Rice Breeding

OPERATIONS MANUAL

of the Philippine Rice Research Institute

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

We want the Filipino rice farmers and the Philippine rice industry to be competitive through research for development work in our central and seven branch stations, including our satellite stations, coordinating with a network that comprises 60 members strategically located nationwide.

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FOREWORD

An operations manual is an indispensable guide for the research and development workers who aspire for excellence in performing their organizational tasks with improved accuracy, consistency, and effectiveness. An operations manual also promotes diligence, efficiency, and safety in the workplace. This highly specialized manual on our rice breeding operations here at Philippine Rice Research Institute (PhilRice) is a welcome material to support our continuous improvement agenda to remain responsive to the needs of our stakeholders and the general public that we serve.

As a technical guide particularly for the human resources in our Plant Breeding and Biotechnology Division (PBBD), this operations manual covers the basic aspects of breeding rice varieties and the associated activities in the field, screenhouses, or laboratories. These procedures also apply to other self-pollinated crops and their seeds. This manual, however, is not about optimization of breeding schemes towards particular product profiles or target market segments in the industry. Rather, our plant breeders and their highly professional teams are encouraged to advance their knowledge of implementing breeding projects.

It is with pleasure to mention that the breeding pipelines developed by the PBBD over the years have not failed to produce commercial varieties that gainfully engaged our farmers, their households, and the value chain players in their enterprises. Our varieties continue to be part of the unprecedented scaling of high-quality certified inbred seeds used by our farmers under the Rice Competitiveness Enhancement Fund (RCEF) and the National Rice Program (NRP). There is endless satisfaction for plant breeders to know this – that their methods and toils under the sun have not been in vain.

JOHN C. DE LEON, Ph.D.
Executive Director
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ACKNOWLEDGMENT

Numerous workshops and meetings were conducted to produce a workable manual not only for the rice researchers but also for the students, practicing plant breeders, farmers, university faculty, administrators, and stakeholders. Finally, the first edition of the rice breeding operations manual of PhilRice, which was conceptualized many years ago, is now published. We would like to express our sincere gratitude to the authors for sharing their expertise. Most of the authors in this manual are already retired or were transferred to other agencies; but the wisdom they contributed here now serves as a legacy to the new breed of researchers.

This manual serves as step-by-step guide to junior breeders and practicing rice breeders in applying its principles. Everything on rice breeding — from the seed preparation, field layout, establishing breeding nurseries, emasculation and pollination, selection, harvest, post-harvest, until description of each trait and ecosystem, are meticulously described in each chapter.

Special thanks to Dr. Tomas M. Masajo †, PhilRice senior consultant, who spearheaded and led the team in writing the breeding procedures and developing this manual. This is wholeheartedly dedicated in his honor.

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EXECUTIVE SUMMARY

A brief history of the rice improvement efforts in the Philippines emphasized the need of increasing rice productivity through new technologies and innovations in breeding. The rice landscape has significantly accelerated the rhythm of varietal improvement from the most primitive selection of naturally occurring variants using the traditional cultivars to hybridization process. Improving rice varieties at PhilRice has defined the breeding themes, techniques, strategies, and achievements as influenced by local and international partnerships and collaborators from 1985 to 2021. The impact of these new breeding methods in farmers' fields has progressed to help meet the challenges related to food production and environmental sustainability.

Components of the chapters explicitly described the step-by-step procedures that can be simply understood and easily followed by rice breeders. Standard operations starting from the required resources, facilities, and personnel until creating or adding genetic variability in breeding populations are thoroughly discussed and presented. The rice plant, which is the primary material, is carefully described and labelled so that all the processes involved in its manipulation can be well understood and carried out. Each growth and development stage were also fully described to determine specific requirements needed by plants and achieve greater productivity.

Breeding methods, selection, and setting the objectives in each trait development were discussed. Identify the traits to improve is important as these affects yield. Grain yield varies depending on the rice ecosystem and other factors. As such, crossing work is one of the most important components in variety development. Each step was meticulously explained so that true F1 seeds can be produced. The breeding nurseries set-up and management including the layout and data required were also presented as a guide on the efficient selection and handling of breeding materials.

Aside from transplanted rice method, breeding for direct seeding was also specified including selection of the most important traits required for direct-seeded rice. These traits include seedling vigor, anaerobic germination, and lodging tolerance. Yield, pest resistance, and grain quality are also considered. These can be selected through the modern biotechnology approach like marker-assisted selection (MAS).

The package of variety development process could not be completed without the screening requirements for biotic stresses such as disease and insect pest resistance. The authors specifically described each selection process involved in the screening of destructive and emerging diseases and insect pests.

Screening methods for cool temperature tolerance, high temperature stress, drought, submergence, Zn deficiency, and salinity tolerance are also included in this manual.

Grain quality is one of the most important traits to be considered in breeding new rice varieties. Screening processes and the selection criteria for grain and eating quality are clearly described. Multi-location testing and regional trials protocols were emphasized because these are the final stages in evaluating the overall performance of the breeding lines. This is also the stage where the best breeding lines are nominated to the National Cooperative Testing (NCT) prior to the recommendation for variety release.

Participatory varietal selection (PVS) is included in the manual as one of the approaches for adopting the best varieties in the farm where farmers select the best variety based on their preference. The concept and process of registration of the varieties through plant variety protection (PVP) and plant breeder's rights (PBR) were also emphasized. Once the varieties are approved and released by the National Seed Industry Council (NSIC), the breeders and institution that developed these varieties will produce the seeds. These varieties are further purified, seed increased, and classified by the National Seed Quality Certification Services (NSQCS) based on seed class. Seeds are classified as breeder, foundation, registered, and certified following a set of criteria for each class.

The last chapter discusses the other methods used in increasing genetic variability in breeding. These methods have been applied in the breeding strategy of PhilRice and have been successful in releasing high-yielding varieties. These methods include mutation breeding using irradiation and chemical mutagens, *in-vitro* mutagenesis (combination of in vitro culture and irradiation), doubled haploid breeding through anther culture, and wide hybridization.

ACRONYM

AC	Amylose Content
ACC	Accession
BLB	Bacterial Leaf Blight
BPI	Bureau of Plant Industry
CES	Central Experiment Station of PhilRice
DA	Department of Agriculture
DAS	Day after Sowing
DAT	Day after Transplanting
DUST	Distinctness, Uniformity, and Stability Test
EC	Electrical Conductance
HI	Harvest Index
IRRI	International Rice Research Institute
MINCER	Micrometeorological Instrument for Near Canopy Environment of Rice
NPK	N-P_2O_5-K_2O
NSIC	National Seed Industry Council
NSQCS	National Seed Quality Control Services
PhilRice	Philippine Rice Research Institute
PS	Preference Score
PSB	Philippine Seed Board
PVP	Plant Variety Protection
QTL	Quantitative Trait Loci
RGA	Rapid Generation Advance
RIL	Recombinant Inbred Lines
RTV	Rice Tungro Virus
SHB	Sheath Blight
SSD	Single Seed Descent
SUC	State Universities and Colleges
UPCA	University of the Philippines College of Agriculture
UPLB	University of the Philippine Los Baños

INTRODUCTION

Rice Varietal Improvement in the Philippines

Thelma F. Padolina

The rice varietal improvement in the Philippines has been focusing on increasing rice productivity to meet the growing demand for rice. The breeding methods and strategies are also evolving to recover from the challenges and constraints besetting the rice industry. Breeding schemes have greatly accelerated the rhythm of varietal improvement from plant introductions to using the most modern scientific plant. Processes have transitioned from the most primitive selection of naturally occurring variants of traditional endemic cultivars to hybridization process based on the laws of heredity. The impact of these new methods is already seen in farmers' harvest and the role of plant breeding in sustainable food production and environment progress is even greater. Amidst these expectations, the ancestral raw materials found in searching for food sources remain to be the building blocks of the future cultivars created using the most modern breeding methods.

Early works

Rice improvement in the Philippines began in 1901 by the Bureau of Agriculture, now the Bureau of Plant Industry (BPI). From 1901 to 1928, the commonly practiced methods were introduction, exploration, selection, and adoption of endemic varieties. In the following year until 1939, rice hybridization and strong selection pressure were undertaken, which produced 61 standard varieties for lowland and upland areas. Information on their suitability to different regions, maturity, yielding ability, and milling quality were known. Some of the outstanding introductions like Kra Suey from Thailand, Ramai from Vietnam, and Seraup Ketchil and Seraup Besar from Malaysia were identified. World War II disrupted the rice improvement work and even the nucleus seeds of the standard varieties were lost. After liberation, seed retrieval and purification on the pre-war designated rice varieties were intensified. These seed stocks became inputs to the new seed schemes in 1950 when rice hybridization was initiated. One of the notable crosses and strains derived from Ramai x Inadhica was approved as Quezon Rice. Other important popular varieties were Apostol, Pinili, Wagwag, and Elon-Elon. Varieties for double-cropped areas were also considered in this period.

In 1952, the seed production program was continuously built-up including the multiplication of nucleus seed stocks of the more important varieties. It was spearheaded by the Technical Cooperation Administration of the USA. This year also landmarked the Philippine rice improvement work. A modest coordinated program of regional variety trials was initiated by the Bureau of Plant Industry (BPI), University of the Philippines College of Agriculture (UPCA), Bureau of Agricultural Economics (BAE), and International Cooperation Administration under the Rice and Corn Improvement Project. In 1954, projects for strengthening the breeding activities were established. Four projects under a memorandum of understanding were signed including the improvement of lowland rice, upland rice, seed production, and distribution (Hayes, 1954, Umali et al, 1956). It gave birth to the National Cooperative Rice Improvement Program.

The creation of the International Rice Research Institute (IRRI) in the 1960s strengthened the rice varietal improvement in the Philippines. Breeding efforts were focused initially on irrigated lowlands with the improvement of yield potential under tropical conditions. Conventional breeding method was used in developing the early modern varieties. The birth of IR8 resulted in quantum leaps in yield potential from 5 to 10t/ha in the tropics and ushered the green revolution in the world

(Chandler, 1969). The improvement of semi-dwarf stature, photoperiod insensitivity, and responsiveness to fertilizer were among the traits improved as compared with the traditional varieties.

The public institutions engaged in breeding such as BPI through the Maligaya Rice Research and Training Center (MRRTC), UPCA, and Philippine Atomic Energy Commission (PAEC) sustained IRRI programs on harnessing the large diversity of germplasm for genetic enhancement. However, the release of IR8 and similar high yielding varieties in the farmers' fields resulted in serious consequences despite its high yielding ability. Poor grain quality, increased disease and insect damages, late maturation, and later in the 1990s, signs of slower production growth became evident. Breeding strategies evolved as the yield of Philippine modern varieties also improved. IR20 and IR22 were developed with better grain quality in terms of translucent grains and high milling recovery. IR36, the first IRRI improved variety with multiple disease and insect resistance, has short growth duration of 110 days and good grain quality. Owing to the desirable combination of these adaptive traits, IR36 became the most widely planted variety in the Philippines.

In later years, IR36 was replaced by IR64, a variety with important grain quality traits, particularly cooking quality, that conformed to the consumer preference of Southeast Asians including the Filipinos. There were 13 varieties identified along with IR64, the first variety with a desirable combination of intermediate amylose content, soft gel consistency, intermediate gelatinization temperature, translucent, and long slender grains. It inherited its superior grain quality from BPI 76, a pre-green revolution Philippine variety, which was extensively used in the early IRRI breeding programs. One of the earliest contributions of UPCA was C4-63G, an excellent grain quality rice, which became the Philippine standard for eating quality. It is also worth mentioning that one variety, PARC 2-2 an irradiated mutant from IR8 from PAEC, now the Philippine Nuclear Research Institute (PNRI), was also developed for grain quality improvement.

Overlapping event during this period was the creation of the Philippine Rice Research Institute (PhilRice) as a government corporate entity under the Department of Agriculture through Executive Order 1061 on November 5, 1985. By virtue of this function, PhilRice became the leader of the national R&D program in the country to help develop appropriate rice technologies so farmers can produce enough rice for the Filipinos. The beginnings of PhilRice were guided by its linkage with UPCA, as an executing committee while searching for the new PhilRice director. The committee laid the groundwork for PhilRice's program plan and operations.

In June 1987, PhilRice operations went full swing with the assumption to office of its first executive director, Dr. Santiago R. Obien. To support him, a PhilRice-UPLB Management Committee was created to assign UPLB experts as seconded staff and lead of PhilRice R&D Programs. However, the committee's functions were gradually reduced as PhilRice became organized and independent in adhering to its mandate of helping respond to the needs of the struggling rice farmers and attain rice self-sufficiency.

The Rice Varietal Improvement Program (RVIP) was one of the biggest thrusts instituted. The spill-over breeding achievements on harnessing the traditional or conventional breeding methods with the BPI and IRRI were nurtured and became the building blocks of the continuing breeding process. As PhilRice developed new varieties, breeding methods were enhanced through improved facilities and skilled human resources.

Technical collaborations and agreements were forged with relevant institutions and networks working on varietal improvement. With the continuing changes in our dynamic farming environments, breeding objectives were reformulated to match the needs of the times. The primary emphasis of rice improvement has been directed towards higher yield potential to attain rice self-sufficiency and sustainable food security in the Philippines. Breeding objectives like the improvement of resistance/tolerance to both biotic and abiotic stresses stabilize the adaptation of newer varieties while enhancing grain quality traits favor the diverse consumer palate. Rice farmers have been benefitting from the updated breeding objectives in terms of productivity and income.

Rising from the challenge

Significant phases on rice varietal improvement occurred three years after the establishment of PhilRice in 1987 under the leadership of Dr. Santiago R. Obien. Under each phase, it allowed PhilRice to continually apply emerging breeding technologies to perfect and support the principles of conventional breeding methods. Various technical collaborations and partnerships were instrumental in carrying out successful undertakings. To summarize these achievements, the breeding highlights are shown in Table 1.

Table 1. Phases of rice varietal improvement at PhilRice: defining the breeding themes, techniques, strategies, and achievements as influenced by local and international partnerships and collaborators from 1985 to 2021.

Phase	Period	Breeding Themes	Breeding Techniques/ Strategies Employed	Technical Partners/ Collaboration	Notable rice varieties released
I	1985-1989	Initiating discipline-based program with the rice varietal improvement as the biggest thrust; continuing the spill-over accomplishments of BPI, IRRI, and UPLB	Classical traditional methods; introduction of non-conventional methods such as anther culture, wide hybridization, induced mutation, and hybrid rice development. Major ecosystems focus on upland, rainfed, and irrigated lowland including special rices and adverse ecosystems such as cool temperature (CT), and saline-prone.	IRRI, UPCA, BPI, PNRI, NCT Network, and INGER	IR8, C4-63G, BPI-76 (NS), BPI-121-407 (mutant), PARC2-2, IR36, RPKn-2, UPLRi-1, BPI Ri-1, IR50, BPI Ri-10, IR64, and IR72
II	1990-1998	Activating the unified Philippine Rice R&D Network expanding more interests from agriculture research organizations including the State Colleges and Universities (SCUs), government and non-government (NGOs) institutions	Utilization of wild *Oryza* for exploring gene sources for stemborer, rice tungro virus, sheath blight, and brown planthopper tolerance; introduction of molecular marker technology with support by the Asian Rice Biotechnology Network (ARBN). Physical mutagenesis as a breeding method was strengthened with the assistance of the Philippine Nuclear Research Institute (PNRI).	PNRI, JICA, YAU, CPD, RCFS, PhilRice R&D Network, NCT Network, China HR, and R&D institutions	(IL) PSB Rc10, PSB Rc18, PSB Rc28, PSB Rc32, PSB Rc34, PSB Rc74, (HR) PSB Rc26H, PSB Rc72H (RF) PSB Rc14, Ma-ayon, Rinara, Chayong, Ennano (UP) PSB Rc1, Ginilingan Puti (CT) PSB Rc44, PSB Rc46 (SAL)PSB Rc48, PSB Rc50
III	1999-2005	Implementing interdisciplinary R&D structure for a more focused, location-specific and problem- responsive ecosystem-based breeding; arresting the narrowing genetic diversity and the slowing down of yield improvements	Wild rices as gene source for biotic resistance; *in vitro* techniques; anther culture for salt tolerance; use of marker technology, molecular breeding; creation of hybrid rice (HR) program	JICA, ARBN, IRRI, UPLB, CPD, RCFS, GRD, and PhilRice R&D Network	(IL) PSB Rc78, PSB Rc82, Matatag 2, Matatag 9, NSIC Rc 122, Angelica, NSIC Rc 130, Mabango 1, (SP) NSIC Rc13, NSIC Rc15, NSIC Rc17, and NSIC Rc19 (HR) NSIC Rc 114H, NSIC Rc 116H, Bigante, SL8 (CT) PSB Rc92, PSB Rc94, PSB Rc96, NSIC Rc104 (SAL) PSB Rc90,PSB Rc108 (UP) PSB Rc7, NSIC Rc9, NSIC Rc11
IV	2006-2010	Gearing towards achieving rice self-sufficiency goal, attaining higher yield potential as major objective	Two and three-line HR breeding and testing; tungro research; unfavorable RF breeding; direct-wet seeding for IL (conventional); beta carotene research (golden rice); wild rice for drought; molecular techniques (alien gene transfer, map-based cloning, QTL mapping) MAS for blast and submergence gene identification	IRRI, DA-NRP, UPLB, DA-RFOs, LGUs, JICA, Dan Forth Plant Center, CLSU-PhilSCAT, NCT Network, China HR, and R&D institutions	(IL) NSIC Rc 142, NSIC Rc 146, NSIC Rc 154, NSIC Rc 158, NSIC Rc 160, NSIC Rc 170, NSIC Rc 172, NSIC Rc 216, NSIC Rc 222, NSIC Rc 226 (HR) NSIC Rc 136H, NSIC Rc 202H, NSIC Rc 204H, NSIC Rc 208H, NSIC Rc 210H NSIC Rc 192, NSIC Rc 194

Acronyms:
IRRI- International Rice Research Institute, UPCA- University of the Philippines College of Agriculture, BPI- Bureau of Plant Industry, PNRI- Philippine Nuclear Research Institute, NCT- National Cooperative Testing, INGER- International Network for Genetic Evaluation of Rice, JICA- Japan International Cooperative Agency, YAU- Yunnan Agricultural University, CPD- Crop Protection Division, RCFS- Rice Chemistry and Food Science, HR- Hybrid Rice, ARBN- Asian Rice Biotechnology Network, GRD- Genetic Resources Division, NRP- National Rice Program, RFO- Regional Field Office, CLSU- Central Luzon State University, PhilSCAT- Philippine-Sino Center for Agricultural Technology

Table 1. Continued

Phase	Period	Breeding Themes	Breeding Techniques/ Strategies Employed	Technical Partners/ Collaboration	Notable rice varieties released
V	2011-2016	Accelerating the use of precision breeding methods for genetic improvement and specific adaptation target; focusing on climate resiliency of incoming released varieties; identifying good specialty rices	Enhancement of adaptation and dissemination strategies; development for value-added traits; enhancement of pre-breeding activities; climate resilient and very early maturing varieties as priority development; genetic improvement using blast, RTV, and BLB R-genes; genetic gain assessment	IRRI, JIRCAS, DA-NRP, and RDA-GUVA	(IL) NSIC Rc 222, Rc 216, Rc 160, Rc 300, and Rc 238
VI	2017-2021	Strengthening breeding system with high selection intensity, wide phenotyping and accurate selection, supported by genomic technology; targeting more location-specific adaptation; sustaining stronger partnerships among public and private partnerships	Micro-nutrient dense and specialty rices; heat tolerance breeding and other climate-related stresses; gene discovery and marker development; pyramiding, population improvement, recurrent selection, genomics, and rapid generation advance (RGA)	IRRI, DA-NRP, DA-RFOs, and LGUs	

Acronyms:
JIRCAS- Japan International Research Center for Agricultural Sciences, RDA- Rural Development Administration, GUVA- Germplasm Utilization for Value Added

Phase I (1985-1989): The conventional breeding method dominated the breeding strategies with the indigenous rice varieties as basic germplasm. Upland and rainfed rice improvement were continued with the establishment of PhilRice. It also infused non-conventional methods such as induced mutation and anther culture techniques. During this period, however, relevant varieties named after the breeding institutions IRRI, UPCA, and BPI-MRRTC were fielded, leading to more significant impact in rice production.

Phase II (1990-1998): PhilRice initiated inter-disciplinary research for a more focused, problem responsive, and location-specific ecosystem-based breeding. This phase is mark by active partnerships with the local and global institutions such as with Japan International Cooperation Agency (JICA) on irrigated lowland improvement and Chinese breeding institutions on hybrid rice development and germplasm exchange. Private sector also offered their hybrid technology to the government. The following first were released: Magat, first hybrid; Burdagol (PSB Rc34), farmer-selected line; PSB Rc72H, hybrid commercialized in 1998; modern varieties Gohang and Sumadel for cool temperature; and Hagonoy and Bicol for saline-prone areas.

Phase III (1999-2005): Ecosystem-based breeding resulted into the development of varieties for all major ecosystems in partnership with IRRI and UPLB. PSB Rc78, a mutant variety was released with PNRI. With our international cooperation with JICA, two PJ lines were approved for cool elevated and irrigated lowland while germplasm exchange diversified the hybrid and cool temperature tolerance breeding programs were prioritized with Yunnan Agricultural University (YAU). Most notable achievements include the release of Matatag series — the BLB resistant varieties, anther-culture derived NSIC Rc 130 from cultivar Wagwag, Angelica variety for low solar radiation, Bigante, and SL8 private sector hybrids.

Phase IV (2006-2010): In this phase, products using innovative techniques in combination with conventional method were developed such as NSIC Rc 142 and Rc 154 with BLB resistance, iron-dense variety NSIC Rc 172, drought resistant NSIC Rc 192, submergence tolerant NSIC Rc 194, tropical japonicas NSIC Rc 170 and Rc 220 as special rices, and the two-line hybrids NSIC Rc 202H and Rc 204H, which were jointly developed by PhilRice and UPLB.

Phase V (2011-2016): As new breeding technologies continue to evolve, the new varieties developed were further customized through enhanced adaptation and dissemination strategies and viable seed system. As such, nationally and regionally recommended varieties were identified and deployed. Among these outstanding varieties were NSIC Rc 222, Rc 216, Rc 160, Rc 300, and Rc 238. New varieties with climate resiliency, multiple biotic and abiotic resistance, nutrient-dense, and higher yielding potential were developed to support rapid variety replacement to help farmers adapt to climate change.

Phase VI (2017-2021): The modern innovative and precise breeding tools were continued to sustain breeding goals. Partnership with IRRI continue to support the varietal improvement at PhilRice. Pre-breeding materials or donor parents were provided using rapid breeding cycles on recipient PhilRice germplasm. Newer varieties from the last decade's efforts were continuously identified and released as NSIC varieties, as we await the products from modern biotechnology. The race goes on because plant breeding is the key mechanism for the needed adaptation of crops to combat the challenges of ever-changing rice ecosystem and landscape.

The need for an operations manual

Rice breeding has been a major research activity of PhilRice since its creation in 1985. Substantial resources are allotted annually on the development and testing of rice varieties. However, there is no standard guide for breeders on the implementation of breeding operations. Generally accepted procedures and practices used in rice breeding exist that when followed, could contribute to the efficiency and better use of resources in developing varieties.

These breeding procedures with technical basis have been used in successful rice breeding programs. Knowing the proven methods of raising breeding materials and selecting agronomic traits is important for a new researcher aspiring to be a rice breeder. This manual outlines the population sizes and the selection criteria used in evaluating segregating materials. Given the appropriate plot sizes and number of replications employed in breeding and in national and multi-locational trials, the guidelines here help avoid the costly oversized plots that do not contribute in conducting more precise experiments.

This breeding manual also facilitates communication and promotes better coordination in a breeding program. With operational procedures known and written, staff could focus on their main tasks, while support staff performs most of the routine work. An operational manual ensures effectivity and efficiency of breeding work at PhilRice while cushioning the institute from the adverse impact of rapid staff turnover.

Objectives

This Rice Breeding Operations Manual was developed and produced to:

a) facilitate the introduction of new research staff to rice breeding,
b) know the requirements of a successfully engaged breeding project,
c) have a generally accepted system of handling breeding materials,
d) effect a better organized and coordinated breeding projects,
e) lessen the impact of staff turnover through operational procedures and well-archived seed and data management, and
f) provide a more sound basis for an equitable allocation of resources among the breeding and its related activities.

Intended users of the manual

The intended audience/users of the Rice Breeding Operations Manual include:

- Practicing rice breeders
- Trained plant breeders but lack rice breeding experience
- Staff in breeding-related disciplines
- Breeding assistants and technicians
- Researchers and students in plant breeding
- Research managers

CHAPTER 1

Resources Required in the Rice Breeding Program

Thelma F. Padolina, Hilario C. de la Cruz, Senen H. Escamos, and Alex T. Rigor

A clearly defined set of objectives must be established in determining the size of a breeding program. To properly develop a set of breeding goals, the breeder must understand the needs of his/her clients: farmers, millers, and consumers. He/she must know the target environment, assess the levels of biotic and abiotic stress that occur in that environment over multiple years, and have sufficient knowledge of the production practices of the farmers, and the economic infrastructure. For instance, are inputs such as fertilizers and pesticides readily available to the producer? The breeder must understand all these things and more if he/she is to implement a successful breeding program - that is to develop a variety that is superior to the farmers' current variety.

1. Adequate functional germplasm collection

The materials discussed in this section are part of the core breeding program component and part of larger rice germplasm collections available in the gene bank. These genetic resources, termed "working or active" breeding materials, include the following:

Samples of all rice varieties commonly grown by farmers in the target production regions of the country. These may include traditional varieties, other materials developed by farmers, bred varieties released by the National Seed Industry Council (NSIC), and when possible, commercial varieties being sold by seed companies and distributors to the growers. Depending on the materials' level of intellectual property protection and extent of availability, these may be used as parents for crosses to create genetically variable selection populations and/or grown as checks in performance trials to evaluate potential new varieties from the breeding program or external sources.

Selected populations produced by hybridizing two or more contrasting parents to create genetically variable populations for selection to develop new parental lines. These include plants in the F_1, F_2, and advanced populations that may include families with varying degrees of homozygosity. These will be used for line development, i.e., new parents for hybrid varieties or parents of inbred varieties.

Pre-breeding or genetically variable populations into which new elite genes are being introgressed through hybridization followed by selection to create variable populations with multiple gene combinations. New lines can be selected here for further development.

Advanced lines for testing and direct use either as parental materials or putative new varieties. These may be materials obtained through agreements with international centers, regional collaborative programs, and breeder-to-breeder exchanges. Advanced fixed lines from external sources are especially important for programs on genetically improved varieties that do not require investment in the crossing and selection activities.

Special materials such as lines considered to be outstanding gene donors or being screened for new alleles, populations of recombinant inbred lines, and other segregating populations used for specialized genetic studies and mutated populations, among others.

Core collections or core sets from larger comprehensive collections of rice germplasm.

These genetic resources should be readily available to breeders. Therefore, close coordination with the institute's gene bank and the breeding programs must be ensured.

2. Staffing

To ensure success in breeding programs, sufficient number of dedicated human resources assigned in administration and management; direction and execution of breeding activities; daily maintenance of field, laboratory, data management, seed processing; and other routine activities must be secured. No breeding program can be executed successfully without adequate qualified breeders and capable support personnel who can set goals, manage resources, and produce the outputs. As such, it is important to regularly gather and evaluate personnel's qualifications, experience, and competence level needed in ensuring that a breeding program will deliver its targets and maintain its capacity.

To support a plant breeding program, an ideal breeding team would include:

Two complementary breeders

A senior staff with sufficient breeding education, knowledge, and experience, who will lead the team and a junior researcher who will serve as an apprentice and eventually, move into a leadership role, are essential in a plant breeding program. The breeders should be well-versed in plant breeding and capable of providing leadership and guidance and contribute expertise in the seed system. The lead, if an experienced breeder, should be knowledgeable of the infrastructure needs and program components required for the variety development of crops for the key target market segments.

The complementary breeders will provide essential continuity in case one of them leaves the program. Program continuity is very important as development of new varieties is a long-term endeavor and the program should avoid disruption to succeed.

The three primary activities carried out by plant breeders are variety development, research on germplasm and breeding methods that contribute to the effective variety development, and education of future plant breeders. Plant breeders also direct/manages breeding program operations and/or facilitates participatory varietal selection and use of farmer-saved seeds through extension workers or other rice specialists.

Technical assistants

A breeding team should include technical assistants, preferably with college level or equivalent training, who can handle seed-related activities, carry out selection and testing, and use data bases for tracking and analytical work. For a large program with field and laboratory requirements, it is necessary to have two assistants — one handling field operations and the other, laboratory work. The types of research and operations stipulated in program will indicate the numbers, qualifications, and specializations of the technical assistants. In programs needing chemical analyses, a qualified assistant must be hired to achieve the targets. As molecular marker-assisted selection is now an integral part of breeding, a person knowledgeable in this technology may be required on a part-time basis or shared with another program.

Technicians and seasonal workers

Personnel who will prepare and maintain field trials for selection and testing are needed. This staff requirement may be supplied by the station where a program is implemented.

Supporting professionals from other disciplines

The multidisciplinary nature of plant breeding and crop improvement often benefits from the expertise of professionals from other disciplines to address issues such as breeding for resistance to diseases (pathology) and insects (entomology), abiotic stress (plant physiology), grain quality, and nutritional or compositional improvement (biochemistry). Often, breeding will require specialized procedures to complement classical breeding and selection such as tissue and cell culture, genetic engineering, marker-assisted breeding (molecular genetics and genomics), and statistics. Having dedicated specialists in these areas may be costly and not justifiable, so the breeding team may instead consider tapping these specialists as participants who will still perform their other professional activities.

Administrative and support personnel

It is ideal to employ staff who will manage the project's budgets and payrolls, human resources, and information technology, particularly those related to electronic databases and internal and external communications. For large and maturing programs, assistance on seed shipments, negotiating contracts and agreements, and complying with regulatory and intellectual property

issues are also needed. This type of support is best provided for multiple programs from a single source at each station, rather than by less specialized staff in each program.

3. Funding for breeding operations and testing

Funding for breeding operations in many instances is not provided in a timely manner. Often, it is assumed that the institute will provide the needed recurring program resources when competitive and supplemental grants to public programs emphasize new science and methodology. However, this is not the usual case, and an otherwise promising program struggles due to insufficient and unreliable operating funds.

The following are required for a functional breeding program:

- Total breeding program annual budget
- Capital outlay
- Maintenance and other operating expenses budget
- Allocation for breeder(s)/staff travel for internal program, i.e., monitoring multi-location trials
- Fund for breeder(s)/staff travel to scientific meetings
- Budget for breeder(s)/staff continuing education

Consideration in funding a plant breeding program:

- Is it a separate program of the institute or part of a larger national program?
- Are salaries, capital outlay, and vehicles included in the budget?
- What proportion of the total breeding program budget is provided by the institute and external grants?
- Does it have a separate budget administered by a senior plant breeder (program/project leader)? Is it administered by another person or unit (Office of the Executive Director/Deputy Executive Director for Research)?
- Who pays for the care and management of field plots including irrigation?
- Does the operational budget (MOOE) funds the multi-location trials and/or on-farm evaluation?
- Are the implementation costs at the branch stations included in the program or in another office? Who decides on budget allocation?

There must be sufficient core funding to assure that breeding program operations are not interrupted. A breeding program that cannot sustain its operations will fail to produce varieties, which is a disservice to the Filipino rice farmers who need these for increased yield and profitability.

4. Physical facilities for breeding operations and testing

The types of facilities needed for a breeding program will depend on the activities being conducted at each location, e.g., core breeding activities, field selection, and evaluation. Activities requiring specialized expertise and costly dedicated equipment should be centralized and shared with other programs or units. It is inefficient for each station to have the capacity to undertake all the breeding program activities. In many cases, it may be more cost-effective to outsource seasonal work than procure expensive, dedicated facilities and equipment.

The physical facilities commonly needed for a breeding program include:

- Adequate field of suitable topography and soil type with dependable water supply and good drainage
- Drying area
- Seed preparation and work room for processing, handling, threshing, cleaning, sorting, weighing, packaging for shipping, and storing dry seeds
- Laboratory/office with a computer database for record keeping and data analyses
- Climate-controlled facility, e.g., refrigerator, walk-in cooler, cool, dry rooms (air-conditioned rooms with dehumidifier) for short-, medium-, and long-term storage of seeds (gene bank) that is free from pests
- Modest grain quality laboratory
- Vehicle to transport labor and materials to and from the fields
- Specialized structures, containers, greenhouses, and screenhouses for crossing and screening populations for insect and disease resistance and abiotic tolerance. Some controlled hybridizations and screening are also done in the open field.

The essential machinery needed to prepare fields and plots, harvesting, and processing include:

- Hand tractor with complete implement for plowing and harrowing
- Plot thresher
- Panicle thresher
- Blower/Seed cleaner

Funds are needed to establish testing sites and ongoing operations. Supplemental irrigation is often a cost-effective way to increase the use of facilities by providing protection from long or intermittent drought, which can ruin small, but important experimental trials critical to the breeding pipeline. Field-testing facilities that are

well-managed and optimally located are also essential to avoid spilling in of plant materials from other resources.

Field sites for testing promising selections are required not only at the central breeding site, but more importantly, at sites in the agro-ecological zones representative of the target production areas, usually in the branch stations. The stations should serve as demonstration facilities for farmers in the surrounding areas to see new materials and provide feedback. These may include:

- Branch stations for early generation selection and testing to meet critical goals
- Branch stations, Department of Agriculture-Regional Offices (DA-RFOs), and State Colleges and Universities (SCUs) for adaptation testing of new potential varieties in critical target environments
- Other testing facilities by SeedNet, LGUs, seed growers, and farmers' field

Participatory breeding and on-farm trials in farmers' fields are desirable alternatives and complement the testing and evaluation activities in branch stations. Although farmers may contribute their land and labor to this joint enterprise, support for the travel of breeders for data collection and other activities away from their work station is required.

- Land and arrangements for farmer-assisted selection and/or testing of new varieties in farmers' fields
- Funds for travel to and information collection at on-farm trials

Depending on technology level used for breeding, relatively sophisticated laboratories that support molecular biology and advanced analytical capability may be required. If access is not available through other collaborative programs, i.e., IRRI, there may be a need to provide these capabilities as part of the breeding program, which will add considerable expenses for establishing and maintaining laboratories for:

- chemical, physiological, and biochemical analyses
- handling DNA samples and marker-assisted breeding (MAB)
- genetic engineering
- cell and tissue culture
- producing doubled haploid plants

5. Scientific information and know-how for crop improvement

What is know-how? When your supervisor asks, "Do you know how to plan, layout, and manage a crossing nursery for producing hybrid rice seed?" If you can satisfactorily accomplish the tasks, then you have the "know-how." If you can't do the task, you need to enhance your knowledge on these.

The acquisition of scientific information on producing hybrid rice seed, for example, is an important "know-how." You also need to produce the seed to start having the sufficient "know-how." Scientific information and know-how are acquired. To use each effectively requires fundamental knowledge about plant breeding, practice, skills, experience, and willingness to learn.

A professional plant breeder needs good basic education about theory and practice, practical field and laboratory training with a knowledgeable breeder working in their specific crop or related crop, access to information from libraries and repositories, interaction with colleagues working with similar crops and breeding goals, and periodic updates of their knowledge and skills at centers of excellence.

Some of the scientific knowledge and information needed to begin a successful career will be acquired by the breeder during their study for a degree or certificate. These topics are relevant to breeding and crop improvement, and should prepare them for leading or participating in a breeding team. With more advanced training, e.g., post-doctorate, PhD, and MSc degrees, the breeder is expected to tackle more difficult problems and provide greater program leadership. Generally, more theory is obtained from advanced education but it does not necessarily provide practical information and experience on developing different crops. To obtain practical information and know-how, it is advised to work as an apprentice for a few years or as an intern with a practicing breeder. It must be noted that an advanced degree is not a pre-requisite for a plant breeder, and that we have successful plant breeders without advanced training but with good practical training and experience in the field.

Plant breeding profession continues to change and evolve, and without continuing participation in professional meetings and periodic updates about new information and technology, practicing breeders may not be aware of new knowledge and applications that will allow career advancement and sustained productivity.

Practical scientific information and know-how critical to operating a breeding program include:

- Compatible hardware and software for collecting, analyzing, storing, and reporting phenotypic and genotypic information and data
- Understanding and manipulating electronic database for record keeping, program management, and breeding operations
- Organization, layout, and planting of field plots suitable for supporting scientific decisions about selection and performance of new lines
- Organization, layout, and maintenance of parents for crossing blocks, breeding nurseries, among other experiment sites
- Good plant cultivation and maintenance practices
- Methods used for pollination, pollination control, and production of experimental selection populations for line and new hybrid production
- Analytical methods used for evaluating important traits to meet program goals and objectives

Methods for extracting and handling plant materials and DNA for analysis in the laboratory or shipment to facilities

Above all, the breeding team must be able to grow good plants in the field and specialized environments. Successful, productive breeding, and excellent science cannot be done with poorly grown plants.

1. Consideration
    - Coverage of envisioned program
    - Target environment
2. Adequate and functional germplasm collection
3. Facilities
    - Experimental farm
    - Field equipment and transport
    - Seed laboratory and seed storage
    - Screen houses
4. Ready access to germplasm collection
5. Staffing
    - Academic training and experience
    - Technicians
    - Labor
6. Funding
    - Amount
    - Timely releases
    - Assurance of continuity

CHAPTER 2

The Rice Plant

Susan R. Brena, Edelweiss E. Sajise, and Democrito B. Rebong

Rice, which belongs to the genus *Oryza,* family *Graminae,* includes two cultivated and about 20 wild species. It is a diploid species with 2n = 24. Its genomic formula is AA. It has wide range of ecological adaptation from dry upland to floating deep-water condition. The two cultivated species, *Oryza sativa* L is commonly known as Asian rice while *Oryza glaberrima* is predominantly grown in Africa (Figure 2.1). *O. sativa,* has three recognized ecotypes – *indica*, *japonica/ sinica,* and *javanica*. Indica type is widely dispersed and adapted to tropical environments while *japonica* (*sinica*) is limited in adaptation to temperate environments. On the other hand, javanica types are generally grown in the cooler mountainous parts of the tropics.

(A)

(B)

Figure 2.1. *Oryza sativa* (A) and *Oryza glaberrima* (B).

Morphology of the rice plant

- The rice plant has round and hollow stems, flat leaves, and panicles at the top of the plant. Rice is a very flexible/adaptable plant that grows well under both flooded and rainfed/water-limited conditions (Figure 2.2).

- The vegetative organs of the rice plant are composed of the roots, stems, and leaves while the reproductive or floral organs consists of the panicle that bore the spikelets or floret.

Vegetative organs

The vegetative organs of a rice plant include the roots, culm or stem, and the leaves. A tiller is the branch of the plant bearing all the culm, leaves, roots, and often has a panicle (Figure 2.3).

Roots anchor the rice plant in the soil and absorb water and nutrients. Like other *Graminae*, the rice root system is fibrous possessing rootlets and root hairs. The roots are relatively shallow, especially under flooded conditions (95% of the roots are found in the top 0.2m of soil).

Culm or stem is composed of a series of nodes and internodes, which main function is to transport water and nutrients and to bring air to the roots. The internodes are hollow with a smooth surface. Each node has one leaf and one bud that can develop into a tiller. From the nodes of the main stem, other stems called secondary tillers, grow and can produce tertiary tillers. The group of tillers produced by a single plant constitutes a rice hill. Tillering ability is a function of a variety, but it is also influenced by growing condition and crop management practices.

The shorter and sturdier culms mainly contribute to the plant's resistance to lodging, favorable grain-to-straw ratio, nitrogen efficiency, and high yield capacity. Likewise, traits related to lodging resistance include culm diameter and thickness of the culm wall.

High tillering and compact plant types are generally preferred and selected in developing new varieties. Compact and moderately erect culms allow increased solar radiation to tillers and minimize shading on plants per unit of land area. Highly tillering varieties, on the other hand, is desirable for maximum productivity of rice cultivated either in moderate or dense population.

Leaf blade or leaves grow alternately on the stem with one leaf per node. The leaves are the growth engine of the plant as they capture solar radiation and produce carbohydrates.

Leaf angle is one of the most important leaf characteristics related to high yielding capacity. In rice breeding, erect leaves are highly preferred as it allows maximum diffusion and more even distribution of light into the crop; thereby, giving higher chances to capture solar radiation for greater photosynthetic activity. Likewise, leaf length is considered important during selection as

Figure 2.2. *Oryza sativa* plant type.

