

Commentary

## Transforming Rice Breeding: Re-Designing the Irrigated Breeding Pipeline at the International Rice Research Institute (IRRI)

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### ABSTRACT

Rice breeding of inbred varieties in the public sector has hardly changed in decades. This has been a cause for concern given that the current rate of yield improvement from new varieties is not considered to be adequate to meet future global demands for rice. In this article, we describe major changes to the irrigated breeding program and former plant breeding division at the International Rice Research Institute (IRRI) headquarters by incorporating modern concepts in plant breeding and practices used in the private sector. These activities were conducted primarily within a five-year research program called “Transforming Rice Breeding” funded by the Bill and Melinda Gates Foundation. These changes were implemented with the specific objectives to increase the rate of genetic gain for yield and improve the effectiveness and efficiency of breeding operations. Key changes in the breeding program included implementing rapid generation advance, earlier multi-location trials, increased selection pressure for yield, an increase use of molecular breeding, and using variety product profiles. Regarding breeding operations, there was a concerted effort to streamline all processes and optimize logistics in order to make breeding like a “factory production line”. The Plant Breeding, Genetics and Biotechnology Division was re-organised into variety

### Open Access

Received: 15 June 2019

Accepted: 18 July 2019

Published: 22 July 2019

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pipelines and trait research teams, with cross-cutting services to support all functions. Considerable benefits and dramatic improvements in efficiency were realized, that are expected to lead to a higher rate of genetic gain for yield in rice. These experiences are also highly relevant to many public sector plant breeding programs, especially in developing countries.

**KEYWORDS:** genetic gain; rapid generation advance (RGA); breeding cycle; marker assisted selection (MAS); breeding efficiency

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## INTRODUCTION

Food security is widely considered to be one of the major global challenges in the 21st century. Rice is one of the major staple food crops and of fundamental importance in the context of global food security [1]. The development of new higher yielding rice varieties with enhanced disease resistance, tolerance to abiotic stresses, and specific quality characteristics needs to be part of the overall strategy towards food security. The demand for rice is projected to increase dramatically and therefore substantial yield improvements are required to meet this demand [1–4], with limitations to increase the rice production areas. This is a cause of concern given the rate of genetic gain for grain yield has been estimated to stagnate at approximately 1% per year [5], which will not be sufficient to meet projected demands for rice. Furthermore, adverse effects from climate extremes are exposing rice crops to more frequent abiotic stresses such as flooding, drought, salinity, heat stress [6], and higher night temperatures [7], limiting rice production in established areas and hampering expansion into new regions. These anticipated challenges have sparked a renewed interest in increasing the rate of genetic gain in the international plant breeding community [8].

It is noteworthy therefore, that rice breeding of inbred varieties in the public sector has remained largely unchanged for several decades. Current breeding schemes and operations have been well described in the seminal and highly influential text book on rice breeding by Jennings, Coffman and Kauffman published in 1979 [9]. For example, IRRI's irrigated breeding program has used a classical pedigree method since the establishment of the institute in the 1960s [10]. Due to the success of this program which led to the release of several key Green Revolution mega-varieties such as IR8, IR36 and IR64 in Asia [11], the pedigree breeding program has been adopted and implemented by many rice breeding programs across Asia. A recent global survey of rice breeders indicated that about 78% of breeders use the pedigree method as their main breeding method [12].

In recent years at IRRI, we critically evaluated and re-designed the irrigated breeding program and organizational structure in the Plant Breeding, Genetics and Biotechnology Division. The major driver was our

