, degree of translucency, and breakage at the basal-ventral end of the grain referred to as the condition of the eye. The above determinations are scored on a 0 to 9 scale according to increasing intensity.

The following scale is used for classifying endosperm chalkiness of milled rice:

Scale	% area with chalkiness
0	None
1	Less than 10%
5	10% to 20%
9	More than 20%

Cooking and eating characteristics

Cooking and eating characteristics are largely determined by the properties of the starch that makes up 90% of milled rice. Gelatinization temperature, amylose content, and gel consistency are the important starch properties that influence cooking and eating characteristics.

Gelatinization temperature (GT)

The gelatinization temperature of the endosperm starch, a useful test of cooking quality, refers to the cooking temperature at which water is absorbed and the starch granules swell irreversibly in hot water with a simultaneous loss of crystallinity and birefringence. Final GT ranges from 55 to 79 °C. Environmental conditions such as temperature during grain development influence GT. A high ambient temperature during grain ripening results in starch with a higher GT (dela Cruz et al 1989). The GT of rice varieties may be classified as low (55 to 69 °C), intermediate (70 to 74 °C), and high (>74 °C).

The physical cooking properties of rice are more closely related to the gelatinization temperature than the amylose content of the starch. Rice with a high GT becomes excessively soft and tends to disintegrate when overcooked. Under standard cooking procedures, rice with a high GT tends to remain undercooked. Rice varieties with a high GT require more water and time to cook than those with a low or intermediate GT. Thus, GT correlates positively with the time required to cook rice.

An estimate of the gelatinization temperature is indexed by the alkali digestion test (Little et al 1958). It is measured by the alkali spreading value. The degree of spreading value of individual milled rice kernels in a weak alkali solution (1.7% KOH) is very closely correlated with GT. Rice with a low GT disintegrates completely, whereas rice with an intermediate GT shows only partial disintegration. Rice with a high GT remains largely unaffected in the alkali solution. In a breeding program, the alkali digestion technique is used extensively for estimating GT.

Although the gelatinization temperature and cooking time of milled rice are positively correlated (Juliano 1967), GT does not correlate with the texture of cooked rice (IRRI 1968). Gelatinization temperature is not associated with other important plant or grain traits except for certain useful correlations with amylose content (Jennings et al 1979). Varieties with a high GT generally have a low amylose content. No varieties are known with a high GT and high amylose content.

A second correlation concerns intermediate GT, which apparently has never been combined with low amylose content. All varieties that have an intermediate GT are either intermediate or high in amylose content.

The low-gelatinizing class has no strict association with low, intermediate, and high amylose contents. Low GT is readily recombined with the three amylose levels.

Steps in determining the rice GT (alkali digestion test):

A duplicate set of six whole-milled kernels without cracks is selected and placed in a plastic box ($5 \times 5 \times 2.5$ cm). Half kernels can be used in the absence of whole kernels. Ten mL of 1.7% (0.3035 M) potassium hydroxide (KOH) solution is added. The samples are arranged to provide enough space between kernels to allow for spreading. The boxes are covered and incubated for 23 h in a 30 °C oven. Samples can be placed outside in the absence of an oven if the ambient temperature is almost the same as what is required. Starchy endosperm is rated visually based on a 7-point numerical spreading scale (Table 1). Standard check varieties of high, intermediate, and low gelatinization types of rice are included for every test.

Amylose content (AC)

Many of the cooking and eating characteristics of milled rice are influenced by the ratio of two kinds of starches, amylose and amylopectin, in the rice grain (Sanjiva Rao et al 1952). Amylose is the linear fraction of starch in the nonglutinous varieties, whereas amylopectin, the branched fraction, makes up the remainder of the starch. Amylose content correlates negatively with taste panel scores for cohesiveness, tenderness, color, and gloss of boiled

rice. Amylose is almost absent from waxy (glutinous) rice. Such rice does not expand in volume, is glossy and sticky, and remains firm when cooked. This rice is the staple food of people in northern and northeastern Thailand and Lao PDR.

A great majority of the rice from Vietnam, Thailand, Myanmar, and the Indian subcontinent has a high amylose content. This rice shows a high volume expansion (not necessarily elongation) and a high degree of flakiness. It cooks dry, is less tender, and becomes hard upon cooling. Low-amylose rice cooks moist and sticky. All of the japonica varieties of temperate regions have a low AC. Varieties grown in the Philippines, Malaysia, and Indonesia have an intermediate AC content. Intermediate-amylose rice cooks moist and tender and does not become hard upon cooling. A survey conducted by IRRI shows that the most preferred varieties in the areas where high-amylose rice is generally grown have intermediate amylose.

Rice varieties are grouped on the basis of their AC into waxy (0–2%), very low (3–9%), low (10–19%), intermediate (20–25%), and high (>25%) (Kumar and Khush 1986).