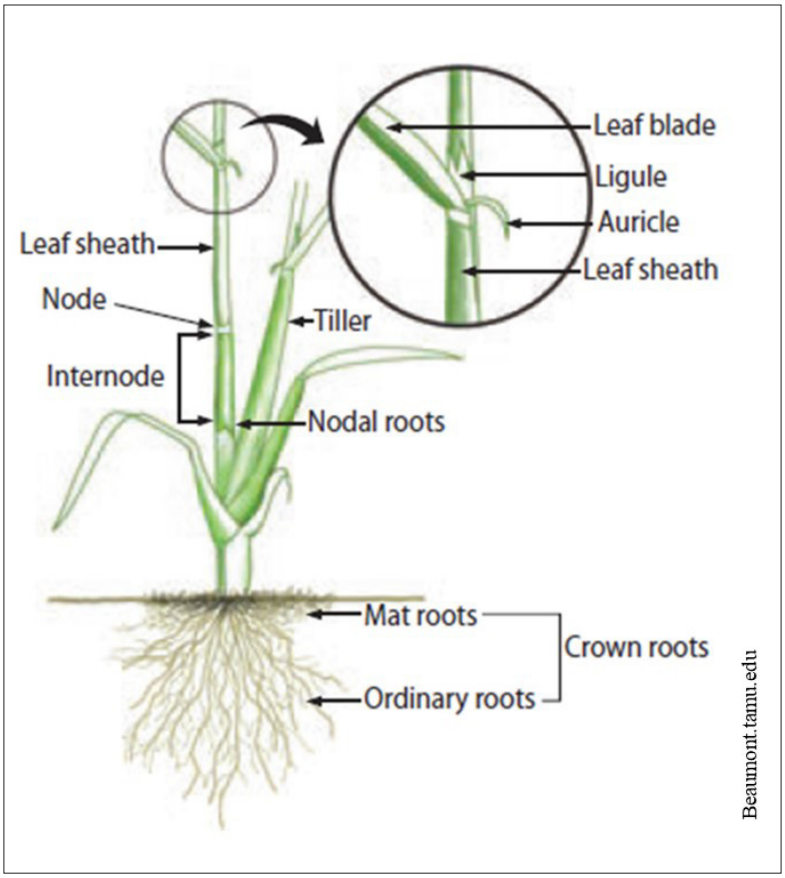

Figure 2.3. Vegetative organs of the rice plant.

it is directly associated to leaf angle. Shorter leaves are more erect and tend to be more uniformly distributed in the plant; thus, shading is reduced. On the other hand, unlike the leaf length, no proven direct relationship has been observed on the leaf width and thickness as these affect the yielding ability of a variety.

Leaf sheath is the lower part of the leaf that originates from the node and wraps the internodes above it and sometimes the leaf sheath and blades of the next internode. The degree of wrapping of the leaf sheaths to the internodes has been associated to the plants resistance to lodging. Two elements are found at the junction point between the leaf and the collar:

The *auricle* is a 2 - 5mm appendix, crescent-shaped, and covered with hair. The auricles are characterized for its presence and absence, color shade that range from whitish, yellowish, or purple hues.

The *ligule* is a membrane with length and shape depending on species and variety. It is long in *O. sativa* while short and round in *O. glaberrima*. Rice is the only *Graminae* possessing both ligule and auricle, which allow distinction from weeds at the seedling stage.

Flag leaf is the topmost leaf below the panicles. This varies in size, shape, and angle among varieties. The flag leaves are important to the yielding capacity of a line or variety because it is the primary source of photosynthates that go directly to the panicle. Erect and moderately long flag leaves may also help protect ripening grains against bird damage.

Table 2.1. Important agronomic and morphological traits useful in breeding.

Character/ Trait	Sample Size (n)	Growth Stage	How to Measure	Scale
Phenotypic acceptability	Score the population	At maturity	Score based on the overall acceptability of the line or variety in relation to the breeding objective	1 - Excellent 3 - Good 5 - Fair 7 - Poor 9 - Unacceptable
Plant height	5	At maturity	Measured from the soil surface to the tip of the tallest panicle (excluding awns if present). Use actual measurements (cm) in whole numbers	1 - Semi-dwarf (lowland: less than 110cm) 5 - Intermediate - (lowland: 110 -130cm; upland: 90 -125cm) 10 - Tall (lowland: more than 130cm; upland: more than 125cm)
Culm or tiller number	5	After heading to near maturity	Entered in data sheets using actual count of the total number of tillers (productive and unproductive tillers)	3 - Low (<10 tillers) 5 - Intermediate (~15 tillers) 7 - High (>20 culms)
Culm diameter	3	Full blooming to near maturity	Measured at the outer diameter on the basal part of the main culm	Recorded as average from the three collected samples
Culm strength		After heading	Gently push the tillers back and forth a few times at about 30cm from the ground to indicate culm stiffness and resilience	1 - Strong (no bending) 3 - Moderately strong (most plants bending) 5 - Intermediate (most plants moderately bending 7 - Weak (most plants nearly flat) 9 - Very weak (all plants flat)
		At maturity	Note the standing position of the plants	Sturdy - upright plants Weak or lodged - plants with bending or buckled culms Brittle - culms break readily
Leaf angle	Score the population	Late vegetative before heading	Estimate the openness of the blade tip against the culm, scored on the leaf below the flag leaf	1 - Erect 5 - Horizontal 9 - Droopy
Leaf length	5	Shortly after heading	Measured on the leaf below the flag leaf of the primary tiller from the collar to the tip of the blade (in cm)	Recorded as average from the 5 collected samples
Leaf width	5	Shortly after heading	Measured on the leaf below the flag leaf of the primary tiller in centimeter of the widest portion of the leaf blade	Recorded as average from the five collected samples
Leaf color	Score the population	Shortly after heading	Visual score based on scale	1 - Light green 2 - Green 3 - Dark green 4 - Purple tips 5 - Purple margins 6 - Purple blotch (purple mixed with green) 7 - Purple
Flag leaf angle	Score the population	At heading	Visual estimate of the angle of attachment of the of the flag leaf blade to the axis of the panicle	1 - Erect (0-30°) 3 - Intermediate (31-60°) 5 - Horizontal (61-90°) 7 - Descending (> 90°)
Leaf senescence	Score the population	At maturity	Evaluate visually the degree of yellowing of mature plants	1 - Late and slow (leaves have natural green color) 5 - Intermediate (upper leaves yellowing) 9 - Early and fast (all leaves yellow or dead)

Reproductive (flower) organs

Panicles form the rice inflorescence (Figure 2.4). It is the top part of the rice plant, carried on the last internode. Panicles are composed of primary ramifications (small branches) that carry secondary branches, which connect to the pedicels where the spikelets emerge. The number of primary and secondary ramifications depends on species and variety. A single panicle can bear 50 - 500 spikelets; however, in most cultivated varieties, the number of spikelets per panicle ranges 150 - 350. Variations in length, shape, angle and on weight and number of spikelets per panicles have been observed among rice varieties.

A rule of thumb on the compensatory association between panicle size and tiller number states that as one increases, the other decreases. As expected, lines with high tillering and longer panicles will produce higher yields. Determining yield is not accounted from the panicle characters alone but also from other subunits or yield components. Fully exserted panicles are selected over those that are partially enclosed as spikelets of enclosed panicle become sterile or partially filled resulting in grain losses.

Spikelet is a unit of inflorescence, most of which develop into grains. Fertile spikelets are contributory to high yield in which 85 - 90% spikelet fertility of a line is considered optimum. Spikelets are consisted of the pedicel and the floret emerged on the primary and secondary branches. The floret includes the lemma, palea, and the flower. The middle nerve of the lemma has a constricted structure at its end called keel which in some varieties are elongated into a thin extension also known as awn (Figure 2.5).

Lines with awnless grains are mostly selected by breeders because the awns are tough, persistent, and cause difficulty in threshing and milling. There are no significant contributions of awns to grain filling, while protection against bird damage has been reported.

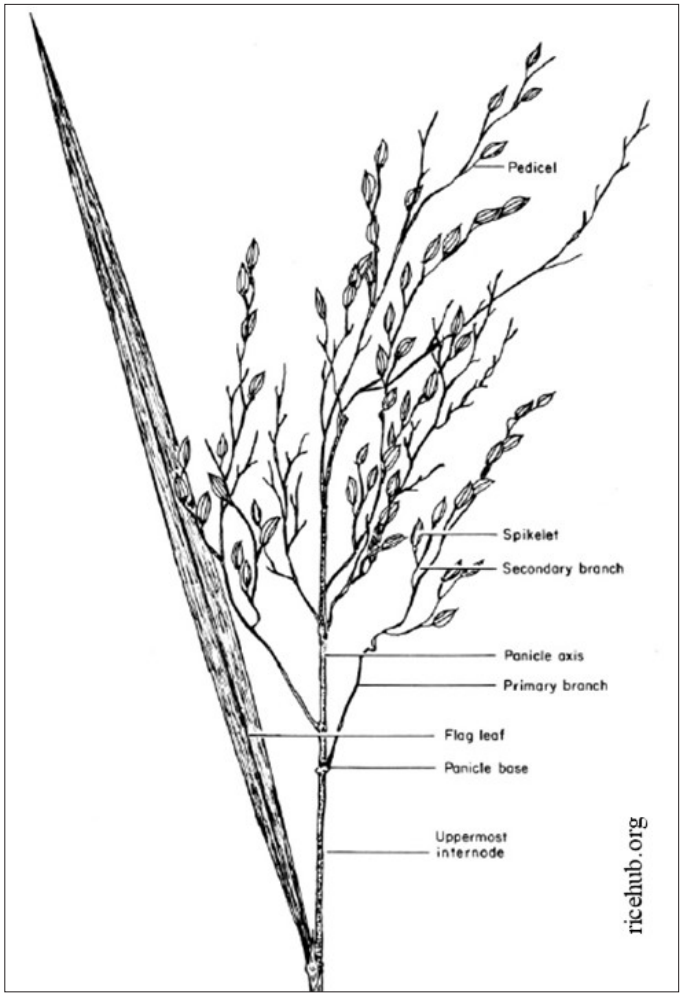

Figure 2.4. Component parts of the rice panicle.

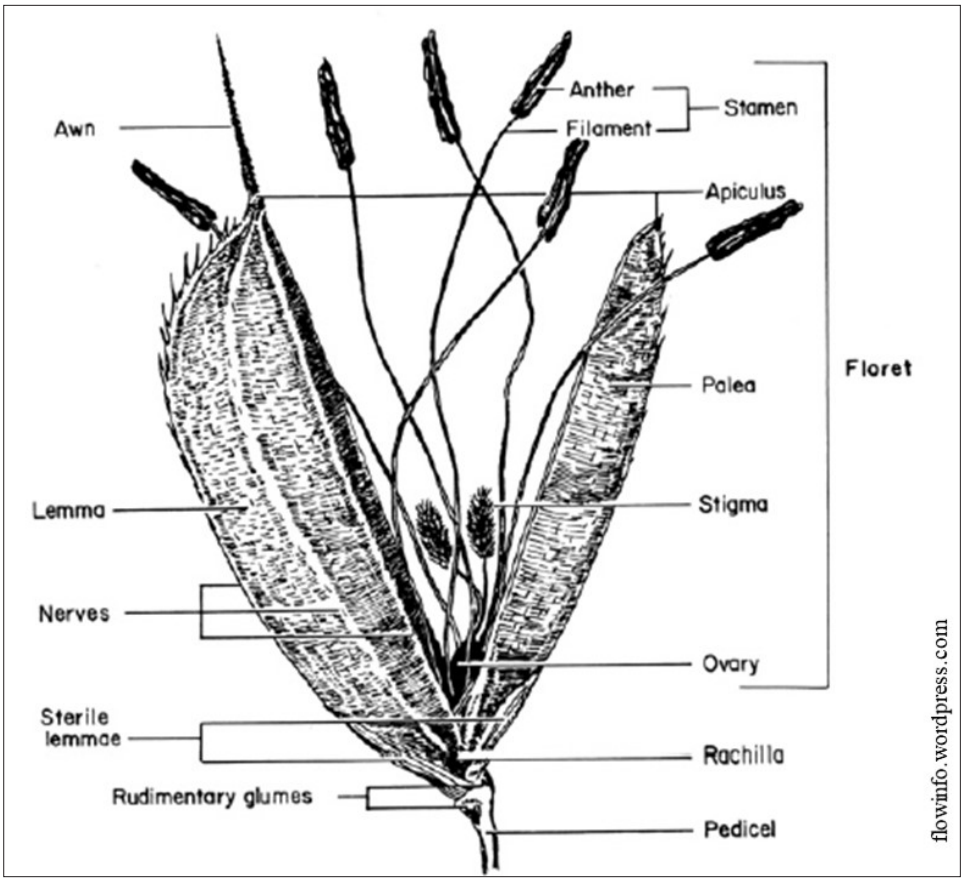

Figure 2.5. Parts of a rice spikelet.

However, partly awned panicles or tip awned grains are tolerable.

Grain shattering is also one of the important characteristics for consideration in breeding. In the Philippines, intermediate-shattering types are desirable because it can be threshed thoroughly with minimum grain losses either manually or by mechanical means.

Flowers are composed of the six stamens or male reproductive organs (the filaments bearing the 2-celled anthers containing the pollen) and a pistil or the female organs (the style, stigma, and the ovary). Rice is highly self-pollinated (autogamous) in which pollen grains pollinate the stigma that comes from the same flower.

Grain is composed of the ripened ovary with the other associated structures that include the lemma, palea, rachilla, sterile lemmas, and awns if present.

The dehulled rice grain or caryopsis is enveloped by two glumes called lemma and the palea. The lemma is a five-nerved bract of the floret that partly encloses the palea. The palea on the other hand is a three-nerved bract of the floret which fits closely to the lemma. Below the palea and the lemma are the sterile lemmas and rudimentary glumes. The pedicel attaches the grain to the panicle branch.

The caryopsis is enveloped by the pericarp layers followed by the tegmen layers and aleurone layers, respectively. The remaining part of the grain is composed of the embryo, which is located at the base on the ventral side of the spikelet and the endosperm that provides nourishment to the embryo and the developing seedling.

Table 2.2. Important floral traits useful in breeding.

Character/ Trait	Sample Size (n)	When to Measure	How to Measure	Scale
Days to heading (DTH)	Score the population	50% heading	Record number of days from soaking until 50% of the plants in the population are heading	Entered as number of days
Maturity	Score the population	At maturity	Record number of days when 85% of the grains on panicle are mature or add 30 days to DTH to get maturity of the line/variety	Entered as number of days
Panicle exsertion	Score the population	Ripening stage	Visual estimate of the panicle exsertion on plants in the population and rate according to the scale	1 - Well exserted 3 - Moderately well exserted 5 - Just exserted 7 - Partly exserted 9 - Enclosed
Panicle shattering	Random sampling of representative panicles in the plot	At maturity	Grasp the panicles of a plant loosely in one hand, squeeze gently, then estimate the percentage of the shattered grains	1 - Very low (<1%) 3 - Low (~3%) 5 - Moderate (~15%) 7 - High (~35%) 9 - Very high (>50%)
Panicle length	10	At maturity	Measure length in centimeters from panicle base to the tip	Recorded as average from the collected samples
Panicle type	Score the population	At maturity	Classify panicle according to mode of branching, angle of primary branches, and spikelet density	1 - Compact 5 - Intermediate 9 - Open
Spikelet fertility	5 Score the population	At maturity	Averaged percentage of filled grains over the total number of spikelets in the panicle or Estimate the percentage of filled grains in the panicle and rate accordingly	1 - Highly fertile (>90%) 3 - Fertile (75-89%) 5 - Partial sterile (50-74%) 7 - Highly sterile (<50% to trace) 9 - (0%)
Awning	Score the population	From ripening until maturity	Note the presence and distribution of the awns along the panicle	0 - Absent 1 - Short and partly awned 5 - Short and fully awned 7 - Long and partly awned 9 - Long and fully awned

Table 2.3. Important grain characteristics useful in breeding.

Character/ Trait	Sample Size (n)	When to Measure	How to Measure	Scale
Grain yield	Harvested from at least $5m^2$/plot	On rough or paddy rice	Compute yield in kilogram per hectare with moisture content adjusted to 14%	Record as the adjusted weight
100-grain weight	100 seeds	After harvest	Weigh 100 whole grains dried to 13% moisture content using precision balance	Record as actual weight
Grain length	10 seeds	After harvest	Measure length of grains in millimeter from the base of the lower most sterile lemma to the tip of the grain. For awned varieties, measure to the point nearest to the tip of apiculus.	Enter as mean of the collected samples
Grain width	10 seeds	After harvest	Measure width of grains in millimeter across the fertile lemma and palea at the widest point of the grain using photo enlarger	Enter as mean of the collected samples
Grain shape	10 seeds	After harvest	Expressed as ratio between grain length and width.	Enter as mean of the collected samples
Brown rice length	10 seeds	After dehulling, before milling	Measure in millimeter from base to tip of the grain in millimeter using photo enlarger	1 - Extra long (more than 7.5mm 3 - Long (6.6 - 7.5mm) 5 - Medium (5.51 - 6.6mm) 7 - Short (5.5mm or less)
Brown rice shape	10 seeds	After dehulling, before milling	Expressed as ratio between brown rice length and width	1 - Slender (over 3) 3 - Medium (2.1 - 3) 5 - Bold (1.1 - 2) 9 - Round (less than 1.1)
Chalkiness, Translucence	10 seeds	After dehulling, before milling	Visual	Refer to Chapter 12, Screening for grain quality
Awning	Score the population	From ripening to maturity	Note the presence and distribution of the awns along the panicle	0 - Absent 1 - Short and partly awned 5 - Short and fully awned 7 - Long and partly awned 9 - Long and fully awned

Growth and development stages of rice

The growth and development stages of rice, regardless of the variety and ecology (Figure 2.6):

The Vegetative Phase

During the vegetative phase, the plant goes through the following stages of development: germination-emergence, seedling growth, tillering, and internode elongation. The duration of the vegetative phase varies according to variety, but could be influenced by temperature and the photoperiod (day-length). In photoperiod varieties, flowering is triggered by short-days or short-day length. The relative length of the vegetative phase will determine whether the variety has a short, medium, or long growing cycle. Most crop management practices are applied during the vegetative phase and the most important are irrigation, weed control, fertilizer application, and insect and disease control.

Stage 0 (G*ermination*). The embryo germinates as soon as it finds sufficient moisture and oxygen and favorable temperature (optimum: 20^0 to 35^0C), marking the start of metabolic activity in the seed. The seedling has germinated with the appearance of the coleoptile from which the first leaf will develop under aerobic conditions or the radicle or the first root under reduced oxygen regime as in water-seeding (Figure 2.7). The germination stage covers the period from the emergence of either the coleoptile or the radicle to the emergence of the first leaf.

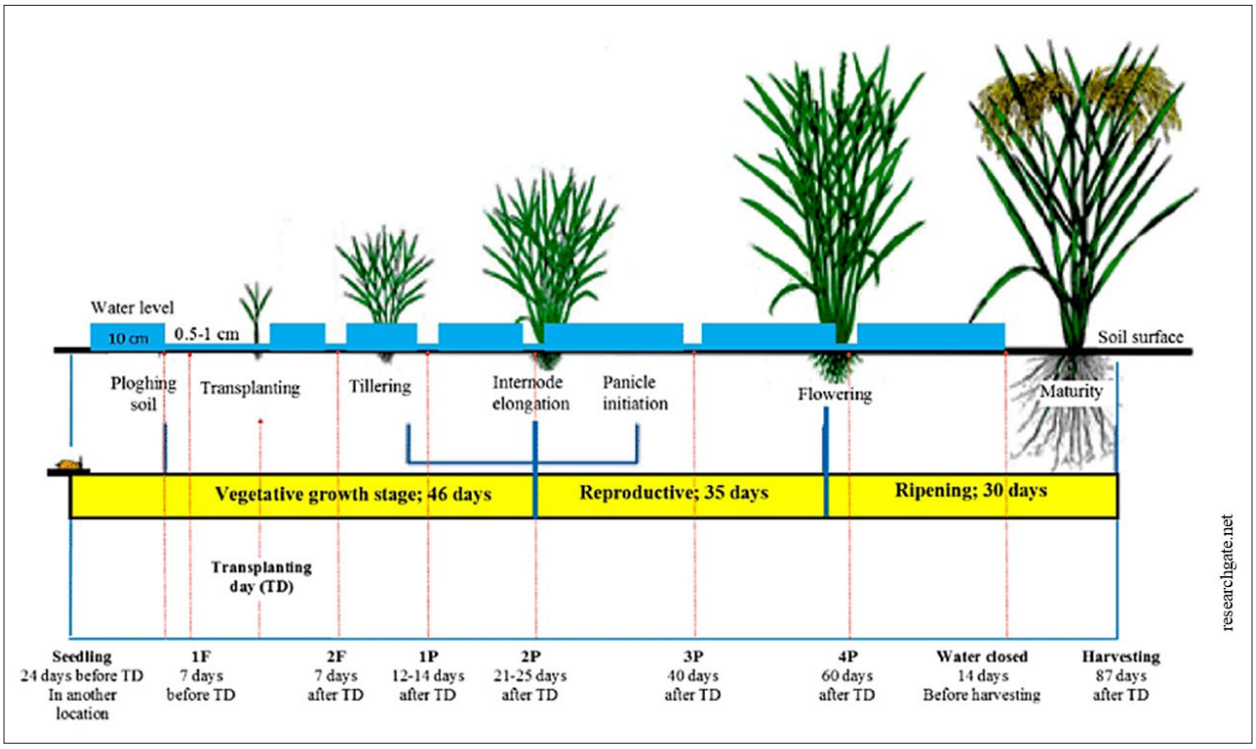

Figure 2.6. Growth and development stages of the rice plant.

Stage 1 (Seedling). This is the period (about 14 days) that follows germination when the young seedling essentially depends on the food reserve in the endosperm. Leaf production follows a rhythm of one leaf every three to four days. The seedling stage covers the period from the emergence of the first leaf to the emergence of the fifth leaf. During this stage the seedling also produces roots.

Stage 2 (Tillering). This is the period during which the seedling produces tillers. This stage starts with the emergence of the fifth leaf. The number of tillers increases until maximum tillering. Thereafter, some tillers degenerate and the number of tillers stabilizes. Tillering is highly influenced by soil fertility. Tillers originate from the auxiliary bud of the third leaf.

Stage 3 (Internode elongation). With the cessation of tillering, the plant's internode starts to elongate leading to increase in plant height.

The Reproductive Phase

This phase is characterized by panicle initiation, development of the spikelets and reproductive organs, heading, and flowering. Its duration is relatively fixed — between 30 and 35 days — regardless of variety or season. The reproductive phase is not affected by

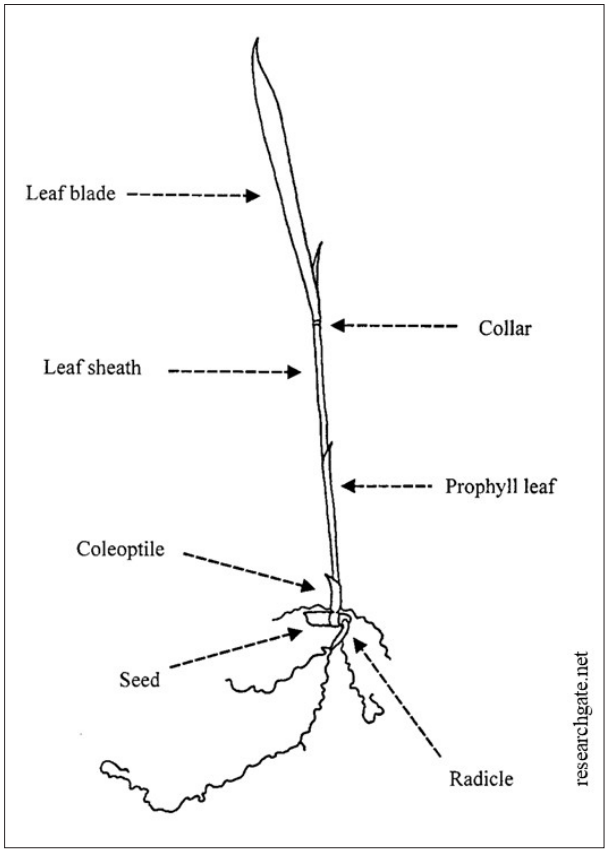

Figure 2.7. Parts of a young rice seedling.

photoperiod, but the stage is very susceptible to adverse temperature, drought, and salinity, which can lead to spikelet sterility and the production of unfilled grains.

Stage 4 (Panicle initiation or PI). The young panicle primordium that develops on the uppermost internode is at first a feathery cone-shaped organ of 1 - 1.5mm, which is only visible if the stem is dissected. The cone becomes visible only about 10 days after it is formed. At this stage, the number of grains in the panicle is already determined. In short-duration non-photoperiod varieties, maximum tillering, internode elongation, and panicle initiation occur almost simultaneously. These stages occur in medium- to long-duration varieties. Timing of panicle initiation in rice is influenced by many factors, among which are variety, temperature, and photoperiod. Panicle initiation is the beginning of the reproductive phase.

Stage 5 (Panicle development). This stage is characterized by the swelling of the bottom of the panicle leaf (flag leaf) due to the developing panicle that is growing upwards. The spikelet and floral organs develop and the panicle grows and move upward until it emerges from the flag leaf sheath.

Stage 6 (Heading and flowering). Heading is characterized by the emergence of the panicle from the flag leaf sheath. Three days after panicle emergence, flowering occurs and the process goes on progressively until the panicle has completely appeared. Flowering means the opening of the spikelets and the dehiscence of pollen from the anther sacs resulting in pollination. In rice flower opening or blooming usually occurs between 9 a.m. and 11 a.m. on bright days, which may be delayed on cloudy or rainy days. Spikelets open with the elongation of the filaments pushing out the anthers and the feathery stigma becoming clearly visible. After pollen dehiscence, the anther sacs dry out and the filaments become pendulous. The pollen tube grows down the style into the ovary and fertilization is affected. The two husks (lemma and palea) close and will not open again. Zygote, the product of fertilization, gives rise to the embryo, which is a diploid tissue. The endosperm is triploid, yielding from the fusion of two male gametes and the egg cell.

The Maturity Phase

This includes the post-flowering stages: milky, dough, and mature stage of the grain. Its duration is also relatively fixed, about 30 - 35 days. Period of grain maturation (grain filling) is shorter under high temperature and longer under low temperature. The crop at grain filling is susceptible to weather extremes like high temperature, strong winds, drought, and prolonged wet periods and low solar radiation.

Stage 7 (Milky stage). After fertilization, the ovary swells and the caryopsis develops until it reaches its maximum size after seven days. The grain (caryopsis) is first aqueous and then reaches a milky consistency, which is perceptible when the grain is squeezed. At this stage, the panicles are still green and erect.

Stage 8 (Dough stage). The milky part of the grain becomes soft and then reaches a hard paste consistency about two weeks after flowering. The panicle begins to droop while the color of the glumes slowly changes color. The moisture content of the grain is above 22%.

Stage 9 (Maturity). The grain is ripe or mature when it has reached its final size and maximum weight. The panicle is droopy from the weight of the grains. Grains become hard and develop characteristic colors from green to straw color or to a color characteristic of the variety (reddish, black, and purple of different shades). Plant is ready for harvest when 85 - 90% of the grains in the panicle are ripe. At this stage, grain moisture content is approximately 20 - 22%.

Mode of reproduction

Rice floral structure favors high level of self-pollination. The floral organs (anther and stigma) are enclosed in a pair of glumes and pollination can occur on the opening of the spikelets or immediately thereafter. Position of the anthers relative to the stigma is a feature conducive to self-pollination.

Blooming habits

Flowering typically begins one day after heading. Flower opening starts from the top of the panicle proceeding downward to the lower spikelets until all florets in the panicle had opened. The duration generally takes 7 days to complete. Anthesis begins with the opening of the florets followed by stamen elongation generally lasting from 1.2 to 2.5 hours between 9 a.m. - 11 a.m. However, anthesis is temperature dependent and can take longer and occur later on cooler or cloudy days. Pollen shedding happens within 9 minutes of floret opening. Pollen usually falls into the stigma of the same floret, resulting is self-pollination. Pollen remains viable for less than 5min and can be wind borne about 100m. Fertilization is completed within 6 hour after pollination.

CHAPTER 3

Breeding Methods Commonly Used in Developing of Inbred Varieties

Thelma F. Padolina, Norvie L. Manigbas, and Alex T. Rigor

Plant breeding is defined as identifying and selecting desirable traits in plants and combining these into one individual plant. Since 1900, Mendel's laws of genetics provided the scientific basis for plant breeding. As all traits of a plant are controlled by genes located on chromosomes, conventional plant breeding can be considered as the manipulation of the combination of chromosomes. In general, there are three main procedures to manipulate plant chromosome combination. First, plants of a given population, which show desired traits can be selected and used for further breeding and cultivation — a process called (pure line) selection. Second, desired traits found in different plant lines can be combined to obtain plants, which exhibit both traits simultaneously — a method termed hybridization. Heterosis, a phenomenon of increased vigor, is obtained by hybridization of inbred lines. Third, polyploidy (increased number of chromosome sets) can contribute to crop improvement. Finally, new genetic variability can be introduced through spontaneous or artificially induced mutations.

Selection

Selection is the earliest and most basic procedure in plant breeding. It generally involves three distinct steps. First, a large number of selections are made from the genetically variable original population. Second, progeny rows are grown from the individual plant selections for observational purposes. After elimination, the selections are grown over several years to allow observations of performance under different environmental conditions for making further eliminations. Finally, the selected and inbred lines are compared with existing commercial varieties in their yielding performance and other aspects of agronomic importance.

Hybridization

The most frequently employed plant breeding technique is hybridization, which aims to bring together desired traits found in different plant lines into one plant line via cross-pollination. The first step is to generate homozygous inbred lines. This is normally done by using self-pollinating plants in which e pollen from male flowers pollinates female flowers from the same plants. Once a pure line is generated, it is outcrossed, i.e., combined with another inbred line. The resulting progeny is selected for combination of the desired traits. If a trait from a wild relative of a crop species, e.g., resistance against a disease, is to be brought into the genome of the crop, a large quantity of undesired traits (like low yield, bad taste, low nutritional value) are also transferred to the crop. These unfavorable traits must be removed by time-consuming back-crossing, i.e., repeated crossing with the crop parent. There are two types of hybrid plants: interspecific and intergeneric hybrids. Beyond this biological boundary, hybridization cannot be accomplished due to sexual incompatibility, which limits the possibilities of introducing desired traits into crop plants. Heterosis is an effect achieved by crossing highly inbred lines of crop plants. Inbreeding of most crops leads to a strong reduction of vigor and size in the first generations. After six or seven generations, no further reduction in vigor or size is found. When such highly inbred plants are crossed with other inbred varieties, very vigorous, large sized, large-fruited plants may result. The term "heterosis" is used to describe the phenomenon of hybrid vigor. For hybrids, farmers must buy new hybrid seed every year because the heterosis effect is lost in the first generation after hybridization of the inbred parental lines.

Polyploidy

Most plants are diploid. Plants with three or more complete sets of chromosomes are common and are referred to as polyploids. The increase of chromosome sets per cell can be artificially induced by applying the chemical colchicine, which leads to a doubling of the chromosome number. Generally, the main effect of polyploidy is increase in size and genetic variability. On the other hand, polyploid plants often have a lower fertility and grow more slowly.

Induced mutation

Instead of relying only on the introduction of genetic variability from the wild species gene pool or from other cultivars, an alternative is the introduction of mutations induced by chemicals or radiation. The mutants obtained are tested and further selected for desired traits. The site of the mutation cannot be controlled when chemicals or radiation are used as agents of mutagenesis. As mutation effects random genetic changes, phenotyping of the mutants for various traits of interest is worth conducting to optimize the use of the induced variability in the mutation products for rice improvement.

Breeding Methods

Bulk Method

The bulk method is one of the methods used in segregating generations. It involves harvesting in bulk and growing a sample of seeds for every generation. When the desired level of homozygosity is reached or where the condition is favorable for selection, plant selection is made. Seeds of each selected plant are grown to a plot as in progeny plot method then record keeping begins.

Procedure for Bulk Method

Main steps in Bulk method

Step	Details
Hybridization	Crossing among selected parents
F_1 generation	F_1 seeds (minimum 20) planted. Bulk harvesting is carried out.
F_2 - F_6 generation	F_2 to F_6 are planted, harvested in bulk. Number of plants should be as large as possible. Generally artificial selection is not carried out.
F_7 generation	Generally, 30,000 – 50,000 F_6 seeds are space planted. Selection is carried out based on phenotype and 1,000 - 5,000 selected ones are harvested separately.
F_8 generation	Individual plant progenies are grown while inferior progenies are eliminated. Harvested in bulk.
F_9 generation	Preliminary yield trials with standard varieties as check. Selection is based on yield.
F_{10} - F_{13} generation	Multi-location yield trials are conducted using standard varieties as check. Performance of lines is evaluated.
F_{14} generation	Seed multiplication for the formal seed system.

Advantages of Bulk Method

- Simple and inexpensive
- Minimal record keeping
- Easy to handle populations as harvest is in bulk
- Natural selection can take place and it may bring about favorable results
- We can wait for opportunity to carry out selection. Selection for some environment-dependent characteristics like disease resistance and lodging resistance can be conducted when the environment is favorable for disease epidemic and severe lodging. The bulk can be maintained easily.

Disadvantages of Bulk Method

- Requires longer time to develop new variety
- Pedigree record is not maintained, so we cannot trace back progeny to the parent plant
- Large number of progenies has to be selected at the end of bulking period
- Natural selection may favor agronomically inferior plants
- Plants in a given generation may be disproportionately represented in the next generation
- Maximum productivity is established in F_2 generation
- No recombination occurrence among superior lines

Applications of Bulk Method

- Homozygous lines can be isolated in less time, automatic increase in homozygosity up to F_6 or F_8 generation after the individual plant is selected
- Natural selection may improve considered character (yield) with minimum expenses

If the purpose of the bulk method is just to advance the generation, space planting is not necessary. The population can be grown in the field or in the greenhouse. Space planting is required for advanced or terminal bulk population with plant selection.

Modified Bulk Method

The bulk method can be modified to include artificial selection. Mild selection for highly heritable characters can be made and seeds of selected plants are bulked. For example, in the F_2 population, semi-dwarf to dwarf early maturing plants are selected and from the bulked seeds of the selected, a sample is taken to represent the next generation. The process is repeated until the desired level of homozygosity is reached (F_4 - F_5). Uniformity for height and maturity increases with selfing generation. Plant selection in the terminal bulk population will focus on other attributes. Note that space planting is necessary for modified bulk.

Modified bulk method employs plant selection as in pedigree method but is free of tedious record keeping and of expensive progeny plots.

Single Seed Descent (SSD) Method

This method is a modification of bulk method. First proposed by Goulden (1939) and later modified by Brim (1966). Instead of bulking whole seed lot of selected plants, a single seed is selected randomly from each selected plant to make bulk. This method involves less record keeping and works better where the main focus is on improving quantitative traits or characters such as yield and earliness, rather than looking into qualitative traits or characters such as flesh color and disease resistance. Selection process is mainly artificial so chances of loosing superior plants are high and it does not eliminate weak plants in the early stage.

All F_2 plants are represented proportionately in succeeding generations until the desired level of homozygosity is reached or favorable environment for plant selection is present. Space planting is only needed when plant selection is followed by progeny plot method.

There are two general ways in which the principle of single seed descent can be accomplished. One approach is to keep the identity of a given F_2 plant in succeeding single plant generation. To ensure that a given F_2 plant will be represented continuously in all generations, at least two seeds of a plant are grown in a hill to safeguard against poor germination or death. When all plants in a hill survive, seeds are collected only from one plant. The second approach does not involve record keeping and population is handled as in bulk method except that equal number of seeds from each plant is bulked. This does not give 100% guarantee that all F_2 plants will be represented in succeeding generations. However, a lot of efforts are saved.

Just like bulk method, SSD is simple and does not require much resources in early selfing generations. It requires less record keeping and minimizes natural selection. SSD can also be modified to include artificial selection as in bulk method. The second approach can be easily subjected to selection if space planting is done. Equal number of seeds from selected plants is pooled. The process is repeated until the desired homozygosity is reached.

Procedure for Single Seed Descent Method

Single Seed Descent method includes stages as described:

Step	Details
Hybridization	Crossing of selected parents
F_1 generation	F_1 seeds grown and bulk harvested
F_2 generation	F_2 seeds grown. One seed from each plant is selected randomly and mixed.
F_3 generation	F_3 seeds are grown and harvested
F_4 and F_5 generation	Similar procedure is carried out
F_6 generation	F_6 seeds are planted. Selection for superior plants is conducted and selected ones are harvested separately. Number of plants could range from 150 to 500.
F_7 generation	The main point in this step is individual plant progenies are grown and selected plants are harvested in bulk
F_8 generation	Preliminary yield trials and quality tests are conducted
F_9 - F_{10} or F_{13} generation	Coordinated yield trials and tests for resistance and quality are conducted
F_{11} of F_{14} generation	Seed multiplication of basic seeds

Rapid Generation Advance (RGA)

Purpose

The Rapid Generation Advance (RGA) is a technique for routine rice breeding of segregating population into line fixation through SSD. SSD is an established breeding method for annual self-pollination of crops, which basically refers to the use of a single seed per line (i.e., one seed from a single plant) in a segregating population to advance one generation by self-pollination and fixing of lines; making the plants genetically homozygous (Collard et al., 2017). RGA technique is a faster version of SSD breeding, in which plants grown in greenhouses are enforced to have earlier flowering and seed setting for several generations or cycles, i.e., from F_2 to F_6

generations. A normal breeding cycle done in normal field conditions takes about 4 years to complete while breeding cycle could be shortened to less than 2 years or a year at best through RGA in greenhouses (Manigbas and Lambio, 2015). A simple room that was converted to an RGA facility was used to speed up generation advance of the breeding materials (Figure 3.1).

The advantages of RGA as a breeding method include the technical simplicity and less requirement of field and labor resources resulting in savings and faster generation advancement (Poehlman and Sleper, 1995; Stoskopf et al., 1993). Record keeping is also not required for RGA. Usually, a seed increase step in the field is required to produce sufficient seed for yield testing in plots. Reported disadvantages include the retention of poor lines during generation advancement, the identity of F_2 plants is not retained, and within-family selection is unfeasible to practice. Breeders also perceive that the genetic variation captured by this method is not as wide as the pedigree method. Furthermore, RGA lines are considered inferior than the other main breeding methods using early-generation visual selection.

Procedure for RGA

Screenhouse system

The screenhouse method uses seedling trays to grow rice plants (Figure 3.2). Seedling trays has 8 rows x 13 columns, 104 cells per tray; 36cm × 56cm) and each cell contained <40cm^3 of soil for a single breeding line (i.e., originally derived from a single F_2 plant). Direct-dry seeding is performed by seeding about 4 - 5 seeds per cell to ensure 95 - 100% germination rate. Plants are later thinned to one plant per cell around 10 - 14 days after seeding (DAS). Minimal fertilizer is applied. Harvesting begins at 90 DAS depending on the population, and completed by 95 - 105 DAS or within a ~2 week window. Once the target generation had been achieved (e.g., F_5 or F_6), the panicles are planted in the field in panicle rows as initial step for seed increase (i.e., usually $F_{5:6}$ seed). Before selection, uniformity of each line is checked for segregation to avoid outcrossing or mixtures. Repeated check varieties are used for observation including comparison with susceptible check varieties to eliminate highly susceptible lines.

Figure 3.1. A simple Rapid Generation Advance (RGA) facility where breeding lines are advanced from F_2 generation until F_6 in just a year.

Figure 3.2. RGA screenhouse system facility at the PhilRice.

Field RGA

Ideally, RGA is best performed in screenhouse or greenhouse facilities where the environment can be easily manipulated. However, when such facilities are not available, or if screenhouse/greenhouse facilities are insufficient, RGA can be performed in the field, and plants can be seeded or transplanted directly into the soil of the designated field area (Figure 3.3). This is possible when the daily temperatures in a location do not fluctuate much during the year. A variant of this was method previously described by Fahim et al., 1998 who used seedling trays and sowed seeds at high density, but did not directly sow seeds into the soil of the field area. In both cases, these breeding populations need to be carefully protected from animals such as rats and birds in a designated field area, and the selected areas must have access to irrigation.