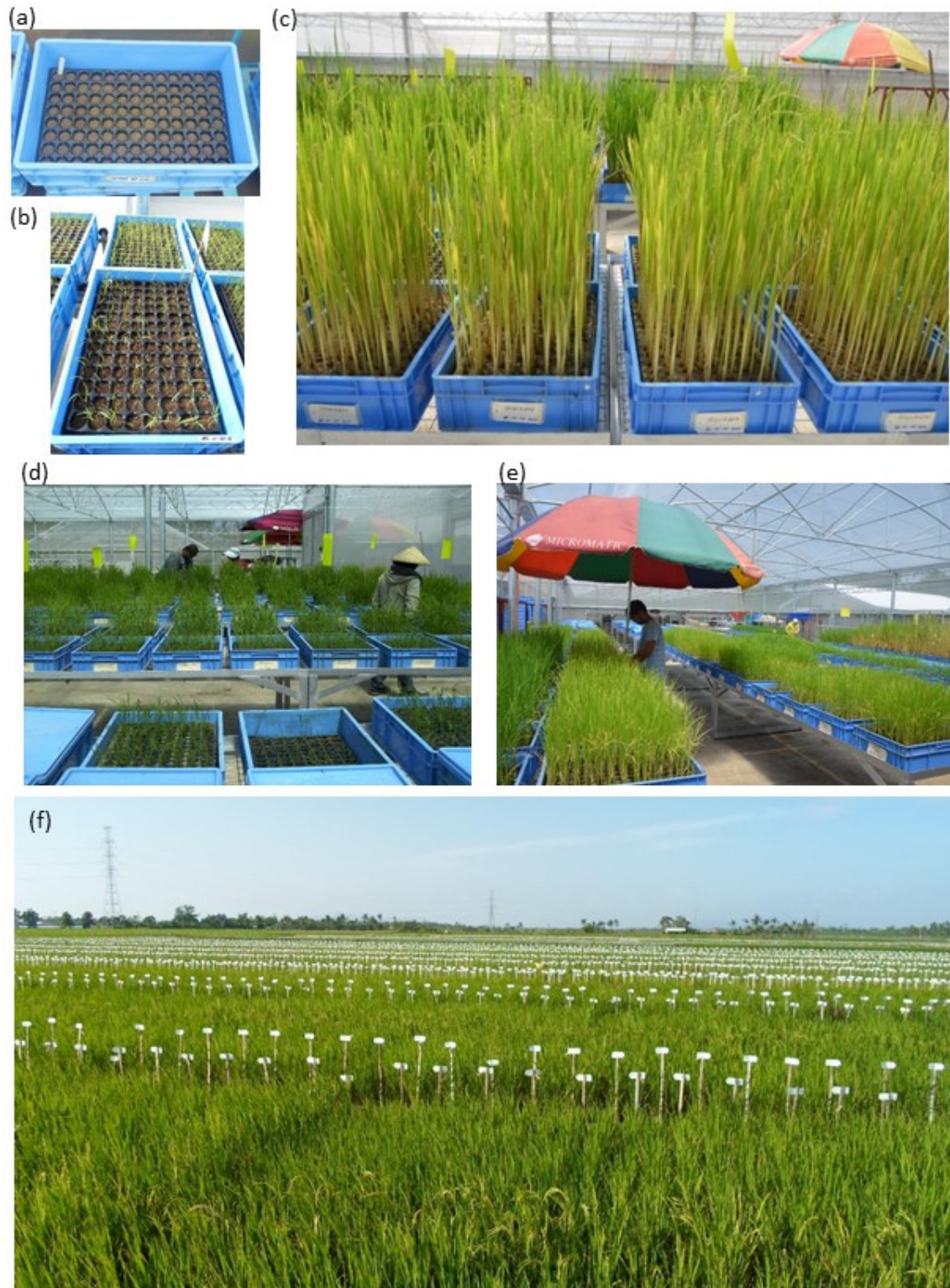
intention to increase the rate of genetic gain for yield. This process involved transforming our breeding activities into more efficient and effective market-driven and product-oriented variety development pipelines, having been influenced by high-performing breeding programs in other crops (especially in the private sector) around the world. As a result, our entire breeding paradigm changed and the irrigated breeding program developed into a “factory production line”. Since 2012 these activities gained considerable interest [13]. In this article, we briefly describe our experiences implementing major changes to the irrigated breeding program and report some technical details as part of a 5-year Bill and Melinda Gates Foundation (BMGF) funded program during 2013 to 2018 called “Transforming Rice Breeding (TRB)”. The article focuses on four main components of the TRB program: breeding methodology, product-oriented focus, efficiency, and breeding support.

## **BREEDING METHODOLOGY**

### **Faster Breeding**

The pedigree method is the most widely-used method in rice breeding in Asia, and has been used by the majority of national breeding programs for many decades. However, alternative breeding methods such as bulk-population or rapid generation advance (RGA) may reduce labour and considerable resources, and RGA in particular can accelerate the development of fixed lines substantially. Another limitation with the pedigree method is that selection usually occurs at a single location up to the  $F_6$  or  $F_7$  generation, limiting the ability to account for genotype by environment interactions.

A major change to the irrigated program was the implementation of single seed descent through RGA as the main breeding method [14]. RGA had been previously used at IRRI for rainfed breeding and for the development of mapping populations; our new objective was to implement it as the main breeding method for the irrigated program on a large scale. The basic principle is to fix lines (*i.e.*, obtain homozygosity) as quickly as possible before testing lines in field trials. In our experience, we were routinely able to produce 3.5 to 4 generations per year in the large-scale RGA system that we established (Figure 1), as opposed to the two generations per year advanced in the field. We also focused on advancing  $F_5$ -derived lines (*i.e.*,  $F_{5;6}$  panicle rows) into the field for seed increase, rather than at the  $F_7$  stage, enabling to test lines in small yield plots more quickly. Observations regarding uniformity were conducted at the  $F_6$  stage using panicle rows for seed increase prior to field trials. The use of RGA shifted the emphasis away from visual selection of single plants, which can be greatly influenced by environmental factors and breeder bias. There was a concerted effort to implement RGA for new  $F_2$  populations as quickly as possible in 2013. However, this required an



**Figure 1.** Overview of rapid generation advance (RGA) system. **(a)** seedling trays (8 rows  $\times$  13 columns) enclosed in plastic trays. **(b)** Early germination. **(c)** post-anthesis stage. **(d)** and **(e)** RGA facility from alternative angles showing different growth stages of rice populations. **(f)** Line stage testing (LST).  $F_{5:6}$  lines (panicle rows) are grown in the field for seed increase and selection. More than 10,000 lines were grown in a  $\sim 1.0$  ha field area.