Intermediate-amylose rice is the preferred type in most rice-growing areas of the world, except where low-amylose japonicas are grown. Therefore, the development of improved germplasm with intermediate AC should be taken into consideration in the grain quality improvement program.

The simplified procedures (AutoAnalyzer and manual method) of Juliano (1971) are used for the AC analysis:

Manual method:
Twenty whole-milled rice kernels are ground in a Udy cyclone mill (sieve mesh size 60), 100 mg of rice powder is put into a 100-mL volumetric flask, and 1 mL of 95% ethanol and 9 mL of 1 M sodium hydroxide are added. The contents are heated in a boiling water bath to gelatinize the starch. After cooling for 1 h, distilled water is added and the contents are mixed well. For each set of samples run, low-, intermediate-, and high-amylose standard varieties are included to serve as checks.

Five mL of the starch solution is put in a 100-mL volumetric flask with a pipette. One mL of 1 M acetic acid and 2 mL of iodine solution (0.2 g iodine and 2.0 g potassium iodide in 100 mL aqueous solution) are added and the volume is made up with distilled water. The contents are shaken well and left to stand for 20 min. Absorbance of the solution is measured at 620 mm with a spectrophotometer such as the Bausch and Lomb Spectronic 20. Amylose content is determined by using a conversion factor and the results are expressed on a dry weight basis. The moisture content of the samples is essentially constant and need not be determined if the relative humidity and temperature of the laboratory are controlled.

For the standard curve, 40 mg of potato amylose (Sigma Chemical Co. or Stein Hall and Co., Inc.) of known moisture content are wetted with 1 mL of ethanol and 9 mL of 1 M sodium hydroxide, heated for 5–10 min in a boiling water bath, cooled, and made up to volume. Solution (1, 2, 3, 4, 5 mL) is placed with a pipette in 100-mL volumetric flasks. The solution is acidified with 1 M acetic acid (0.2, 0.4, 0.6, 0.8, and 1.0 mL, respectively) and treated as above. The absorbance values are plotted at 620 mμ against the concentration of anhydrous amylose (mg) and the conversion factor is determined. The dilution factor of 20 for the samples is included in the conversion factor.

Starch solutions (100 mg 100 mL^{-1}) prepared by the manual method can be automatically analyzed with an AutoAnalyzer. Portions of the starch solutions are transferred into the sample cups of the AutoAnalyzer and run at 70 samples h^{-1}. A standard curve is made using rice samples of predetermined amylose content by the simplified manual method at 620 mμ. A fresh working iodine solution (1.0 mL 1 M acetic acid and 3.0 mL stock iodine solution diluted to 100 mL) is prepared daily. Results are expressed as percent apparent AC in milled rice weight. Apparent AC is used since, at an amylose concentration of more than 25%, amylopectin shows increased iodine binding instead of amylose (Perez and Juliano 1978). These authors proposed a constant factor of 2.0% to convert apparent AC to absolute AC based on methanol defatting.

Gel consistency (GC) test

A rapid, simple test, complementary to the test for amylose content, was developed based on the consistency of a cold 4.4% milled rice paste in 0.2 M KOH (Cagampang et al 1973). GC is measured by the length of the cold gel in the culture tube held horizontally for 0.5 to 1 h.

Varietal differences in GC exist among varieties of similar amylose content (>25%). The GC of rice with less than 24% amylose is usually soft. The GC test is based on the consistency of the rice paste and differentiates among varieties with high AC. The test separates high-amylose rice into three categories:

1. Very flaky rice with hard GC (length of gel, 40 mm or less).

2. Flaky rice with medium GC (length of gel, 41 to 60 mm).
3. Soft rice with soft GC (length of gel, more than 61 mm).

Medium or soft GC is preferred over hard GC in almost all regions of Asia.

Steps for the gel consistency test:
Make certain that all the samples are stored in the same room for at least 2 days so that the moisture content is similar. Place 10 whole-milled rice grains in the Wig-L-Bug amalgamator and grind for 40 sec to give a fine flour (100 mesh).

One hundred mg (±1 mg at 12% moisture) of powder is weighed in duplicate into the culture tubes (13×100 mm). Hard, medium, and soft gel rice varieties are included as checks. Ethyl alcohol (0.2 mL of 95%) containing 0.025% thymol blue (alcohol prevents clumping of the powder during alkali gelatinization, while thymol blue imparts color to the alkali paste to make the gel front easier to read) is added and 2.0 mL of 0.2 M KOH is added with a pipette. The contents are mixed using a Vortex Genie mixer with speed set at 6. The test tubes are covered with glass marbles (to prevent steam loss and to reflux the samples). The samples are cooked in a vigorously boiling water bath for 8 min, making sure that the tube contents reach 2/3 the height of the tube. The test tubes are removed from the water bath and left to stand at room temperature for 5 min. The tubes are cooled in an ice-water bath for 20 min and laid horizontally on a laboratory table lined with millimeter graphing paper. The total length of the gel is measured in mm from the bottom of the tube to the gel front.