There are several advantages of field RGA. First, this method can be used when screenhouse/greenhouse facilities are unavailable. Second, plants grown in the field may avoid artificial micro-climates often encountered in greenhouse or screenhouse facilities, which can reduce risks of pest outbreaks. Third, temperature may be more suitable than RGA in greenhouses without adequate temperature control or shading. The disadvantages of field RGA include genetic drift and possible extensive loss of material due to field pests or natural disasters such as typhoons, although this is also possible for material grown in screenhouses/greenhouses.

At the International Rice Research Institute (IRRI), the field RGA method was further modified by inserting the seedling trays directly into the soil. The plastic trays are used to constrain plant growth by restricting root development and keep the plants smaller and with fewer tillers than these would have if planted directly into the soil using 20cm x 20cm spacing. This also minimized edge effects. Seeds are pre-germinated in seedling trays before placing on top of the soil, which permitted roots to grow into the ground from the hole in the cells of the

Figure 3.3. Field RGA at the PhilRice.

seedling tray. At harvests, only a single panicle is taken with sufficiently filled spikelets and processed in the same way as the screenhouse method.

Materials required:

Consumables

- Coin envelopes (6-inch diagonal)
- Brown paper bags (size 16)
- Labelling tapes, waterproof pen markers
- Brown wrapping papers (Manila paper)
- Filter papers or paper towels
- Packaging materials (e.g., cartons) for turnover of F_6 seeds to the client/s

Chemicals/Fertilizers

- Iron Sulfate ($FeSO_4$), in powder form
- Ammonium Sulfate ($(NH_4)2SO_4$), in granular form
- Complete compound (basal) fertilizer with N:P:K ratio of 14-14-14, in granular form

Greenhouse and laboratory wares

- Minoru® trays (104-celled)
- Plastic crates with removable lids (l*w*h dimensions: 60-40-16.5cm) to house the
- Minoru trays
- PVC pipes (63mm diameter)
- Manual single hole puncher (see image in section 4.4)
- Framed screen mesh, 3 or 5mm mesh size, for soil sieving
- Shovels
- Transport carts (for the crates)
- Plastic wares (big trays) for preparing and mixing fertilizer with soil
- Small plastic scoops
- Plastic buckets
- Petri dishes
- Wash bottle
- Spray bottle

Soil and seeding tray preparation

Soil sieving

Sterilized fine soil must be used as the growth medium for the seeds as soil aggregates with an effective diameter of 5mm or smaller. Thus, coarse soil is sieved through a screen mesh, sized 3 or 5mm. The fine soil is then collected in plastic buckets or trays; making sure that the sieved soil is dry prior to sterilization.

Recurrent Selection

Recurrent selection means repeated cycles of selection and breeding aimed at gradual genetic improvement of a few key traits in a single species. The benefits of breeding are cumulative in each cycle or the generation of improvement builds upon advances made in prior generations. Genetic gain in selected traits results in the changes in frequencies of alleles at loci controlling expression of those traits, with favorable alleles increasing in frequency. As most commercially important traits are polygenic (i.e., controlled by many loci), gene frequencies change slowly at any single locus and these changes are generally unknown to breeders. Progress is measured by mean performance for target traits (e.g., greater harvest yield, reduction in disease incidence, or increased wood density). Performance for non-target traits should only have minor changes, as long as the traits are controlled by different loci rather than the target traits. Starting with larger founding populations and infusing unrelated material into the population also extend the number of cycles before a selection plateau is reached.

Pedigree Method

In pedigree method, detailed records of the origin of the selected individuals or lines are maintained. As such, we know from which F_2 plant the selected progeny originated. It is the most extensively used method to handle segregating generations from crosses.

A general procedure for pedigree method is presented. However, it may vary according to various conditions.

Step	Details
Hybridization	The first step involves crossing between selected parent plants
F_1 generation	Seeds obtained by hybridization (F1 seeds) are planted with proper sowing distance. Seeds of about 20 - 30 plants are harvested in bulk and forwarded to grow F2 generation.
F_2 generation	Selection is the main process carried out in this step. About 1,000 - 2,000 plants are grown from F1 generation seeds (F2 seeds). With application of selection process, about 500 plants are selected and harvested separately.
F_3 generation	About 30 or more progenies are raised from each of the selected plant of F2 generation. About 100 - 400 superior plants (the number could be anything, preferably less than those selected in F2 generation) are selected.

F_4 generation	Seeds from F3 generation are space planted. Plants with desirable characters are selected in number much less than those selected in F3 generation.
F_5 generation	Individual plant progenies planted in multi-row (three or more) plots so that superior plants (about 50 - 100) can be selected by comparison.
F_6 generation	Individual plant progenies planted in multi row (three or more) plots. Plants are selected based on visual evaluation. Progenies showing segregation can be eliminated.
F_7 generation	Preliminary yield trials with minimum three replications and a check. Quality tests are conducted.
F_8 - F_{12} generation	Multi-location yield trials with replications are conducted. Tests for quality and disease resistance are conducted.
F10 or F13 generation	Seed multiplication for distribution

Applications of Pedigree Method

- Most commonly used in handling segregating generations from crosses in self-pollinated crops
- Suitable for improving high heritability characters that are quick and easy to measure such as disease resistance, plant height, maturity
- Used to improve an established variety for some specific character

Advantages of Pedigree Method

- Excellent method for improvement of easily observable, high heritability characteristics
- As pedigree record is maintained, information regarding inheritance pattern of characters can be easily obtained
- Each plant can be traced back to its parent plant
- Only progeny lines containing plants with desired characters are selected for next generation
- Progeny is tested. Thus, it is based on genotypic value rather than phenotypic value.
- Increased breeding efficiency by early identification of superior heterogeneous populations
- Scope for transgressive segregation occurs for the characteristics like yield
- New variety development is shorter than the bulk method

Disadvantages of Pedigree Method

- Costly
- Labor intensive
- Requires skilled person as selection is practiced
- Record maintenance is time consuming
- Selection for yield or other characteristics in F_2 and F_3 is ineffective
- Genetic variation available for selection decreases in later generations due to the individual plant selection carried out earlier

Backcross Method

Backcross method is used to incorporate desired gene, either dominant or recessive, to the highly productive, commercially successful variety, which lacks that specific gene.

Backcross method is named as "backcross breeding," which has been used by plant breeders to transfer specific character into elite lines. The variety, which receives gene is the recipient parent and the variety where the gene came from is the **donor parent.** Recipient parent is used repeatedly; hence, also called the recurrent parent. Donor parent is non-recurrent parent. Repeated backcrossing leads to homozygosity at the same rate as selfing.

Use of Backcross Breeding Method

- Backcross to transfer dominant gene
- Backcross to transfer recessive gene

How dominant gene is transferred with Backcross Breeding?

Let variety X the well-adapted and high-yielding variety.

Variety Y is another variety resistant to a specific disease, which is governed by a dominant gene. We wish to transfer this dominant gene form variety Y to variety X. So, variety X is the recurrent parent (♀) and variety Y is the donor parent (♂).

The **steps in** backcrossing are as follows:

1. **Hybridization** – The two varieties X and Y are crossed. Recipient/recurrent variety is used as female parent.
2. **F_1 generation** – Plants from F_1 seed are back crossed with recipient variety X. Selection for disease resistance is not performed.
3. **First backcross generation (BC_1) –** Selection is done for disease resistance and selected ones are back crossed with recurrent parent X.
4. **Second to fifth backcross generation (BC_2 - BC_5)** – Segregation for disease resistance occurs in every back cross generation. Plants are selected based on similarity with the recurrent parent and resistance to disease. Selected ones are back crossed with recurrent parent X.

5. **Sixth backcross generation (BC_6)** – Disease resistant plants are selected. They are self-pollinated and harvested separately.
6. **BC_6 F_2 generation** – Individual plant progenies are grown from seeds of BC_6 generation. Plants are selected based on similarity with the recurrent parent and resistance to disease. They are harvested separately.
7. **BC_6 F_3 generation** – Individual plant progenies are grown from seeds of above cross. Plants are selected based on similarity with the recurrent parent and resistance against disease but harvested in bulk.
8. **Yield trials** – Yield trials are replicated with recurrent parent as a check. The newly-constituted variety should be similar with variety X for most of the important characteristics. Seeds are multiplied for distribution.

How recessive gene is transferred with Backcross Breeding?

Let variety X the well-adapted and high-yielding variety.

Variety Y is another variety resistant to a specific disease (e.g., rust disease resistance), which is governed by a recessive gene. We wish to transfer this recessive gene from variety Y to variety X. So, variety X is the recurrent parent (♀) while variety Y is the donor parent (♂).

When the desired character, i.e., disease resistance is governed by a recessive gene, back crosses are not performed in continuous manner as in dominant gene transfer method. After first back cross and then after every two back crosses, F_2 must be raised to test rust resistance. Only F_2 is tested for rust resistance as all F_1 and back cross progenies are heterozygous and susceptible to disease.

The steps for this backcross breeding are as follows:

1. **Hybridization** – The two varieties X and Y are crossed. Recipient/recurrent variety is used as female parent.
2. **F_1 generation** – F_1 plants are back crossed with the variety X.
3. **BC_1 generation** – Plants raised from seeds of above cross are selfed. As disease resistance is controlled by recessive gene, all the plants will be susceptible to disease, so disease resistance is not tested for this generation.
4. **BC_1 F_2 generation** – Disease resistance is evaluated. Disease resistant plants with similar plant characteristic to the recurrent parent are selected and back crossed with recurrent parent.
5. **BC_2 generation** – Plants are grown from seeds of above cross. Plants with similar plant characteristic to the recurrent parent are selected. Resistance is not tested.
6. **BC_3 generation** – Plants are selfed to grow F_2. Resistance is not tested for selection, rather for determining the resemblance to the variety.
7. **BC_3 F_2 generation** – Selection for resistance is conducted and selected plants are back crossed with variety X.
8. **BC_4 generation** – Back cross of plants selected above with recurrent parent. Disease resistance is not evaluated.
9. **BC_5 generation** – Plants are selfed to raise F_2. Disease resistance is not evaluated in this generation.
10. **BC_5 F_2 generation** – Disease resistant plants with similar plant characteristics to the recurrent variety are selected. Selfed and seeds are harvested separately.
11. **BC_5 F_3 generation** – Individual plant progenies are grown. Disease resistance and resemblance to variety X are selected. Seeds of selected plants are bulked to constitute the new variety.
12. **Yield trials** – Yield trials are replicated with variety X as check. The newly-constituted variety should be similar with variety X for most of the important characteristics. Seeds are multiplied for distribution.

CHAPTER 4

Breeding Objectives and Selection Criteria for Desirable Traits

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It is important to identify the traits to improve before understanding a breeding program for rice. As the country aims for rice sufficiency, rice breeders should consider looking at traits or characters that contribute to yield. The traits affecting yield include the number of productive tillers per unit area, the number of grains per panicle, percentage filled spikelets, and the weight of 1,000 grains. The ecosystem where rice crop is grown and other factors affect grain yield. It is difficult to select for yield alone as a quantitative trait is governed by many genes.

Agronomic Characters

Lodging resistance

Resistance to lodging is a requirement for stable high grain yield. Height and strength of straw or culm are the major traits affecting lodging. Semi-dwarf to intermediate height (80 - 115cm) is generally more resistant to lodging than tall plants. Plant stature of varieties could depend on target ecology. In general, semi-dwarf varieties are bred for irrigated lowland and intermediate varieties for rainfed conditions. Stiff-strawed varieties with the leaf sheath tightly enclosing the culm and with erect rather than droopy leaves are good indicators of lodging resistance. In general, transplanted varieties are better established in the soil than similar varieties that are direct-seeded. Hence, stronger lodging resistance is sought for varieties intended for direct- seeding than in transplanting.

Leaf characteristics

Breeders discriminate long and droopy leaves over more erect leaf angle. Thick green leaves are also preferred over thin and pale leaves. Erect leaves generally allow deeper penetration and more even distribution of sunlight resulting in increased photosynthesis and minimized lodging. Breeders developing varieties for rainfed conditions consider height and leafiness as factors that will help rice against weed competition. Glabrous leaves (without pubescence) are generally preferred when rice is grown under mechanized system (combine harvesting) to avoid skin irritation and inconvenience in harvesting. This also happens in manual harvesting and mechanical threshing. It is important for early maturing varieties to have sufficient leaf area at maximum tillering. For this reason, it is recommended for very early maturing varieties to be direct-seeded for dense planting and to minimize transplanting shock. Long and near erect flag leaf (20 - 30^{o}) are often observed in high yielding varieties. Panicle position is preferably below the top canopy.

Dormancy

Ripening grains that are continuously drenched during the wet season germinate prematurely especially if the variety has no dormancy period. Aside from causing heavy yield losses, lack of dormancy period is also a problem as rice crop has to be dried immediately. However, long dormancy period is not preferred because it takes more time to use the harvested seeds. Dormancy period of 2 - 3 weeks is generally preferred. For seed production and distribution, information on seed dormancy is especially important to guide seed producers and farmers on the appropriate time to sow the seeds.

Milling recovery

Milling recovery of at least 65% is considered acceptable. However, breeders should develop varieties with more than 65% milling recovery as this is a determinant of

actual yield. There are genetic differences in milling recovery. The trait is influenced by age at harvest, prevailing temperature and humidity during ripening, post-harvest operations like threshing and drying, moisture content, and the type of milling machine. Breeders should select grains that are medium long, slender, and translucent (less chalky) for milling. Long grains are preferred by some consumers; but in general, they break during milling resulting in lower percent head rice. Chalky grains are also more prone to breakage during milling. Percent total milled rice is greater than or equal to 65.1% while percent total head rice is greater than or equal to 48% based on amount of rough rice.

Threshability

Varieties differ in threshing characteristics. Some are difficult to thresh while others are easily shattered from the panicles even with slight pressure. Breeders disregard the shattering varieties and those that are difficult to thresh. Grain losses during harvesting and handling are higher in shattering varieties. However, in very hard threshing varieties, some grains still remain in the panicle and are thrown away with the straw during threshing. Breeders generally select varieties with moderately firm threshing result. Breeders measure shattering during field selection by pressing the mature panicles in the palm of the hand. Panicles with most dislodged grains are considered shattering. Varieties are considered hard threshing when few grains are dislodged from the panicle. Varieties are more shattering in cold than in warm environment.

Photoperiod

Rice is a short-day plant; however, there are varieties that flower depending on daylength. Varieties are either strongly photoperiod-sensitive, weakly sensitive, or non-sensitive. Many modern varieties are non-photoperiod sensitive. Strongly photoperiod sensitive varieties only flower when the length of day is short. In the development of the varieties, genotypes that are non-photoperiod sensitive or flowering regardless of daylength are selected. Growth duration of non-photoperiod sensitive varieties remain nearly the same regardless of planting date. Varieties differing in growth duration based on planting date signal photoperiod sensitivity. Photoperiod-sensitive varieties are useful only under certain growing environment.

Sterility (empty spikelets)

In some varieties, spikelets at the basal part of the panicle are unfilled in varying amounts that could go up to 15% and in some cases up to 30% during the wet season. Causes of empty spikelets could be inherent to the variety or due to environmental stress like disease, low and high temperature, and drought, among others. Breeders should ignore varieties with high percentage of unfilled spikelets.

Response to fertilizer

Good fertilizer response is an essential trait of modern varieties. Breeding lines are usually evaluated for fertilizer responsiveness before these are recommended to the farmers. Fertilizer trials are conducted to determine the efficiency in converting nutrients into grains and the effect on yield. It is also important to know whether the increase in fertilizer could yield higher harvest without the effects of lodging and insect and disease susceptibility. This is especially true for applied nitrogen, which is largely related to plant height — a trait that is also connected to lodging resistance and grain-straw ratio. High nitrogen application may also increase yield but relative amount of increase is low; hence, not economical. Phosphorous and potassium are also supplied in varying amounts.

New varieties are released for commercial cultivation if they are better in one or more desirable attributes than existing varieties. These attributes include grain yield, growth duration, resistance to major insect pests and diseases, good milling characteristics, acceptable cooking and eating quality, and good response to applied fertilizer.

Resistance to insect pests and diseases

Diseases

Breeding for disease resistance is not solely a breeder's work. It requires an interdisciplinary approach in which geneticists, molecular biologists, agronomists, pathologists, and biometricians have a full grasp of the problems and find the right solutions. An efficient, accurate, and reliable screening procedure in the field and laboratory is the key to the successful identification and selection of resistant genotypes. The major diseases include rice tungro virus, bacterial leaf blight, blast, and sheath blight. Tungro virus is transmitted by the insect green leafhopper (*Nephotettix virescens*). Rice plants resistance to insect alone does not ensure protection from virus infection but it can reduce green leafhopper population and avoid outbreaks of tungro. Bacterial leaf blight caused by *Xanthomonas oryzae* usually occurs when high nitrogen rates is applied, rainfall is heavy, dense planting is practiced, and susceptible varieties are used. Blast is caused by *Pyricularia oryzae* and considered one of the most destructive diseases in rice.

Several genes had already been identified and could be used in the breeding program. Sheath blight caused by a fungus *Rhizoctonia solani* is one of the most widespread diseases, which cause severe yield losses.

Identification of improved gene source and donors should contribute to a new, high level of stable resistance to these diseases as the level of rice resistance seems to weaken in recent years despite improved breeding. One of the reasons is that diseases occur as a result of changing climatic conditions. Another is the improper cultural management practices and indiscriminate use of pesticides, which led to disease recurrence and proliferation of more virulent pathogens.

Gene transfer through biotechnological means is already being practiced using donor genes from the wild type and genetic engineering. Molecular breeding approach using linked molecular markers and polygenic transformation system should contribute significantly towards generating stable disease resistance along with high yield potential and good grain quality. The development of transgene-pyramided stable elite rice lines that are resistant to a particular disease could be applied by conventional breeding. Integration of different approaches to control disease problems remains a challenge to scientists to develop rice varieties with stable resistance to major diseases.

Pests

Insect pests normally transmit virus diseases to rice plants while others cause damage by directly feeding on the plant. The most destructive insect pests of rice are the brown planthopper (*Nilaparvata lugens*) and green leafhopper (*Nephotettix virescens*), which transmit virus to the plants. Different biotypes had been identified with brown planthopper and green leafhopper; making these insects more difficult to control. Breeding strategy for multi-genic and durable resistance can address this problem.

Stem borers and white-backed planthoppers cause damage by feeding and sucking the sap of the plants that ultimately lead to substantial loss in yield. White-backed planthoppers attack rice seedlings while stem borers cause damage in older plants by feeding inside the stem, drying the leaves, and producing white heads or empty white panicles at reproductive stage. Efficient screening techniques and incorporation of multiple resistance genes to new cultivars with diverse backgrounds should be the aim of the breeding program.

Tolerance to abiotic, physical, and chemical stresses

Salinity and Drought

Identifying target areas is critical in setting the objectives of the breeding program for these environments as the source of genetic materials or genes is extremely useful. In the past, breeders try to simulate the problem soil conditions of an area under laboratory or *ex-situ* conditions to have a good control in conducting the experiment. However, this technique presents problems because of unrealistic evaluation of the rice genotypes, which more often leads to failure of the breeding program. Breeding for salinity and drought is difficult because of the complexity of the environment and its interaction with the genotypes. Currently, the most practical method is simply plant in the fields where the problem occurs. In this way, the breeder can readily distinguish the rice genotypes that exhibit differences in their ability to survive and obtain yield under these conditions.

Submergence

Prolonged submergence of the rice plant at any growth stage could reduce yield. It is important that before screening genotypes for submergence tolerance, characterization of the target area should be considered. Characterization includes type of soil, topography, agro-climatic conditions, weather data, history of pest and disease occurrences, source of flood water, and most importantly, flooding regimes or duration of floods. Incorporating submergence tolerant gene, *sub-1*, to high yielding varieties has improved rice yields under this environment with submergence up to 14 days during vegetative growth stage.

High and Low temperature

Rice is most susceptible to high temperatures during reproductive stage. Temperature above 35^{o}C during daytime and above 29^{o}C at night can significantly reduce yields by 5 - 10%. Heat stress affects pollen and floret fertility during flowering. High temperature also makes the pollen grains sticky; thus, grains have difficulty shedding, which ultimately cause defective pollen release. Genetic resources for high temperature tolerance have been identified and gene incorporation will improve the yield performance of rice under heat stress conditions.

Low temperature tolerance is important in high elevated areas. Temperature at 18°C below during the day and night can significantly increase sterility; thus, reduce yield. Depending on location, low temperatures can occur at any growth stage of the rice plant and screening only at a certain stage may not be useful. It is important that genotype screening is done in an actual environment so that selection can be effective. Sources of tolerance for this adverse condition are already available worldwide

Zinc deficiency

Continuous rice cropping and high application of phosphates and urea fertilizers resulted in soil zinc deficiency. Efficient zinc-using rice genotypes or tolerant to zinc deficiency is an approach in developing cultivars for this environment. Cultural and management practices such as application of zinc sulphate to the soil and dipping roots to zinc oxide solution will correct this deficiency.

Peat soils

Soils with high content of organic matter are called peat soils. These are also used for growing rice; however, peat soils have been generally recognized as problem soil with marginal agricultural productivity. They are poorly drained and waterlogged throughout the year or within the rice growing period; thus, nutrients are deficient and unavailable for rice crop and the soil is toxic and acidic. Rice improvement under these soil conditions is a challenge for breeders. Large number of germplasm should be screened under field conditions so that selection of desirable phenotypes could be achieved.

Grain quality

Good grain quality is an important characteristic to producers and consumers. Medium to long white translucent grains is most preferred by consumers in tropical countries including the Philippines. Rice with medium to long, white translucent grains, and good grain expansion when cooked, commands a good price in the market. Intermediate amylose and gelatinization temperature are important so that the cooked rice is soft and slightly sticky after it has been cooked for long hours. Aromatic grains are also preferred because aroma increases palatability. However, aromatic rice varieties may sometimes be difficult to grow because they are prone to pests and rodents attack.

Rice consumers nowadays are willing to pay extra amount for the type of rice they wish to buy. The rice should not get spoiled within 5 hours after it has been cooked and its characteristic good quality is maintained. Eating quality should also be highly acceptable.

Rices for special market

Rice for special market draws premium price not because of yield, but due to its excellent quality. Usually, these rices are low to intermediate yielding cultivars marketed in malls, large outlets, or other countries. These rices could be traditional cultivars or landraces with specific growing areas. They are not widely grown because of specific adaptation to climate and farming practices.

Nutrient-rich rice

To address malnutrition and add more value to rice, breeding high nutrient-dense rice is very promising. Micronutrients such as iron and zinc had been incorporated in the rice grain of high yielding cultivars together with other important traits. Vitamin A-rich rice has been developed to address vitamin A deficiency in infants, children, and pregnant women. Source of genes had been identified and successfully used in the breeding program.

CHAPTER 5

Hybridization

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Hybridization involves crossing of two individuals to create genetic variability and to combine desirable traits into a single genotype. Rice is a highly self-pollinated crop with outcrossing reported at less than a fraction of one percent. As rice is a highly autogamous crop, in which the pistillate and staminate parts are enclosed in a pair of glumes (lemma and palea), producing crossed seed is more difficult and cumbersome than other crops of different floral structure. In hybridization, however, only small quantities of crossed seeds are sufficient.

Choice of parent

Choice of parents depends on breeding objectives. Generally, breeders would choose a parent with improved genotypes. Choice of the second parent is to complement traits lacking in the other parent. The traits of interest should be intensely manifested in the parents. Crosses are made to improve one or more traits such as improved plant type, yield potential, resistance or tolerance to biotic and abiotic stresses, and preferred grain quality. Objectives of crosses may include improving resistance to tungro virus, blast, and leaf and plant hoppers. Improving rice milling recovery and eating quality is also a common objective in making crosses.

Establishing the parent

Make a list of the parents to be used in crosses during the season. In the list, group the parents based on outstanding traits such as plant type, yield potential, resistance to pests and diseases, and grain quality. In irrigated lowland direct-seeded rice, tolerance to anaerobic condition is a valued trait. Incorporating tolerance to submergence is increasingly becoming important in irrigated and rainfed rice. Seeds can be sourced from the program's elite lines and released varieties, germplasm bank materials, lines from international yield and screening nurseries, traditional varieties, wide hybridization, and pre-breeding derived lines.

Seed preparation

1. Prepare list and assemble seed of varieties/lines chosen as parents for the season.
2. Prepare in a coin envelop three sets of 3-gram seed per set of each parent.
3. Break dormancy by placing the seeds in an oven at 50 - 55^{0}C for 4 - 5 days.

Seeding

1. Seedlings could be raised in seedbed in the field or in the screenhouse in concrete bed or seed trays.
2. Seed the parents on three dates at 7 - 10 days interval to synchronize flowering.
3. Make furrows in the seedbed at 60cm long and 10cm apart.
4. Furrows are labelled. Seed envelopes are distributed and plot number are double checked before seeding. Labels should be written in black indelible ink.
5. Distribute the seeds evenly in the entire length of the furrow ensuring that seeds are contained in the furrows.
6. Cover the furrows with fine soil. Coir or saw dust are good substitutes.
7. Seedbed should be kept wet but not flooded.
8. Seedlings are ready for transplanting 21 days after seeding.
9. Seedbed is flooded to soften the soil before pulling the seedlings.
10. Labels should be tied with the seedling bundle.

Transplanting

1. Transplant the seedlings 21 days after seeding. Transplant at least 20 - 25 plants per parent for each date at 25cm between hills and 30cm between rows.
2. Transplant only one seedling per hill.
3. Replant missing hills 5 - 7 days after transplanting.
4. The crossing block is managed as in other breeding nurseries.
5. Observe and record the following: days to heading, plant height, tillering capacity, grain size and shape, reaction to major pests and diseases, and overall phenotypic acceptability at maturity.

Emasculation and Pollination

Emasculation

Emasculation is the process of removing the anthers from the florets or rendering the pollen non-functional by physical treatment without damaging the female reproductive parts (Figure 5.1). The objective of emasculation is to avoid self-pollination. Plants intended as female parents in crosses are emasculated.

Selection of female parent

In principle, either of the two parents could be used as female or male in a particular cross. However, in choosing the direction of the cross, the parent with obvious recessive traits if present are used as female to identify true hybridity. For example, tall plants are normally used as male parent and short plants as female parent. True F_{1s} are expected to be taller than the female parent. Pubescent or pigmented genotypes are also used as male parents. Choice of parents, however, is more governed by the objectives rather than morphological marker for the identification of true F_{1s}.

1. Make a list of intended cross combinations and number of F_1 seeds required to be produced. Number of florets and panicles to be emasculated depend on the amount of seeds needed. Twenty crossed seeds are already enough for single crosses with two inbred parents. Two to three emasculated panicles will normally yield this amount of seeds.
2. For topcrosses and three-way crosses, at least 60 seeds should be produced and more seeds are needed for double crosses.
3. Generally, a breeder is not confined to materials grown in the crossing block. He/she may wish to use the materials in the field breeding nurseries like pedigree nurseries, observational trial, replicated yield trials, or other trials.
4. Plants are ready for emasculation when about 50% of the tiller have emerged panicles.
5. Take the whole plant by cutting the soil around the base with a sickle ensuring minimal damage to the root system (Figure 5.2). Bring to the screenhouse and place in 8- or 9-L plastic bucket. Add soil leaving 5cm space for water. Lifting of plants is better done early in the morning or at least 6 hours before emasculation for the plants to recover.
6. Label the plant with the crossing block number and with variety or line designation (Figure 5.3A).
7. Trim off excess leaves for ease in emasculation. Expose the whole panicle from the leaf sheath prior to emasculation (Figure 5.3B).

Figure 5.1. Machine emasculation.

Figure 5.2. Collecting plant for emasculation.

(A) (B)

Figure 5.3. Plant (A) and panicles (B) ready for emasculation.

Materials needed for emasculation and pollination (Figure 5.4)

- Sharp pointed scissor (small)
- Black marking pen and pencil
- Paper tag of appropriate size
- Glassine bag of sizes #1/2 or #1 or cellophane bags
- Fine forceps
- Paper clips or cotton twines
- Plastic bucket (8 - 9L) or earthen pots
- Vacuum emasculator
- Stools

Emasculation Methods

A. Clip Method – This is the simplest and most efficient emasculation technique.

1. Under normal conditions of good sunshine, clip spikelet in the afternoon starting at 4 p.m. to ensure that there are no dehiscing anthers. Some breeders emasculate early in the morning (6 a.m. – 7 a.m.), but this practice is seldom used.
2. Using sharp scissors, cut off all spikelets, which have undergone anthesis and young immature florets (whitish) of the selected panicles.
3. Cut about 1/3 - ½ part of each spikelet obliquely (45^0) to expose the anthers. Clipping this way is helpful to facilitate anther extraction while retaining a bigger part of the glumes (Figure 5.5).
4. With a vacuum emasculator (Figure 5.1), pass the pipette tip over the clipped floret to extract the anthers (Figure 5.6A). Anthers could also be removed using fine forceps (Figure 5.6B). Rice spikelet has six anthers in a. Re-check the emasculated spikelets to ensure that no anthers or fragments are left to avoid selfing.
5. Cover the emasculated panicles with glassine or thin cellophane bags to prevent pollen contamination. Clip the bags to keep them in place (Figure 5.7).
6. Label the panicle with the line or variety name or code number.
7. Emasculated panicle is ready for pollination the next day. Emasculated plants should be kept in the screenhouse to protect them from rain.

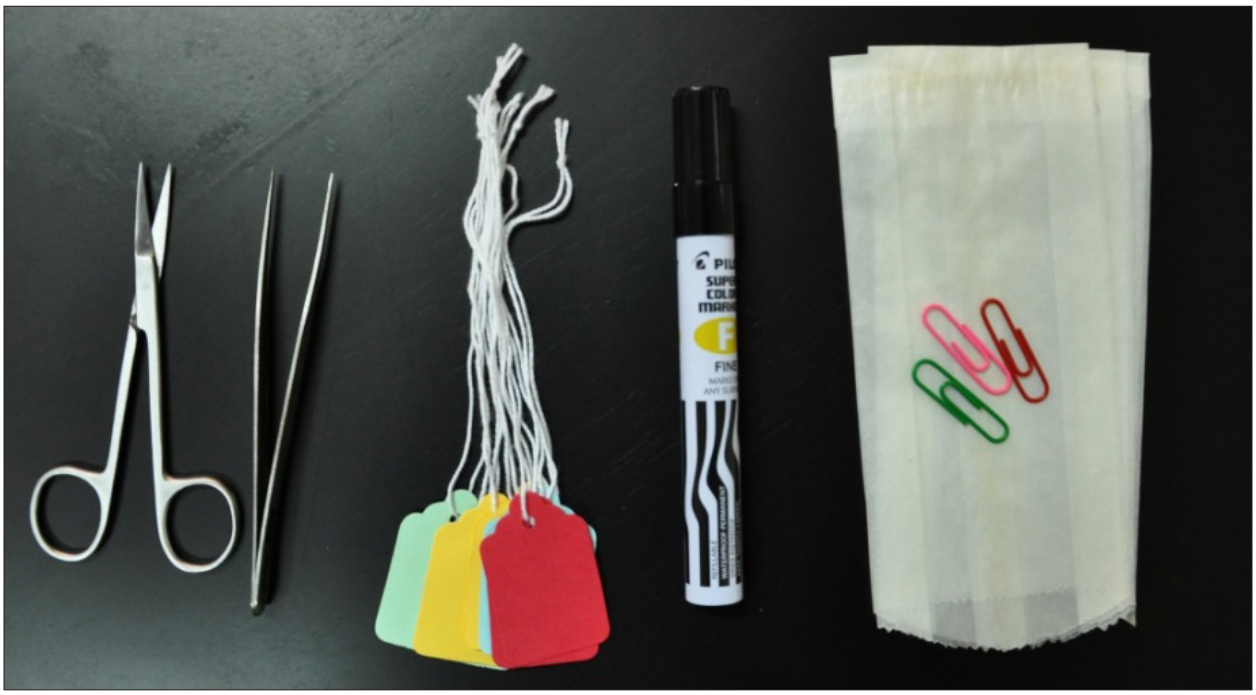

Figure 5.4. Materials for emasculation and pollination.

Figure 5.5. Clipping part of the spikelet using scissor to expose the anthers.

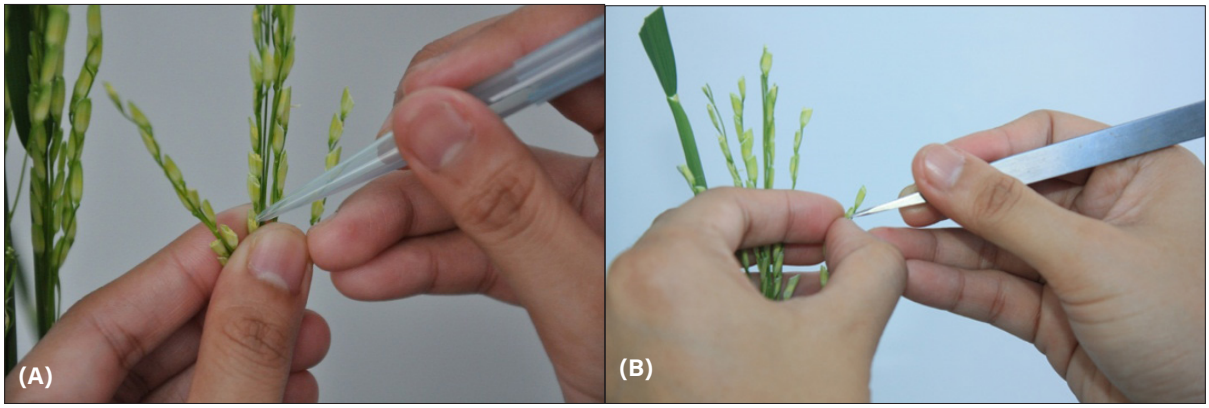

Figure 5.6. Removal of anthers using vacuum (A) and forcep (B).

B. Hot water method

1. When there is a special need for whole F_1 seeds with glumes intact, the heat treatment (hot water or hot air) is used to kill the pollen.
2. Plants are lifted, placed in bucket, and the leaves trimmed.
3. Remove dehisced and immature spikelets.
4. Spread the panicles on a basin and pour 40 - 44^{0}C hot water in the basin submerging all panicles for 5 - 10 min.
5. Cut off all unopened spikelets then remove the extruded stamens by forceps or by shaking the panicles to facilitate pollination.
6. Pollinate immediately after removing the unopened spikelets. Pollinate by dusting pollen from the male parent.
7. Cover the pollinated panicle with glassine or cellophane bag and keep the bag in place with a clip.

Figure 5.7. Panicles with large number of blooming florets (with extruded anthers).

Pollination

Pollination is the transfer of pollen grains from a desired male parent to the emasculated flower. It is normally done a day after emasculation between 9 a.m. to 12 noon. Anthesis time will vary depending on weather condition.

Method of Pollination

A. Dusting method

1. Collect 5 - 10 panicles of designated male parent with anthers about to extrude from the glumes before pollination. Cut panicles about 8 - 9cm from the panicle base.
2. Put designated label (line/variety, code).
3. Immerse the panicle stem in a pot containing tap water to avoid stress.
4. Wait until the spikelets open and the anthers are out of the glumes (Figure 5.8).
5. Handle dehiscing panicles gently to keep pollen from shedding.
6. Dust the pollen on top of the emasculated spikelets (Figure 5.9).
7. Cover the panicles with glassine bag indicating the cross and pollination date (Figure 5.10).
8. Fold the bottom of the bag and fasten with paper clip.

Figure 5.8. Gently shake or dust the panicle over the emasculated florets.

Figure 5.9. Covering with glassine bag after pollination and fastening with the clip to secure the cover.

Figure 5.10. Matured F_1 seed in an emasculated panicle.

Types of Crosses

- Single cross – is a cross between two varieties or lines. It is used when parents are both improved and have desirable traits that could complement deficiency in another.
- Backcross – is a cross of an F_1 to one of its parents. This is done if one parent of the single cross is more improved or desirable than the other.
- Topcross or three-way cross – is a cross of an F_1 with another variety or line. This method is used when both parents of a single cross are good combiners but lack one or more important trait.
- Double cross – is a cross between two F_1 hybrids. Use double cross if both parents of the single cross are reasonably good performing but lacking in important traits that cannot be provided by a single parent.

Processing and storing crossed seeds

1. Harvest the F_1 seeds when mature, usually 25 days from pollination (Figure 5.11).
2. Harvest, keep the panicles enclosed in the glassine bag, and air dry for 2 - 4 days before sun drying.
3. Thresh F_1 seeds (Figure 5.12) by hand and remove the glume remnants.

Figure 5.11. The F_1 seeds or naked seeds after successful crossing.

4. Count the dehulled seeds and place them in small coin envelopes.
5. Mark the envelopes with the corresponding cross, date of pollination and harvest, and number of seeds.
6. Break dormancy by placing the seeds in a dry-air oven for 5 days at 50 - 55°C.
7. If crossed seeds will be stored, place in a cold room or in a refrigerator without removing the hulls.

Recording crosses

Record the following for each cross: varietal names or pedigree of both the female or pistillate parent and the male or staminate parent, pollination date, and number of crossed seeds obtained.

Record the crosses by writing the female or pistillate parent first followed by the male or staminate parent in a cross.

Designating crosses

These are assigned individual cross number designated by the breeding institution. Normally, assignment of cross numbers is in order by season, ecology, and breeding project. This will ensure that individual crosses are unique and no two crosses have the same number. Cross numbers will facilitate identification of parents, main purpose of the cross, and crossing date. Assign a new cross number should the breeder wishes to repeat a cross.

Crosses are recorded in the crossing book using this method:

Single cross	A/B	NSIC Rc 222/ Gayabyeo
Backcross	A2/B	NSIC Rc 600*2 / FL478
Three-way or top cross	A/B//C	PR43200-3-2-1-B/ Matatag 9//PSB Rc 18
Four-way or double cross	A/B//C/D	PSB Rc 82/ Hanareumbyeo 2// NSIC Rc 480/N22

Record the crosses by listing the female parent first followed by the male parent. Indicate the variety names or pedigree of both the male and female parents. PhilRice crosses carry the prefix PR while UPLB with C.

Seeding F_1 seeds

Seeds should be dehulled and the dormancy broken. Treat the seeds with suitable fungicide prior to seeding. Arrange the seeds in petri dishes lined with clean filter paper or paper towel. Seeds should be arranged with spaces in between to avoid fungus infestation. Use distilled water in a wash bottle for watering the seeds. The filter paper should be dripping wet but not soaked or flooded. Keep petri dishes on well-lighted table tops. Observe aseptic techniques in handling germination. Transfer the seedlings into seed boxes 7 days after seeding. Seedlings are ready for transplanting 21 days after seeding.

CHAPTER 6

Breeding Nurseries and General Management

Danilo J. Lalican, Emily C. Arocena, and Thelma F. Padolina

A. Breeding Nurseries

1. Crossing block

The greatest single factor that facilitates rice improvement is the extra-ordinary varietal diversity of *Oryza sativa* and its close relatives. Wide variability is the cornerstone of successful varietal improvement programs. Hence, collection of germplasm with diverse genetic make-up is necessary and success in rice breeding depends largely on the choice of parents. The crossing block is the assemblage of diverse parents for hybridization and is usually grown in the field.

1.1 Materials

a) The crossing block is organized in groups based on program objectives.
b) Parents for crossing work include NSIC-released varieties, traditional varieties, elite lines derived from local breeding programs, varieties with known tolerance and resistance to biotic and abiotic stresses, genotypes/ varieties with excellent grain quality traits and accessions sourced from germplasm banks, introductions, and international nurseries.
c) It is customary that breeders should have preliminary data on the possible parents to direct crossing work.
d) Collecting and varietal characterization can augment and strengthen one's knowledge in parental selection.

1.2 Number of seedlings and establishment

a) Sow the seeds in three planting dates each season at 7 - 10-day intervals to ensure simultaneous flowering of the male and female parents and for continuous supply of male and female parents.
b) If possible, sowing of parents should be timed with the sowing of the breeding nurseries as these nurseries are also sources of potential parents.
c) Apply 50g of complete fertilizer (14-14-14) and 30g urea (46-0-0) per m^2 10 days after sowing and 1 week before transplanting, respectively.
d) Pull the seedlings 21 days after seeding. Saturate the seedbed to soften the soil.
e) Transplant the seedlings of each parent at one seedling to a hill in 2-row plots 5-m long. Use spacing distance of 30cm between rows and 25cm between hills (Figure 6.1).
f) In case several breeders opt for the establishment of a common crossing block, the number of rows for each parent could be increased to three rows to ensure adequate number of both male and female parents.