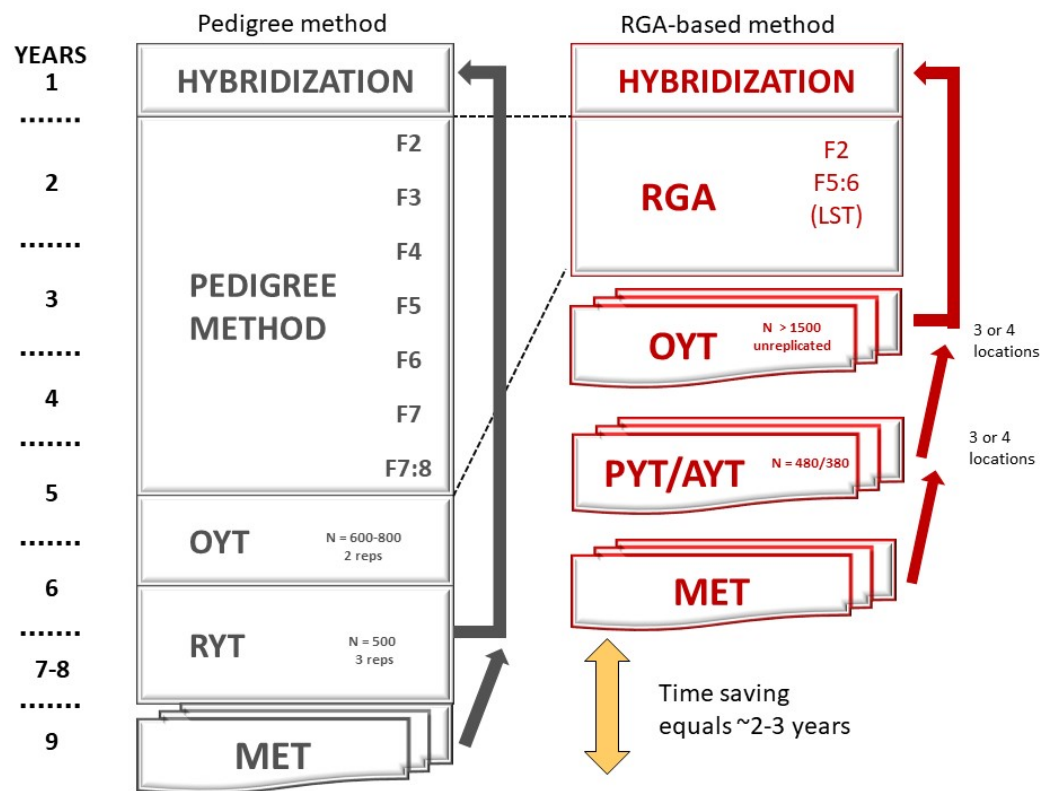
overlapping transition period during which some pedigree breeding continued for F<sub>3</sub>–F<sub>5</sub> material, and the bulk population method was temporarily adopted to reduce labour costs. Using the RGA method, we were able to develop an equivalent number of advanced breeding lines (*i.e.*, several thousand) as were generated using the pedigree method, but more quickly and cheaply. A review of literature on the use of RGA in rice and detailed descriptions of our methods and preliminary results was published in Collard *et al.* [14].

One of the main drivers for change to the irrigated breeding program was our intention to accelerate the rate of genetic gain ( $\Delta G$ ). One of the simplest and most effective methods to increase genetic gain is to shorten the breeding cycle by adopting quicker breeding methods (based on the “breeder’s equation”):

$$\Delta G = (i H^2 \sigma_P)/L \quad (1)$$

where  $i$  is the selection intensity,  $H^2$  is the trait heritability,  $\sigma_P$  is the square root of the phenotypic variance, and  $L$  is the length of the breeding cycle or generation interval [15–17]. An alternative version of this equation includes selection accuracy ( $r$ ) and specifies the additive genetic variation within the population [8]:  $\Delta G = (i r \sigma_A)/L$ .