Grain elongation

Some varieties expand more in size than others upon cooking. Lengthwise expansion without an increase in girth is considered a highly desirable trait in some high-quality rice. Basmati rice of India and Pakistan, Bahra of Afghanistan, Domsiah of Iran, Bashful of Bangladesh, and D25-4 from Myanmar elongate 100% upon cooking.

This characteristic is being incorporated into improved germplasm. Evaluation for this characteristic commences with the F_3 generation. Only the lines originating from crosses involving the parents having this trait are evaluated. Grain elongation appears to be a quantitative trait. Preliminary experience indicates that only a few hybrid lines approach the parents in degree of elongation. The method of Azeez and Shafi (1966) is used for evaluating the degree of elongation.

Procedure
The elongation test consists of measuring 25 whole-milled kernels that are soaked in 20 mL of distilled water for 30 min. The samples are placed in a water bath and the temperature is maintained at 98 °C for 10 min. The cooked rice is transferred to a petri dish lined with filter paper. Ten cooked whole grains are selected and measured in a photographic enlarger. The proportionate elongation is the ratio of the average length of cooked rice grains to the average length of raw rice grains.

Aroma

Scented or aromatic rice is preferred in some areas of Asia and draws a premium price in certain specialty markets. Middle East consumers prefer rice with a strong aroma. They believe that rice without a distinctive aroma is like food without salt. For consumers in Europe, a trace of aroma is an objectionable trait because for them any scent signals spoilage and contamination (Efferson 1985).

Most of the high-quality preferred varieties in the major rice-growing countries are aromatic. Examples are the Basmati rice of India and Pakistan, Dulhabhog of Bangladesh, Khao Dawk Mali and Leuang Hawn of Thailand, Azucena and Milfor of the Philippines, Rojolele of Indonesia, Sadri varieties of Iran, Barah of Afghanistan, and Della of the United States. Long slender grains, intermediate AC, intermediate gelatinization temperature, high elongation ratio, and strong aroma characterize these varieties.

A simple laboratory technique to evaluate rice for the presence of aroma was developed at IRRI in 1971. One gram of freshly harvested milled rice is placed into a centrifuge tube (50 mL, round bottom) and 20 mL of distilled water is added. The tubes are then covered with aluminum foil. The samples are placed in a boiling water bath for 10 min. The cooked samples are allowed to cool and the presence of aroma is determined for every sample. Brown rice may also be used with the cooking time increased to 30 min. The samples are scored as strongly aromatic, moderately aromatic, slightly aromatic, and nonaromatic. A strongly scented variety is used as a check for comparison.

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Table 1. Numerical scale for scoring gelatinization temperature.

Score	Spreading	Alkali digestion	Gelatinization temperature
1	Kernel not affected	Low	High
2	Kernel swollen	Low	High
3	Kernel swollen; collar complete or narrow	Low or intermediate	High-intermediate
4	Kernel swollen; collar complete and wide	Intermediate	Intermediate
5	Kernel split or segregated; collar complete and wide	Intermediate	Intermediate
6	Kernel dispersed; merging with collar	High	Low
7	Kernel completely dispersed and intermingled	High	Low

Table 2. Numbers of samples run through the various tests of the grain quality laboratory of the Plant Breeding, Genetics, and Biochemistry Division in the last three years (Normita dela Cruz, Oct. 2002). I have no record of rainfed, upland, hybrid, and Korean materials; thus, they were not included in the computation.