1.3. Preparing parents for emasculation and pollination

a) Parents for emasculation should have at least 50% of their panicles emerged from the flag leaf sheath.
b) Same materials are lifted from the crossing block by balling and placed in the plastic buckets for ease in handling.
c) During pollination, panicles of male parent are cut and placed in a container with water and brought near to the emasculated females ready for pollination when the anthers are about to shed pollen. Alternatively, the male plant is placed beside the female plant while the panicles are placed inside the plastic bag with the emasculated panicles before anthesis.

Figure 6.1. Transplanting of the parentals in the crossing block using 30cm x 25cm spacing.

1.4 Observations

d) Evaluate repeatedly the purity of the parents through visual observations before doing any crossing work.
e) Rogue off-types as soon as they appear.
f) Record data on days to heading, plant height, phenotypic acceptability, field reaction to prevailing insect pests and diseases, lodging, and other distinguishing characters for reference.

1.5 Harvesting

a) Harvest uniform plants from each of the parentals and label them properly.
b) Thresh the seeds, clean, and place in a properly labeled coin envelope or perforated plastic bags or nylon net bags, and sundry to 14% moisture content.
c) Place the seeds in a box and keep in a dry place at room temperature. If seeds will be stored for extended period, keep in a cold storage room.

2. F_1 nursery

The F_1 hybrids are products of crosses between two or more parents. Seeds are carefully handled as they are the starting materials from which succeeding generations are produced (Figure 6.2). It should be noted that F_1 plants from a single cross are generally uniform while F_1 plants from multiple cross segregate.

2.1. Materials

a) F_1 nurseries are usually composed of four types of crosses, namely, single crosses, three-way crosses, backcrosses, and double crosses.
b) For single crosses, it is necessary that female parents are planted before every cross to serve as check for plants that self-fertilized.
c) Harvest the F_1 seeds 21 - 25 days after pollination together with the female parent.
d) Manually thresh the F_1 seeds and the female parent separately with proper labels and place in a coin envelope.
e) Place the label of the F_1 seeds of each cross indicating the female and male parent. Staple the coin envelope of the female parent with the F_1 seeds and number the envelopes accordingly to identify the parent from the F_1.
f) Sundry to proper moisture content. Oven-dry at 50°C for 3 days to break seed dormancy of both the female parent and the F_1s. Assign the cross number and cross designation using the ICIS program. The F_1s of the same female parent are numbered consecutively.

Figure 6.2. Overview of F_1 nursery.

2.2. Seeding and seedling establishment

a) Prior to seeding, treat the F_1 seeds lightly with fungicide to prevent infection as they lack the protection of the hull.
b) Pre-germinate the seeds of the female parent for single crosses and the F_1 seeds separately in petri dishes lined with filter paper before seedbed seeding.
c) Transfer the pre-germinated seeds in the seedbed to allow vigorous growth.
d) Apply 50g of complete fertilizer (14-14-14) and 30g urea (46-0-0) per square meter 10 days after sowing and one week before transplanting, respectively.
e) Transplant the seedlings in the field 21 - 25 days after sowing with spacing distance of 30cm between rows and 25cm between hills.
f) Transplant the F_{1s} at one seedling to a hill side by side with the female parent particularly for single crosses for comparison and to facilitate identification of plants that self-fertilized and the true hybrids.
g) Plant one row only for each female parent while for the F_{1s}, the number of rows should depend on the number of seedlings germinated.
h) Replant 5 - 7 days after transplanting (DAT) and employ the recommended cultural management practices required for this nursery.

2.3. Observations

a) In the field book, make a listing of all the F_1s and record in separate columns opposite each cross the traits to combine.
b) Evaluate all plants of each cross regularly and closely monitor all growth stages for selfing. It is easier to detect selfed plants during flowering stage. Selfed plants are those that are agro-morphologically similar to the female parent.
c) Discard plants that have self-fertilized.
d) Before harvesting, evaluate the plants' agro-morphological characteristics in comparison with the female parent.
e) Gather data on days to heading, growth habit, field reaction to prevailing insect pests and diseases, lodging, plant type, tillering ability, and other distinguishing characters. F_1 plants that exhibited good vigor and better agronomic characteristics than the female parent are advanced to F_2.

2.4. Harvesting

a) Harvest the true hybrids and the female parent separately in a net bag with proper identification.
b) Thresh manually and sundry to proper moisture content.
c) Bulk harvest the seeds to produce each F_2 population and label properly.
d) Clean the seeds using mechanical seed blower and place the required seeds in a coin envelope.
e) Keep the seeds in a safe dry place.

2.5. Cross number and cross designation

a) Assign a cross number for each F_2 population and arrange the coin envelope/nylon net bag with the F_2 seeds consecutively.

b) In the book of crosses, indicate the breeding program, year and season the crosses were generated, and enter the female and male parent of the cross in the corresponding cross designation.
c) For uniformity and convenience in cross designation, use the IRRI second system, which is simpler and adopted in rice breeding programs. The symbol "x," which means 'cross with' is replaced by the symbol "/" and the crosses are numbered chronologically.

Example:	First System	Second System
Single cross	A x B	A / B
Three-way cross	(A x B) x C	A / B // C
Double cross	(AxB) x (CxD)	A/B//C/
Backcross	A/2 x B	A^2 / B

3. F_2 populations

The main purpose of hybridization is to create genetic variation for wider selection. Genetic variability is highest in the F_2 generation; but it is when the best individual plant is most difficult to identify (Figure 6.3). Plants in a population are genetically different and competition affects or obscures the true performance of an individual plant. The size of an F_2 population may range from 1,000 to 2,500 individual plants. F_2 populations are generally subjected to single plant selection but can be bulk planted with minimal selection for undesirable characters.

3.1 Establishment

A. F_2 population for single plant selection

a) Chosen area should have good irrigation and could be drained at will. Soil should be uniformly fertile and free from dropped seed. Avoid shaded areas or along field boundaries that are prone to rats and birds.
b) Construct seedbeds about 60cm wide and raise 7cm from the base. Ensure that levees are high enough so the beds could be flooded when needed.
c) Seedbed should be level, smooth on top, and the sides straight.
d) Make furrows using a handy wooden template about 4cm-deep spaced, 10cm apart, and perpendicular to the longer side.
e) Sow 4g seed per row, which is equivalent to a seeding rate of 50g/m^2. Avoid seed dropping outside the furrows.
f) Cover the furrows with covering materials like fine dry soil, cocopeat (coir dust), sawdust, or carbonized rice hull, whichever is locally available, and apply fertilizer at the rate of 80-30-30kg/ha N, P_2O_5, and K_2O, respectively.
g) Gradually irrigate and let the water seep into the beds. Seedlings will grow on wet but unflooded seedbed.
h) Broadcast a small amount of granular insecticide in the seedbed to prevent birds from feeding on sown seeds.
i) Fence the seedbed area with plastic sheet barrier to manage rats.
j) Cut the grass on levees and keep the seedbed weed-free.

B. F_2 bulk

a) Pre-germinate 75g from each population and direct-seed in a wet bed of 1m x 2m plot. Each plot should be separated by a 0.10m levee and 0.40m canal in between blocks.
b) Fallow the area to be used for one season to allow dropped seeds to germinate and to minimize volunteer plants during seeding.
c) Always follow an S-type orientation in placing bamboo stakes during field lay out. Lower plot numbers should start from the left going right and continue across strips.
d) The plots are sprayed with molluscicide after seeding to protect the seeds from golden apple snail damage.
e) Irrigate the plot 10 DAS or until the soil cracked.

Figure 6.3. F_2 population.

f) Molluscicide should be re-applied if needed.
g) Apply basal fertilizer at the rate of 20-20-20kg/ha NPK and 10kg N/ha 30 DAS to minimize lodging.

3.2 Field layout

The field should be well-prepared (Figure 6.4) before transplanting. Organic matter should be completely decomposed and mud and water are thoroughly mixed. It should be properly leveled characterized by absence of low and high spots. Levees should be repaired and well-plastered.

a) Layout the field based on the selected F_2 populations and the required population size.
b) Allow 0.5m side levees from both ends of the field. Use *abaca* twine to straighten the marker bamboo stake markers to be placed.
c) Place bamboo stakes with labels to mark each F_2 population. Use a planting guide, which could either be a guide wire or knotted nylon twine of desired row spacing. Measure the width of each F_2 population by marking the needed number of rows to accommodate the required population size (about 1,000 - 2,500 plants).
d) Do the same for succeeding plots of other F_2 populations.
e) Do not forget to mark the end of each plot using a bamboo stake.
f) Always follow an S-type orientation in placing bamboo stakes during field lay out. Lower plot numbers should start from the left going right and continue across strips.
g) Make sure all bamboo stakes with labels face the levees and alleys. This will facilitate field inspection and observation and easy identification of each F_2 population plot.
h) Distribute the seedlings following the S-type orientation as seen in the field lay out. This will be very helpful in renumbering of plots in cases of lost tags.
i) Place the tied seedlings at the base of each bamboo stake with label ensuring these are intact and the roots are attached to the soil to prevent further stress due to hot weather and delays in transplanting.

3.3. Transplanting/replanting

For single plant selection, hybrid populations can be transplanted either manually or through a mechanical transplanter.

a) For manual transplanting, pull the seedlings 21 DAS. Flood the seedbed a day before pulling to soften the soil and to make the task easier.
b) If seedlings are too long, clip about 1/4 - 1/3 of the leaves to prevent them from touching the soil and to avoid excessive transpiration during distribution.
c) Transplant the seedlings using the same guide wire or knotted nylon twine and planting board one seedling to a hill and in straight rows. Use spacing distance of 30cm between rows and 25cm between hills.

Figure 6.4. Plant breeding field nursery should be properly levelled and prepared before layout and transplanting.

d) For mechanical transplanter, seedlings are raised in a seed tray specific for the machine. Sow single seed in each of the hole in the tray. Numbers of trays depend on the required population size of each of the F_2/F_3.
e) Collect remnant seedlings for each F_2 population and place these near the plot labels to be used for replanting.
f) Replant 10 DAT. Inspect each row. Look for missing hills and use remnant seedlings as replacement.

3.4 Irrigation and water management

a) Maintain shallow water flooding (3 - 5cm) during the early stage of crop growth to control weeds and to enhance efficacy of soil-applied herbicides.
b) Practice intermittent irrigation to lessen drainage problem and lodging.
c) When selection is done in some F_2 populations nearing maturity, irrigation is still required for the considerably large number of medium to late maturing plants for further selection. Practice flush flooding and drain system.

3.5 Control of pests and diseases

a) Plants of F_2 populations should be grown unprotected.
b) Occurrence of insect pests and diseases helps identify susceptible from resistant plants during selection.

3.6. Observations

a) Observe regularly each F_2 population during the entire growing season. This will be important in deciding the populations to retain or to reject especially if there are only desirable plants to select.
b) Observe growth duration, plant height, field reactions to major insect pests and diseases, and grain type.

3.7. Selection and harvesting

a) Observe for variation within the population. Breeders make the most in selection progress with large differences among plants in a population (Figure 6.5).

Figure 6.5. The breeder (R) and assistants selecting in F_2 population.

b) Selection should always be based on single plant performance or population performance under bulk method.
c) Selection pressure should be high against insect pests and diseases.
d) Selected plants from each F_2 population are harvested individually. Tie selected plants from one population with the corresponding label.
e) Observe for variation in maturity, reaction to prevalent insect pests and diseases, lodging, plant type, and other agronomic characteristics if modified bulk is used. Rogue undesirable plants from each population like very tall and very late maturing ones.
f) At maturity, the breeder will decide whether a population will be subjected to selection or will be discarded based on observations. Selected desirable population will be bulk harvested and placed in nylon net bags with proper identification.
g) Sundry for about 3 days, thresh using a self-cleaning panicle thresher, clean, and place in plastic bag.
h) Sort chronologically according to each population and store in a safe place for next planting season.
i) Harvested population will be advanced in the modified bulk nursery (MBN) for single plant selection.
j) Employ individual plant selection for populations identified with good segregation/ variation by tying the individual plants with the desired characteristics such as uniformity in height and maturity, good plant type, good tillering ability, sturdy culm, slow senescence rate, long flag leaf, long and dense panicle, good panicle exsertion, uniform grain ripening, resistant to moderate field reaction to prevalent insect pests and diseases, good grain filling, good ripening color, high grain fertility, and shattering trait.
k) Label the selected plants properly and tie together accordingly.
l) Harvest and sundry the selected plants per population until 14% moisture content are reached. Store the selected plants in a safe place.
m) Evaluate the physical grain appearance of the selected desirable individual plants Traits initially worth-looking into include uniformity in grain size and shape, translucency/ opaqueness, and pigmentation.

4. Pedigree nursery

The pedigree method in handling segregating generations is the most popular and widely used in rice varietal improvement. Breeders can eliminate undesirable plants rapidly and concentrate on the most favorable materials later in the selection process. Selection is based on individual plant performance. Progenies and individual plants within the progenies are also selected.

4.1. Materials and seed preparation

a) Pedigree nurseries are usually composed of F_3 - F_8 generations. In this method, seed from the individual F_2/F_3 plants are sown in single–row plots to form the F_3/F_4 generation. The seed of the best plants from superior F_3/F_4 rows are harvested individually to produce the F_4/F_5 lines. The process is continued until lines become uniform (usually F_6 - F_8) in terms of maturity, plant height, and grain type, and consequently harvested for testing in larger plots.
b) Immediately sundry the selected panicles from individual plants for three days.
c) Use a self-cleaning panicle thresher to separate the grains and clean the grains by blowing lightly to remove the unfilled grains.
d) Clean the panicle thresher after each plant is threshed to avoid mixtures.
e) Place the seeds of each plant in a coin envelope of appropriate size labeled with the row number where it was harvested.
f) Staple the coin envelopes to secure the seeds.
g) Do the same process for selected panicles of other generations using new coin envelops that are labeled properly.

4.2 Seedbed preparation, sowing, and labeling

a) Follow the procedures in seedbed preparation as described in sowing of F_2.
b) Mark the seedbed furrows in numbering sequence of 25 row intervals for ease in labeling and counting.
c) Mark the furrows using bamboo stakes of 50cm length stapled with appropriately labeled shipping tags or 6-inch painted bamboo labels until the numbering for all selections in each generation are completed.
d) If shipping tags are used, dip the tags in melted paraffin wax to prevent labels from being washed by rains.

e) Sow about 3 - 4g of seed per furrow, which is sufficient to plant one row in the field.
f) Include check varieties during sowing.
g) Cover the furrows with the locally available covering materials (fine dry soil, coco peat, sawdust, or carbonized rice hull) and apply fertilizers at the rate of 80-30-30 kg/ha N, P_2O_5 and K_2O, respectively.

4.3 Field lay out, plant spacing, and transplanting

Field layout should be made prior to transplanting, following the planting plan. Proper field layout will prevent misplacement of seedlings during distribution.

a) Pull the seedlings of each selection 21 DAS. Flood the seedbed a day before pulling to soften the soil.
b) Tie every seedling bundle with corresponding label with plastic twine.
c) Always follow an S-type orientation in placing bamboo stakes at 10-row interval during field layout where lower row numbers start from the left going to the right and continue across strips.
d) Reserve all rows ending in "0" for check varieties.
e) Al labels must face the levees and alleys for ease in identification during field inspection.
f) Distribute the seedling bundles to the corresponding assigned plots.
g) Untie the seedlings and place them near the base of the labeled bamboo stake, ensuring these are intact and the roots are attached to the soil.
h) Transplant the seedlings strictly at one seedling to a hill and in straight rows using the guide wire or knotted nylon twine used in the layout.
i) Use 25-cm spacing between rows and 25cm between hills.
j) Collect remnant seedlings and place these near the plot labels to be used for replanting.
k) Replant all missing hills within 7 - 10 DAT and bury the excess seedlings in the soil.

4.4 Plot size and number of plants

a) For each selection, use single row-plots 5m long. This length can accommodate 20 plants.
b) Using single row plots affords the breeder the convenience of identifying readily superior rows for selection.

4.5. Observations

a) Observe regularly all the plants in the pedigree nurseries from tillering to flowering.
b) Record the agronomic traits that are highly heritable such as days to heading, plant height, panicle type, grain size, and shape. Heading date should be taken in each line when about 50% of the panicles have emerged from the flag leaf sheath.
c) Record field reactions to major insect pests and diseases. Consider the severity or level of incidence of an insect pest or disease.

4.6. Selection and harvesting

a) Selection process in the pedigree nurseries is almost similar in the F_2s except that it is stricter because more information is available regarding line behavior.
b) In most nurseries, heading dates differ substantially among lines. Select first from the lines nearing maturity.
c) Make other rounds of selection preferably in weekly intervals if a range of heading dates is desired until the latest acceptable maturity is reached.
d) Pedigree selection can be continued up to F_6 or F_8 generation. Select desirable plants from superior rows and place harvested individual plants in paper bags and sundry for three days.
e) It is strongly recommended to harvest three plants from each selected line for ease in evaluation and field layout for the next generation.
f) Thresh each plant separately, blow lightly to remove unfilled grains, then place in coin envelope.
g) Starting from F_6 until succeeding generations, harvest in bulk all superior uniform lines for yield testing. If the breeder opts to commence bulk harvesting at F_5, exercise caution as the level of heterozygosity is still high. Examine carefully each uniform line and remove off-types before harvesting. Place harvest in nylon net bags and sundry.
h) Evaluate kernel qualities as in the F_2 - F_3 plant selections. At later generations, milling recovery and milled rice appearance can be evaluated with the availability of enough seeds.
i) Thresh and clean the seeds.

5. Bulk population nurseries

Bulk method of handling segregating generations is an alternative to pedigree method. This method avoids the disadvantages commonly experienced in pedigree breeding. Pedigree breeding is laborious and requires

a larger space to accommodate all segregating materials and resources.

Bulk method is most appropriate in populations with parents that are more or less similar in height. It allows the breeder to handle large number of individual plants at least cost.

5.1. Materials

a) In bulk population nurseries, F_1 hybrids are allowed to self-pollinate and each succeeding generation starting from the F_2 is harvested in bulk until homozygosity (uniformity) is attained in the F_5 - F_8 generation.
b) Natural selection reduces or eliminates plants with lower survival value while artificial selection is practiced to rogue out obviously inferior types.
c) In the final bulk generation, in which traits are already fixed, single plant selections are then made and evaluated in the same way as in pedigree method.
d) Uniform lines are immediately entered in yield trials.

5.2. Plant spacing

Bulk population nurseries could also be planted with spacing distance of 20cm between rows and 20cm between hills as in pedigree nurseries.

5.3. Plot/population size

An F_2 population consisting of approximately 800 plants will be sufficient to start a bulk population nursery. A 30-row plot, 5.75m long, can accommodate this number of plants. At maturity, the entire plot is harvested in bulk and the seeds used to establish the next generation in a similar plot the following season.

5.4. Observations

a) Observe regularly all the plants in the bulk nurseries from tillering to flowering.
b) Record agronomic traits like growth duration, plant height, grain type, and absence of leaf diseases.

5.5. Selection and harvesting

a) In retaining plants for the bulk population nursery every season, selection is usually based on traits like duration, grain type, plant height, and absence of leaf diseases.
b) In advancing about 800 plants per generation, it is advisable to harvest single panicle per plant and bulk the harvested plants.
c) Sundry for three days, thresh, and clean.

6. Observational nursery (ON)

The observational nursery (ON) is established to serve as starting materials for initial yield evaluation.

6.1. Materials

Seeds from the panicles of the superior uniform lines selected from F_6 to F_8 generations. Maturity check varieties are also included in the nursery for comparison.

6.2. Seedbed preparation, sowing, and labeling

a) Follow the procedures in raised seedbed or wet bed preparation.
b) For raised seedbed, mark the seedbed furrows with numbering sequence of 5-row intervals for ease in labeling and counting.
c) Mark the furrows using bamboo stakes of 50cm lengths stapled with appropriately labeled shipping tags or 6-inch painted bamboo labels until the numbering for all entries are completed.
d) Sow ungerminated seeds in five separate furrows, enough to plant five rows in the field.
e) Include maturity check varieties during sowing.
f) Cover the furrows with locally available covering materials and apply fertilizers at the rate of 80-30-30 kg/ha N, P_2O_5 and K_2O, respectively.
g) For wet seedbed, sow 50g pre-germinated seeds in a 1.0m x 0.5m plot.

6.3. Field lay out, plant spacing, and transplanting

Field layout should be made prior to transplanting, following the planting plan. Proper field lay out will prevent misplacement of seedlings during distribution.

a) Pull the seedlings of each entry 21 DAS. Flood the seedbed a day before pulling to soften the soil.
b) Tie every seedling bundle with corresponding label with plastic twine.
c) Always follow an S-type orientation in placing bamboo stakes at 5-row interval during field layout where lower row numbers start from the left going to the right and continue across strips.
d) Labels should face the levees and alleys for ease in identification during field inspection.

e) Distribute the seedling bundles of each entry to assigned plot labels.
f) Untie the seedlings and place near the base of the labeled bamboo stake ensuring these are intact and the roots are attached to the soil.
g) Transplant the seedlings strictly at one seedling to a hill and in straight rows using the guide wire or knotted nylon twine used in lay out.
h) Plant a check variety in frequent intervals to compare the lines being tested with the variety in the nearest check plot.
i) Use 20cm spacing between rows and 20cm between hills.
j) Remnant seedlings should be placed near the plot labels to be used for replanting.
k) Replant all missing hills within 7 - 10 DAT and bury the excess seedlings in the soil.

6.4. Plot size and orientation

Entries in the observational nurseries are usually evaluated in 5-row plots, 5m long, and spaced 20cm apart. Plots are unreplicated but are generally laid out in augmented design. The plots should be laid out in east to west orientation to avoid mutual shading between plant rows. An indicator is that when you are standing in front of the plots, the sun is either in front of you or at your back, depending on the time of the day.

6.5 Fertilizer application

a) It is always necessary to follow fertilizer rate based on location recommendations.
b) During the final harrowing or leveling, apply the first dose of nitrogen and all of phosphorous and potassium and incorporate into the soil.
c) Split application of nitrogen is preferred as the element is mobile and can easily be lost.

6.5. Observations

a) In a field book, record traits such as uniformity, heading date, plant height, yield, spikelet sterility, grain shattering, lodging, and field reactions to pests and diseases.
b) Evaluate the entries based on the vegetative vigor, the rate at which lines close the space between rows, and increase in leaf area.
c) At maturity, rate each entry based on phenotypic acceptability score. This is subjective but is very helpful in testing large number of entries.
d) Rogue off-types as soon as they appear in the plots.

6.6. Selection and harvesting

a) Evaluate the test entries for one dry season (DS) and two wet seasons (WS). However, test entries that show promising performance over the checks may be advanced even without completing the required season of evaluation. Inferior plots identified before and during harvest should not be harvested, provided back-up seeds are available and stored for evaluation of other traits of interest.
b) For the selected promising plots, harvest only the three inner rows and exclude the hills at the end of each row.
c) To have high quality seeds for the next cycle of yield trial, select about 50 panicles from the border rows of uniform plots.
d) Thresh and sundry the harvested grains. Clean, weigh, and convert the yield to kg per hectare. If necessary, adjust the yield to standard 14% moisture content.
e) Retain or advance test materials with yields higher than or comparable with the corresponding maturity check varieties and with better resistance to prevalent insect pests and diseases. Otherwise, entries should be dropped from the trial.
f) Allot about 500g - 1kg of seed sample to determine milling quality traits such as milling recovery, head rice recovery, chalkiness, and grain size and shape. Discard lines with low percentage of milling recovery.

7. Preliminary yield performance trial (PYT)

Preliminary yield trials (PYT) are replicated and generally composed of entries selected from the ON for continued evaluation. Entries are fairly uniform in growth duration but may be grouped in maturity classes. As in the ON, maturity check varieties are included for comparison in terms of yield and other desirable attributes.

7.1. Seedbed preparation

a) Follow the procedures in raised seedbed or wetbed preparation as described in the sowing of ON.
b) Sow 50g of seeds per entry in 12 furrows of raised seedbed or 100g pre-germinated seeds in a 1m x 1m plot for wetbed, which is sufficient to plant 24 rows in the field. One replication is composed of 8 rows with 5m length.
c) Include check varieties as test entries during sowing.

d) Cover the furrows with fine dry soil or cocopeat (coir dust) and apply fertilizers at the rate of 80-30-30 kg/ha N, P_2O_5 and K_2O, respectively.

7.2. Experimental design and field layout

a) A randomized complete block design (RCBD) with three replications should be used in the experimental layout.
b) In laying out the trial, a replication should be as square as possible.
c) A 30-entry yield trial with three replications requires two sub-blocks for each replication. A sub-block is equivalent to a replication for a 15-entry yield trial.
d) Separate each replication by a 1m wide alley.
e) Make sure that all plots in a replication are located in the same paddy.
f) Number the plots consecutively by placing labeled bamboo stakes at 8-row interval from left to right direction in all replications.
g) Labels must face the levees and alleys for ease in identification during field inspection.

7.3. Plot size, plant spacing, and transplanting

a) Each entry should be planted in 8-row plots, 5m long with 20cm between rows and 20cm between hills spacing distance.
b) Pull the seedlings of each entry 21 DAS. Flood the seedbed a day before pulling to soften the soil.
c) Divide the seedlings into three bundles and tie each bundle with corresponding plot and replication labels with plastic twine.
d) d) Distribute the seedling bundles of each entry to assigned plot labels.
e) Place the seedlings near the base of the labeled bamboo stake ensuring these are intact and the roots are attached to the soil.
f) Transplant the seedlings strictly at one to three seedlings to a hill and in straight rows using the guide wire or knotted nylon twine used in layouting.
g) Collect remnant seedlings and place them near the plot labels. They will be used for replanting.
h) Replant all missing hills within 7 - 10 DAT and bury the excess seedlings into the soil.

7.4. Observations and harvesting

a) In a field book, record the days from sowing to heading, plant height, number of productive tillers, phenotypic acceptability rating, and field reactions to pests and diseases.
b) For yield determination, harvest at maturity the six inner rows excluding the hills at the end of each row.
c) Thresh using a self-cleaning plot thresher, place in nylon net bags and sundry for about three days.
d) Clean using a mechanical seed blower and place in paper bags.
e) Determine moisture content of each sample and convert the plot yield into kg per hectare adjusted to 14% moisture content.
f) From the same seed sample, obtain a reasonable amount of seed to evaluate other traits like milling quality and physico-chemical properties in the laboratory.
g) Evaluate the test entries for 1 DS and 2 WS. However, test entries that show promising performance over the checks may be advanced even without completing the required season of evaluation.

8. Advanced yield trial

a) The advanced yield trial (AYT) is composed of promising lines identified in the PYT.
b) It is conducted with the same procedures as in PYT except that it has a bigger plot size (at least 10 rows per plot).
c) It is usually laid out in RCBD with three replications. The data gathered at maturity are the same with PYT. Yield performance is always the major consideration in identifying superior lines for advancement into the National Cooperative Testing (NCT).
d) Evaluate the test entries for 1 DS and 2 WS. However, test entries that show promising performance over the checks may be advanced even without completing the required season of evaluation.

B. General Management of Breeding Nurseries

1. Seed preparation

Seed preparation is a basic operation required for an effective rice breeding program. Thorough knowledge of the procedures involved in seed preparation minimizes errors during implementation (Figure 6.6).

a) Seeds should be thoroughly clean, free from weed seeds, inert matter, and off-types before placing in coin envelopes.
b) Seed packets should be properly labeled indicating the time of harvest, rows from which they were harvested, and nursery from where they came from.
c) Break seed dormancy by heat treatment in an oven at 50^0C for 3 days.
d) Arrange the individual plant selections numerically according to the field numbers of the selected rows and check the number of plants against the number recorded in the field book during selection. Use plastic trays as these are convenient to use, light, and durable.
e) Do the same arrangement for all entries in the ON and PYT.

2. Preparing the field book and label

Field books and labels are a must for data gathering and recording. They also serve as ready reference during field inspection and for observing and evaluating breeding materials (Figure 6.7).

a) Use field books that are easy to carry. These could either be improvised or computer-generated, prepared using the Breeding Management System platform.
b) It should be small enough to fit in the pocket of a field jacket. Covers must be hard and waterproof.
c) Improvised field book for pedigree nurseries may measure 21.5cm x 14cm with 300 pages, each containing 29 lines.
d) Use two pages for record keeping and data gathering. The left page should contain previous and current plot numbers, the cross number, and pedigree while the opposite page contains the heading date, tiller number, plant height, reaction to insect pests and diseases, and other morphological traits, e.g., flag leaf angle, lodging, threshing result, and awning.
e) A pair of pages can accommodate 14 selections. Leave one line blank for numbering and proper notation. This will also minimize confusion during reading and recording.
f) For ON and PYT, use a field book composed of pre-formatted standard mimeo paper (A4) showing all the relevant data to be observed and gathered. Use comb puncher and plastic ring binder for binding and convenience in going over the pages.
g) Use of automatic numbering machine is recommended to avoid errors.

3. Type of seedbed, rate of seeding, and age at pulling

a) It is recommended to use wetbed in raising seedlings but raise seedbed can also be used. Choose a part of the field that is near the water source and has good drainage (Figure 6.8).
b) Make beds of about 60cm wide and raise 7cm from the base. Provide about 40cm between seedbeds to keep irrigation water and as alleyways. Ensure that levees are high enough to flood the beds when needed.
c) Seedbeds should be level on top and the sides straight.
d) For raised bed, make furrows using a wooden template about 4cm-deep spaced, 10cm apart, and perpendicular to the longer side.
e) Sow 4g seed per row, which is equivalent to a seeding rate of 50g/m^2. Avoid seed dropping outside the furrows.
f) Cover the furrows with fine dry soil and apply fertilizer rate of 80-30-30kg/ha N, P_2O_5, and K_2O, respectively.
g) If fine dry soil is unavailable, use coco peat (coir dust), sawdust or carbonized rice hull in covering the furrows.
h) Water the beds when needed. Flood the alleyways to maintain moisture in the beds. Water will gradually seep into the beds.

Figure 6.6. Seed preparation.

Figure 6.7. Field book and labels are prepared before seed sowing.

Figure 6.8. Recommended wet-bed method in raising seedlings.

i) For wetbed, prepare 1m x 1m seedbed plot and sow 50g pre-germinated seeds.
j) Drain the water in the plot 24 h after sowing. Irrigate when the soil cracked.
k) Broadcast a small amount of granular insecticide (Furadan 3G) in each seedbed to prevent birds and rats from feeding on sowed seeds.
l) Always maintain cleanliness in the seedbed area.
m) Pull the seedlings 21 DAS. Flood the seedbed a day before pulling to make the task easier.
n) If seedlings are too long, clip about 1/3 of the leaves to prevent them from touching the soil and to reduce excessive transpiration during distribution.

4. Field layout

The field should be well-prepared before transplanting. Organic matter should be completely decomposed, and mud and water are thoroughly mixed. It should be properly leveled characterized by absence of high or low spots. Levees must be repaired and plastered.

a) If the field is divided equally into 1,250m^2 blocks including bunds or levees, (100m long and 12.5m wide), two active strips can be allowed, each measuring 99m long and 6m wide.
b) Allow 0.5m from the side levees at both ends of the field. Use *abaca* twine or guide wire to straighten the bamboo stake markers to be placed. *Abaca* twine/guide wire will also serve as the baseline.

c) Measure the plots from the baseline. Mark each plot with bamboo stakes. Use a planting board and planting guide, which could either be a guide wire or knotted nylon twine during the layout. Measure the width of each plot by marking the rows.
d) Always follow a serpentine or an **S-type orientation** in placing bamboo stakes during field layout. Lower plot numbers should start from the left going to the right and across strips. As much as possible, orient the plots in east-west direction.
e) Labels must face the levees and alleys to facilitate field inspection and observation (Figure 6.9).

5. Seedling distribution

This activity includes transfer of seedlings from the seedbed to the field. Avoid interchanging plot numbers and arrangement problems before seedlings are transplanted.

a) Distribute the seedlings in a galvanized iron or wooden tray following the serpentine or **S-type orientation** as seen in the field layout. This systematic plot arrangement will help in re-labelling of plots in case of lost tags.
b) Untie the seedlings and attach the tags to the bamboo stakes.
c) Place the seedlings at the base of each labeled bamboo stake ensuring they are intact and the roots are attached to the soil to prevent further stress due to hot weather and transplanting delays (Figure 6.10).

6. Transplanting/replanting

a) Pull the seedlings 21 DAS. Flood the seedbed a day before pulling to soften the soil and make the task easier (Figure 6.11).
b) If seedlings are too long, clip about 1/3 portion of the leaves to prevent them from touching the soil and to reduce transpiration during distribution.
c) Transplant the seedlings using the same guide wire or knotted nylon twine during the layout at the rate of one seedling to a hill and in straight rows. Use spacing distance of 30cm between rows and 25cm between hills for the hybrid populations and pedigree lines and 20cm x 20cm to advance generations in the yield trials (Figure 6.12).
d) Collect remnant seedlings for each corresponding plot and place near the plot labels for replanting.
e) Replant missing hills within 7 - 10 DAT.

7. Field management of breeding nurseries

7.1. Fertilizer application

a) Soil analysis is important in deciding the rate and type of fertilizer to apply. Fertilizer application must always be based on recommended rates.
b) Apply and incorporate the first dose of nitrogen and all of phosphorous and potassium during the final harrowing or leveling.
c) Split application of nitrogen is preferred as the element is mobile and can easily disperse.

Figure 6.9. Field layout (before and after transplanting) with bamboo stakes and corresponding labels of the breeding entries.

d) First topdressing of nitrogen should be done at 21 DAT or at maximum tillering stage.
e) Second topdressing should be done at approximately panicle initiation stage.

7.2. Irrigation and water management

a) Good irrigation and effective drainage are key factors for good water control. During land preparation, water level should be maintained to keep the soil moist prior to transplanting.
b) Maintain shallow water flooding (about 3 - 5cm) during the early stage of crop growth to control weeds and to enhance efficacy of applied pre-emergence herbicides.
c) Practice intermittent irrigation throughout the growing season to lessen drainage problem.
d) When selection is done on considerable number of rows nearing maturity, irrigation is still required for late maturing plants. Practice flush flooding and drain system.

7.3. Control of pests and diseases

a) Regularly monitoring the field for occurrence of major insect pests and diseases throughout the growing season.
b) Incidence of viral disease like tungro is very critical. To prevent extensive damage, uproot immediately and bury infected plants.
c) To minimize damage of golden apple snails, apply molluscicide at least three days before final leveling or right after transplanting. Handpick the golden apple snails.
d) At the reproductive stage, apply insecticide on a need-based level to minimize damage by rice bugs.
e) Incidence of insect pests and diseases in the field during the growing season helps identify the susceptible from resistant plants.

Figure 6.10. Seedlings placed at the base of each labelled bamboo stake during seedling distribution.

Figure 6.11. Seedlings are carefully pulled to avoid damage.

Figure 6.12. Transplanting of breeding populations using guide wire at the rate of one seedling per hill.

7.4. Weed management

Weeds should be managed during the first three weeks after transplanting as they compete for sunlight, water, space, and nutrients of the growing seedlings.

a) Herbicides should be applied accurately.
b) Apply pre-emergence herbicide after transplanting.
c) If applied as chemical spray, sprayer should be properly calibrated.
d) Too much chemical spraying may injure the crop. This method could also be wasteful and costly. Note also that minimal spraying may not be effective.
e) Practice good water control to suppress weed growth and enhance nutrient absorption.
f) Use rotary weeder when weeds start to grow and perform spot weeding at later stages.

7.5. Rats

a) Rats attack all stages of the rice crop. Implement a rat control program as early as possible.
b) Practice sustained baiting using recommended rodenticides.
c) Always maintain cleanliness in the experimental areas. Clear the surroundings, canals, and levees and destroy possible breeding places.

7.6. Birds

a) Birds are usual problems during the start of the grain-filling stage. As a control measure, employ bird watchers from 6 a.m. to 6 p.m. for about 35 days or if resources permit, protect the plots using bird's net.
b) Use devices to scare off birds such as scarecrow, sound-creating device or shiny tapes, and swaying plastic buntings and empty cans.

8. Data gathering

Except for quantitative traits like yield, days to maturity, plant height and number of productive tillers per hill, and the rating scale as contained in the Standard Evaluation System for Rice (SES) should be followed in gathering most of the qualitative characters like phenotypic acceptability.

9. Fertilizer computation

When given the recommended fertilizer rare and available fertilizer materials, the rice breeder or technical person involved in managing the breeding nurseries should be able to weigh the exact amount of appropriate fertilizer materials to apply in the experimental areas. Knowledge of computing these materials leads to applying the required amount and enables him/her to decide on the cheapest materials and fertilizer combinations to apply.

Fertilizer rate is usually expressed in kilograms nitrogen (N), phosphoric acid (P_2O_5), and potash (K_2O) per hectare. Another expression is the number of fertilizer bags per hectare. To compute for the right amount of fertilizer materials, follow this formula:

$$\text{Weight of fertilizer} = \frac{\text{Weight of nutrient recommended x area (ha)}}{\text{\% Nutrient in the fertilizer material}}$$

10. Calibrating sprayer

Sprayers must be calibrated periodically to obtain accurate and reliable results especially for pesticides and herbicides in liquid formulation. The rate of pesticide application depends on the following:

a) Pressure in the spray tank – must be kept as constant as possible. Use of pressure gauge or pressure regulator will be very useful.
b) Size of the nozzle orifice – regulates the amount of liquid passing through the nozzles. Pressure and nozzle orifice affect the volume of spray material per unit time.
c) Uniform spray swath – affected by the distance between nozzle tip and top of the plant or ground level.
d) Walking speed of spray handler – affects the area covered per unit time

To calibrate, follow these simple steps:

a) Prepare the sprayer. Fill the sprayer with clean water, remove and clean the nozzle, check for leaks by applying pressure, and replace nozzle and strainers. Refill with clean water and apply pressure.
b) Determine walking speed of the spray handler, which could be determined in field condition:
   b.1. Mark starting point with a stake.
   b.2. Carry the sprayer on back and operate as in actual spraying. One hand pumping and the other directing the nozzle within a spray swath and walk at constant speed
   b.3. Monitor the spray handler as he/she walks using a stopwatch.
   b.4. Stop walking after one minute.
   b.5. Mark the stop point with a second stake.

b.6. Measure the distance between the two stakes in meters and express the walking speed in meters per minute.
b.7. Repeat at least three times and get average walking speed.

c) Determine the width of swath or spray in meters. Maintain constant pressure in the tank and proper distance between nozzles and ground level.
d) Calculate area sprayed in one minute. If the swath is 1.5m and the walking speed obtained in step b, the area of spray per minute is:

Area of spray per minute = Spray swath (1.5m) x walking speed per minute

11. Field plot techniques

Knowledge of field plot techniques is essential in minimizing experimental error in conducting field experiments.

a) Effect of plot size, shape, and orientation on the precision of field trials
a.1. Small plots yield unreliable results
a.2. Experimental error decreases as plot size increases
a.3. Unnecessary big plots waste time and resources
a.4. A square-shaped plot exposes the least number of plants to border effect
a.5. Plot orientation oftentimes change the effect of fertility gradient in the field

b) Five major factors important in choosing plot size, shape, and orientation for field experiments
b.1. Type of experiment
b.2. Soil heterogeneity
b.3. Border effects
b.4. Availability of seeds
b.5. Measurement of traits aside from yield

c) Four general rules to reduce the effect of soil heterogeneity
c.1. Use square plot if fertility gradient is unknown.
c.2. Use rectangular plot if gradient is known.
c.3. If gradient is present, orient longest dimension of the plot in the direction of the greatest variation.
c.4. Use larger plots for patchy-type heterogeneity.

d) Guidelines in using small plots
d.1. Select special experimental design if magnitude of differences in plant characters among the test selections is significant.
d.2. Use refined field techniques even if it is costly.

e) Reasons for using larger plots
e.1. To accommodate sampling for various characters
e.2 To have reasonably effective harvest area from biased plant competition due to plant removal

f) Two factors to determine number of replications
f.1. Magnitude of expected experimental error – consider coefficient of variation (CV)
f.2. Degree of precision desired – consider standard error of treatment means and magnitude of treatment difference

g) Determining number of replications
g.1. Use appropriate standard statistical table.
g.2. Ensure that number of replications provides at least 10 degrees of freedom for experimental error.

h) Five common sources of error requiring special field techniques
h.1. Border effect
h.2. Missing hills
h.3. Residual effect of fertilizers
h.4. Off-types
h.5. Insect pest and disease damage

i) Minimizing border effects
i.1. Avoid unplanted alleys.
i.2. Plant a border variety around the unplanted borders.
i.3. Choose a design to group homogeneous varieties.
i.4. Construct levees between plots with different fertilizer treatments.
i.5. Handle fertilizer-applied plots properly.

j) Coping with missing hills in rice field experiments
j.1. Do not collect data on hills adjacent to missing hills.
j.2. Do not determine plot yield and regard it as missing data if missing hills exceeds 20% of the total.

k) Effects of off-types on experimental results
   k.1. Increases heterogeneity among plants.
   k.2. Introduces undesirable plant competition.
   k.3. Remedial measure not generally possible.

l) Remedial and preventive measures against off-types
   l.1. Rogue off-types as soon as they appear and replant.
   1.2. Exclude from harvest and adjust plot yield if detected late.
   l.3. Use pure seeds.
   l.4. Maintain seedbeds free of dropped seeds.
   l.5. If possible, use separate seedbeds for different varieties.
   l.6. Uproot volunteer plants.

m) Minimizing effects of insect pest and disease damage on experimental results
   m.1. Do not get observations from damaged plants.
   m.2. If damaged plant loss exceeds 20%, exclude damaged plants from harvest and make necessary adjustment in yield data.

n) Guidelines in selecting experimental materials and methods
   n.1. Use pure seeds.
   n.2. Use chemical from the same formulation.
   n.3. Keep materials uniform.
   n.4. Label seeds and chemicals properly.
   n.5. Keep remnant samples.
   n.6. Record source of seeds and chemicals.
   n.7. Allocate enough space for field experiments.