The time savings by shortening the breeding cycle (*i.e.*, reducing  $L$  in the breeder’s equation) are directly proportional to increases in the rate of genetic gain, assuming all other factors remain constant [18]. Cutting the breeding cycle in half, effectively doubles rates of genetic gain. Shorter breeding cycles are also preferable in a rapidly changing climate as new recombinants are tested under conditions more similar to future production environments [18]. In our experience, we shortened the line development time and breeding cycle by at least 2 years using RGA, compared to the previously-used pedigree method (Figure 2). We also saved considerable resources, especially labour, and the dramatic reduction in workloads was greatly appreciated by all technical staff! These cost savings were extremely beneficial as they coincided with funding cuts to the Consultative Group for International Agricultural Research (CGIAR) centres that greatly reduced operational budgets for breeding. From an economic perspective, the quicker release of new improved varieties results in significant economic benefits to farmers and the industry [19].



**Figure 2.** Comparison of old (pedigree) and new (RGA-based) breeding schemes. The re-designed scheme was several years quicker and enabled shorter breeding cycles (indicated by arrows on the right side). For the RGA-based scheme, RGA-derived lines are first evaluated in the field as  $F_{5;6}$  lines at the LST stage. Yield testing in 4 to 5 m<sup>2</sup> plots begins at the OYT stage. Multi-location “pre-MET” trials were also a key novel feature of the new irrigated pipeline. Figure adapted from Bert Lenaerts (unpublished). Abbreviations: LST, line stage testing; OYT, observational yield trial; RYT, replicated yield trial; PYT, preliminary yield trial; AYT, advanced yield trial; MET, multi-environment testing program.

### Increasing Selection Pressure for Yield

First and foremost, we increased our emphasis and selection pressure for grain yield (*i.e.*, grain yield per se measured in plots). We ceased to impose selection during the segregating generations and limited the selection to fixed lines ( $F_{5;6}$ ). We became more stringent in the rejection of new test entries based on height, flowering time, and grain type and did not allow advancement to the yield trial unless these criteria were met nor did we allow re-testing of material that did not perform well in the initial yield trial. In other words, we highly scrutinized the selection of entries for inclusion in the next trial stage or for re-testing, and we were more aggressive in discarding material. The consistent inclusion of industry-standard checks was critical for this purpose. Any new test entries that were below par for yield compared to check varieties (*i.e.*, dominant varieties or popular varieties) sometimes referred to as “industry standards”), would never be superior to existing varieties even in future yield trials, and therefore would never be released.

We further increased the number of entries at the observational yield trial stage (OYT) from between 600–800 to about 1500 entries with plot sizes of 4 or 5 m<sup>2</sup>. Great care was taken to ensure trials were well managed, experimental design criteria were adhered to, and data collection was as accurate as possible. This included the introduction of barcoding to minimize risks of mislabeling and human error. By ensuring the trial data was of high quality, heritability ( $H^2$ ) was maximized and thus confidence in selections improved.

One novel aspect was the introduction of multi-location evaluation at the OYT and preliminary yield trial (PYT) stages. Previously, multi-location trials were only performed after replicated yield single-location trials on IRRI campus. We were able to expand the testing effort by coordinating and co-locating with the newly established IRRI multi-environment trial (MET) system which collaborated with national agricultural research and extension services (NARES) in target countries. Our target was to establish 4 locations (minimum of 3) (3 in the Philippines and 1 in Myanmar) for the OYT and PYT stages. These trials were located at research stations. In practice, the number of locations will be determined by resources and access to off-station field testing sites. Increasing the number of testing locations increases  $H^2$  by better sampling the environmental variance in the targeted population of environments (TPE) [16].  $H^2$  is calculated as follows:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{n_E} + \frac{\sigma_\epsilon^2}{n_E n_r}} \quad (2)$$

Where  $\sigma_G^2$  is the genotypic variance,  $\sigma_{GE}^2$  is the genotype-by-environment interaction variance,  $\sigma_\epsilon^2$  is the error variance,  $n_E$  is the number of locations and  $n_r$  is the number of replications within a trial [16,20]. Importantly, some of these locations were deliberately chosen to be disease “hotspots” to enforce selection pressure for critical biotic traits and eliminate susceptible lines.

For data analysis, a two-stage model was employed where in stage 1 entries were considered as fixed effects and adjusted based on spatial analysis. Spatially adjusted best linear unbiased estimates (BLUES) for each line at each location are then used in stage 2 where genotypes are considered random effects and environmental main effects and genotype × environment interaction variance components are calculated. The resulting best linear unbiased predictors (BLUPs) for each genotype are then used for selection [21]. A partially replicated (p-rep) design was used for the PYT stage, which has been shown to be an effective design for multi-location trials [22]. From a practical point of view, these designs require less space and resources, but provide comparable data to fully replicated trials [22]. At the time, the most promising lines from the PYT were advanced to IRRI’s multi-environment testing (MET) program, but this was subsequently modified and became the advanced yield trial (AYT) stage.