Nursery[a]	Year	Grain quality traits							
		Amylose content	Gel. temp. (alkali test)	Gel consistency	Aroma	Grain elong. ratio	Size, shape, and appearance	Milling yield	Grand total
Jan. PN	2000	4,668	5,109		564				
May PN	2000	5,020	8,691		2,403	2,403			
July PN	2000	3,794	7,278		126				
Nov. PN	2000	6,568	8,843		1,258	1,258			
HB (DS)	2000	162	162	162			162		
RYT (DS)	2000	400	400	400			400	400	
OYT (DS)	2000	355	355		186	186	355		
HB (WS)	2000	162	162	162			162		
RYT (WS)	2000	400	400	400			400	400	
OYT (WS)	2000	493	493		261		493		
Total		22,022	31,893	1,124	4,798	4,108	1,972	800	66,717
Jan. PN	2001	3,165	7,200		63				
May PN	2001	6,345	8,715		774				
July PN	2001	2,465	5,836						
Nov. PN	2001	5,208	7,723		1,359	609			
HB (DS)	2001	162	162	162	–		162		
RYT (DS)	2001	400	400	400	–		400	400	
OYT (DS)	2001	653	653		422	422	653		
HB (WS)	2001	162	162	162	–		162		
RYT (WS)	2001	450	450	450	–		450	450	
OYT (WS)	2001	707	707		369	369	707		
R OYT (WS)	2001	486	486				486		
Total		19,717	32,008	1,174	2,987	1,400	2,534	850	60,670
Jan. PN	2002	2,229	6,760						
May PN	2002	4,311	6,645		1,041	810			
July PN	2002	4,819	8,586						
Nov. PN	2002	–	–	–	–	–	–		
HB (DS)	2002	162	162	162			162		
RYT (DS)	2002	450	450	450			450	450	
OYT (DS)	2002	901	901		402	402	901		
HB (WS)	2002	162	162	162			162		
RYT (WS)	2002	350	350	350			350	350	
OYT (WS)	2002	376	376		376	376	376		
Total		13,760	24,392	1,124	1,819	1,588	2,401	800	45,884

[a]DS = dry season, WS = wet season, PN = pedigree nursery, HB = hybridization block, RYT = replicated yield trial, OYT = observational yield trial.

Apendix B. Summary of methods used at Yanco Agricultural Research Institute, New South Wales, Australia

The fully automated quality evaluation program of Dr. Melissa Fitzgerald is run from April to September, and then the laboratory is used for research the rest of the year.

Physical

Paddy (150 g) is dehulled, weighed, milled, and weighed again. Brokens are separated and then the whole white grain is weighed. The scales deliver the weight directly to a spreadsheet. On the spreadsheet, whole grain percent is calculated.

A subsample of the brown rice is used for length and width. An image is collected of 50 grains and then a computer program counts the grains and reports the length and width of each. We then calculate the average and the standard error (for uniformity).

A subsample of the white rice is used to measure chalk, which is also determined by image analysis. Color (yellowness index) is determined by a handheld spectrophotometer.

All the data from these four stations are written directly from the equipment onto a spreadsheet located on the Institute's common server.

Cooking quality

We conduct amylose analysis and measure gelatinization temperature on harvested F_4s. We determine amylose by molecular markers (Bergman et al 2001). The microsatellite is fabulous! The data are extremely illuminating and very, very easy. We also measure amylose by the iodine method but only on advanced lines.

Gelatinization temperature is measured by the differential scanning calorimeter

We use the relative viscosity analyzer (RVA) on advanced lines and we do gel texture by penetrometer on the same lines, in particular Japanese ones. We will be doing elongation for basmati by marker very soon once we are comfortable that the marker segregates with elongation.

Appendix C. List of method sheets provided by the U.S. Department of Agriculture-Agricultural Research Service, Beaumont, Texas

Amylose by autoanalyzer for milled rice
Amylograph for milled rice
Surface lipid for milled rice
Total lipid in brown or milled rice
RVA Series 3D—Standard operating procedure
Polymerase chain reaction protocol
Quantification of 2-acetyl 1-pyrroline by gas cromatograph with flame ionization detector (GC-FID)
Moisture determination—convection and vacuum oven methods
Phenolics in rice bran—methanol-HPLC
Dry matter loss for parboiled rice
Satake milling meter protocol
Protein analysis by Leco combustion analysis
Cooked grain elongation procedure
Minimum cooking time
Alkali test for white milled (head) rice

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About the author

Robin Graham took degrees in chemistry and soil science before joining the University of Adelaide in 1970 as a lecturer in agronomy and he has now held the position of professor in plant science there since 1995. He has been adjunct professor in soil and crop science at Cornell University since 1993. He is the scientific coordinator of the CGIAR Micronutrients Project, which is conducted in four CGIAR centers and 14 countries. He was a Fulbright Senior Professional Fellow in 1995 and 1998 and is a fellow of the Australian Institute of Agricultural Science and Technology. His research interests include micronutrients in the food chain; cultivar differences among staple-food crops in ability to extract micronutrients from soils, especially soils of low general fertility, and the genetics and molecular biology of such traits; cultivar differences in ability to load micronutrient into food grains, and the genetics and molecular biology of such traits; the role of adequate micronutrient nutrition in resistance to disease; product quality in general; and the sustainability of cropping systems. Nutrients of particular interest are iron, zinc, copper, manganese, selenium, iodine, and boron, as well as cereal-grain carotenoids. With his wife, Norma, he runs a grazing property an hour from Adelaide that is based on Hereford cattle and Wiltipol sheep. Other interests are sailing, diving, and fishing.

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IRRI
INTERNATIONAL RICE RESEARCH INSTITUTE
DAPO Box 7777, Metro Manila, Philippines