12. Harvest and post-harvest management

Great care is required in harvesting experimental breeding materials. The optimal stage to harvest is when 80 - 85% of the grains have turned yellow and the grains in the lower part of the panicle are in the hard dough stage.

12.1 Threshing

a) Use a self-cleaning panicle thresher (Figure 6.13).
b) In yield trials, use a self-cleaning mechanical plot thresher (Figure 6.14).

12.2. Drying, seed storage, and safekeeping

a) Rice seeds should be dried to less than 14% moisture immediately after threshing.
b) If seeds will be stored for extended periods, these should be dried to about 12% moisture content or less and placed in a sealed container.
c) Seeds must be protected from insects and rodents.
d) Seeds must be protected from reabsorbing moisture either through rain or surrounding atmosphere.
e) Always keep back-up seeds for re-evaluation.
f) Valuable parents should be kept for not more than two years.
g) Discard old and insect-damaged seeds.

Figure 6.13. Self-cleaning mechanical panicle thresher.

Figure 6.14. Self-cleaning mechanical plot thresher.

C. Plot Numbering Scheme for Plant Breeding Trials

A universal plot numbering scheme, composed of seven characters, must be followed for an organized pedigree breeding. The first character mentions the ecosystem and purpose for which the breeding line is being developed. The code for each ecosystem and purpose is shown in Table 6.1. The second character states the year in a particular decade. It is represented by numbers 0 - 9. Number 2 for example denotes 2012 for this decade and 2022 in the next decade. The third number is the cropping season with codes D for dry season and W for wet season. The fourth code denotes the type of nursery shown in Table 6.2. For yield trials such as preliminary yield trials, the fifth character indicates the replication or blocking. The last three characters of the plot code are represented by numbers 0 - 9, which refer to numbering of entries in the field. For F_3 families where the number of entries could be more than 10,00 families in one season, the last three characters are not enough; hence, two nursery codes should be assigned.

For example, plot number A2WH001 means, the first entry (A2WH001) in the first batch of the hybridization block (A2WH001) for irrigated lowland breeding (A2WH001) during the wet season (A2WH001) of 2012 (A2WH001). Plot number U9DQ124 means the 24th entry (U9DQ124) in replication 1 (U9DQ124) of the upland (U9DQ124) multi-location advanced yield trial (U9DQ124) during the dry season (U9DQ124) of 2019 (U9DQ124). The codes and their values are given in Table 6.3.

Table 6.1. Codes used for breeding objectives.

Code	Description
A	Transplanted irrigated lowland breeding
D	Direct-seeded irrigated lowland breeding
S	Specialty rice breeding
H	Hybrid rice breeding
R	Breeding for rainfed lowland ecosystem
C	Breeding for stress-prone irrigated lowlands
U	Upland and aerobic rice breeding

Table 6.2. Breeding nurseries and corresponding codes.

Nursery	Codes
Hybridization block	H (first batch), I (second batch), J (third batch)
F_1 populations nursery	A
F_2 populations nursery	B
F_3 families nursery	C (001 to 999, e.g., C001), D (1000 to 9999, only last three digits are to be affixed after "D," e.g., D001)
F_4 lines nursery	E
F_5 lines nursery	F
F_6 lines nursery	G
Observation nursery	N
Preliminary yield trial	P
Multi-location advanced yield trial	Q

Table 6.3. Summary of codes for plot numbering scheme.

Code number	Particulars	Values	Remarks
1	Ecosystem, breeding goal	A, D, S, H, R, C, U	Refer to Table 1
2	Year	0 – 9	Year in the particular decade
3	Season	D, W	Dry season (D), Wet season (W)
4	Nursery code	H, I, J, A, B, C, D, E, F, G, N, P, Q	Refer to Table 6.2
5	Numeric code 1	0 – 9	Entry numbering or blocking for replicated trials
6	Numeric code 2	0 – 9	Entry numbering
7	Numeric code 3	0 – 9	Entry numbering

CHAPTER 7

Direct-seeded Rice Breeding

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Direct seeded vs. Transplanted

Direct seeding is becoming increasingly important in the Philippines due to increasing labor cost on seedbed preparation, care of seedbed, pulling, and transplanting and the availability of pre- and post-emergence herbicides at more economical prices. Development of high yielding early maturing varieties is also a factor in the wide acceptance of direct seeding practice. Direct-seeded crop compared with transplanted is generally 7 - 10 days earlier in maturity, which is another attractive feature of the system.

Direct seeding is practiced in irrigated and rainfed farms. Practices range from (1) pre-germinated seeds on puddled soil, (2) dry seeds on puddled soil, until (3) dry seeds on dry soil. In irrigated farms, broadcasting pre-germinated seeds on well-levelled puddled soil is the general practice. The use of dry seeds, which is either drilled or broadcasted on puddled soil, is uncommon. Under rainfed condition, the use of pre-germinated and dry seeds on puddled soil is common. In rainfed farms, farmers' use of dry seed or pre-germinated seed on dry or puddled soil depends on soil moisture condition.

The breeding principles in developing transplanted and direct seeded varieties are essentially similar. There are some differences in breeding procedures associated with the nature of transplanted versus a direct seeded crop. In crop established by transplanting, individual genotypes are easily identified so that plant selections are readily accomplished. In direct seeded crop, the plant breeder is generally limited to panicle selection in the early breeding generation. More seeds are also required in establishing breeding nurseries and performance trials. In addition, weed management in breeding trials for direct seeding is not as easy as in transplanted nurseries.

Irrigated

Additional major traits considered desirable in direct seeded system include: anaerobic germination, greater seedling vigor, early growth duration, and lodging resistance.

Selection of parents and type of crosses

Genetic donors or parents to be used for hybridization are chosen based on the major desirable traits already stated. The germplasm database is reviewed based on the characteristics for direct seeding and donors are identified and evaluated for anaerobic germination and seedling vigor. Other important traits are also considered. The type of crosses is similar to transplanted.

Selection in the F_1 and F_2

In breeding, F_1 and F_2 are handled through transplanting. However, F_2 seeds (approximately 2,000 - 4,000 seeds) are sown directly into the saturated puddled soil and irrigated with 5cm water depth after sowing. Surviving 3-leaf stage seedlings (i.e., 21 - 25 days old) are transplanted one plant per hill in the field breeding nursery. Selection is similar to transplanted individual rice plants.

Selection in the F_3 - F_6 or F_7

In handling the pedigree nursery, F_3 - F_6 or F_7 or until the lines are fixed, the seeds are directly seeded in rows, 5m long, and 25cm between rows at the rate of 5 - 10g seeds per row, which is equivalent to 40kg seeds per hectare. Pedigree rows are established following systematic arrangement with plots ending in "0" reserved for check varieties. One row per line is sufficient in the F_3 - F_7 pedigree nursery. In selection, pedigree nurseries are

reviewed, the best lines are identified, and three panicles from each selected line are collected. At F_4 and later generations, the remaining plants in the selected lines can be harvested for other tests and screening trials. Desirable uniform lines are normally bulk harvested starting at the F_6 generation.

Observational, preliminary, and advanced yield test

Observational nurseries, preliminary, and advanced yield trials are conducted using the same plot size, design, and number of replications as in breeding for transplanted rice. The following observations are emphasized in direct seeded trials: (1) germination ability and seedling vigor on puddled soil, (2) days to heading, (3) culm strength or lodging resistance, (4) tillering, and (5) phenotypic acceptability at maturity. However, the number of tillers is determined based on measured segment of the row, which is usually one meter.

Unfavorable Rainfed

The major traits are considered desirable in direct-seeded rice in addition to those already described under transplanted include: (1) anaerobic germination in puddled soil, (2) seedling vigor, (3) drought resistance, (4) early growth duration, (5) yield stability, (6) adaptation to varying moisture regimes, (7) high level of resistance to blast, and (8) weed competitiveness.

Selection in the F_1, F_2, and advanced generation

F_1 and F_2, and F_3 - F_6 (in pedigree nurseries) or until the line are genetically fixed, are grown transplanted. Pedigree nurseries are selected on plant basis until these are bulked when they are uniform. Observational nursery, preliminary, and advance yield trial are conducted under stressed and non-stressed conditions. Trials under stressed condition are directly seeded and trials under non- stressed condition are transplanted, which are conducted to select genotypes that will perform well under stressed condition and will show better results than other rainfed varieties under favorable condition. The major consideration is stable yield under varying rainfed conditions. While the pedigree is the general breeding method of handling segregating generations, combination of pedigree and bulk method are also practiced. The wet season trials are considered critical, but the breeding materials are grown under transplanted condition during dry season to advance generation and to select obvious deficiencies.

Establishment and selection in location-specific environments

Sampling the diverse rainfed environments is important in developing rainfed variety. Preliminary selections on-site are done in at least two sites regarded as selection environments (SE). Currently, we have six SE majority, which are located inside research stations, at PhilRice branch stations, and collaborating research institutions. Initial selections for location specific adaptability are based on the performance in the multi-environment trials (MET) conducted in the SE outside of the breeding institution, representing the target population of environments (TPE). The preferred MET locations are research stations in the TPE where the trials can be well managed for accurate data collection from which preliminary line selections will be based. The MET for a set of entries is conducted in at least two wet seasons with the first trials (MET1) consisting of 200 - 300 entries in un-replicated augmented experimental design, where each entry is established in at least $5m^2$ plot size with 4 rows of 1m x 5m length, 25cm apart between rows, and check varieties planted after every 10 - 20 entries.

Selections from MET1 are advanced to MET2 with 80 - 100 entries in augmented or randomized complete block design (RCBD), or alpha-lattice design, depending on the degree of site heterogeneity and available resources, with at least three replications and at least $5m^2$ plot size for each entry in a replication. The selections of 15 - 20 elite lines from MET2 are evaluated in the researcher-managed trials in the farmers' field for adaptation and acceptability. Each entry is established in at least $10m^2$ plots with 8 rows of 2m x 5m length, 25cm apart between rows, in RCBD with at least three replications. Each farmer's paddy may serve as replication with small-sized but contiguous paddies. All entries including check varieties must be contained in one paddy or replication. Strategically, the number of replications can be reduced for a more effective selection of wide adaptation and stability given scarce resources for multi-location testing. This could be complemented by increasing the number of test locations.

Farm walk through Participatory Variety Selection (PVS) is conducted in the farmers' fields to identify entries acceptable to the intended farmer beneficiaries. One to five kilograms of one to three elite lines, which were identified as farmers' most preferred entries in each location, are evaluated on-farm in the farmer-managed trials for comparison with the farmers' varieties. The selected lines are potential candidates for entry in the NCT.

The minimum data for collection include plant height at maturity, heading/maturity, number of panicles per linear meter, biomass at maturity, yield and yield components, harvest index, field reaction to prevailing pests under stressed and non-stressed conditions, drought score, and panicle exertion under stressed condition.

Screening for anaerobic tolerance

F_2 plants, entries in the ON, and new introductions are screened for anaerobic germination tolerance and seedling vigor. Anaerobic germination tolerance and seedling vigor include screenhouse and field screening methods. Survived seedlings in the field are seed pulled for transplanting from the screening area to the corresponding nursery. As the seedbed screening area is always flooded with water during screening, it is easy to pull the seedlings that survived the condition. However, care should be always observed because few seedlings could survive the screening. Always handle the seedlings with extreme care because these seedlings have acquired the tolerance to anaerobic germination. Seedlings handled gently during uprooting and transporting recover faster when transplanted than the materials that were crushed, bruised, or allowed to dry out. Seedlings should be pulled gently at about 45 degrees angle to avoid root damage. Care must be observed so as not to detach the roots of the seedlings. Avoid putting too much stress on the seedlings. If they are not to be planted immediately, keep them in water. Do not tighten the string used to bundle the seedlings. See to it that labels are not mixed up.

Screenhouse Anaerobic Germination Evaluation

1. Prepare the seeds for planting. Label each entry accordingly. Break dormancy by subjecting the seeds to 50°C for 5 days. Prepare the trays. Make sure they are leveled on the ground and have no leaks.
2. Prepare puddled soil obtained from the field and mix thoroughly in pail or large container. Place sufficient amount of soil in recyclable plastic cups. Do not fill the cup fully. Allow 1.5cm space from the top of the cup to the soil surface where the seeds are to be sown to give space for the garden soil to be added on top of the seeds; thereby, submerging the seeds at exactly 1.5cm.
3. Use 10 pre-germinated seeds in each plastic cup and cover with garden soil (10 seeds/cup: 5 cups/entry/replication) (Figure 7.1). Put muddy/murky water (coming from irrigation canals) in the tray where the cups are arranged and level it to 5cm depth above the cup surface (i.e., soil surface) (Figure 7.2). Maintain the 5cm water level for 14 days. Use three trays as replication (Figure 7.3).
4. Determine the percentage anaerobic germination by counting the number of seedlings that emerged from the 5 cups per replication divided by 50 multiplied by 100. Record the data in the worksheet provided in this protocol.
5. Determine the classification of the entry (R, I, S) using rating system below. Compare the entries with the check varieties: Khao Hlan On (R), PR36951-B-B-12 (R), IR 42 (S).

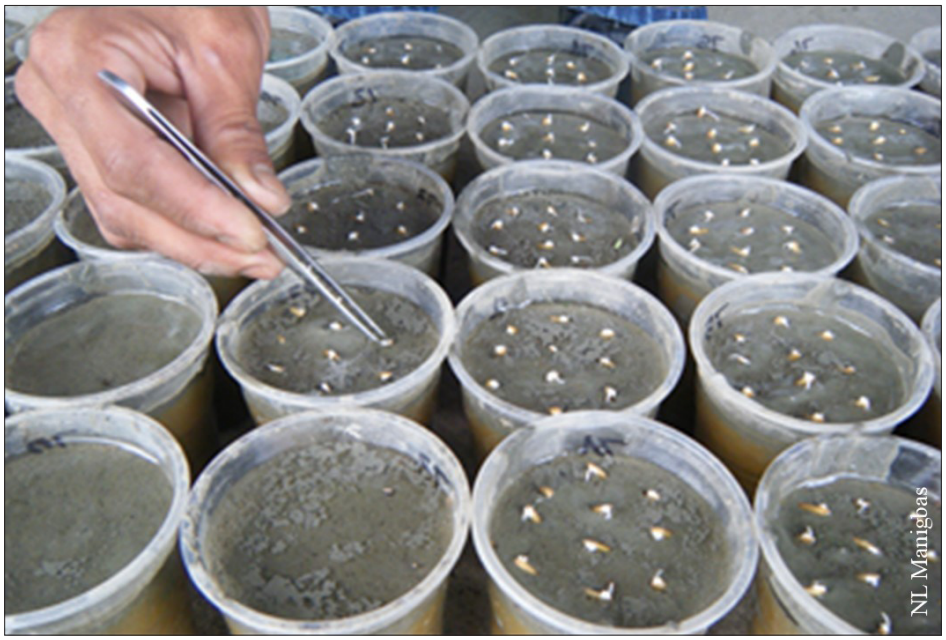

Figure 7.1. Sowing of 10 pre-germinated seeds in each plastic cup. (Source: NLManigbas)

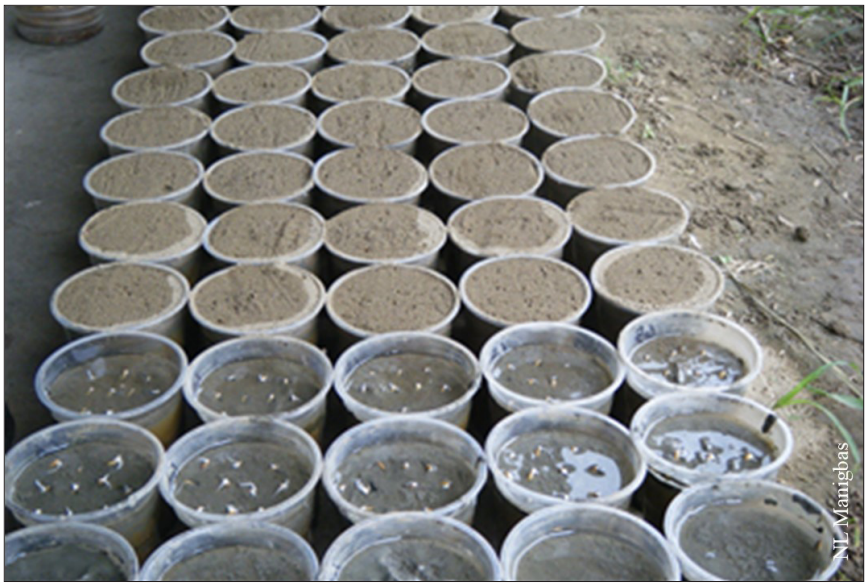

Figure 7.2. Plastic cups are covered with garden soil after sowing germinating seeds.

Figure 7.3. Screenhouse anaerobic germination screening.

% Anaerobic Germination	Classification:
0 - 40	Susceptible (S)
41 - 70	Intermediate (I)
71 - 100	Resistant (R)

Seedling Vigor Evaluation

6. Prepare coin envelopes and write the entry number of the materials being tested.
7. Pull carefully the seedlings and wash them thoroughly with clean water. Measure the root and shoot length (i.e., in millimeters). Record accordingly. Cut with scissor the basal part of the seedlings so that the root and shoot are separated. Place them in the coin envelope with the right entry label. Conduct this in three replications.
8. Put the root and shoot of 5 plants in the coin envelope (one envelope per entry) and place in the drying oven. Dry the samples for 5 days in 50°C oven. Weigh the samples repeatedly or after 5 days until the constant dry weight is obtained. Record the final weight of the root and shoot separately.
9. Determine the seedling vigor by comparing the biomass of the entries with the varieties used as checks, namely:
   - *Italica Livorno* (R)
   - IR72 (S)
   - IR64 (S)

10. Record the data in the worksheet or separate excel file for analyses.

Figure 7.4. Field anaerobic germination screening.

Field anaerobic germination tolerance screening

1. Broadcast the ungerminated seeds (200g) of the test materials in flooded seedbed (Figure 7.4).
2. Screen for anaerobic germination underneath water (5cm) for 7 - 10 days.
3. Transplant survived seedlings in the field (i.e., one seedling per hill) spaced at 20cm x 20cm.
4. Observe the survived seedlings for phenotypic acceptability, seedling vigor, lodging susceptibility, maturity, and general field reactions to insect pests and diseases (Figure 7.5).
5. The field should not be too inundated or flooded as this would make transplanting more difficult and encourages gas infestation. Around 1cm or 2cm of water is enough. Distance between hills is 20cm x 20cm in the dry and wet season. Always remind the transplanters that only one seedling should be transplanted per hill.

1	Extra vigorous (very fast growing); plants at 5 - 6 leaf stage have 2 or more tillers in majority of the population)
3	Vigorous (fast growing; plants at 4 - 5 leaf stage have 1 - 2 tillers in majority of the population)
5	Normal (plant at 4-leaf stage)
7	Weak (plants somewhat stunted; 3 - 4 leaves; thin population, no tiller formation)
9	Very weak (stunted growth; yellowing of leaves)

6. Maintain the breeding nursery free from weeds and ensure adequate supply of irrigation water. Drain the fields a week before harvest. Harvest and properly label the selected plants with desirable characteristics based on the breeding objectives.

Figure 5. Field anaerobic screening showing survived seedlings

Figure 6. Manual furrowing for direct wet-seeding

Figure 7. Row direct wet-seeding.

CHAPTER 8

Marker-assisted Selection

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In a breeding program, plant breeders normally use markers in selecting lines and parents for crossing to develop breeding populations. Breeders use their "breeder's eye" to pick out a desirable genotype by visual inspection to simplify the selection process. In essence, breeders use morphological markers such as panicle length and spikelet density to infer the overall and specific performance of a plant. But the ultimate interest is grain yield. In a segregating population, breeders compare lines or individual plants and select those that are relatively semi dwarf-statured and with sturdy culm in contrast to very tall plants with weak culm. Morphological markers are commonly used in conventional breeding. They are genetic markers that are inherited in a recognizable pattern and can be easily classified or scored. For example, short round grains are associated with *japonica* types while long slender grains are associated with *indica* types. Breeders sometimes cross *indica* and *japonica* types to recover desirable properties from both. However, *indica* types are generally more adapted for irrigated lowland in the Philippines and grain shape can be used as marker. Aside from morphological markers, there are also biochemical (isozymes) and DNA-based markers.

In marker-assisted selection (MAS), breeders refer the word 'marker' to DNA-based markers. For purposes of this chapter, marker and DNA marker, from here on, will mean the same. DNA markers are fragments or sequences of nucleic acid that differ among plants or lines in a population. Breeders look for variation in the DNA that corresponds to variation in phenotype. A marker can be part of the gene of interest itself or tightly linked to it; thus, being inherited together with the gene most of the time. MAS relies on the assumption that marker variation translates to phenotype variation. MAS uses marker data in selecting the desired plants or breeding lines in conjunction with trait data. Marker scores are used as signals to determine the presence of a trait or gene in an individual. It is a form of indirect selection.

Types of DNA markers used in MAS

A DNA marker is like a landmark in the genome. Hence, markers are a valuable resource in tagging genes that can be used in tracking down specific gene introgressions in breeding populations. The most common marker types used in marker-assisted selection are simple sequence repeats (SSR), sequence tagged sites (STS), cleaved amplified polymorphic sequence (CAPS), and single nucleotide polymorphisms (SNPs). Use of MAS in rice breeding enhances efficiency and accuracy of selecting plants carrying the gene of interest. Knowledge of whether a marker is dominant or co-dominant and gene-based or linked is critical in its usefulness in plant breeding.

Dominant vs. co-dominant markers. Classification of DNA markers into dominant and codominant types is very important in the application of MAS in plant breeding. When a DNA marker detects only two types, the presence and absence of a DNA band or fragment, the marker is said to be *dominant*. This type of marker cannot detect the heterozygous (presence of both alleles from parents 1 and 2, assuming that the two alleles are different in molecular weight or size) genotype usually present in the F_1. Examples of dominant markers are RAPD, ISSR, and PCR-based SNP. In contrast, co-dominant DNA markers can detect all three marker genotypes at a bi-allelic locus: (a) homozygous for parent 1 allele, (b) homozygous for parent 2 allele, and (c) heterozygous. In plant breeding, co-dominant markers are usually preferred because they can differentiate homozygous from heterozygous plants.

This step is critical when detecting introgressions in segregating populations (i.e., F_2, BC_1F_1).

Linked vs. gene-based markers. In marker-assisted selection, it is critical to define the gel bands that are diagnostic for specific traits. The type of DNA markers used is important to avoid selection of 'false positives,' in which the diagnostic band is present but the corresponding phenotype is absent or is not shown in the plant. A marker is said to be linked when it is only associated with the trait and is identified using linkage analysis. This type of marker is developed when the gene controlling the specific trait is not yet known or identified. When the gene has been identified and the physical location in the genome is known, a marker can be designed from the sequence of the gene. This type of marker is called gene-based or functional marker.

Advantages and disadvantages of MAS

MAS offers a lot of advantages over conventional selection alone. For traits that are difficult to measure and observe such as root traits, which need destructive sampling, molecular markers may be used. For traits that require selection pressure like biotic and abiotic stresses, markers make selection for resistant or tolerant plants possible even when the stress is absent.

MAS may also be cheaper than the conventional method. Breeding for nutrient use efficiency, for example, would require chemical inputs and sophisticated devices. In dealing with a few samples, it is possible that the screening cost is not substantial. However, when thousands of samples are needed to be analyzed, which is the usual case when dealing with complex traits, MAS would the more practical option, although a high initial investment is necessary.

MAS may shorten the breeding process. When the trait is only observed during the latter stages of plant growth, selection can be carried out earlier by employing DNA markers. Breeding lines may also be evaluated through MAS in the early filial generations. Relatively, it is also more efficient than conventional methods. When the marker is the gene itself (functional marker), MAS becomes direct selection for the trait.

Most economically important traits are quantitative and selection using the trait is inefficient without the proper protocols and set-up. Even with the best protocol, selection based on phenotype is limited because quantitative traits are highly affected by the environment. Markers for quantitative trait loci (QTL) together with phenotype data can provide a more accurate basis for selection, especially markers for QTLs with large effects. For quantitative traits, different genes can confer the same phenotype, which could limit conventional selection for that trait. For example, in disease resistance, two different genes can yield similar resistance response. MAS will allow stacking or pyramiding of these different genes resulting in a more robust plant, which cannot be done easily with conventional breeding. The trait is sometimes controlled by a recessive gene; thus, complicating the conventional selection process. As such, marker-assisted selection is the more practical option. When aiming for recovery of recurrent parent genome such as in backcrossing, genotyping using markers around the genome and calculating the similarity between progeny lines and the original parent will give a more definitive guide on the progress of recovery. This is better than just relying with theoretical basis regarding the number of backcrosses to recover certain percent of the recurrent parent genome.

MAS, on the other hand, has limitations. It is not applicable to all kinds of traits and sometimes population-specific. MAS is not ideal for traits that are not well-established, meaning there are few studies and literature about using markers for these traits. Although marker studies can be done, it is not the immediate concern of most breeders. Marker discovery is also not an easy task. When we say population-specific, it means that the available markers have been developed for a particular population only, and may not be applicable to your breeding population. This is mostly true for linked markers. It is necessary to first conduct polymorphism survey to check whether the variation in marker scores can discriminate the donor from the recipient and this should be carried out using a segregating population (i.e., F_2). The background genome also matters. It is possible that even if the transfer of gene is successful, the desired trait will not be expressed because of background effects and other factors that inhibited the transcription and translation of the gene in the recipient line. For traits that are controlled by several genes, the success of MAS also lies in the accuracy and reliability of mapping studies.

Requirements and considerations for a MAS program

There are several things to consider when employing MAS in a breeding program. Resources are one of the main considerations. Employing MAS means you need to have the proper equipment, tools, facilities, chemical inputs, and human resources. It is essential to learn the knowledge and skills about genotyping. Researchers should be familiar with techniques for DNA extraction, amplification, and DNA band detection and analysis. The equipment needed include freeze dryer, tissue homogenizer, pipettors, water bath, centrifuge, -20°C freezer, thermocycler, gel electrophoresis apparatus,

and gel documentation system. Chemical reagents needed are DNA extraction buffer, PCR kits, agarose or polyacrylamide gel, electrophoresis buffer, and DNA dye. Although one of the advantages of MAS is cheaper screening or selection, the cost of setting up a genotyping laboratory is quite high. A huge initial investment is necessary; however, once the requirements are in place, the advantages of MAS can be fully explored. It is also worthy to note that the cost of genotyping is decreasing. It is important to plan the breeding scheme in which MAS will be applied. It can be applied in a backcrossing, marker-assisted pyramiding, selection in the early generation, and a combination of these. The selection of a marker system to be used is also critical. PCR-based markers are the more practical option.

General procedures in genotyping using DNA markers

Leaf sample collection

Leaf samples can be collected as early as 3-leaf stage of rice. A systematic approach should be adapted and individual containers with proper labels should be provided to prevent mislabeling of samples. When sample collection can be carried out over a short period of time, the sample can be brought to the laboratory afterwards. However, if there are many entries to collect from, sample should be stored temporarily in containers with ice and brought to the laboratory as soon as possible. Leaves collected should be green and healthy.

DNA extraction

Leaf tissue is freeze-dried, then grinded using a mechanical homogenizer (Figure 8.1). Liquid nitrogen may also be used for grinding leaf tissue with mortar and pestle. The resulting powder (Figure 8.2) is mixed with the extraction buffer (Figure 8.3). DNA is precipitated by centrifugation (Figure 8.4), rinsed with ethanol, and suspended in DNA buffer. The extracted DNA must be in good quality.

DNA amplification

Amplification of DNA is necessary to attain a detectable amount. The DNA extract is mixed with a solution of polymerase enzymes, primers for the marker of interest, free nucleotides, and buffer (Figure 8.5). The resulting solution is subjected to a thermal cycle by which DNA is denatured, annealed with primers, and synthesized repeatedly (Figure 8.6). The desired quantity can be attained by setting the number of cycles to at least 30.

Detection of bands and data analysis

PCR products are fractionated by running on a gel exposed to an electric field (Figure 8.7). DNA fragments migrate towards the positive field at a speed depending on their size. The gel is stained and viewed under a gel documentation system (Figure 8.8). Scoring is done by comparing banding patterns of different genotypes.

Advanced Breeding Methods Employing DNA Markers

Genomewide Selection

In the traditional sense, MAS was originally designed using only one or two markers in selecting for a trait with simple genetic inheritance such as bacterial blight resistance conferred by *Xa21*. When it comes to quantitative traits such as grain yield, there are too

Figure 8.1. Tissue homogenization using a mechanical grinder.

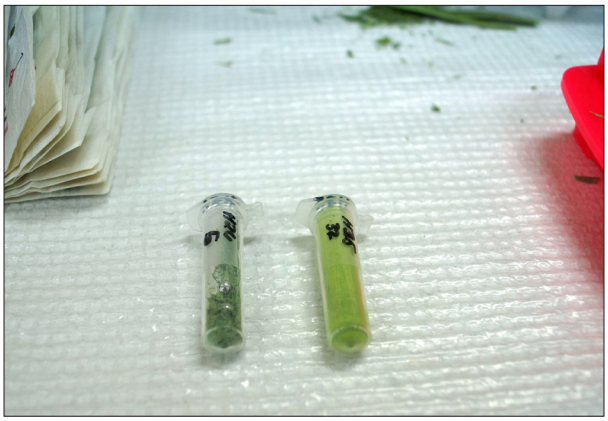

Figure 8.2. Centrifuge tube with leaf samples before (L) and after (R) mechanical grinding.

Figure 8.3. Addition of extraction buffer into ground leaf samples.

Figure 8.4. Precipitation of DNA by centrifugation.

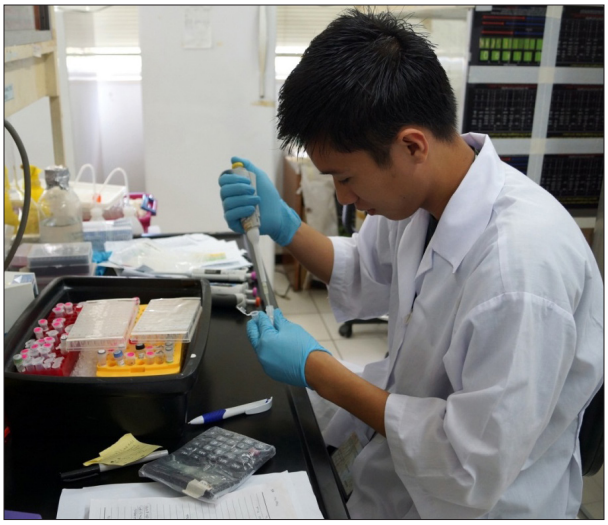

Figure 8.5. Preparation of PCR mix containing DNA template, PCR primers, deoxynucleotides (dNTPs), polymerase enzyme, magnesium chloride, buffer, and water.

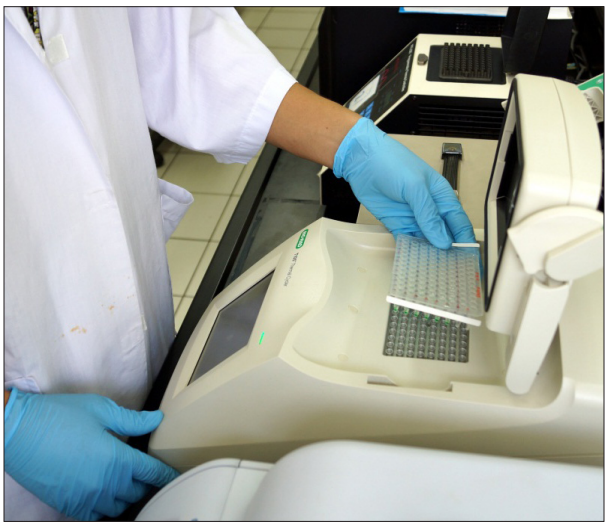

Figure 8.6. Setting up the PCR mix in a thermocycler for PCR amplification.

Figure 8.7. Loading of PCR amplification products in a polyacrylamide gel for electrophoresis.

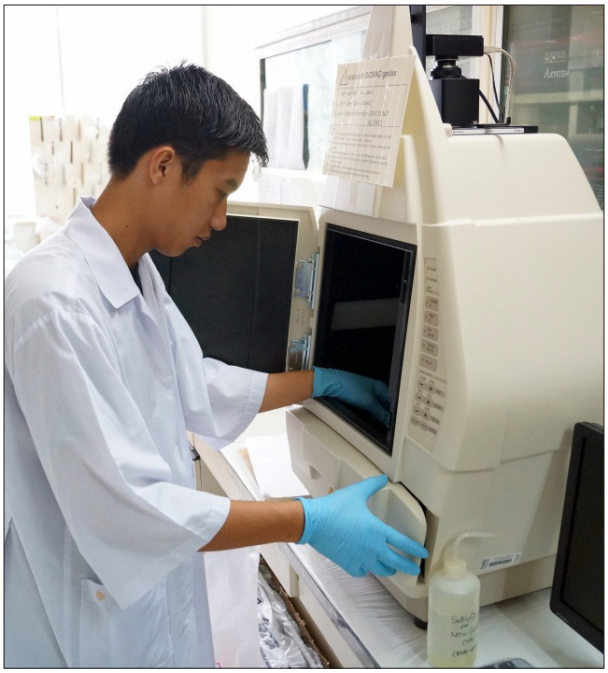

Figure 8.8. Loading a gel onto the UV box after staining to visualize DNA bands.

many genes involved and so the number of markers to be used becomes unmanageable. In genome-wide selection, a set of markers that sufficiently covers the 12 chromosomes of rice is needed without any assumption for known genes or QTLs. For each breeding population, recombinant inbred lines are developed, evaluated for the quantitative trait, and genotyped with bi-allelic markers. Using the phenotype and genotype data, additive effects for each marker are estimated in this population. The F_1 is self-pollinated, then the F_2 plants are grown as a single breeding population. The F_2 plants are genotyped, and the top 10% are inter-mated based only on the total additive effects across the genome-wide markers (i.e., marker-based selection). This select-self-recombine cycle may be carried out once or twice before finally self-pollinating the best plants to extract inbred lines. The purpose of having two to three cycles of recurrent selection is to accumulate favorable alleles across the many loci responsible for the quantitative trait.

Prediction of Performance in Untested Hybrids

In hybrid rice breeding, the main interest is to identify specific parent line combinations that will produce superior F_1. In a pool of promising parents, testcrossing is routinely conducted to produce F_1 for progeny testing. A large volume of crosses is necessary to effectively identify promising parents every season, but the number of testcrosses that can be produced is limited by the available resources. The genetic merit of the parents is determined by testing the F_{1s} in the following season; thus, taking two seasons (i.e., testcrossing in the first season, then evaluation of the F_1 in the second season). The capability to predict the performance of untested crosses is therefore highly valuable. By genotyping the breeding line pool with genome wide markers, the genetic relatedness can be determined, which in turn may be used to predict the yields of untested hybrids in conjunction with yield data from tested hybrids through a methodology called best linear unbiased prediction (BLUP).

CHAPTER 9

Screening for Disease Resistance

Fe A. dela Pena, Alfredo Sinohin, and Juliet P. Rillon

Attack of diseases gravely affect rice production; thus, breeding disease-resistant rice cultivars is one of the goals of rice improvement programs. Breeding for disease resistance is geared towards the incorporation of durable resistance into improved rice varieties. Pest management in rice, then depends to a great extent on the genetic resistance of rice cultivars. The most simple and economical approach to disease management is the use of improved varieties with resistance to major diseases to minimize crop losses. However, varieties released as resistant became susceptible after only few seasons or few years of cultivation due to pathogen evolution and adaptation to cultivated varieties. Thus, breeding for disease resistance is a continuous challenge to rice breeders and plant pathologists.

The success of evaluation for disease resistance depends on the presence of the pathogen in abundance, susceptible host, and the environmental conditions favorable for the development of the disease. The relative resistance of a plant is its maximum pathologic response to a pathogen. This is evaluated comparatively with that of a known susceptible check plant, which detects the presence of the disease.

The true limit of disease resistance is not frequently attained when one or more of the factors that favor disease development are not operating in its optimum conditions. In such case, the plant's true response is not manifested and leads to wrong assessment. The result: instability of the presumed resistance. Hence, favorable condition for the disease development is a must in evaluating disease resistance.

An effective evaluation method is essential in breeding for disease resistance. It should be simple and closely simulate the local environmental conditions. It is also efficient so that evaluation would not be slow and should be less expensive.

Blast, sheath blight, bacterial blight, and tungro are destructive rice diseases endemic in most rice growing regions of the Philippines. They also occur in epidemic proportion and cause considerable damage. The causal organisms can be introduced artificially (induced method) to the test plants or the test plants are naturally infected (natural method) under field conditions. The latter can be applied whenever disease pressures are adequate to give consistent results.

As these diseases do not occur at high level in some cropping seasons, intervention in the evaluation method in some NCT sites is made to favor disease development. This simulates a condition similar to a disease outbreak. In all sites, however, the natural occurrence and spread of the disease take its course under low to moderate level of infection; seldom reaching epidemic proportion.

1. BLAST

Pathogen: *Pyricularia oryzae*
(teleomorph: *Magnaporthe oryzae*)

Factors favorable for disease development:

a) Low soil moisture
b) High leaf wetness or long dew period
c) High relative humidity
d) High amount of nitrogen fertilizer
e) Frequent periods of rain shower
f) Presence of the blast spores in the air throughout the year
g) Upland rice environment
h) Cloudy skies
i) Aerobic rice environment where drought stress is prevalent

Symptoms:
The disease is found throughout the whole growth stages of the rice plant. Leaf blast lesion (Figure 9.1) is at first, minute brown spots, then expands rapidly to spindle-shaped lesions; center of the spot is usually gray or whitish with brown margin. Node blast has black rotten node and breaks easily. Neck blast may be confused with "white heads" caused by stem borer. Both injuries showed empty, erect white gray panicle. However, unlike injury caused by stem borer, in which the entire stem can be pulled readily, neck blast causes only injury at the neck and does not extend further into the leaf sheath.

Figure 9.1. Blast-infected plants showing symptoms on leaves.

Table 9.1. Steps and key points to prepare for blast screening.

Steps	Key points
1. Select area	Select an upland field or well drained site not subjected to flooding and have a good windbreak such as planting of banana trees, corn plants, cassava, sugar cane, and *ipil-ipil* surrounding the blast nursery to avoid the escape of the inoculums and infect other rice crops in nearby fields.
2. Prepare land	Prepare beds: 1.2m wide, 0.1m high, and of a convenient length. Divide the bed into two for replication.
3. Apply fertilizer	Before seeding, apply 50 - 100kg N/ha and other nutrients, if necessary. Fifteen days after seeding, top dress 50 - 100kg N/ha.
4. Seed the test entries from F2 to F6 and spreader rows	Sow the 5g seed (stored in coin envelope no. 0)/entry in a 50cm row. For every 10 rows of test entry, assign one row for the standard S-check, one row for resistant R check, and one row for the local S check. Have a 10cm space between rows. Cover the seeds with fine soil (Figure 9.2).
5. Manage the nursery	After emergence and throughout the duration of screening, mist irrigation is recommended to maintain high relative humidity.
6. Introduce inoculum	Collect blast infected leaves, chop to 2 - 5cm long and broadcast over the border rows 15 days after sowing. Dry, blast infected leaves from previous screening will be used for inoculation. Blast infected seedling may also be transplanted in-between spreader rows.
7. Aid spore production	Cover the plots with polyethylene sheets at sunset each day. Support the sheets with bamboo sticks and leave plots covered until 9 a.m. the next day.
8. Evaluate	Score at 30 - 35 days after sowing or when the susceptible checks are severely infected using the 1 - 9 standard scale below.