## FOCUSING ON PRODUCTS

### Defining Breeding Targets—Product Profiles

“Variety product profiles” are commonly used in the private sector for defining target variety characteristics and establishing breeding objectives [8]. Importantly, they specify the target traits in comparison to check varieties providing ideal and realistic trait values or ranges, and define criteria for the replacement of varieties. In the private sector, product profiles are commonly designed using marketing information and business intelligence that are constantly updated based on annual sales and consumer feedback.

IRRI’s variety development pipelines were structured to ensure that breeding products developed meet the demands of rice producers and consumers. These product profiles were based on a large amount of data including surveys on consumer preference for rice traits in 24 key cities in South and Southeast Asia, focus group discussions with farmers and interviews with millers and other rice value chain actors to understand supply chain constraints and opportunities [23–26]. In addition, digital product profiling was conducted with farmers in some regions to elicit their preferences for varietal trait improvements through an interactive App which simulates the investment market for public rice breeding. Findings from such surveys and activities provide breeders guidance in setting priorities and incorporating improvements in grain quality traits in response to market demand along with agronomic and stress tolerance traits which are relevant to farmers.

In our experience, product profiles permitted numerous opportunities for discussion and feedback among breeders and scientists. Product profiles were used to ultimately determine and scrutinize the crossing and advancement decisions that were made. Importantly, product profiles enabled optimal resource allocation for breeding objectives. Examples of product profiles defined for South and Southeast Asia are shown in Table 1.

**Table 1.** Variety product profiles for SE Asia (developed about 2014). **(a)** Bangladesh (*boro* season). **(b)** Philippines. It’s noteworthy that product profiles undergo continuous improvement and are constantly updated.

Characteristic	Requirements	Benchmark	Breeding notes
<b>(a) Bangladesh (<i>boro</i> season)</b>			
Yield	Yield potential: minimum average 7.5 t/ha	>5% greater than BRRI dhan28/BRRI dhan29	
Agronomic traits	Duration: 130–150 days		Want shorter duration ( $\leq$ BRRI dhan28 or $\leq$ 130–140 days) for intensive cropping systems
	Height: 100–110 cm		



**Table 1.** *Cont.*

Characteristic	Requirements	Benchmark	Breeding notes
<b>(a) Bangladesh (<i>boro</i> season)</b>			
Disease resistance	Bacterial leaf blight Blast resistance	≥BRR1 dhan28	Key target genes for MAS: <i>xa5</i> , <i>Xa21</i>
Quality	Grain length: medium to long slender	BRR1 dhan28 for all attributes.	The government has recently introduced a requirement that the amylose content for new varieties must be >25% except for special purpose varieties.
	Amylose content: intermediate to high (>25%)		
	Gelatinization temperature = intermediate		
	Gel consistency = moderate		
Desirable (“win”) traits	Cold tolerance	BRR1 dhan36/BRR1 dhan55	
	Salinity tolerance	BRR1 dhan47/BRR1 dhan67	
	Enhanced BPH and GLH resistance		
<b>(b) Philippines</b>			
Yield	Average: >6 t/ha (7 t/ha dry season; 5 t/ha wet season)	>5% over NSIC Rc222/NSIC Rc216	
Agronomic traits	Duration: 110–120 days		Also want shorter duration ≤90–105 days like earlier varieties (≤PSB Rc10) for intensive cropping systems
	Height: 110–130 cm		

**Table 1.** *Cont.*

Characteristic	Requirements	Benchmark	Breeding notes
<b>(b) Philippines</b>			
Disease resistance	Bacterial leaf blight	≥NSIC Rc222	Key target genes for MAS: <i>xa5</i> , <i>Xa21</i>
	Blast		
	Tungro virus	≥NSIC Rc222/NSIC Rc238/“Matatag” varieties	Critical trait—should be screened at early stage. Select for <i>eIF4G</i> resistance allele by MAS
Quality	Grain length: medium to long slender		
	Amylose content: intermediate (21%–22%)	IR64/NSIC Rc160 = for all cooking and eating quality attributes	
	Gelatinization temperature = intermediate		
	Gel consistency = moderate		
Desirable (“win”) traits	Submergence tolerance	=PSB Rc68	Select for <i>Sub1</i> by MAS
	Enhanced BPH and GLH resistance	≥NSIC Rc216	

### Breeding Scale and Financial Management

A major change for IRRI’s breeding organization was the implementation of a full-cost recovery model (around 2013) for all internal trait screening services, the costs of which had previously been absorbed by the institute. Around the same time, IRRI experienced major reductions in funding leading to substantial financial pressure on breeding operations. Both factors instantly forced breeders to review their breeding schemes and develop the most cost effective strategies adapted to the reduced annual budgets. It became paramount for all IRRI breeders to carefully manage operational budgets. This is common in the private sector but less common in the public sector where detailed understanding and monitoring of operational funds is not always applied.

At the same time, we realized that the scale of the program was larger than could be comfortably managed by technical staff. Based on this observation and the need to carefully manage finances, we significantly optimized our breeding activities. Our field footprint was reduced by many hectares by abandoning the resource intensive pedigree method for

the more cost efficient RGA. This allowed us to not only consolidate and cost-optimize the breeding nursery but also permitted an initial field evaluation of lines at the post-RGA seed increase stage prior to the yield trial. This allowed for more careful population planning by starting with the target number of entries intended for inclusion in the MET program and extrapolating the required initial populations to meet this target working “backwards”. This ensured that the breeding pipeline was streamlined, because we only developed and advanced the amount of material that was required and could be comfortably managed from a practical perspective with the allocated resources (especially staff).

Another example was optimizing the number of crosses. Several hundred crosses would be routinely performed each season prior to TRB, however we reduced this number to about 100 crosses/year. Each cross was determined from a specific variety product profile and information regarding pedigree, genetic distance and segregation of known genes/QTLs were carefully considered. Crosses that would have a high chance of producing progeny that did not match profile targets (e.g., both parents with high amylose when low or medium was required) were not made. In other words, our paradigm regarding the number of crosses shifted from quantity to quality, which had been previously advocated by other rice breeders [27].

Implementation of these changes freed up time and resources that could be allocated to data analysis and interpretation, as well as crossing design and selections. Ultimately this provided more time to focus on breeding objectives and product delivery. Despite the reduction in the size of the irrigated breeding pipeline, we were still able to develop a large enough number of new improved breeding lines that were superior to check varieties.

## **BREEDING EFFICIENCY**

### **Data Collection and Management**

Breeding programs are large-scale logistical operations involving substantial amounts of data to be collected. While computerized systems for data collection are used in the majority of public and private sector breeding programs in developed countries, these are often lacking in the public sector programs of developing countries.

To facilitate efficient and accurate data collection, we implemented simple computerized systems to capture field data (*i.e.*, flowering time measurement), including electronic field books and barcodes. The barcoding of all plots and rows represented a landmark change in IRRI's breeding operations. The biggest gains in efficiency were achieved by using these systems to measure yield from plots after harvesting. Combine harvesters are routinely used in temperate cereals in developed countries, but due to challenging field conditions they are difficult to be utilized in tropical rice fields. We piloted the use of computerized stationary

threshers, which were able to rapidly process plot samples, and record accurate plot yields (with moisture tests) in the field. In order to increase efficiency and accuracy of rice breeding, computerized systems need to be adopted [13]. A simple cost-benefit analysis indicated that this system was far more time and cost efficient compared to the previous method.

Given the critical importance of data, an in-house breeding data management system called “Breeding-for-Results” (or B4R) was developed for the institute. This system continues to be developed at IRRI (<https://riceinfo.atlassian.net/wiki/spaces/ABOUT/pages/326172737/Breeding4Results>) and is targeted for expansion across the CGIAR system. This renewed emphasis on data represented another fundamental change in our breeding philosophy. In the past, plant breeding has been largely influenced by subjective factors and hence has always been considered an “art and science”. While we still acknowledge that there is a creative element in breeding; in our factory concept, breeding decisions were first and foremost based on data.

### **Streamlined Breeding Operations**

Overall, we made a concerted effort to improve and optimize the efficiency of all core breeding operations (*i.e.*, processes and logistics). Our “streamlining” process simply tried to reduce cost, aggregate demand, and subsequently increase the rate of genetic gain per dollar within our available fixed resources. This was an iterative process, which included a critical review of all activities at the end of each season with a view to incrementally optimize and improve processes and operations for the coming season. This concept is referred to as “continuous process improvement”.

In accordance with practices routinely used in factory production lines, we implemented strict metrics to monitor breeding operations. These metrics were divided into three general categories:

- Program metrics—used for monitoring the size of the core breeding operations (e.g., number of trials and plots, field area, number of crosses)
- Progress metrics—used for indicating improvement and genetic gain (e.g., how the best new lines compare with current check varieties)
- Financial metrics—used for monitoring expenditure and budgeting (e.g., cost of operations and activities)

We believe that the above approaches are ideal for implementing the “factory production line” paradigm and reflect best practices for modern rice breeding.

### **Molecular Breeding**

Molecular markers have been used increasingly in crop breeding over the last few decades [17]. There is enormous potential in rice because of the availability of extensive publicly available genomics resources [28,29].

Using DNA markers will continue to improve the effectiveness and efficiency of rice breeding by providing increased efficiency for trait screening and improving selection accuracy. Currently the most attractive targets for irrigated rice breeding are major genes/QTLs for disease resistance and abiotic stress tolerance. Major genes or QTLs for grain quality are also potential candidates for marker assisted selection (MAS) because the cost of marker genotyping may be significantly cheaper compared to routinely used chemical tests (e.g., involving “wet lab” evaluation).