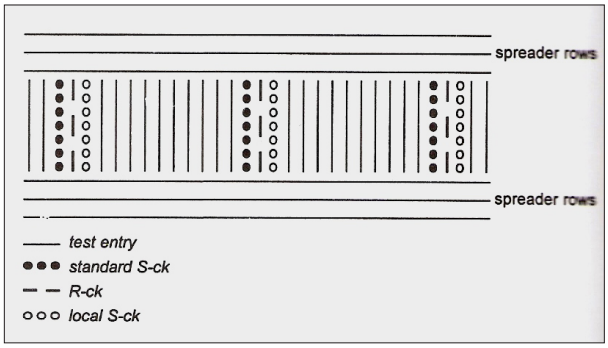

Figure 9.2. Blast nursery layout.

Disease Rating Scale:

Scale	Description
1	Few small brown specks of pinhead size
2	Larger brown specks
3	Small, round, necrotic gray spots about 1 - 2mm in diameter with brown margin
4	Elliptical lesion 1 - 2cm long, usually confined to the area of the two main vein, average of one to five lesions on a leaf
5	Average of 6 - 10 lesions on a leaf or less than 10% of leaf area infected
6	Average of 11 - 25% leaf area infected
7	Average of 26 - 50% leaf area infected
8	Average of 51 - 75% leaf area infected
9	Average of above 75% leaf area infected

Scale 1, 2, 3 Resistant (R)
Scale 4, 5, 6 Intermediate (I)
Scale 7, 8, 9 Susceptible (S)

2. SHEATH BLIGHT

Pathogen: *Rhizoctonia solani*
(perfect stage*: Thanatephorus cucumeris*)

Factors favoring the disease development:

- Temperature (28 - 32°C) and relative humidity (85 - 100%) of the crop canopy
- High nitrogen fertilizer application
- High seeding rate or closer plant spacing
- Dense canopy
- Low light intensity

Symptoms:
Grayish-green lesions may enlarge and coalesce with other lesions, mostly on lower leaf sheaths, but occasionally on the leaves (Figure 9.3).

Figure 9.3. Sheath blight-infected plants showing symptoms on sheath.

Table 9.2. Steps and key points to prepare for sheath blight screening.

Steps	Key Points
1. Prepare test plants	Sow seeds in wet bed nursery. Transplant 20 - 25 days-old seedlings at 2 - 3 seedlings per hill in rows of 8 - 10 hills, spaced 20cm x 25cm. Assign a test entry for each row. For every ten rows of test entries, assign 1 row of standard S-check (IR29) and 1 row of the local S-check.
2. Manage plots	Follow the recommended fertilizer application for wet and dry seasons. Control weeds and insect pests as needed.
3. Prepare inoculum	Culture the causal organism on sterilized rice grain and rice hull mixture (1:3) and incubate at room temperature for 2 - 3 weeks.
4. Inoculate seed entries	Inoculate each entry at 45 - 50 days after transplanting by placing about one teaspoon of the cultured organism in between tillers.
5. Evaluate	Score at 2 - 3 weeks after inoculation using the 1 - 9 standard scale below.

Disease Rating Scale (based on relative lesion height)

Scale	Description
0	No infection observed
1	Lesions limited to lower 1/4 of leaf sheath area
3	Lesions present at the lower 1/2 of leaf sheath area
5	Lesions present on more than 1/2 of leaf sheath area. Slight infection on lower 3rd or 4th leaves
7	Lesions present on more than 3/4 of the leaf sheath. Severe infection on upper leaves
9	Lesions reaching top of tillers; severe infection on all leaves and some plants died

Scale 0, 1, 3 Resistant (R)
Scale 5 Intermediate (I)
Scale 7, 9 Susceptible (S)

3. BACTERIAL LEAF BLIGHT

Pathogen: *Xanthomonas oryzae pv. oryzae*

Factors favoring disease development:

a) High nitrogen fertilizer rate. Generally more than 120kg N/ha.
b) Cloudy and humid conditions
c) Temperature range of 28 - 35°C
d) Continuously flooded field

Symptoms:

Lesions start at the edge near the tip of the leaves. The young lesion expands along the vein forming yellow streak. The edge of the lesion is usually irregular or wavy and has a yellow margin. As the disease advances, the lesion covers the entire blade, turn straw colored and later become grayish from the growth of various saprophytic fungi (Figure 9.4)

Table 9.3. Steps and key points to prepare for bacterial leaf blight screening.

Steps	Key Points
1. Prepare test entries	Sow seeds in wetbed nursery. Transplant 20 - 25 days-old seedlings at 2 - 3 seedlings per hill in rows of 8 - 10 hills, spaced 20 x 25cm. Assign a test entry for each row. For every ten rows of test entries, assign 1 row of standard S-check (IR24) and 1 row of the local S-check.
2. Manage plots	Follow the recommended fertilization for wet and dry seasons.
Control weeds and insect pests as needed.	
3. Prepare inoculum (Figure 9.5)	
a. From infected leaves	Collect naturally infected leaves. Remove old brown lesions and save those with young, advancing lesions. Weigh and cut infected leaves into small pieces. Mix 50g infected leaves per 500 ml of water (distilled/rain) and let stand for 20 min. Filter mixture in 2-3 layers of cloth.
b. From pure culture	Streak 2 loops of 2-3h old culture on agar plates Invert and incubate the plates at room temperature for 48 - 72h. Pour 10mL of sterile distilled water on the plate and scrape the bacterial growth. Prepare 100mL suspension to have a population of about 10^8 cells/mL
4. Inoculate (Figure 5)	a. With inoculation clippers: Place suspension in the container of the clipper. Clip the leaves 6cm from the tip. b. With scissors: For each entry select the two front hills, dip the scissors into the suspension and cut leaves 6cm from the tip.
5. Evaluate	Evaluate at 14 days after inoculation following the 1 - 9 standard scale below.

Figure 9.4. Bacterial leaf blight-infected plants showing symptoms.

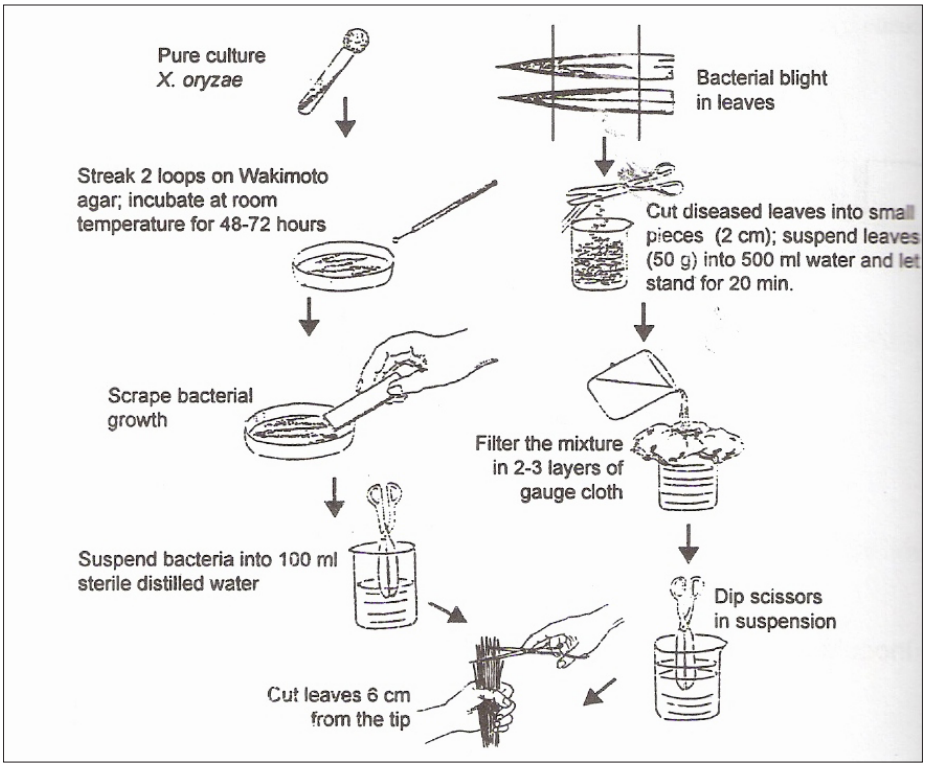

Figure 9.5. *Xanthomonas oryzae* inoculum production and inoculation.

Disease Rating Scale (based on lesion area):

Scale	Description
1	Lesions from cut tip cover 1 - 5% of the leaf 0 - 5% lesion area
3	Lesions from cut tip show blight chlorotic symptoms 6 - 24%
5	Downward length of lesion from cut tip may extend covering 1/4 to 1/2 leaf area showing chlorotic symptoms 25 - 50%
7	Downward length of lesions from cut tip may extend 3/4 of leaf showing chlorotic symptoms 51 - 75%
9	Lesions cover > 75% of the leaf and reaching the leaf sheath >75%

Scale 1, 3	Resistant (R)
Scale 5	Intermediate (I)
Scale 7, 9	Susceptible (S)

4. TUNGRO

Pathogen: Rice tungro virus

Factors for development:

a) Presence of GLH and inoculum source
b) Age and susceptibility of the host plant
c) Favorable environment

Symptoms:

Rice plants infected by both RTBV (Rice Tungro Bacilliform Virus) and RTSV (Rice Tungro Spherical Virus) show typical tungro symptoms: stunting and yellow or orange-yellow discoloration of the leaves (Figure 6). Panicle of tungro infected plants is poorly exserted and produce empty or partially filled grains. Plants that are infected with RTBV alone develop symptoms that are similar to but milder than those by both RTBV and RTSV. Plant infected with RTSV alone may be symptom less or exhibit only very mild stunting.

Figure 9.6. Rice tungro virus-infected field showing symptoms.

A. Modified field screening

Steps	Key points
1. Prepare test plot	Transplant 2 weeks old susceptible variety (IR64) and tungro infected plants on the spreader rows at 2 - 3 weeks before planting the test entries.
2. Prepare test plants from F_5 onward	Follow the wetbed method of raising seedlings. Transplant test entry in a row of 20 hills spaced 20 x 20cm at one seedling/hill.
3. Evaluate	Score at 45 and 60 days after transplanting. Evaluate disease reaction by taking the percent infection.

$$\text{Percent infection} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Percent infection	0 - 20 %	Resistant (R)
Percent infection	21 - 40 %	Intermediate (I)
Percent infection	41 - 100 %	Susceptible (S)

Entries showing intermediate to resistant reaction will be subjected to tungro induced greenhouse evaluation.

B. Induced greenhouse evaluation

Steps	Key points
1. Prepare test plants	Sow seeds of test entries and susceptible check, Taichung Native 1 (TN1) every 10 test entries in a compartment box. Thin out seedlings into 10 - 15 per selection after 10 - 12 days.
2. Prepare viruliferous green leafhopper	Collect adult hoppers from cages and allow them to feed on infected plants for 4 days (Figure 9.7).
3. Inoculation	Cover the seed box with a screen to prevent the escape of insects. Collect hoppers and release into the seed box at 3 - 4 hoppers per seedling. Cover with black cloth to prevent attraction of insects to light. Allow the insects to feed for 24h. After feeding, retrieve the hoppers. The inoculated seedlings are transplanted in lowland plot.
4. Evaluate	Evaluation should be taken at 3 - 4 weeks after transplanting.

Use the disease incidence in evaluating the disease reaction:

$$\text{Percent infection} = \frac{\text{No. of infected seedlings}}{\text{Total no. of seedlings}} \times 100$$

Percent infection	0 - 20 %	Resistant (R)
Percent infection	21 - 40 %	Intermediate (I)
Percent infection	41 - 100 %	Susceptible (S)

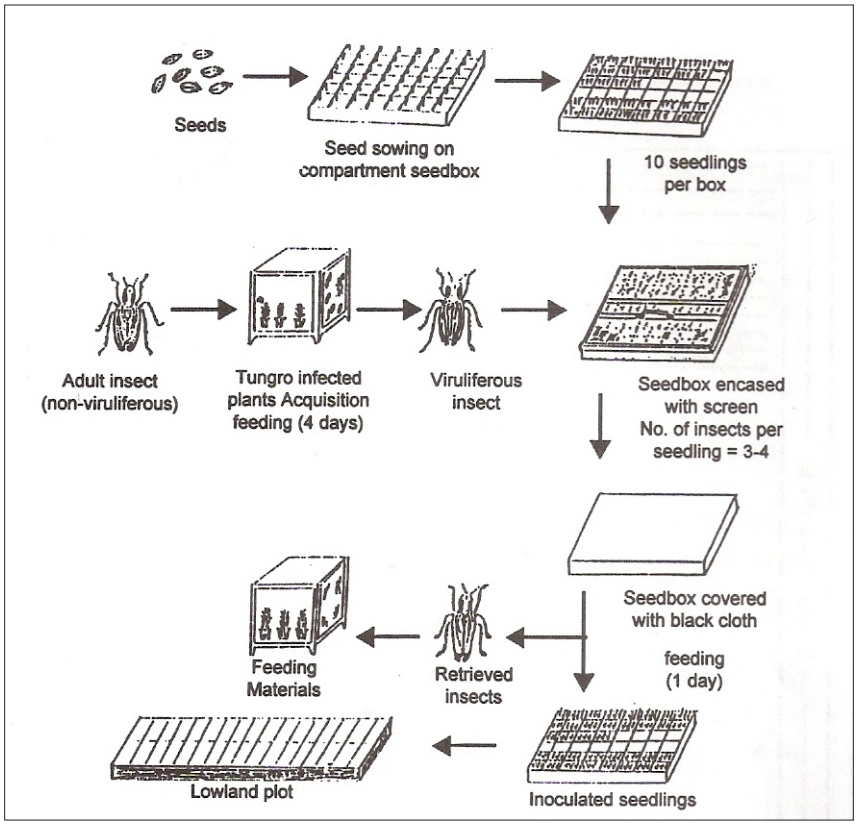

Figure 9.7. Preparation of viruliferous green leafhoppers for tungro evaluation.

CHAPTER 10

Screening for Insect Pest Resistance

Ester A. Magsino and Gilely DC. Santiago

With the shift of insect pest control from the conventional method of insecticide use to the more environment-friendly and sustainable approach of insect pest management, incorporating resistance had become a major objective in crop breeding programs. According to Painter (1951), host plant resistance includes heritable characters that enable a plant to reduce the degree of damage it suffers. This could be due to either the non-preference or antibiosis response of the insect to the plant or the tolerance response of the plant to the insect. Heinrichs et al. (1985) cited the following reasons for the importance of host plant resistance, especially in rice: low production income and high expenditures for input lower the farmer's profit; the cost of chemical insecticide increases faster than the price of palay; farmers' lack of knowledge on insecticide use based on threshold levels, prophylactic applications are wasted; insecticides may cause poisoning accidents as these contaminate the environment and kill non-target organisms; and varietal resistance is compatible with other control tactics – chemical, biological, and cultural control.

Identifying sources of resistance is the initial step in breeding for insect resistance. Germplasm accessions and other materials, as parentals in hybridization work, have to be characterized in terms of their reaction to insect pests. While it identifies the desired level of resistance, evaluating progeny lines would eliminate entries that are highly susceptible; thereby, reducing the bulk of the materials being handled at the early stage of varietal development. When done in advance lines, evaluating insect resistance will determine a variety's resistance level. In case of a high yielding variety with relative susceptibility to an insect pest, its resistance level will aid in identifying the insect pest management approaches that will allow maximum expression of its yield potential.

Setting priorities for screening for insect resistance and its breeding program

Criteria must be considered when deciding on the resources to be spent and insect pests to be included in evaluating insect resistance and its breeding program. The extent of economic losses caused by the various insects, distribution within the geographic area concerned, periodicity of outbreaks, and the prevailing species in the locality should be known. Insects, which may cause only moderate yield losses, but are widely distributed and have regular outbreaks may cause more financial loss than an insect, which has a higher damage potential but seldom reaches economic proportions or occurs only in isolated area. Furthermore, an insect, which causes direct damage by feeding, and in addition, transmits a virus would have higher priority than those which only cause feeding damage. Four species of stem borer exist in the country. The stripe (*Chilo suppressalis*) and yellow stem borers (*Scirpophaga incertulas*) are the predominant species in Luzon, white stem borer (*S. innotata*) in Visayas, and the pink stem borer (*Sesamia inferens*) predominates in Mindanao. Stem borer resistance should be screened against the species present in the area.

The choice of the screening methodology is another important aspect of evaluation for resistance to insect pests. Evaluation under greenhouse conditions would require an efficient insect mass rearing protocol and an appropriate greenhouse. On the other hand, sufficient insect pest pressure must be ensured when relying on natural infestation for field evaluation.

Another requisite is an efficient evaluation rating system, which would distinctly separate one resistance reaction from the others. Ratings used in rice insect resistance

screening programs are based mostly on degree of plant damage, but in some cases, insect numbers are also used. The Standard Evaluation System for Rice (IRRI, 1996) was devised for this purpose and includes most of the major rice insects. All rating scales describe degree of damage on a 0 - 9 scale (0 = no damage; 9 = severe damage). To compare entries, either or both the numerical or/and adjectival ratings could be used.

Plant reaction and subsequent damage rating depend on the number of insects per plant, plant vigor, plant age, and environmental factors such as temperature. The insect population used should also give distinctly different reactions for the susceptible and resistant entries. When insect populations are too high, all entries may appear susceptible; when too low, all entries may appear resistant. Plants that lack vigor because of nutrient deficiencies maybe mistakenly identified as susceptible.

Screening procedures had been developed for insect pests of rice (Heinrichs et al., 1985). In Asia and South America, evaluating resistance for leafhoppers, planthoppers, and stem borers (Heinrichs, 1994) were emphasized. Among the insect pests of rice in the country, the brown planthopper (BPH), green leafhopper (GLH), and stem borers are likewise identified as the most destructive, and as such, this manual focused on the resistance evaluation of these pests.

Methodology

1. Screenhouse Evaluation

A. Maintenance of hopper cultures

- Prepare basic materials needed in the collection and maintenance of the hopper cultures:
  a) Insect cages (Figure 10.1)
  b) Sweep net (Figure 10.2)
  c) Aspirators used in collecting reared hoppers (Figure 10.3).
  d) Potted TN1 or IR8 plants in size 4 pots (rim of pot is 11cm in diameter).

- Collect test insects from the field (Figure 10.4).
- Maintain the culture using the host plants. Collect from the field hopper populations (Figure 10.4) after every two seasons (4 generations). Introduce these to the culture to minimize inbreeding and to strengthen the culture. Purify first the brown planthoppers before introducing to the corresponding cultures. Refer to Section B on purifying BPH biotypes.
- Maintain a steady supply of host plants by planting in a staggered manner. Use clay soil collected from the paddy. Produce required amount of seedlings in typical *dapog* style.
- Apply basal a pinch of urea (46-0-0) per pot. Keep the host plants in water pans or ponds inside screenhouses, if these facilities are available. Otherwise, ensure that the plants are properly irrigated.
- Always provide fresh potted host plants in the rearing cage. Remove completely wilted host plants and replace with fresh ones (Figure 10.5). To ensure that the fresh host plants to be used are free from other insects, dip the whole potted plant in a pail of water, remove old and dried leaf sheaths, and brush clean the pots.
- Collect the BPH or GLH egg infested test plants to produce test insects of uniform age. Lightly tap the plants inside the rearing cage to dislodge the nymphs and adults. Transfer collected plants in clean cages for egg emergence. Take note of the date of the emergence of the nymphs from the eggs (Figure 10.6).
- Allow the nymphs to develop up to the second or third instar, the required age for infestation. This is a week after hatching.
- During the cold season of the year, install a 50-watt incandescent bulb inside the cage and kept lit at night. This will increase the temperature and minimize fungal infection of the test insects.

B. Purifying BPH biotypes

There are five BPH biotypes. Populations in the area should be screened regardless of the biotypes. However, when the objective is to screen for a specific biotype, field collected populations should be purified.

- Place 10 pairs of hoppers in a cage provided with potted TN1 plants.
- From this population, produce F_3.
- Purify the F_3 progenies by transferring a gravid female to a caged potted host plants. Following are the differential varieties for the desired biotype:

Biotype	Variety, in which females have developed a population of approximately 250 individuals/female or Variety fed upon and killed by the population being purified
1	TN1
2	Mudgo, IR26, IR29, IR30, IR34
3	ASD7
4	Rathu Heenati
5	Babawee

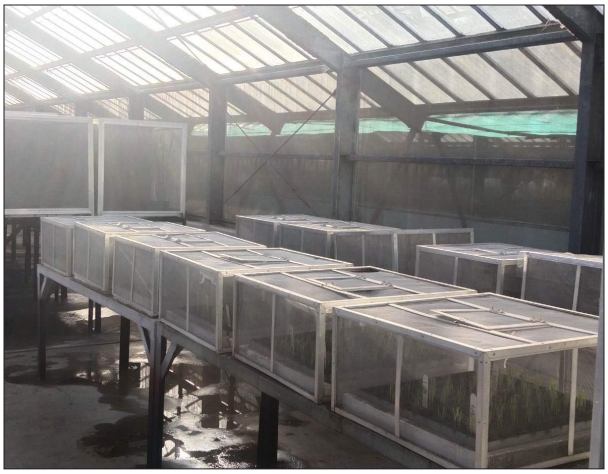

Figure 10.1. Insect cages.

Figure 10.2. Sweep net used to collect insects.

Figure 10.3. Aspirator used in collecting reared hoppers.

Figure 10.4. Collection of test insects.

Figure 10.5. Host plants inside rearing cages: (A) completely wilted plants ready to be removed; (B) fresh host plants. The bottom of the cage should hold water to create a humid condition for host plant maintenance and a favorable environment for the development of the test insects.

Figure 10.6. Hopper eggs between the leaf sheath and stalk of the host plant.

C. Screening of test entries

Basic material requirements for screening of test entries

- Compartment seed box with cover (preferably aluminum)
- Fine garden soil
- Labels
- Marking pens

C1. Green leafhopper/Brown planthopper

- Conduct the seedling test for resistance to the brown planthopper inside the screenhouse. If different biotypes are maintained, conduct the screening in the same screenhouse where the biotype is mass reared to avoid biotype mix-up.
- Use a compartment seed box with cover (Figure 10.7). Label the opening on top of the cover. Invest in seed box and cover made of aluminum material that are rust proof and have a longer service life.
- Randomly allocate the entries each to a compartment with the two middle rows assigned to the resistant (TKM6) and susceptible (TN1) check varieties (Figure 10.8). Prepare a randomization lay out before sowing the seeds in the compartment seed box.
- Middle rows are planted to TN1 (susceptible check) and IR 54 (resistant check).
- Collect, dry, pulverize, and sieve garden soil. Fill the compartment seed box with the prepared soil.
- Sow about 30 seeds of each entry directly on each compartment. Cover them with a thin layer of soil.
- Water the seeds and the developing seedlings. Keep the compartment seed box covered.
- Take note of the date of seedling emergence.
- At 7 days after seedling emergence, uniformly introduce 3 - 5 nymphs per seedling. While keeping the compartment seed box covered, put the potted host plants with the test insects inside the seed box through the opening on the top of the cover. Lightly tap the host plants to dislodge the nymphs.
- Regularly water the seedlings to prevent wilting and keep the environment humid for the insects. Secure the test insects from ants.
- Keep the seed box covered to prevent escape of the test insects and infestation by other insects.
- If screening for a specific BPH biotype, refer to the table on differential varieties for the susceptible checks to use.
- Rate the entries for their resistance reaction when the susceptible check is completely wilted or killed (Figure 10.9) using the following scale:

Index	Rating	Description of damage
1	R	Very slight damage
3	MR	First and second leaves of most plants partially yellowing
5	I	Pronounced yellowing and stunting or about 10 to 25% of the plants wilting
7	MS	More than half of the plants wilting or dead and remaining plants severely stunted or drying
9	S	All plants dead

C2. Stem borers

Stem borers are usually screened in the field but may also be evaluated in the greenhouse provided there is an area sufficient for the mass rearing of the test insects and for the screening. Unlike the screening for hoppers, which is done using compartment seed box, entries screened for resistance to the stem

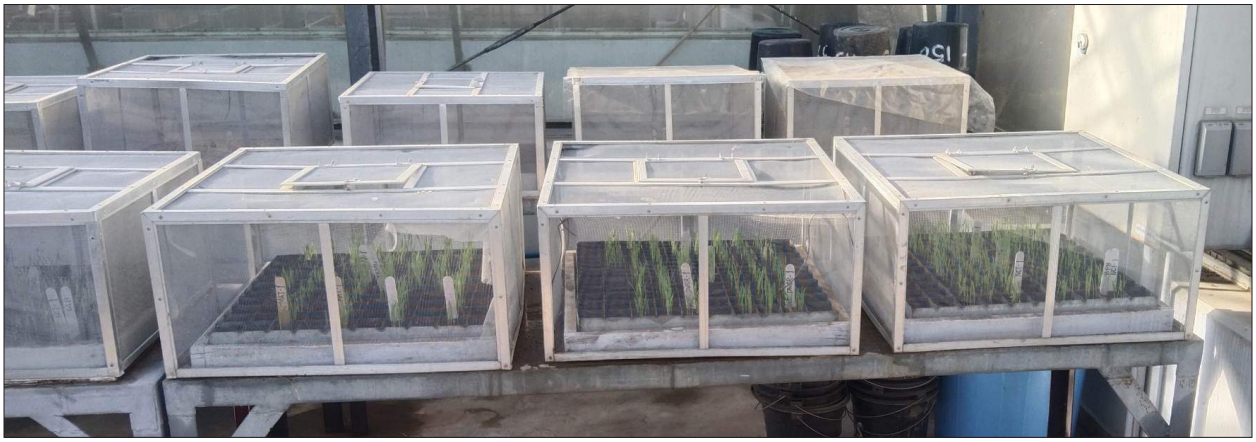

Figure 10.7. Aluminum compartment seed box and cover used in screening for hopper resistance.

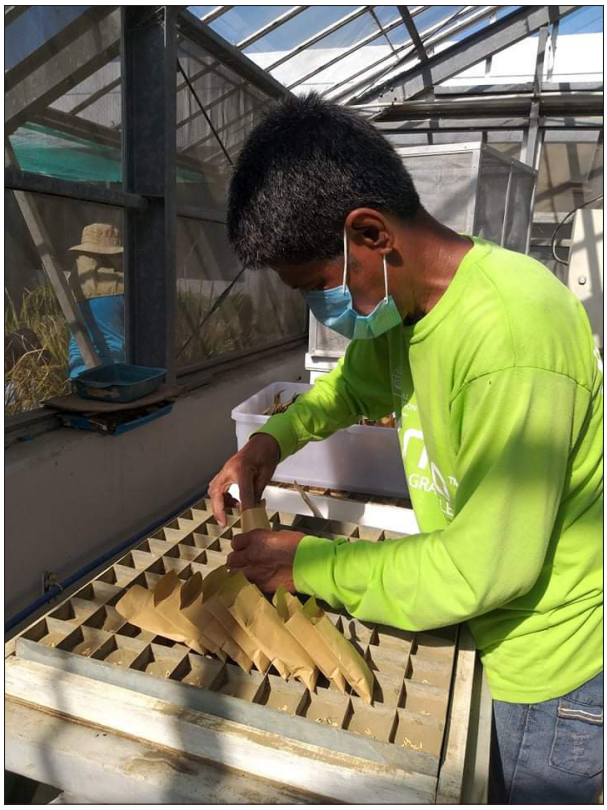

Figure 10.8. Compartment seed box.

borers are either planted in pots or directly sown in specially constructed paddies inside the screenhouse. Protocols for the screening for stem borer resistance are available (Heinrichs et al., 1985).

2. Field Evaluation

A. Seedling establishment, transplanting, and cultural practices

- Sow the entries using the wetbed method.
- Prepare a randomization layout in RCBD with at least three replicates for the nursery to be evaluated. Include in the randomization the regular check varieties for stem borer, IR 8 (susceptible check), and TKM6 (resistant check) to be planted along with the test entries.
- Plant five rows of 21-day old susceptible variety (preferably TN1 and/or IR 8) as border rows 15 days before transplanting the test entries (Figure 10.10).
- Start evaluating progeny lines at F_5. Rate the entries in the yield trial if seeds are not sufficient to plant a separate set-up for insect screening. In the absence of the susceptible check in the yield trial, record the actual percent damage. If an insect screening set-up will be planted, an unreplicated one row plot with 15 hills per entry is enough for evaluating the progeny lines considering the limited number of seeds.
- Transplant 21-day old seedlings at one to two seedlings per hill with 20cm spacing between hills and rows. Each plot should consist of three 3-m rows such that there will be 15 hills per row or a total of 45 hills per plot.
- Follow all recommended cultural practices except that no insecticides will be applied. Fertilization rates depend on the soil analysis of the area.

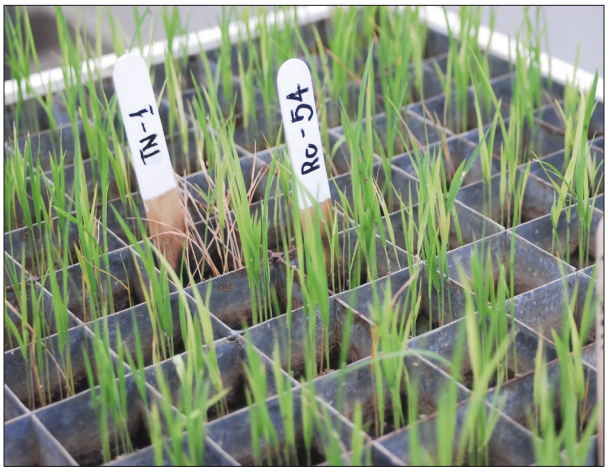

Figure 10.9. Entries ready for evaluation. TN1 is completely wilted.

B. Screening for stem borers

- Schedule the planting date so as to hit the peak of stem borer population in the locality.
- Make the damage rating 35 and 50 days after transplanting for deadhearts and 10 days before harvest for whiteheads.
- Compute for percent deadheart or whitehead using the formula:

$$\%\ \text{deadheart or whitehead/hill} = \frac{\text{number of deadheart or whitehead}}{\text{total number of tillers or panicles/hill}} \times 100$$

- Percent deadhearts and whiteheads in susceptible

Figure 10.10. Area ready for transplanting of the test entries. IR8, transplanted 15 days earlier, line the border rows.

check should average at least 20% and 10%, respectively, for the test to be considered valid. However, take reading even if the stem borer damage is less than the above figures.

- Convert percent deadheart and whitehead into scale or adjectival rating using the following:

Index	Rating	% Deadhearts	% Whiteheads
1	R	1 - 10	1 - 5
3	MR	11 - 20	6 - 10
5	I	21 - 30	11 - 15
7	MS	31 - 60	16 - 25
9	S	61 and above	25 and above

C. Screening for Brown Planthopper

In case of natural BPH field infestation, evaluate the entries against this insect in the field. The hopper population should be uniformly distributed across the field with at least 10, 25, and 100 hoppers per hill at 10 - 15 days after transplanting, maximum tillering, and early booting stages, respectively, for the evaluation to be considered valid. Rate the middle row of each plot and use the following scale to determine the resistance reaction of the entries:

Index	Rating	Description
1	R	Slight yellowing of a few plants
3	MR	Leaves partially yellow but with no hopperburn
5	I	Leaves with pronounced yellowing and some stunting or wilting and 10-25% of plants with hopperburn, remaining plants severely stunted.
7	MS	More than half the plants wilting or with hopperburn, remaining plants severely stunted.
9	S	All plants dead

CHAPTER 11

Screening for Resistance/Tolerance to Abiotic Stresses

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Screening for Cool Temperature Tolerance

Thelma F. Padolina

Rice varieties differ significantly in their capacity to tolerate low temperature stress or chilling injury (below 15 - 18.8^0 C) at various growth stages. The usual symptoms of low temperature damage at early growth are poor and delayed germination, stunted seedling growth, and leaf yellowing. Meanwhile, inhibited rooting and tillering ability are evident at the vegetative stage. During the reproductive stage, particularly at booting stage or young microspore stage, the rice plant is most sensitive to low temperature, which disrupts the development of pollen grains, preventing fertilization; hence, causes higher spikelet sterility (Nishiyama, 1984). At near maturity, low temperature induces poor grain filling and rapid leaf senescence. Consequently, these symptoms bring about significant yield loss (Lee, 2001).

Breeders aim to develop rice germplasm that can produce high and stable yields despite low temperature stress. In the Philippines, high-elevation areas ranging from 500 to 2,000 meters above sea level (MASL) have low temperature patterns affecting rice productivity. These areas can be found in the Cordillera Administrative Region (CAR), where rice is also grown as a major staple. Cool temperature stress is experienced under two different conditions in the rice fields in CAR. The main crop (November - January planting) requires varieties tolerant to low temperature at seedling stage while the off-season crop (July - August planting) needs varieties tolerant at the reproductive stage. Therefore, appropriate screening protocols must be available for breeding.

Natural field screening

Location-specific target sites provide the best opportunity to identify desirable and adapted cultivars and offer the cheapest alternative to artificial screening. Screening under natural conditions with medium to high elevation (500 - 2,000 MASL) is the easiest and most inexpensive way to reproduce cool temperature damage incidence. In this method, the experimental plants are raised under natural conditions where the critical growth stage of the rice plant sensitive to low temperature coincides with the natural cool temperature period. The features of the screening are:

1. Use of resistant and susceptible checks,
2. Careful observations based mainly on visual scoring on the traits associated with growth stages, and
3. Use of the IRRI's Standard Evaluation System (SES) for rice, which provides a qualitative evaluation of traits associated with low-temperature stress tolerance and/or susceptibility.

Target testing site

The target testing sites should have temperatures between 15 - 18.8^{o}C during germination/seedling stage and booting to full anthesis stage. The qualified sites are medium- to high-elevation areas in Benguet (La Trinidad or Tublay), Nueva Vizcaya (Kayapa), or Ifugao (Banaue or Hungduan). For seedling cool temperature tolerance, the best screening time is between November - February,

while for the reproductive cool temperature tolerance, the planting time is from July to December during which the low critical temperature coincides with the booting stage.

Sowing date

The sowing date is very important so that the appropriate growth stages will coincide with the critical low temperature to affect stress. For seedling cool temperature tolerance, sowing must be done from November to December when the temperature is less than the optimum temperature of 20°C but not lower than 18°C. For the reproductive cool temperature tolerance, off-season planting from July to December is recommended so that the critical temperature will coincide with the booting or microspore stage, when variation of reactions to cool temperature stress is manifested by the test entries.

Materials for testing

The number of entries that can be accommodated for testing depends upon the size of the experimental area. It is important to group the materials according to maturity based on the days to 50% heading or flowering data observed previously. Entries flowering in 85 days or less from sowing are classified as early, and medium if the flowering takes more than 85 days. Appropriate resistant and susceptible check cultivars as reference varieties must accompany the test entries. Suggested check cultivars are shown in Table 11.1.

Table 11.1. Resistant and susceptible check varieties for cool temperature test.

Group	Resistant Check	Susceptible Check
Indica	Gohang/Sumadel	IR72/PSB Rc2
	K39-96-1-1-1-2	
Japonica	Barkat/Stejaree 45	MS11
	NSIC Rc104	

Experimental design

For advance generation and fixed lines, employ the systematic arrangement of entries without replication. Plant each entry in a plot with 4 rows and 3-m long, with one seedling per hill and space planted at 25cm x 25cm between hills and rows.

Cultural management

Follow the farmers' common and best practices in the area for land preparation and nutrient, water, and pest management. Follow other cultural practices as well.

Assessment of cool temperature tolerance

Good growth volume and fertility are the two most important indicators of cool temperature tolerance. Visual observations on traits associated with growth stage must be carefully recorded in comparison with the checks.

Traits to be evaluated

Vigor or Growth volume: Several factors such as tillering ability, plant height, and survival rate may interact, influencing vegetative vigor, which is rated using the following score system (Table 11.2):

Table 11.2. Scoring system in evaluating the seedling vigor or growth volume.

Score	Description
1	Extra vigorous (very fast growing; plants at 5-leaf stage, have two or more tillers in majority of population)
3	Vigorous (fast growing; plants at 4 - 5 leaf stage, have 1 - 2 tillers in majority of population)
5	Intermediate or normal (plants at 4 - 5 leaf stage)
7	Weak (plants somewhat stunted; 3 - 4 leaves; thin population; no tiller formation)
9	Very weak (stunted growth; yellowing of leaves

Leaf color: Scale differs between seedling, tillering, and reproductive stages; yellowing is a cold susceptibility symptom.

Tillering ability: The score should represent most plants within the plot (Table 11.3).

Table 11.3. Scoring system in evaluating the tillering ability of entries.

Score	Description
1	Very prolific (more than 25 tillers/plant)
3	Good (20 - 25 tillers/plant)
5	Medium (10 - 19 tillers/plant)
7	Poor (5 - 9 tillers per plant)
9	Very poor (less than 5 tillers per plant)

Plant height (cm): Measurement from soil surface to the tip of the tallest panicle, excluding the awn

Heading days: Number of days from sowing to 50% flowering.

Spikelet fertility score: Actual counting of filled and unfilled grains or use SES.

Panicle exsertion score: Use the SES scale. Poor exsertion is an indicator of cold susceptibility (Table 11.4).

Table 11.4. Scoring system in evaluating panicle emergence and panicle exsertion.

Panicle emergence index	% Panicle Exsertion	Description
1	100	Well exserted
3	95 - 99	Moderately well exserted
5	75 - 94	Just exserted
7	50 - 74	Partly exserted
9	<50	Enclosed

Phenotypic acceptability score: This is the most valuable information usually taken at maturity. This score reflects the overall acceptability of the variety where it is grown (Table 11.5).

Table 11.5. Scoring system in evaluating phenotypic acceptability.

Score	Description
1	Excellent
3	Good
5	Fair
7	Poor
9	Unacceptable

Grain yield. Get seed yield weight and % moisture content and compute yield per hectare at 14% moisture content. Compare yield of test entries with the check varieties.

Get the harvest area (crop cut sample) excluding the border rows, thresh, clean, weigh, and compute yield as follows:

$$\textit{Grain Yield at 14\% MC (kg/ha)} = \textit{Grain weight (kg)/ Crop cut sample (m}^2\textit{)} \times \textit{10,000m}^2\textit{/ha x (100-MC)/86}$$

Weather data
- Temperature
- Relative humidity
- Solar radiation and duration

Induced screening at different growth stages
Artificial or induced stress conditions are used in specific instances such as the characterization of new donor parents, and confirmation of cool temperature tolerance.

The general steps involved in this screening for cool temperature tolerance are as follows:

1. Germination — The seeds of rice varieties germinate within different temperature ranges. There is a minimum, an optimum, and a maximum temperature for germination, which values differ among varieties. Screening for cool temperature tolerance at germination is conducted at 10 - 15°C for a varying duration of up to 10 days. Include tolerant check varieties (Gohang at vegetative and Sumadel at reproductive stage) and susceptible cultivars (IR72 or PSB Rc2) for comparison.
   - Break seed dormancy
   - Sterilize and wash seeds
   - Incubate the seeds at 10 - 15°C
   - Count the germinated seeds after 5 days of incubation and do a final count after another 5 days

2. Early seedling — Most farmers ordinarily soak and incubate the rice seeds in a warm place so that the growth of pre-germinated seeds in the seed bed is affected by low temperature. When the temperature fluctuates to around 10°C, the pre-germinated seeds may either be killed or retarded in growth; thus, cool temperature tolerance at this stage is necessary (Li, Vergara, and Visperas, 1980).
   - Soak around 20 uniform seeds per genotype for 24h using plastic vials (3cm diameter, 5cm height)
   - Drain the water, wash the seeds thoroughly, and cover the seeds with moist tissue paper.
   - Incubate the seeds for 3 days under room temperature, and then remove the tissue paper. Add fungicide solution into the seeds to prevent fungal growth. Place the vial with the pre-germinated seeds in the refrigerator (4-5°C) for 10 days. The reaction to cool temperature treatment varies. Discrimination between cool temperature-tolerant and susceptible cultivars is easier if the seeds subjected to cool temperature treatment have well-developed coleoptiles of about 5mm long and with traces of chlorophyll pigment. Add water occasionally to prevent drying.
   - Remove the seeds from the refrigerator and place in a warm room for a day before placing the vials under the sunlight.
   - Score the entries for survival 10 days after removal from the refrigerator using the following criteria (Table 11.6):

Table 11.6. Scoring system in evaluating early seedling stage after cool temperature treatment.

Score	Criteria
1	All seedlings alive, leaves green
2	Less than 30% of the seedlings dead
3	30 - 50% seedlings dead
7	Over 50% dead
9	100% dead

3. Seedling-transplanting stage – The retardation of seedling development is a common problem with cool water temperature. Seedling mortality is based mainly on seedling vigor (IRRI method). This method uses an automated cool temperature treatment chamber.
   - Sow the pre-germinated seeds in a tray with soil, maintaining the same density for each tray. The tray contains 3kg garden soil fertilized with 4g N, 2g P_2O_5, and 3g K_2O.
   - At 2 - 3 leaf stage (about 10 days-old), place the tray in a cool temperature treatment chamber for 10 days.
   - Score the seedlings using the criteria in Table 11.7.

Table 11.7. Scoring system in evaluating seedling-transplanting stage after cool temperature treatment.

Score	Criteria
1	No drying of leaves or only little drying on leaf tips
3	Having few top green leaves
5	No top green leaves but all plants alive
7	Only a few plants alive
9	All plants dead

At booting to heading stage, the use of cool water was found to be effective for screening because it inhibits grain ripening. However, it is best evaluated in the field of cool areas, particularly in mountainous or high-elevation areas. The best indicator of cool water injury is to measure the average sterility per plant. The effect is parallel to that of cool air. At this stage, laboratory screening is impractical and should be done in the field.

Screening for High Temperature Tolerance

Norvie L. Manigbas

Heat stress is one of the most important abiotic factors that affect rice plants. Temperature above 35°C during daytime and more than 29°C at night can induce adverse effects on rice growth and development affecting yield performance. Rice plants are susceptible to heat stress during reproductive stage. It was reported that yield can be reduced by up to 10 - 14% under heat stress environments. The wide uncertainty with regard to local and regional climate change means that it is difficult to rule out negative possibilities for any given area. Rice yields could be affected as climate change may give a significant addition to future stresses and maybe beyond the capability of the existing rice cultivars to adapt to the conditions. Therefore, breeding for high temperature tolerance is necessary to improve rice varieties that can cope with changing climatic conditions like heat stress.

Field screening

This approach of screening for high temperature tolerance is the most appropriate because it represents the actual field conditions where rice is grown. Breeding nursery can be established in locations where high temperature occurs. In the Philippines, these areas are mostly located in Nueva Ecija, Isabela, and Cagayan where temperature during dry season can reach from 37 to 40°C. Usually, these temperatures are achieved during April - May.

The flowering date or days to heading of rice genotypes is important in screening for heat tolerance under field conditions. The sowing date is critical so that booting to flowering stage will coincide with the hottest temperature of the season. Staggered planting is done to achieve desirable results. During these stages of rice growth, plants are most susceptible to heat stress. Plants are evaluated based on percent sterility or fertility after harvest. Temperature above 35°C affects anther dehiscence of the rice flower; thus, reduces the number of filled spikelets in the panicle. The heat intolerant genotypes are eliminated at this temperature while temperature 38°C and above can discriminate tolerant genotypes.

The check varieties [N22 (Acc. 03911)-tolerant, IR64-intermediate, IR52-intolerant] are designated every 20 rows and evaluated for spikelet fertility or sterility together with the breeding lines at the end of the season. Early segregating populations (F_2 - F_5) are handled in the field and selected under heat stress conditions until homozygosity is attained. Advanced lines are further screened under high temperature in the glasshouse or controlled growth chamber.

Glasshouse screening

Temperature inside the glasshouse can reach up to 42°C depending on the time of the day and season. In the absence of growth chambers, screening for heat tolerance is done in the glasshouse. During anthesis, which usually occurs from 9 a.m. to 11a.m., high temperature up to 38°C can be reached and increasingly rise up until 2 p.m. This is the time when the plants are exposed to high temperatures and could be used to screen rice genotypes. At this temperature, the tolerant genotypes can be identified. Similarly, entries in the high temperature screening are conducted side by side in the non-heat stress environment. This serves as the control. The rice genotypes are allowed to grow in normal conditions where heat stress is absent. Plants are also evaluated based on percent sterility or fertility after harvest. Check varieties are similar to those under field and growth chamber conditions.

MINCERs (Micrometeorological Instrument for Near Canopy Environment of Rice) are installed inside the glasshouse and the control to monitor the temperature and relative humidity every 2min for 24/7. At harvest, five plants are selected every row and three panicles are subjected to fertility or sterility count. Selection of desirable plants with heat tolerance is evaluated based on higher percentage fertility. Other characters are observed such as panicle exsertion, insect pest and disease reaction, presence or absence of awns, color of the grains, and plant height. Plants with undesirable traits are discarded.

Controlled growth chamber

The use of a controlled growth chamber using pot-grown plants is another approach to screen advanced rice lines or genotypes for heat tolerance. However, only limited number of populations (i.e., homozygous lines) can be accommodated in a test. Plants are grown in a normal environment and during reproductive stage, these are subjected to high temperature condition. Panicles are tagged during treatment so that at maturity, the panicles are evaluated for percent fertility or sterility.

The growth chamber is usually equipped with automatic settings for temperature, relative humidity, CO_2, and solar radiation. These parameters can be programmed so that desirable temperature and other factors can be achieved during treatment. The temperature is set to 38°C, 78 - 82% RH, 200ppm CO_2 content, and 1,200 µmol/m^{-2}/ sec solar radiation. At this temperature, rice genotypes could be segregated into heat-tolerant, intolerant, and intermediate.

The following procedure is used to screen rice genotypes for heat tolerance under growth chamber conditions:

- Grow healthy seedlings in seed box until 21 days including check varieties.
- Transfer three healthy seedlings in a pail then maintain two plants in an open condition. Fertilize as needed.
- At reproductive stage during heading, tag one panicle of each plant and place the pail inside the growth chamber.

Set the growth chamber condition using the protocol (adopted from IRRI, 2012) (Table 8):

Table 11.8. Growth chamber condition during high temperature tolerance screening.

Time	Temperature (°C)	RH (%)	Light
5:30 – 7:00 a.m.	30	70 - 80	ON (low)
7:00 - 8:00 a.m.	32	70 - 80	ON (high)
8:00 - 3:00 a.m.	38	70 - 80	ON (high)
3:00 - 5:30 a.m.	32	70 - 80	ON (high)
5:30 - 6:30 a.m.	25	70 - 80	ON (low)
6:30 - 5:30 a.m.	25	70 - 80	OFF

Take out the plant after the tagged panicles have fully emerged. Maintain the plant in open area until maturity. Protect the plant from damage.

Harvest the tagged panicle and count the filled and unfilled grains when the grains are fully matured. Compute for percent empty panicle and compare the test entries with the check varieties.

Pollen staining

Pollen staining by I_2KI (Iodine Potassium Iodide) solution is another procedure by which pollen sterility or fertility can be determined. Further observed rice breeding lines with accepted pollen fertility in the field. Follow the procedure:

1. Weigh 1g of I (Iodine), 2g of KI (Potassium Iodide), dissolve in 70% ethanol, then store for 24h. Add 100mL water and keep the solution in a brown-colored bottle in a dark place.

2. Sample a panicle, which is newly flowered or ready to be flowering in the testing day. Selected spikelets are assumed to be flowering within the testing day.
3. The fresh sample is taken into the laboratory for examination. If many samples are collected and cannot be examined in a short period, the spikelet can be stored in vials containing 70% ethanol under room temperature.
4. The sample panicle or spikelets are brought to the laboratory for slide preparation prior to microscopic examination.
5. Five to ten spikelets are opened using needle and forceps and 2 - 3 anthers are taken randomly per spikelet. The anthers are placed on a microscope glass slide.
6. Drop 3% IKI solution to the slide. Anthers are crushed using needle or forceps to force out pollen grains from the anther sacs.
7. Put the slide under the microscope. Observe three fields in the entire slide and count any stained or partially stained pollen.
8. Compute for percent dark-stained pollen.
9. Classify the samples using the scale in Table 11.9.

Table 11.9. Rating based on pollen staining of entries.

% of Darkly-stained Pollen	Category	Rating
0%	Completely sterile	CS
1-5%	Sterile	S
> 5%	Partially sterile	PS
> 80%	Complete fertile	F

Percent empty spikelets

Percent empty spikelet is determined by taking five random plants from each entry and three panicles are chosen for the number of filled and unfilled spikelets count. The check varieties are sampled and compared with the breeding lines. Percent empty spikelets or fertility is computed. Diseased plants due to stem borer damage are not included or other diseases that may have caused sterility. To economize, only breeding lines with accepted pollen fertility shall only be incorporated in the field screening.

RIL development using RGA

Rapid generation advance (RGA) is a procedure in plant breeding to shorten the growth duration of segregating populations and increase the turnover of plant generation. This method uses dark room facility to control day-length so that rice plants are exposed to short day and long dark period to enhance flowering at a shorter time. Under RGA, four generations are possible in one year while two generations per year are segregated under field conditions. This technique makes it possible to develop RILs within 1 ½ year.

F_2s of selected rice breeding populations for heat tolerance are subjected to RGA treatment. Seeds are harvested and planted in bulk following the modified single seed descent (SSD) method. Each population comprises of 2,000 plants planted at very close spacing and less fertilizer application. Only short panicles are produced and 1 - 2 seeds are harvested, which is enough for the next planting. This process is done until homozygosity of the population is attained in the F_6 generation. RILs are used for QTL mapping studies and evaluation for heat tolerance using more advanced populations.

Weather data

Important weather data such as temperature, relative humidity, wind speed, and solar radiation are collected through the nearest weather station. MINCER (Micrometeorological Instrument for Near Canopy Environment of Rice) can be installed in the field or glasshouse to monitor the temperature and relative humidity above and within canopy of the rice plants. This instrument is equipped with a data logger that can automatically give information every minute or hour depending on the setting by the user.

Screening for Drought Tolerance at Seedling to Early Vegetative Growth Stage

Nenita V. Desamero

It is a common knowledge that breeding for new improved varieties of rice or any other crop is a "number game." Wide genetic variability in the base population or gene pool is a must for effective selection of breeding lines containing genes for the traits of interest. Conventionally, breeding starts with generation of segregating populations, with thousands of progeny plants at early generation (F_2 or $F_{2:3}$) from various types of cross combinations (single, three-way, double cross) between parents with complementary traits. Breeding for quantitative trait such as tolerance to drought stress involves a number of minor genes, warranting large number of segregating plants to comprise the families at the initial generation. Selections from among and within families generate breeding lines for further evaluation, selection, and advancement to subsequent generations until homozygosity is achieved in the resulting fixed breeding lines.

Drought tolerance at seedling to early vegetative stage is important in the purely rainfed rice areas where drought episode occurs during the establishment or in the early growth stage of the rice crop. Limited water coming from the rain during crop establishment most likely results in crop failure if there is no inherent tolerance in the variety being cultivated. Effective and efficient mass screen for selection of families and breeding lines with tolerance is a must for a successful breeding program for drought stress tolerance at seedling and early vegetative growth stage, intended for areas with episodes of early drought, and no sources of supplemental irrigation, other than rainfall.

The following mass screen protocols for rice are applicable to early segregating populations, fixed lines, accessions, introductions, and other genotypes, targeting identification and selection of putative tolerant plants or breeding lines to induced or managed drought stress at seedling to early vegetative growth stage.

Seeding tray preparation

1. The facility consists of galvanized iron (GI) sheet-made trays measuring 3m length x 1m width x 0.4m depth (Figure 11.1a), and 5m length x 1m width x 0.6m depth (Figure 11.1b), anchored on the 0.35m and 0.22m high "framed stand," respectively. The GI tray contains 30cm thick garden soil:sand mix (1:1 by weight) as the growing medium at the top layer, lined with 10cm thick sand layer at the bottom of the 3m long tray, 10cm pebbles, and 10cm sand at the bottom layers of the 5m long tray.
2. Puddle the garden soil:sand mix (Figure 11.1b) to plow under any germinated rice or weed seeds. Soak in water the garden soil:sand mix for easy cultivation and removal of unwanted roots. Let the growing medium stand at soil moisture saturation for 1 - 2 weeks to allow germination and removal of unwanted remnant rice seeds and weeds. Repeat the process as needed.
3. Level and smoothen the garden soil:sand mix well for effective water and nutrient management resulting in uniform growth of healthy seedlings.
4. Make rows or "furrows," 10cm apart, parallel to the width, that is, perpendicular to the length of the tray (Figure 11.1a). There are 30 rows for the 3m long tray, and 50 rows for the 5m long tray. The three terminal rows from each end of the tray are allocated for drought tolerant check variety serving as border rows alongside the width of the tray. There are 24 and 44 effective internal rows allocated for the experimental materials or entries, in 3m and 5m long tray, respectively.
5. Label the rows with corresponding consecutive numbers using any appropriate label materials.
6. Poke or mark shallow holes along each row at 1.8cm apart, leaving the two 5cm terminal ends, to serve as borders alongside the length of the tray. The internal 90cm row length accommodates 50 holes or "hills" at 1.8cm distance apart.
7. Each seeding tray accommodating the corresponding entries serves as one replication. Screening each set of entries is done in two replications. Table 11.10 summarizes the capacity of the 3m long tray for mass screen of fixed breeding lines.

Figure 11.1a. Galvanized iron (GI) sheet-made 3-m long seeding tray without water outlet for draining. (Note: Pre-germinated rice seeds direct seeded in rows spaced 10cm apart).

Figure 11.1b. Galvanized iron (GI) sheet-made 5m long seeding tray with valve opening or water outlet for draining, containing plowed garden soil:sand mix (1:1 by weight).

Table 11.10. Capacity of the 3m-long tray facility for drought tolerance mass screen from seedling to early vegetative growth stage of fixed breeding rice lines.

Particular	Number	Remarks
Total no. of rows per 3m long tray, 10cm apart	30	
Row allocation:		
Tray end borders (3 rows each end)	6	Allocate for tolerant variety
Susceptible check variety row	1	Assign row in the middle section of the tray
Resistant check variety row	1	Assign row in the middle section of the tray
Total rows per tray allocated to fixed breeding lines in a mass screen set-up per replication (rep)	22	Allocate 1 row per fixed breeding line per rep
No. of hills or holes per row, 1.8cm apart	50	Translates to 50 seedlings per row per rep of fixed breeding line mass screen
No. of trays with 22 breeding lines per set-up for 2 replications: one tray is one rep	2	With 50 hills/rep x 2 reps, gives 100 hills (or seedlings) per breeding line, seeded in 2 trays
Total no. of fixed breeding lines for mass screen using the available 10 trays (22 fixed lines per tray constitutes one rep; 5 sets of 22 breeding lines per mass screen set-up of 2 reps)	110	Allocate 2 trays per set of 22 breeding lines to constitute 2 reps
No. of months as per duration of one mass screen set-up, from seed to seed, including clean-up and preparation of the seeding tray	3	10 weeks from seeding to termination; 2 weeks allotted for soil cultivation and seed preparation.
No. of fixed breeding lines for evaluation per season or 6 months	220	110 fixed lines screened per 3 months x 2 set-ups per season or 6 months
No. of fixed breeding lines for evaluation per year or 12 months	440	110 fixed lines screened per 3 months x 4 set-ups per year or 12 months

Seed preparation, sowing, and seedling maintenance

1. Prepare the list of experimental materials, data sheet with corresponding entry code for each genotype, and adequate amounts of seeds of each entry.
2. Break seed dormancy as needed. Keep at room temperature for a day.

   Seed dormancy in rice results in poor seedling establishment for direct-seeding and poor seedling emergence in nursery boxes for transplanting (Shiratsuchi et al., 2017). Rice seeds remain dormant during storage under low temperature (Roberts, 1961).

   Break rice seed dormancy with the use of dry heat, hot water, high humidity, steam, or chemical treatment. As reviewed by Shiratsuchi et al. (2017), the following treatments have been reported to break rice seed dormancy in different laboratories:
   - Dry heat treatment with 45°C for 7 days; 50°C for 5, 5 - 7 or 4 - 10 days; 55°C for 7 days
   - Hot water treatment at 60°C for 10min; 40°C for 24 - 72h; moisture-saturated seeds at 40°C for 1 - 2 days
   - High humidity treatment with 80% relative humidity (RH) and 45°C for 1 - 3 days; 95% RH and 40°C for 7 days; 40, 60, and 80% RH and 25°C for 1 - 5 weeks humidification; and 100% RH and 35°C for 88h
   - Steam treatment using steam nursery cabinet at 40°C for 7 days for highly dormant seeds; 40°C for 5 days for less dormant seeds. Steam treatment is not necessary for non-dormant seeds (Shiratsuchi et al., 2017)
   - Husk removal and use of hydrogen peroxide and calcium cyanamide

3. Prepare the petri plates or any appropriate alternative container to be used in germinating the seeds of experimental genotypes. Put entry label at the bottom of the petri plate. Alternatively, label can be written with pencil or any marker at the bottom side of the filter paper (or any alternative water absorbent material) lining the petri plate.
4. Germinate seeds of experimental genotypes in petri plates lined with moist filter paper.
5. Place 60 - 70 clean, healthy seeds in each petri plate. (Note: Each row in the seeding tray accommodates 50 pre-germinated seeds. With 85% germination rate, 60 seeds meet the required 50 pre-germinated seeds per row in the seeding tray).
6. Put water in the petri plate soaking the half portion (horizontally) of the seeds, and keep in the dark at room temperature for 24h. Seed germination is enhanced at 30 - 35^{0}C in a convection oven or incubator.

7. Drain the excess water from the petri plate, rinse the seeds (now at seed break), and incubate for another 24h, resulting in the protrusion of 3 - 4mm coleoptile. Incubation keeps the seeds warm, increases the growth of the embryo, and results in uniform germination.
8. Place the 50 pre-germinated seeds of each genotype in a row of the seeding tray, one seed per hole at 1.8cm apart along each row in the seeding tray.
9. The 3m and 5m long tray can accommodate 24 and 44 entries, respectively, including the susceptible check IR64 and tolerant check PSB Rc14. Note that inclusion of a dynamic check variety, that is, new and better performing variety, is considered whenever available, replacing the old check variety.
10. Maintain 1cm water depth above the garden soil:sand mix in the seeding tray. Apply fertilizer as needed within 7 - 10 days after seeding (DAS). The plants are at the two to three-leaf stage, and of 8 - 10cm height.
11. At 21 DAS, initiate drought imposition. The seedlings are at five-leaf stage, and of 15 - 18cm height. Make sure that the seedlings to be exposed to drought stress are healthy and robust.
12. It is best that non-stress entries be set-up at the same time as the entries included in the drought stress mass screen set-up. Compare the plant response under both non-stress and stress set-up to generate information on the effect of drought stress to seedling growth and development.

Drought imposition and drought development

1. Impose drought stress at 21 DAS by withdrawing the water in the seeding tray (Figure 11.2). For the 5m long seeding tray equipped with valve that regulates the opening or water outlet, partially open the valve to drain the water slowly until the garden soil:sand mix moisture saturation is reached. Water saturation is reached within 4 - 6h from opening the valve. With the 3m long seeding tray without water outlet, withhold irrigation water at 16 DAS during dry season (or 5 days before 21 DAS) and 13 DAS in the wet season (or 8 days before 21 DAS) to attain the garden soil:sand mix saturation at 21 DAS. Take note of the date when the water is withdrawn and when saturation is reached, that is, no more standing water, and the garden soil:sand mix is still soaking wet. For the 3m long seeding tray without water outlet and contained in the screenhouse with glass roofing, it takes approximately 5 days in the dry season and 8 days in the wet season to reach the garden soil:sand mix moisture saturation.
2. During mass screening, it is important to note and record the prevailing environmental conditions including temperature, relative humidity, and solar radiation at the outside and inside of the screenhouse or glasshouse where the drought screen facilities are contained, as these factors are determinants of the rate of induced drought development. The data for the external environmental conditions can be

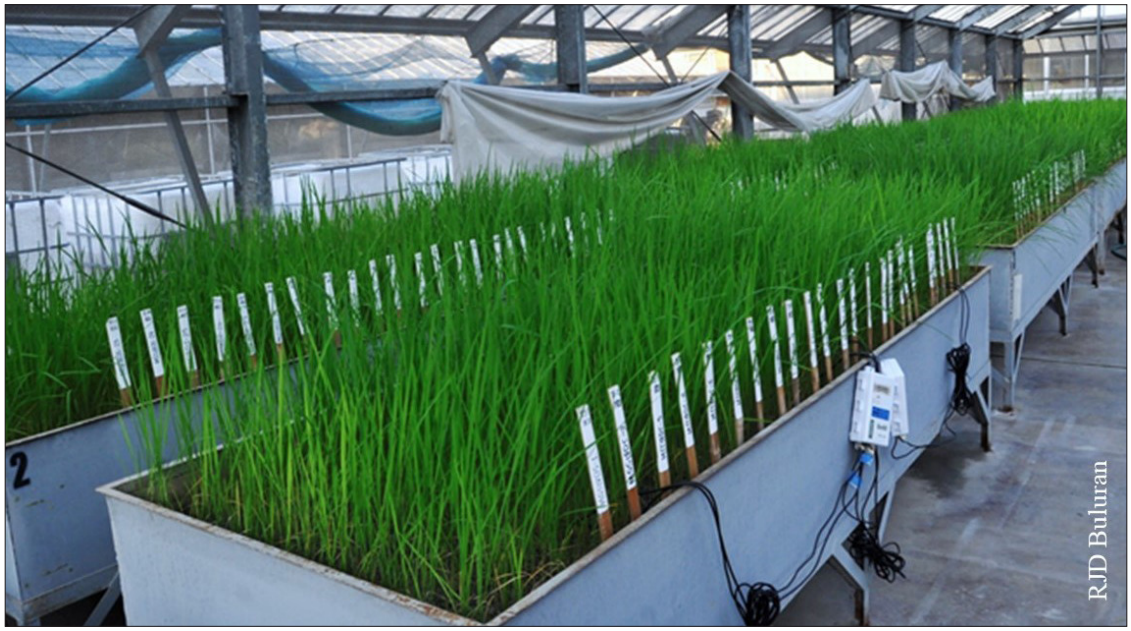

Figure 11.2. Drought imposition. Irrigation water is withdrawn at 21 DAS of fixed breeding lines. (Note: Soil moisture meter and data logger installed. Garden soil:sand mix moisture content at initial water withdrawal in the set-up is 23%).

sourced out from the institute's weather station. The internal environmental conditions are measured and recorded from the installed portable instruments used to measure the temperature, relative humidity, and solar radiation inside the screenhouse or glasshouse. If a phytotron is available for mass screening, the measurements of the internal environmental conditions are automated.

3. Monitor the garden soil:sand mix moisture dynamics commencing at moisture saturation, progressing towards drought development, until termination of induced drought stress. This is done either by installing soil moisture meters and data logger in the seeding tray for automatic monitoring and recording or by gravimetric method for manual garden soil:sand mix moisture determination.
   With gravimetric method, take samples of the garden soil:sand mix at regular intervals using auger, initially at saturation and progressively until drought stress termination. Determine the fresh weight of the samples, then dry the samples in the convection oven at 70^0C, re-weigh, and compute the moisture content. Repeat the drying and reweighing step until constant value is obtained. In the dry season, initially air dry the garden soil:sand mix samples before oven-drying. The samples are ready for oven-drying when they turn from brown to light brown in color, have hard texture and cracks, and crumble when pressure is applied.
   The soil moisture content (% SMC) on dry weight basis is calculated as follows: % SMC = [(soil fresh weight – soil dry weight) / soil dry weight] x 100. The procedure is repeated with subsequent mass screen set-ups until consistent results are obtained, and the soil moisture dynamics in the facility is established.
4. Record the garden soil:sand mix sampling dates and the corresponding moisture content in relation to the garden soil:sand mix moisture dynamics, drought stress development, and corresponding seedling growth response to the stress. The duration of drought development and corresponding seedling growth response to the stress is influenced by the ambient environmental conditions inside and outside of the containment facility and by the seedling growth rate and development.

Plant response to induced drought stress and upon release from stress

1. Prepare the appropriate data sheet for recording the observations on the plant response to induced drought stress. Record the data and information relating to leaf rolling and leaf drying (Table 11.11) during stress development, seedling growth in terms of green shoot occurrence, seedling vigor (Table 11.12), and seedling recovery or survival (Table 11.13).
2. The onset of drought stress in the seeding tray varies with the ambient environmental condition and seedling growth. Determine the occurrence and duration of the induced drought stress based on the response of the susceptible or intolerant check variety.
3. Observe the initial drought stress development by taking note and recording of the date when the leaves of the susceptible check IR64 start to roll.
4. Observe the progression of drought stress by taking note and recording of the date when the leaves of the tolerant check PSB Rc14 start to roll.
5. Observe further development of drought stress by taking note of the date of occurrence of leaf drying of the susceptible and tolerant checks, with a score of 1 or 3.
6. Determine the leaf rolling (LeR) and leaf drying (LeD) scores (Table 11.11, Figure 11.3) of all entries when the susceptible check IR64 reached the score of 7 or 9. The scoring of the LeR and LeD response to drought is based on IRRI's Standard Evaluation System (IRRI, 2014) for rice.
7. Release experimental materials from drought stress: Terminate the drought stress when the susceptible check IR64 reached the LeD score of 7 or 9. At this point, rewater the tray slowly (flush irrigation) until saturation (Figure 11.4). It takes 5 days for the set-up to reach saturation. Abrupt waterlogging may induce decay or disintegration of droughted plant tissues.
8. For a more stringent selection, extend drought stress duration by 1 week or so before rewatering the seeding tray, but not to the extent that all plants will no longer regrow upon release from the stress. (Note: As observed, it takes 25 - 39 days from drought imposition or start of irrigation water withdrawal until leaf drying of the susceptible check variety with LeD score of 7 or 9).
9. Observe, take note, and record the date of occurrence of the first green shoots for each entry. This observation serves as one of the indicators of the relative vigor and initial recovery from stress of the droughted experimental materials. Once the green shoots with newly developed leaves appeared, intermittently add water in the seeding tray, and maintain it at 1cm depth. Closely observe the growth of the surviving plants and record any unexpected or unusual observations.
10. Score all entries for green plant vigor (Table 11.12) and plant recovery or survival (Table 11.13) within 5 - 21 days from rewatering or release of the set-up from drought stress.

Figure 11.3. Rice plant response in terms of leaf rolling (LeR) and leaf drying (LeD) to the induced (managed) drought stress at seedling to early vegetative growth stage.

(0)	(1)	(3)	(5)	(7)	(9)	(0)	(1)	(3)	(5)	(7)	(9)
Leaves healthy	Leaves start to fold (shallow V-shape)	Leaves folding (deep V-shape)	Leaves fully cupped (U-shape)	Leaf margins touching (O-shape)	Leaves tightly rolled	No symptom	Slight tip drying	Tip drying extended up to ¼ length in most leaves	One-fourth to ½ of all leaves fully dried	More than 2/3 of all leaves fully dried	All plants apparently dead
Leaf Rolling (LeR) Score (1, 3, 5, 7, 9)						Leaf Drying (LeD) Score (1, 3, 5, 7, 9)					

Table 11.11. Leaf rolling (LeR) and leaf drying (LeD) score: Response to the induced (managed) drought stress *(Standard Evaluation System for Rice, IRRI, 2014)*.

Scale	Description	
	Leaf Rolling	**Leaf Drying**
0	Leaves healthy	No symptoms
1	Leaves start to fold (shallow V-shape)	Slight tip drying
3	Leaves folding (deep V-shape)	Tip drying extended up to ¼ length in most leaves
5	Leaves fully cupped (U-shape)	One-fourth to ½ of all leaves fully dried
7	Leaf margins touching (O-shape)	More than 2/3 of all leaves fully dried
9	Leaves tightly rolled	All plants apparently dead

Table 11.12. Seedling vigor score system *(Standard Evaluation System for Rice, IRRI, 2014)*.

Scale	Description
1	Extra vigorous (very fast growing; plants at 5-leaf stage have 2 or more tillers in majority of population)
3	Vigorous (fast growing; plants at 4 - 5 leaf stage have 1 - 2 tillers in majority of population)
5	Intermediate or normal (plants at 4-leaf stage)
7	Plant less vigorous than normal (plants somewhat stunted with 3 - 4 leaves; thin population and no tiller formation)
9	Plants very weak and small (stunted growth and yellowing of leaves)

Figure 11.4. Rewatering the mass screen set-up for fixed breeding lines when leaf drying (LeD) of susceptible check variety IR64 reached the score of 7 or 9. (Note: It takes 25 - 39 days from drought imposition or start of irrigation water withdrawal until leaf drying of the susceptible check variety to reach the LeD score of 7 or 9. Garden soil:sand mix moisture content in the set-up is 0.7%).

Table 11.13. Plant survival (recovery) as a function of tolerance to drought stress induced at seedling to early vegetative growth stage in rice *(Standard Evaluation System for Rice, IRRI, 2014)*.

Plant survival (%)	Drought Stress Tolerance Level
90 – 100	Highly tolerant (HT)
70 – 89	Tolerant (T)
40 – 69	Moderately tolerant (MT)
20 – 39	Susceptible (S)
0 – 19	Highly susceptible (HS)

11. As an option, biomass (shoot and root dry weight) may be determined for each entry at the termination of the mass screen. This is applicable only to the materials from which seed harvest from the recovered plants is not needed.
12. Consider all data and information gathered in the mass screen in the final selection of putative drought tolerant breeding lines. Base the final selections on the consolidated data for leaf rolling, leaf drying, plant vigor, and plant survival from induced drought stress.

Growing surviving plants to maturity for seed recovery

Should the seeds need to be recovered for seed increase or generation advance, rescue the surviving stressed plants and grow to maturity for further observation and seed harvest.

Mass screen of fixed lines

Mass screening of fixed lines identifies and selects lines putatively tolerant to drought stress at seedling to vegetative growth stage. Rescuing and growing the surviving plants to maturity provides the opportunity to assess the effect of drought stress at seedling to early vegetative growth stage of the breeding lines at the reproductive stage. The seeds to be harvested from the surviving stressed plants may serve as the initial seed source of the selected putative tolerant breeding lines.

It is best to consider a non-stress set-up to comparison the drought stress mass screen set-up. Evaluating the pre-selected fixed lines under managed drought field condition will confirm tolerance and yielding ability. Mass screen in the screen/glasshouse effectively reduces the number of breeding lines for field evaluation, resulting in savings in resources.

To know the effect of the induced stress at seedling to vegetative growth stage to the performance of the surviving plants at reproductive stage, observe further the fixed lines identified and selected as putative drought tolerant from the mass screen. Grow the surviving putative tolerant plants to maturity in the screenhouse,

glasshouse, seed boxes, or in pots, where the plants can be monitored and taken care of for further observation and seed harvest. Characterize the selected surviving materials agronomically (tillering, flowering, maturity, height at maturity, other agronomic traits. Gather data on yield and yield components such as panicle, spikelet, and seed. Compare the data and information gathered from stress and non-stress set-up. Use the information generated to substantiate further the selection of putative tolerant breeding lines. Where applicable, analyze the data statistically to support a sound breeding line selection.

Grow the seeds recovered from the surviving putative tolerant plants for seed increase before use in subsequent confirmatory evaluation and selection.

Mass screen of segregating population at early generation

Early selection for abiotic stress tolerance can be employed with segregating progenies of crosses at F_2 or $F_{2:3}$ generation and with mutant populations at M_2 generation.

The mass screen of segregating population at F_2 or $F_{2:3}$ generation (Figures 11.5, 11.6, 11.7, 11.8) is best conducted in the 5m long seeding tray equipped with water outlet for better control of drought imposition and water management. The minimum target for mass screen per segregating population is 1,500 F_2 or $F_{2:3}$ derived plants. As a quick guide, refer to the summary describing the capacity of the 5m long tray for mass screen of early segregating populations, F_2 or $F_{2:3}$ generation (Table 11.14).

For each tray with 50 rows spaced at 10cm apart, allocate one terminal row at each end of the tray to a tolerant variety to serve as border rows, and three rows each for the susceptible and resistant checks, dispersed at left, middle, and right sections of the tray. The remaining 42 rows are allocated to the segregating population. Each row can accommodate 20 holes or hills 5cm apart. The two 5cm terminal ends of the rows serve as borders, leaving 18 hills per row for seeding. A total of 756 hills per tray are available for the segregating population. It requires two 5m long trays to accommodate one mass screen set-up for one population consisting of 1,500 segregating plants at F_2 or $F_{2:3}$ generation. It takes 4 months to complete the mass screen, from seed to seed, with tray clean up and seeding tray preparation included. With 10 available 5m long seeding tray, 15 segregating populations can be screened in 12 months.

Direct seed the pre-germinated seeds from segregating population along the rows 5cm apart. Subject the population to drought stress following the protocols described earlier. With mass screen of segregating population at F_2 or $F_{2:3}$ generation, identify the putative tolerant plants (Figure 11.8) and grow them in the mass

Table 11.14. Capacity of the 5-m long tray facility for drought tolerance mass screen at seedling to early vegetative growth stage of early segregating population of rice at F_2 or F_3 generation.

Particular	Number	Remarks
1. Total no. of rows per 3m long tray, 10cm apart	30	
Row allocation:		
2. Susceptible check variety row	1	Assign row in the middle section of the tray
3. Resistant check variety row	1	Assign row in the middle section of the tray
4. No. of trays with 22 breeding lines per set-up for 2 replications: one tray is one rep	2	With 50 hills/rep x 2 reps, gives 100 hills (or seedlings) per breeding line, seeded in 2 trays
5. No. of months as per duration of one mass screen set-up, from seed to seed, including clean-up and preparation of the seeding tray	3	10 weeks from seeding to termination; 2 weeks allotted for soil cultivation and seed preparation.
6. Tray end borders (3 rows each end)	6	Allocate for tolerant variety
7. Total rows per tray allocated to fixed breeding lines in a mass screen set-up per replication	22	Allocate 1 row per fixed breeding line per replication
8. No. of hills or holes per row, 1.8cm apart	50	Translates to 50 seedlings per row per rep of fixed breeding line mass screen
9. Total no. of fixed breeding lines for mass screen using the available 10 trays (22 fixed lines per tray constitutes one rep; 5 sets of 22 breeding lines per mass screen set-up of 2 reps)	110	Allocate 2 trays per set of 22 breeding lines to constitute 2 reps
10. No. of fixed breeding lines for evaluation per season or 6 months	220	110 fixed lines screened per 3 months x 2 set-ups per season of 6 months
11. No. of fixed breeding lines for evaluation per year of 12 months	440	110 fixed lines screened per 3 months x 4 set-ups per year of 12 months

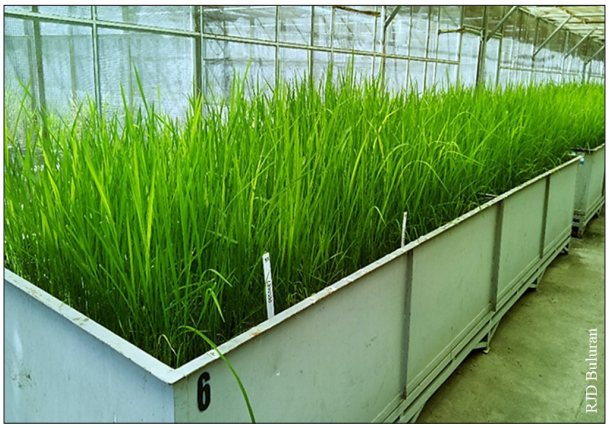

Figure 11.5. Drought imposition. Irrigation water is withdrawn at 21 DAS of the $F_{2:3}$ segregating population with initial garden soil:sand mix moisture content in the set-up of 22.3%.

Figure 11.6. Drought stress development: leaf rolling and leaf drying in the segregating plants from the $F_{2:3}$ population 28 days from irrigation water withdrawal with garden soil:sand mix moisture content of 2.4%.

Figure 11.7. Drought stress progression at advance stage: leaf drying in the plants of the $F_{2:3}$ segregating population 39 days from irrigation water withdrawal with garden soil:sand mix moisture content of 0.9%.

Figure 11.8. Release from drought stress: seedling regrowth of putative drought stress tolerant plants from $F_{2:3}$ segregating population at 10 days after rewatering. (Note: Irrigation water was withheld for 39 days before rewatering).

screen tray until maturity for seed harvest. Maintain the garden soil:sand mix moisture at saturation or with minimal irrigation or no standing water condition, depending on the growth condition of the surviving plants. Take note and record the flowering and maturity of the individual plant selections from which the seeds are to be harvested. One panicle harvest from a surviving plant suffices the need for generation advance, although a back-up panicle is desirable to serve as remnant seeds. The seeds recovered from the surviving putative tolerant plants are used for subsequent generation advance, breeding line development, and further evaluation and selection. Follow an organized record keeping system for traceability of the breeding lines developed from the drought stressed breeding populations.

Take note that each selected individual F_2 or $F_{2:3}$ plant is considered a family where the breeding lines within the family are generated and developed from the harvested F_3 or F_4 seeds. Grow the harvested seeds the following season for generation advance and further selection for other traits of interest.

Where applicable, with segregating population, conduct fingerprinting or marker-aided selection from the plants that survived the induced drought stress in the mass screen facility for genotype confirmation. This entails populations from crosses between parents having the genes of interest to be studied for associated and available molecular markers.

Mass screen for drought stress tolerance at seedling and early vegetative growth stage is also applied to early generation (M_2) mutant populations for early plant selection, followed by subsequent mutant line development, evaluation, and selection for other traits of interest. The use of molecular markers, when available, enhances the selection efficiency for the traits of interest.

Screening for Drought Tolerance at Reproductive Stage

Tahere Sigari, Nenita V. Desamero, Roel R. Suralta, Jonathan M. Niones, Dindo Agustin A. Tabanao, and Arnel E. Pocsedio

A large portion of the world's poor live and farm in rainfed system where water supply is unpredictable and droughts are prevalent. Permanent or temporary drought limits the growth, yield, and production of all crops including rice. Drought, as a meteorological term, is defined as the absence of rainfall for a period of time long enough to result in depletion of soil water and injury to plants. In reference with crop plants, we mostly deal with water deficit, which is the situation where plant water potential and turgor are reduced enough to interfere with plant normal functioning.

Screening for drought tolerance during the reproductive stage under lowland conditions

In breeding for drought tolerance under lowland conditions, screening is generally conducted in fixed lines (F_6 or higher generations). Promising entries from the observation nurseries and preliminary yield trials are entered in the drought screening. Conventional selection is conducted in early generation lines. Selection is based on other important traits such as insect pest and disease resistance and grain quality.

- The screening trial should be conducted in well-leveled field with good drainage. Drought tolerant and drought susceptible checks are included as test entries. Entries can be divided into 3 - 5 maturity groups with a maximum of 7-day difference and planted in staggered so that all genotypes flower at the same time. This is not practical when lines of early breeding generation are screened.
- Alpha lattice design in three replications is used to take care of soil variation and minimize neighboring effects. Each plot is composed of four rows of 2 - 2.5m length. Use the augmented design for large number of entries.
- Prior to land preparation, soil samples are collected from the field to determine the soil properties (soil pH, texture, organic matter content, NPK, and cation exchange capacity). Minus-one Element Technique can also be used for bio-assessment of soil deficiencies.
- Piezometers are installed after land preparation, layouting or just after seeding/transplanting (Figure 11.9). At least three piezometers are installed along the field slope.
- Field should be irrigated at uniform depth and water level is maintained at around 5 - 7cm. Dikes can be constructed to maintain uniform standing water within the plots. Fertilization (basal and top dress) is based on the result of soil analysis.
- Crop establishment is scheduled such that reproductive stage of crops coincides with low rainfall (delayed planting in the wet season or early planting in dry season). Missing hills are replanted within a maximum of 10 days after transplanting.
- Soil drying is scored in each plot. This score can be used to adjust for field differences. Entry of water from adjacent wet area is prevented. Testing site is isolated by putting up plastic barrier at the border sides. Removal of water should be done at the desired time only (Figure 11.10).
- Drought is imposed during panicle initiation (based on the maturity of entries) to effectively inflict water stress at booting stage. Water is withheld for a maximum of 25 days. Irrigate when soil moisture content at surface (0 - 15cm) reaches 20% (gravimetric method) or soil water potential is -20 KPa. This cycle of drought and rewatering can be continued until two weeks before harvest.
- The data to be collected include leaf rolling and leaf drying, days to 50% heading, delay in flowering (among entries of the same maturity group), plant height before and after stress, tiller number, yield and harvest index, panicle fertility score and panicle exsertion, spikelet fertility, and culm length. When large number of entries is evaluated, the basis for comparisons will be panicle fertility and panicle exsertion. These two parameters can be either scored (SES) or can be quantified by exact measuring.
- Soil status is monitored by sampling or tensiometer readings. The amount of rainfall is measured daily using a rain gauge while daily standing water is measured using a slopping gauge

Screening for drought tolerance during the reproductive stage under upland conditions

F_1 and F_2 populations generated for upland rice breeding are grown in lowland conditions. In the F_2, plant selections are carried out based on visual morphology. Plants with dense and long panicles, erect flag leaf, and are relatively tall and with high biomass are favored. F_3, F_4, and F_5 lines, on the other hand, are planted in the upland ecosystem. Plants that can adapt well under these conditions are selected. Late generation populations (F_6 onwards) are screened for drought tolerance. For the early generation population, the objective is to select for lines with high yield potential. Screening of the early generation lines is not discouraged. Screening

Figure 11.9. Piezometer installed after land preparation along the field slope.

can be conducted either during the wet or dry season. When irrigation facilities (preferably sprinkler irrigation system) are in place, dry season screening is preferred for a more reliable drought imposition.