Significant investments were made during the TRB program to optimize marker applications within the IRRI irrigated breeding program. These efforts aimed at decreasing costs, increasing throughput and improving turn-around time, while enhancing robustness as well as accuracy of marker calls. Sampling and processing was accomplished through the development and implementation of consistent barcoding and rapid sampling approaches and integration of a laboratory information management system (LIMS) to ensure “chain of custody” for each sample from the field to final marker scores. Furthermore, DNA extraction was outsourced to a commercial provider and we initiated a shift from leaf-disc based sampling to seed-based sampling [30]. Regarding trait-specific marker genotyping, this was accomplished by replacing Simple Sequence Repeat (SSR), Cleaved Amplified Polymorphic Sequences (CAPS) and insertion-deletion (InDel) markers with Single Nucleotide Polymorphism (SNP) markers. This allowed for a shift from laborious in-house gel-based assay systems to high throughput fluorescence-based platforms, both in-house and outsourced (first using the Fluidigm platform, and later using the KASP (Kompetitive Allele Specific PCR) system) [31]. The KASP method continues to increase in popularity and KASP markers are increasingly used in rice [32–34].

In addition to converting existing trait markers to KASP, we capitalized on the comprehensive existing SNP variation information accumulated from efforts such as the 3000 rice genomes data mining [35] and the Cornell\_6K\_Array\_Infinium\_Rice (C6AIR) genotyping [36] of IRRI breeding lines to develop a minimal 10 SNP quality control set with optimized allele frequencies to distinguish any two *indica* lines by at least one polymorphic SNP. This quality control set found widespread application in parental verification prior to crossing, hybridity verification post crossing, and line variation throughout the breeding and seed production pipelines, minimizing risk of misidentification or carry-over of accidental selfs in crossing and backcrossing schemes.

Our main principle was to use MAS for forward breeding of critical traits (for which reliable markers were available) as early as possible in the breeding scheme [8,29]. This ensures that poor breeding lines (*i.e.*, lines that did not inherit favorable alleles at these loci from their parent(s)) that will never become a new variety are eliminated as early as possible to maximize efficiency (*i.e.*, before extensive and costly field testing). Traits

must be prioritized accordingly based on the importance for the variety product profile. Consideration should be given to the benefits of using markers versus conventional screening (e.g., cost and time required for conventional phenotypic assay) and the resources (*i.e.*, for marker screening) available for MAS.

The RGA breeding method is highly suitable for integrating MAS because screening can be performed during line fixation and be used to reduce population sizes. Our scheme was also designed to over-lay genomic selection in order to further increase the rate of genetic gain [37]. Genomic selection (GS) (also called “genome-wide prediction”) is a recent molecular breeding method for crops that complements MAS and is more suitable for quantitative traits such as yield. The first GS pilot in rice using a breeding population of irrigated *indica* breeding lines was reported in 2015 [38]. This method requires medium-density genotyping (*i.e.*, hundreds to thousands of markers) and exploits novel statistical methods and powerful computational ability [39,40]. As the name implies, selection is based on whole-genome marker information called ‘genomic estimated breeding values’ (GEBVs) which is a single score for each trait, rather than on individual genes or QTLs [41].

For genomic selection to become economically viable, the total cost of genotyping must be cheaper than the combined cost of phenotyping for the respective traits of interest. In order to achieve this, a robust and cheap high throughput platform is necessary. Since neither array based systems such as the C6AIR [36] nor traditional genotyping-by-sequencing (GBS)[42] methods were ideal, we developed a custom amplicon-sequencing-based approach. The 1000 SNP Rice Custom Amplicon (1kRiCA) panel was optimized for maximum informativeness across the IRRI irrigated breeding program and delivers a low-density DNA fingerprint plus trait marker information at a fraction of the cost of comparable platforms [30]. Based on exciting recent developments in this area, GS will surely become more widely used in rice breeding in the near future.

#### **DIVISIONAL CHANGES: MAXIMISING BREEDING SUPPORT**

There were dramatic changes to the Plant Breeding, Genetics and Biotechnology division of IRRI from 2012 onwards. In brief, there was a concerted directive to make the IRRI’s largest division more efficient and effective. This was also in response to cuts to core funding provided to the institute. Fundamentally, the activities of the entire division were re-organized [43,44]. Several breeding pipelines were established based on region and ecosystem and cross-cutting breeding support teams were established to provide services to all breeding pipelines. Breeding pipelines were to be focused on product delivery rather than R & D; research teams (*i.e.*, trait research teams) were separated from breeding pipelines enabling them to service multiple pipelines. Services included: centralized crossing, trait screening, genotyping services, breeding informatics and seed production. Previously, all programs tried to do all

activities independently, except for crossing. This is usually referred to as a “matrix” structure in the private sector. It is worth pointing out that this was highly effective given the large size of the division (>400 staff). However, this configuration may not be as effective in smaller institutions.

One highlight was the creation of centralized services for abiotic stress tolerance (salinity, submergence and drought) and biotic (bacterial leaf blight (BLB) and blast) resistance screening operated with standardized protocols. Prior to transplanting in field plots, F<sub>5</sub> and advanced materials were routinely screened against blast in the centralized blast nursery. Efficiency of mass screening of lines is made with inoculation of large numbers of lines with the blast inoculum, covering with fine nets, and using an irrigation system to optimize infection, all of which is handled by a few technicians. Only resistant seedlings were transplanted in the field plots. A few plants in each line in the field plots were also inoculated for BLB screening, where leaves are clipped with application of the inoculum provided by pathologists. Data were immediately reported back and visual assessment was made by the pathologist. Breeding lines screened for salinity or submergence tolerance were selected through high-throughput genotyping using markers in the laboratory and candidate lines are further validated with centralized stress phenotyping in the phytotron for salinity and using a deep-water tank for 14 days submergence. Results were available two weeks post-treatment. Centralized drought screening was conducted under controlled environment conditions and needed continuous monitoring for soil moisture testing and timing of irrigation. The centralization of activities provided considerable improvements in efficiency (especially cost savings) by exploiting economies of scale.

### **CONCLUDING REMARKS**

Our activities spanning more than five years represented a major change to the irrigated rice breeding program at IRRI. Fundamentally, our entire philosophy on rice breeding changed with a new focused approach on product development. We focused on key elements of best practice plant breeding programs including genetic gain, efficiency and product development. However, we acknowledge that there are additional key components of plant breeding programs that must be integrated to achieve increases in genetic gain. These components include: sufficient additive genetic variation among elite lines, sufficiently large population sizes with defined selection intensities, improved accuracies through efficient phenotyping systems, and efforts to reduce cycle time further by recycling parents as soon as a reasonable prediction can be made. Furthermore, targeted and applied pre-breeding activities are required by the International Rice Breeding Community to develop new germplasm and to validate and implement new technologies. To achieve synergy and guarantee yield increases, agronomy research, and strong extension and seed systems are also essential.

The TRB program revived considerable interest in the concept of genetic gain in the international rice breeding community. We sincerely hope that this project will serve as a case study for other breeding programs, and that the concepts and components presented in this article can be implemented by NARES. Given that current rates of genetic gain in rice are not sufficient to meet future demands, the development and adoption of innovations and new technology in rice breeding will be essential to ensure the development of improved rice varieties which ultimately lead to sufficient global rice production.

#### **AUTHOR CONTRIBUTIONS**

All authors were actively involved in the TRB program and contributed to the key ideas and writing of this paper.

#### **CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

#### **FUNDING**

This research was funded by the Bill and Melinda Gates Foundation (BMGF), Global Development Grant Number OPP1076488.

#### **ACKNOWLEDGMENTS**

First and foremost, we thank Dr. Gary Atlin whose vision inspired global interest in improving genetic gain in crops. Special gratitude goes to the irrigated team at IRRI who implemented changes in the irrigated pipeline: Vit Lopena, Rhylyx Mendoza, Ron Santilices, Joseph Beredo and Holden Verdeprado; and to Richievel Ibanez, Marlyn Rala and Vina Alvarez for administrative support. Special thanks to various teams including the Market Analysis Cluster at IRRI (Matty Demont, Marie Claire Custodio, Maria Lourdes Velasco, Jhoanne Ynion and Neale Paguirigan) and the database team led by Marko Karkkainen. We thank our many IRRI colleagues who were involved in the planning or implementation of various components of the TRB program: M.S. Ramesha, Alexis Ndayiragije, Rosemary Murori, Maria Ymber Reveche, Joong Hyoun Chin, Casiana “Nollie” Vera-Cruz, Bo Zhou, Ricardo Oliva and many others. For assistance and collaboration of multi-location trials, we thank Ed Redoña, Varoy Pamplona, Mary Jean Du, R.K. Singh and Norvie Manigbas (PhilRice). For valuable discussion, we thank Russell Reinke, Guoyou Ye and Nese Sreenivasulu; for collaboration on RGA, we thank Bert Lenaerts (Hasselt University). Last but certainly not least, we thank Dr. Achim Dobermann (former Deputy Director General—Research at IRRI) for his unwavering support and bold vision for modernizing rice breeding.



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How to cite this article:

Collard BCY, Gregorio GB, Thomson MJ, Islam MR, Vergara GV, Laborte AG, et al. Transforming Rice Breeding: Re-Designing the Irrigated Breeding Pipeline at the International Rice Research Institute (IRRI). *Crop Breed Genet Genom.* 2019;1:e190008. <https://doi.org/10.20900/cbagg20190008>