- The upland screening trial should have a granular soil, drained freely, and is not influenced by subsurface ground water. There should be no irrigated lowland trials near it, but if this cannot be avoided, the screening trial should be situated at a higher elevation.
- At least two drought tolerant and two drought susceptible checks should be included as test entries. Entries should be of similar maturity or planted in staggered. Prior information about the maturity of the entries is important.
- There should be two set of trials, one is the irrigated trial (non-stress) and the other is the managed drought trial (stress).
- Each trial should be laid out in an alpha lattice design with at least three replications to better manage soil variation. Use RCBD for few test entries.
- The standard plot size is 4 rows of 4m length. Breeders can opt to use a plot of 2 rows with 2m length for more than 30 entries.
- Entries are dry-direct seeded in the soil and irrigated after. The trial is irrigated up to field capacity and this is done until canopy closure or more than a month after seeding.
- Basal fertilization can be done 0 - 2 days after emergence. Top dressing is done before drought imposition. Fertilization should be similar in both trials in terms of quantity, quality, and timing.
- Irrigation is withdrawn in the managed drought set-up until the desired intensity of drought is attained. This can be based on the wilting of the susceptible check or tensiometer reading (about -50KPa at 30cm depth).
- Drought tolerance related parameters such as leaf drying and leaf rolling are measured.
- Re-irrigate to prevent overstressing the plants after the drought period.
- The drought imposition or re-irrigation cycle should be done repeatedly until harvest. Data gathering is conducted every cycle.
- Yield and harvest index should be determined. Record data on days to flowering and plant height.
- Yield reduction between the non-stress and stress trials should be about 80%.
- Record climate data such as daily rainfall, daily maximum and minimum temperature, and relative humidity. Other observations such as pest and disease damage should also be recorded.
- For wet season screening, crop establishment is delayed to coincide infrequent rainfall with the entries' reproductive stage. A single trial (stressed) is already enough.

Figure 11.10. Field under drought at a certain period for scoring at a given time.

Root-based drought tolerance screening

Roel R. Suralta

The high variability of drought stress and insufficient understanding of its complexity hinder the identification of specific physiological traits required for improved crop performance under drought, consequently limiting plant breeding efforts to enhance crop drought tolerance. Therefore, it is important for breeders to utilize an efficient screening system. The system should consider uniform application of stress and it should also be repeatable. The following systems are useful in screening for drought tolerance:

I. Root Box Pin Board Method

This method enables the breeder to collect the whole root system with minimum impairment or disturbance to its structure, evaluate response of root system development and plant water use precisely, and regulate the target soil moisture conditions. With this, the root system development of potential parents to be used for crosses under drought will be fully characterized.

1. Preparation of root box. The root box is made of transparent PVC of 5mm thickness. The dimensions are 25cm × 2cm × 40cm (L × W × H). They are put together with a bonding agent. Only a wall of one side of the box (26cm × 40cm) is removable. Carefully spread the silicon sealant on the contact point between the sliding wall and the main box. Mount the wall to the main box, tape tightly, and bind with six clips per box. After the sealant is thoroughly dried, fill the root box with 2.5kg of air-dried sandy loam soil, which are sieved through 3mm mesh and mixed with desired amount of fertilizer. The bulk density of the soil should be uniform at 1.25g/cm3. The soil in the box is first submerged overnight in water at 5 - 10cm above the root box, which is enough to fully saturate the soil. Remove the root boxes from water then drain for another 24h to stabilize soil moisture.

2. Cultural management of plant in the root box. Sow three pre-germinated seeds in a root box, which is placed in the vinyl house to protect from rainfall. Thin the seedlings to one per box after plant establishment. Water the top of the root box whenever necessary. For drought treatment, weigh each root box daily and replenish the amount of water loss, which is recorded as evapotranspiration. Some root boxes without plants will also be prepared to measure the amount of evaporation from the soil surface so that the amount of water lost through transpiration (water use) of each plant will be estimated. It takes about one month for root system of rice to fill the root box of this size.

3. Preparation of root sampling. Drill stainless nails (2.7cm length) vertically on a plywood board (45cm × 35cm × 7mm, L × W × T) at equal intervals of 1cm to make pin board. Screw the other board as backing. Hole a transparent polyethylene bag (45cm × 30cm × 0.08mm, L × W × T) using a puncher of 5mm diameter at equal intervals. Cut both sides of the bag to make the folio of holed sheet.

4. Root sampling. Place the root box on a desk or any flat surface so that removal of the wall is in upside position. Take off the clips, gummed tapes, and silicon sealants, and then the removable wall by sliding towards the top of the box. The pin board with one side of the plastic sheet mounted on the nails is pressed against the soil in the box. After pressing, turned the box upside down and then downward. This leaves the soil profile attached to the pin board. Gently spray tap water to wash away the soil on the pin board. The root system is sandwiched between the sheets and then the sheet will be removed from the pin board together with whole root system.

5. Root digitizing. Two options for root digitizing are available depending on the availability and convenience of the researcher:
   - Taking photograph. Each root sample sandwiched by the sheet is stained in 0.25% Coomassie Brilliant Blue R aqueous solution for 48h. This staining procedure is indispensable in taking high-resolution digital photographs of the entire root system including fine lateral roots. Rinse the stained root samples with tap water to remove excess stains. One side of the plastic sheet will be opened up to expose the root system and put on a lighted box for digital photographing using a digital camera at adequate resolution (300dpi). Download the digital images in jpeg format in a computer and convert into tiff format for total root length measurements.
   - Computer scanning. The procedure for preparing root samples for digitizing is similar with taking photographs except that a scanner attached to a computer is used. The root system embedded in a sheet is put on top of the scanner and the whole root system is scanned at a resolution of at least 300dpi and an output TIFF format of 256 grey scales.

6. Traits that are measured in this system include root traits (root length density, total lateral root length, linear frequency, root dry weight, and specific root length); shoot traits (shoot dry weight, plant height, number of tillers, leaf number, and leaf area); and water use.

7. Available computer software program such as a macro program on NIH developed by Kimura et al. (1999) and Kimura and Yamasaki (2001), commercial software WinRhizo and freeware ImageJ (Tajima and Kato, 2011) can be used in root trait analysis.

8. Precautions regarding uniform soil filling and compaction of the soil should be observed in all the root boxes. There should be no water leaks from the root box to measure water use more precisely. Silicon sealant must be carefully put between the removable side wall and the box to fill gaps where water may pass through. It should also be noted that measurement of scanned root system as sampled tends to underestimate the root length because of overlapping of roots, especially fine ones like lateral roots.

II. Line Source Sprinkler System

This screening system can be used for rapid evaluation of germplasm and genotypes in response to various intensities of drought stress. This will identify the extent of drought tolerance of potential parents for development of cultivar suited to drought stressed conditions.

1. A watertight soil bed with line source sprinkler system under a rain-out shelter is to be constructed.

2. The size of the soil bed will be 25m × 3.6m. The length however can be shortened or increased depending on the number of genotypes that will be evaluated.

3. A PVC pipe with nozzles will be installed at the center of the field. Water mists coming out from the nozzles will be used for wetting the soil; therefore, it is possible to create gradient in soil moisture, in other words, different intensities of drought stress.

4. The soil moisture contents (SMC) at 5 points on each side of the PVC pipe (total of 10 points) will be monitored using soil moisture sensors (EC-10 Decagon, Utah, USA), so as to adjust the amount of irrigation to maintain the moisture gradient.

5. Seeds of each genotype will be grown in the nursery boxes under well-watered conditions for 21 days before transplanting. Twenty-one-days-old seedlings of each line will be transplanted perpendicular to the PVC pipe (line source) so that they will be exposed to and grown under various intensities of drought.

6. Each genotype will be replicated thrice (three rows) with 1.8m in length and adopting a spacing of 45cm × 20cm, which are randomized. Each row will contain eight plants of a certain genotype that will receive different amounts of water from the PVC pipe. Hence, the closest genotype receives the most while the farthest gets the least amount of water. Keep the fields well-watered through a sprinkler irrigation for one week after transplanting and then impose drought stress.

7. Yield and harvest index should be determined and analyzed according to the intensity of drought stress.

III. Raised Bed System

This screening system is used for screening genotypes with deep rooting ability, which are suited under upland rice conditions.

1. In the raised bed system, plants are grown under rainfed conditions throughout the growth period, relying exclusively on natural rainfall. The beds are prepared over a 5cm-thick layer of gravel laid on the soil surface to prevent capillary rise of water into the raised bed. The roots of the rice plants can penetrate the gravel and reach deeper levels in the soil. Thus, plants with good performance under this system are assumed to have deep rooting ability.

2. Three or four seeds will be sown in each hill (20cm × 15cm) and plants will be thinned to one per hill after seedling establishment.

3. Plants will be grown to maturity.

4. Yield and harvest index should be determined.

Screening for Submergence Tolerance

Nenita V. Desamero

Low lying rice areas are prone to flooding, which may result in crop damage, the degree of which, depends on the time and duration of the flooding episode, flood water quality, water current, and depth, among other factors. Flooding of the rice paddy may result from too much rainfall, spill over from paddies in the higher toposequence, and poor drainage. Flooding can be transient, lasting for a short period of time, as in 1 - 2 weeks, with at most 100cm water depth, referred to as submergence; stagnant flooding, lasting for longer period, as in months, with at most 50cm water depth; and deep-water flooding, with more than 100cm water depth, and extended over the cropping season. Tolerance of rice to the various flooding events is regulated by different sets of genes.

Intolerant rice varieties completely submerged in murky flood water for 3 - 4 days deteriorate, decay, and eventually die. Tolerant varieties can survive up to 14 days of complete submergence. The first NSIC registered varieties with tolerance to complete submergence are PSB Rc 68 (Sacobia), released for commercial cultivation in dry-seeded, rainfed lowland in 1997; NSIC Rc 194 (Submarino 1) and NSIC 2020 Rc 590 (Submarino 2), released for cultivation in flash flood-prone rice areas in 2004 and 2020, respectively. These varieties possess the *sub1* gene from FR13A, a land race from Orissa, India, conferring tolerance to continuous complete submergence for up to 14 days at seedling and vegetative stage, the duration depending on the flood water quality, current, and depth, and environmental conditions, among other factors. The level of expression of the *sub1* gene is also influenced by the genetic background or the genotype into which the gene is introgressed.

The mass screen for submergence tolerance is conducted at seedling and vegetative stage under managed-submergence stress condition.

I. Screening at the concrete tank for seedling submergence tolerance

The concrete tank for submergence tolerance screen at seedling stage being used at PhilRice CES was used to be the facility for evaluating cool temperature tolerance. Although already defunct and non-operational for the intended original purpose, it serves the objectives for complete submergence mass screen at seedling stage of rice (Figure 11.11). The details of the capacity of the cemented tank for mass screen are provided below.

Screening capacity of the existing cemented tank at PhilRice CES.

Particular	Description
Size of cemented tank	3.97m (L) x 2.97m (W) x 1.18m (H)
Tank capacity: number of trays accommodated	40 – 35
Tray capacity: number of entries per tray	14 4+2
Total number of entries per screen in the tank	560 (14 entries/tray x 40 trays) with 3 replications = 187 entries with 2 replications = 280 entries

Materials:

- seed trays with 14 x 32 holes or plastic or polypropylene seed boxes measuring 15 x 21 x **y** inches (L x W x H) or 38 x 53 x **y** cm (**y** size may vary depending on what is available locally)
- clay or plastic pots
- labeling materials and marking pens
- concrete tank with height that can maintain ≥1 m deep flooding water, preferably screened
- growing medium – 1:1:1 garden soil:carbonized rice hull:coir dust mix (or any suitable mix of growing medium) pure paddy soil – change every set up
- seeds of test genotypes, IR42 (susceptible check), FR13A (tolerant check)

Establishing the entries for screening

- Assemble properly labeled seeds of entries and break dormancy by incubating seeds at 50°C for 4 - 5 days.
- Prepare layout of entries and entry label duplicates.
- Pre-germinate seeds by incubating in petri plates lined with moist filter paper for 36-48h, at 30°C in the dark.
- Fill seed trays or seed boxes with soil mix growing medium, label properly according to the entry layout.
- Sow at least 30 well-germinated seeds per entry per replication, singly in each hole of the tray or along the row in the seed box, not more than 1cm below the soil surface with 1cm spacing between seeds. Cover the seeds with moist coir dust or sieved garden soil.
- Fill in three 5 pots with soil mix and sow 5 well-germinated seeds per pot of the susceptible check IR42. This is used as indicator in determining the time when to drain the tank.
- As the seed tray contains shallow soil depth, place the seeded trays on the concrete tank, lined with 10 - 15cm paddy soil layer. On the other hand, seeded

boxes are maintained in well-lighted area for better seedling growth, and then transferred in the tank 18 - 21 days from seeding, ready to be submerged. Do not fertilize the seedlings.

- The 18 - 21 days old seedlings are ready for submergence stress exposure. Record the total number of seedlings per entry before submerging. Assemble properly labeled seeds of entries and break dormancy by incubating seeds at 50°C for 4 - 5 days.

Submergence stress imposition and response evaluation

- Optional: To measure plant elongation, take the seedling height of 5 random plants per entry from the soil base to the tip of the longest leaf, before and after submergence.
- Start the submergence treatment by filling in the tank with water from 11 a.m. to 2 p.m. to allow the plants to photosynthesize first before imposing stress.
- Check the water depth daily, add water as necessary to maintain complete submergence of the plants.
- Monitor screenhouse and floodwater temperature and other floodwater condition such as, pH, EC, dissolved O_2, and light penetration, as necessary.
- Observe the susceptible check IR42 by pulling out a pot planted to IR42 starting at day 6 of submergence and check whether the seedlings become soft at the growing point or shoot-root junction until such time that deterioration, as manifested by foul smell, is apparent. Sample susceptible check every other day until 14 days from complete submergence.
- Drain the tank by opening the outlets at the time when 70 - 80% of the IR42 seedlings exhibits stress injury. Start de-submerging preferably after noontime until late afternoon, at around 5 p.m., when light intensity is low, which minimizes post-submergence injury. To avoid lodging, slowly drain the tank by closing one of two drainage outlets, when the water level reaches the seedling height.
- Count and record the number of surviving plants per entry 10 days after de-submergence. Surviving plants will regenerate at least one new green leaf. Compute % survival (S) of each test entry and the resistant check entry such as the FR13A, PSB Rc68, or NSIC Rc 194 as follows:

  % Survival (S) = (*number of surviving seedlings / number of seedlings before submergence) x 100*

- Compute for % comparative survival value as follows:

  % Comparative survival value = *(% S of entry / % S of resistant check) x 100*

- Translate the % comparative survival value into a scale of 1 - 9 as presented in Table 11.15.

Table 11.15. Scoring system in evaluating percent comparative survival after submergence.

Score	% Comparative Survival Value	Classification
1	100	Highly tolerant
3	95 - 99	Tolerant
5	75 - 94	Moderately tolerant
7	50 - 74	Moderately Intolerant
9	0 - 49	Intolerant

Reference: Standard Evaluation System (SES), IRRI, 2014

II. Screening at the field tank for vegetative submergence tolerance

The on-station induced or managed-submergence stress screen allows expression of tolerance at tillering stage and determines comparative effect on the agronomic response and grain yield under stress.

Materials

- Seeds of test entries, susceptible check (IR42), tolerant checks (FR13A, PSB Rc68, NSIC Rc 194)
- Labels
- Wooden stakes
- Marking pens
- Panting wire
- Molluscicide
- Fertilizer

Experimental design

The entries, which include the *sub1* gene source FR13A, two tolerant check varieties — PSB Rc68 and NSIC Rc 194, and one intolerant check variety, IR42, are arranged in RCBD with three replications. The replications are laid out across or perpendicular to the variability gradient, if it exists. The block is subdivided into plots corresponding to the number of entries. Each entry is randomly assigned in each plot within a block. Treatment is randomly assigned for each block or replication.

The seedlings of each entry are transplanted in 1m (5 rows) x 3m (15 hills) plot, with a plot area of $3m^2$/entry, and plant spacing of 20 x 20cm between rows and hills.

Field Layout

- Levees between treatments and replications are not necessary. However, the whole experimental area should be enclosed by levees to prevent contamination from the adjacent area.

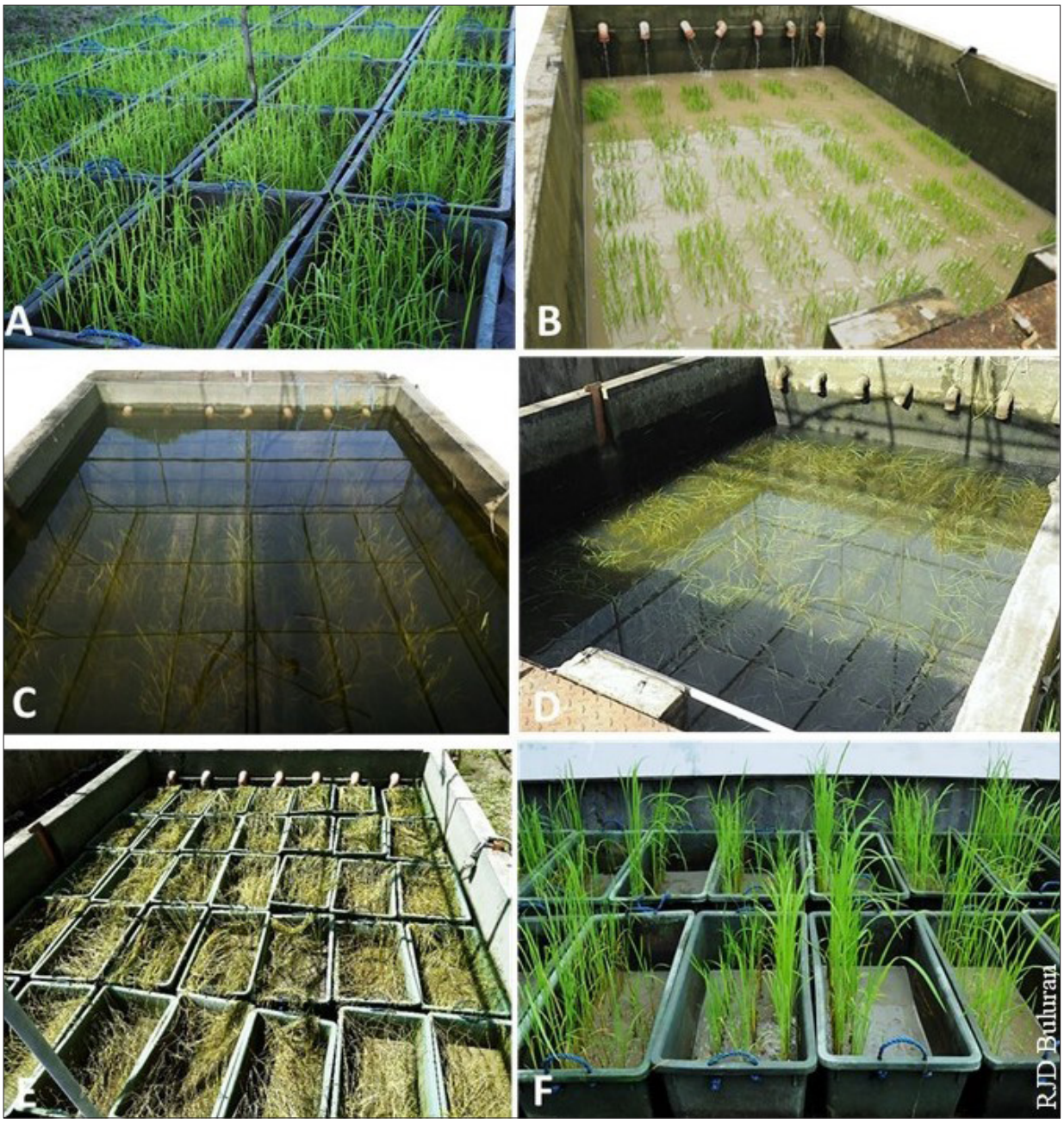

Figure 11.11. Mass screen for complete submergence tolerance: (A) 21 days old rice seedlings to be submerged; (B) seedlings in the cemented tank being filled up with water; (C) tank with seedlings completely submerged for 10 days and (D) 14 days; (E) stressed seedlings after de-submergence, 14 days from treatment; and (F) regrowth of tolerant check FR13A seedlings at 21 days after de-submergence.

- Before transplanting, layout the field following the planting plan.
- Arrange the rows per plot from left to right and orient in the east-west direction.
- Follow the zigzag, left to right direction of layouting the plot that corresponds to each entry.
- Mark each plot corresponding to the assigned entry by placing a 1m bamboo stake (or any appropriate material) in the first row of each plot.
- At transplanting, distribute the labeled bunched seedlings of each entry in the assigned experimental plot.

Seedbed management for raising robust seedlings

Regardless of the plant stage for screening, raising healthy and robust seedlings is vital as this affects the survival and recovery from submergence stress, and may increase yield by up to 40%.

- Determine seedbed size from the amount of seeds provided for each entry. Use a seeding rate of 50 - 75g seeds per square meter of seedbed
- Prepare raised seedbed with a height of 4 - 5cm above the soil level. Alternatively, seedlings can be raised in seed boxes for better control and management.
- Add carbonized rice hull (CRH) at the rate of 1.25kg/m^2 seedbed and mix with the soil to improve soil porosity. This is equivalent to 20 bags CRH per 400m^2 seedbed for a hectare of rice crop. One bag contains 25kg CRH.
- Spread 313g (~300g) organic fertilizer (OF) per square meter, on top of seedbed (not mixed with soil). This is equivalent to 5 bags OF per 400m^2 seedbed. One bag contains 25kg OF. Water the seedbed at saturation.
- Broadcast pre-germinated seeds on the seedbed or drill along rows 10cm apart. Cover seeds with sawdust or coir dust.
- Apply urea (46-0-0) at the rate of 30g/m^2 seedbed at 10 DAS, equivalent to 12kg per 400m^2 seedbed. Alternatively, in place of urea, apply ammonium sulfate (21-0-0) at the rate of 37.5g/m^2, which is equivalent to 15kg per 400m^2 seedbed.
- Maintain shallow water and adjust level with the age of seedlings. Avoid drying of soil. Maintain 2 - 3cm water level in the seedbed from 3 to 5 DAS and increase to 5cm a day before pulling the seedlings.

Seedling pulling, Zn treatment, and transplanting

1. Carefully pull the seedlings 18 - 21 DAS. Avoid damaging and breaking the roots when pulling the seedlings. Properly label the seedlings with the tags prepared earlier. Ensure proper identity of the entries with correct labels.
2. Dip the roots of the seedlings briefly (10 seconds) in 2% Zinc Oxide or Zinc Sulphate solution after pulling.
3. Transplant 2 - 3 seedlings per hill and place the remnant seedlings at the end of the last row in the plot for use in replanting. Replant missing hills within 7 DAT.
4. Transplant seedlings of the intolerant check IR 42 in 5 pots with 2 seedlings per pot. Place the pots at the side of the pond at transplanting time for easy access. Use the potted plants to determine the time of draining or de-submerging the field tank.

Field operation

Land preparation

Prepare the land thoroughly as recommended and level it before transplanting so that no low and high soil spots are observed after the final leveling.

Fertilizer management before and after submergence

1. Do not apply basal fertilizer.
2. After de-submergence or draining the flooded water, apply fertilizer only after the plants are fully recovered, which is usually 7 - 14 days after draining and repeat application at panicle initiation.
3. Use half bag of urea at the rate of 23kg N per hectare, in split application, i.e., 12.5kg of urea 14 days after de-submergence and 12.5kg at panicle initiation.
4. Alternatively depending on the status of the crop after de-submergence, apply the type and amount of fertilizer based on soil analysis and recommended rate for the area only when needed.

Imposing submergence stress and pond water management

1. Before submerging the field tank (Figure 11.12), install at least two vertical water level gauge at strategic locations for monitoring pond water depth.
2. Manage the golden apple snail. Apply molluscicide after transplanting and two days (12 DAT) before increasing the water level in the pond.
3. Count the total number of plants per plot per entry before submerging the pond. *(Optional)* Measure the plant height of five random plants from each entry.
4. Increase the water level of the pond to one meter 14 DAT. If some of the leaves of the test entries float 5 - 10 days after submergence, increase the water level to 1.5m to prevent escape of the tall entries.
5. Monitor the water quality (murkiness), water temperature, pH, EC, and dissolved O_2 if measuring tools are available.

6. Sample intolerant (susceptible) check by pulling the pots of IR42 to observe their condition starting five days after submergence until such time that deterioration, as manifested by foul smell, is apparent. In case of severe submergence, the plants are 70 - 80% chlorotic and the stems are very soft. This condition is expected within 10 - 14 days or more of submergence depending on flood water quality and environmental conditions, signaling the termination of the stress.
7. Totally drain the field tank after the submergence stress treatment and let the soil without standing water for at least 3 days. Rewater the field when the plants start to recover, about 10 days from de-submergence with an initial depth of 2 - 3cm of water. Increase the water level to 3 - 5cm and maintain until hard dough stage.
8. Drain the field 1 - 2 weeks before harvest at 85% maturity to facilitate easy harvesting.

Insect pest and disease management

Follow integrated insect pest and disease management practices. Manage the weeds especially before flooding the pond to avoid competition for nutrients. Golden apple snail is a major problem in the submerged paddy and t should be managed before, during, and after flooding.

Harvesting

Harvest when 85% of the grains are already yellow and 15% of the grains at the bottom of the panicle are at hard dough stage.

Post-Harvest

Thresh the samples immediately after harvest, dry to less than 14% MC, and store in a cool dry place. (Note: 10 - 12% MC will suffice for seed short-term storage).

Data collection

Survival data

Record the actual number of plants surviving per plot or entry per replication and compute for the % of plants [(actual no. of hills with surviving plants/total hills per plot)*100] that survived (S) in a plot (SES, IRRI, 2002), at 7, 14, 21, and 28 days from de-submergence, and at harvest.

Agronomic data

- Days to 50% heading — number of days from seeding to 50% heading.
- Maturity — number of days from seeding to 85% grain ripening (85% of grains on panicle are mature).
- Plant height (cm) — the length from the ground level to the tip of the longest panicle, excluding the awns if any, collected from 5 random plants.
- Culm length (cm) — measured from the ground level to the panicle neck of the longest panicle, taken from 5 random plants.
- Panicle length at maturity (cm) — the length from the panicle neck to the tip of the longest panicle, collected from 5 random plants.

Yield component data on a per plant basis (optional)

- Number of tillers per hill at maturity — count the number of productive and unproductive tillers from 5 random plants with adjacent plants
- Number of tillers per hill at maturity — count the number of productive and unproductive tillers from 5 random plants with adjacent plants
- Number of panicles per hill — count the number of panicles from 5 random plants
- Filled, unfilled, and total grains at maturity — harvest 5 primary tillers from 5 random plants per entry per replication. Count the filled and unfilled grains and record the total. Compute % filled grains per panicle based on the total grains per panicle
- 1,000-seed weight (g)

Per plot (entry) yield component data

To address the effect of submergence stress on the crop, the following measurements are taken on a per plot basis:

- Total number of surviving plants at maturity (or at harvest)
- Total productive tillers of all harvested plants
- Total unproductive tillers of all harvested plants
- Total number of panicles with seed set
- Total number of panicles without seed set
- Total weight of filled (g) and unfilled grains per plot (g) – compute % filled and unfilled grains
- 1,000-seed weight (g)
- Total number of filled and unfilled grains – this can be calibrated by taking small seed samples of known weight and number of filled and unfilled grains. Calibrate total grain number through ratio and proportion computation. Alternatively, total number of filled grains can be calibrated using the 1,000-seed weight and total filled grain weight data in the computation.

Figure 11.12. Screening at field tank for submergence tolerance at early vegetative stage: (A) set-up ready for complete submergence at 14 DAT, (B) tank submerged with 90cm water depth, (C) drained tank 8 days after complete submergence, (D) regrowth of tolerant plants 14 days after de-submergence, (E, F) deteriorating intolerant check, IR42, 8 days after de-submergence, and (G) regrowth of tolerant check, FR13A, 3 days after de-submergence.

Grain yield data

Per plot grain yield is taken from all surviving plants. For each entry, record the number of surviving plants per plot (entry) per replication.

Harvest all surviving plants per plot and compute the yield on a per plot basis. Take the grain moisture content at the same time when the grain yield weight is taken. Compute for the adjusted grain yield at 14% MC and grain yield per hectare as follows:

Adjusted plot grain

$$\text{Adjusted plot grain yield (g at 14\% MC)} = \text{plot grain yield (g)} \times \frac{\text{100-MC}}{\text{86}}$$

$$\text{Grain yield/ hectare (kg/ha)} = \frac{\text{Adjusted plot grain yield (g)}}{\text{Harvested area (m}^2\text{)}} \times \frac{\text{10,000 (m}^2\text{)}}{\text{1ha}} \times \frac{\text{1kg}}{\text{1,000g}}$$

Data analysis

Where applicable, the analysis of variance, mean separation by Tukey's, and mean comparison with the check by Dunnett shall be performed for the collected data.

Screening for Salinity Tolerance

Nenita V. Desamero

Successful breeding program requires reliable and repeatable screening techniques. A reliable screening technique can segregate susceptible and resistant reactions among genotypes. An ideal mass screening technique should be rapid, reproducible, easy, and affordable.

Rice is salt-sensitive, but its sensitivity to salt stress varies with growth stage. It is sensitive during early seedling and at reproductive stage, i.e., from panicle initiation and exsertion to flowering (pollination), while tolerant during germination, active tillering, and towards grain ripening. Management options in salt-affected areas include flushing with fresh and good quality irrigation water, providing good drainage for easy washing away of salts, and observing the dynamics of salt intrusion for timing of crop establishment such that the sensitive growth stages do not coincide with the occurrence of high-level stress. Growing salt tolerant varieties is still the most efficient and practical management option for saline-prone areas.

Saline soil characteristics

Coastal saline soils are dominated by sodium cations (Na^+) and soluble chloride (Cl^-) and sulphate (SO_4^{-2}) anions. For rice, soil salinity beyond ECe (electrical conductivity) ~4dS/m is considered moderate, while it is high with ECe more than 8dS/m. Saline soils has pH<8.5 and exchangeable sodium percentage (ESP) <15. The EC is high in dry season and early wet season and decreases during the monsoon season. Salinity is compounded by mineral deficiencies such as P and Zn, as well as toxicities of Fe, Al, H_2S and organic acids.

Mechanism of salinity tolerance

It has been established that salt injury in rice is due to excessive Na^+ uptake while Cl^- as a neutral anion is tolerated over a wide range of concentration. Typically, the salinity tolerance mechanism in rice is Na^+ exclusion or reduction of Na^+ uptake and increased absorption of K^+ to maintain a good Na^+-K^+ balance in the shoot. The ideal salt tolerant variety can withstand high amount of Na^+ (high tissue tolerance), good excluder (minimum Na^+ per day uptake), high K^+ per day uptake, low Cl^- uptake, low Na^+-K^+ ratio, good initial vigor, agronomically superior plant type and grain quality, and with high yield potential.

Indicators of salinity tolerance at seedling stage

The Na^+-K^+ ratio is a good indicator of salinity tolerance in rice but its determination is tedious and expensive. With hydroponic-based mass screen at seedling stage (Figure 11.13) in breeding, it is adequate to use visual symptom rating in determining the level of tolerance of a large volume of test genotypes. The visual symptom rating has been shown to correlate well with yield performance in salt stressed field.

Rice is very sensitive during early seedling stage at 2 - 3 leaf stage and gains tolerance at vegetative growth stage. Reduction in effective leaf area is the first visible symptom of salt injury manifested in the first leaf, followed by the second, and finally on the growing leaf. The oldest leaf starts to roll then die, followed by the next older, and so on. The surviving seedlings have the old leaves losing vitality while the youngest leaf remains green. Salt stress suppresses leaf elongation and formation of new leaves. Plant height, root length, emergence of new roots, and dry matter decrease significantly in salt stressed plants.

Indicators of salt injury in the field

Agro-morphologically, the salt injuries in the rice crop are manifested in the following salt stress symptoms:

- white leaf tip followed by tip burning (salinity)
- leaf browning & death (sodicity)
- white leaf blotches
- stunted plant growth
- low tillering
- leaf rolling
- poor root growth
- patchy growth in the field
- change in flowering duration
- spikelet sterility (papery spikelets)
- low harvest index
- less florets per panicle
- less 1,000 grain weight
- low grain yield

Salinity tolerance at seedling stage mass screen protocol

1. Creation of seedling floats
   Fabricate rectangular styrofoam float with 100 holes (10 x 10), with nylon net bottom and 2.5cm thick frame pasted on top. The frame helps fit the float to an improvised carbouy trays with 14L capacity.

2. Preparation of stock solutions

   Macronutrients
   - Weigh the required amount of reagent (Table 11.16) and transfer to a 500mL beaker and do initial mixing with about 375mL distilled water.
   - Pour the mixture to 1L volumetric flask then add distilled water and make up volume to 1L.
   - Stir the mixture for 15min using a magnetic stirrer and transfer to stock solution dark bottles.

   Microelements
   - Dissolve each reagent (Table 11.16) separately.
   - Use 25mL distilled water to dissolve each reagent except for ferric chloride, which must be dissolved in 50mL distilled water.
   - Mix all solutions together in 500mL distilled water using 1L capacity volumetric flask.
   - Add ferric chloride solution to the mixture just before citric acid and stir the mixture for 15min using a magnetic stirrer.
   - Add 50ml sulfuric acid to the mixture and make up volume to 1L.
   - Stir for another 10min and store in dark bottle.

3. Salinization of nutrient solution
   - Mix 100mL each of stock solution to tap water and make up volume to 80L (60mL stock solution + 740mL tap water).
   - Salinize the nutrient solution by adding NaCl while stirring up to the desired EC (3g and 6g NaCl/L nutrient solution gives an EC of 6dS/m and 12dS/m respectively).
   - Fill up the trays with the solution high enough to touch the nylon net bottom of the styrofoam.

4. Management of nutrient solution
   - Maintenance of the nutrient solution is very important. Monitor and maintain the pH of the culture solution to 5 daily.
   - Change the nutrient solution every 8 days.

Table 11.16. Nutrient component and preparation of stock solution for hydroponic medium.

Element	Reagent (Analytical Reagent Grade)	Amount (g/L)
Macronutrient		
N	Ammonium nitrate (NH_4NO_3)	91.40
P	Sodium phosphate, monobasic monohydrate ($NaH_2PO_4.H_20$)	35.60
K	Potassium sulfate (K_2SO_4)	71.40
Ca	Calcium chloride, dihydrate ($CaCl_2.2H_20$)	117.35
Mg	Magnesium sulfate, 7-hydrate ($MgSO_4.7H_20$)	324.00
Micronutrient		
Mn	Manganouse chloride, 4-hydrate ($MnCl_3.4H_2O$)	1.50
Mo	Ammonium molybdate, 4-hydrate [$(NH_4)6Mo_7O_{24}.4H_20$]	0.074
Zn	Zinc sulfate, 7-hydrate ($ZnSO_4$, $7H_2O$)	0.035
B	Boric acide (H_3BO_3)	0.934
Cu	Cupric sulfate, 5-hydrate ($CuSO_4.5H_2O$)	0.031
Fe	Ferric chloride, 6-hydrate ($FeCl_3.6H_2O$)	7.70
	Citric acid, monohydrate ($C_6H_8O_7.H_2O$)	11.90

Source: Adapted from Yoshida et al (1976)

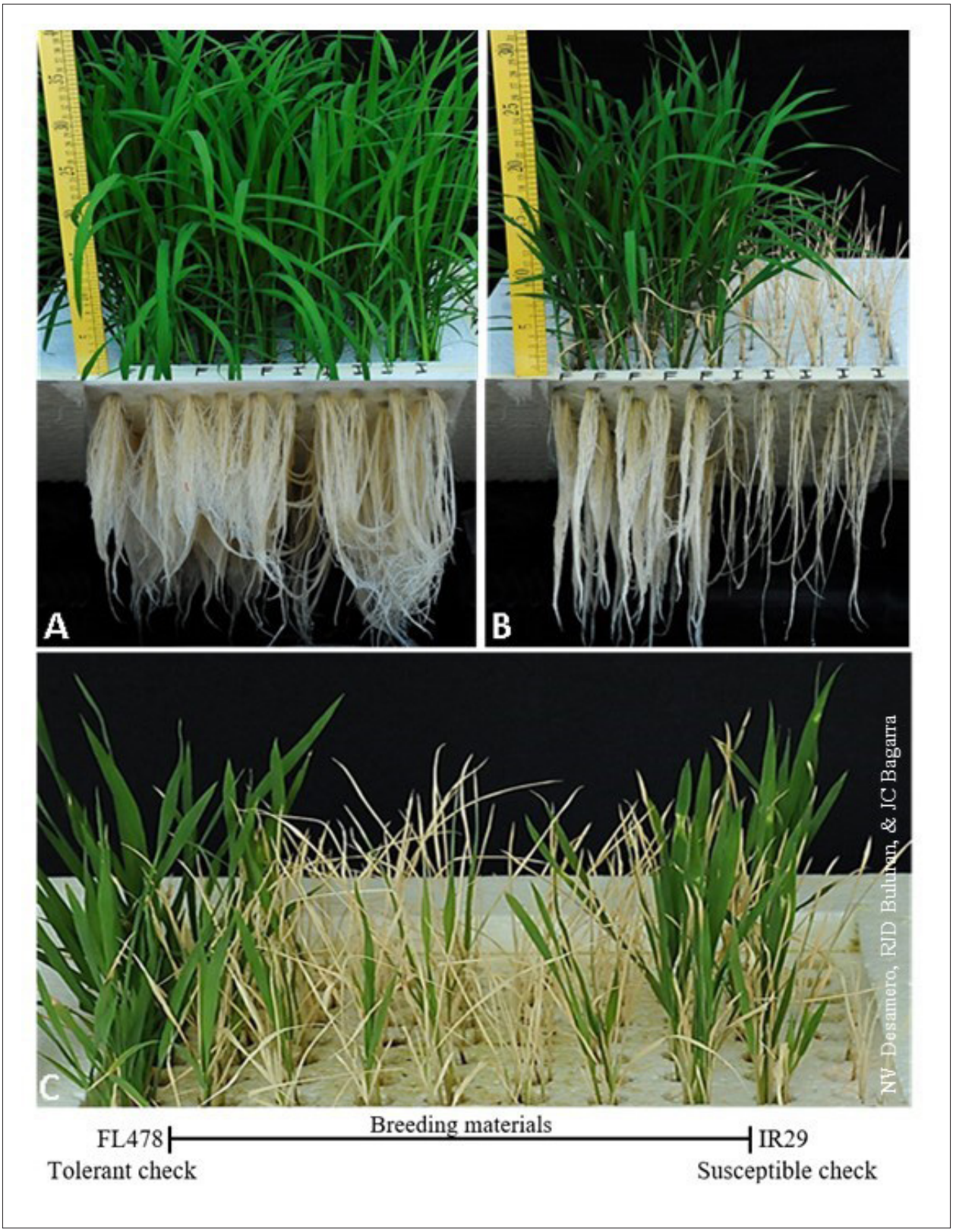

Figure 11.13. Mass screen for salinity tolerance at seedling stage using sodium chloride to induce salt stress in hydroponic growing medium: seedling growth of tolerant (FL478) and intolerant check (IR29) varieties under non-stress (A) and salt-stressed medium (B); comparative response of check varieties and breeding lines to salt stress at EC=16dS/m.

5. Handling of seedlings and salt stress imposition (Table 11.17)
   - Break seed dormancy at 50°C for 4 - 5 days.
   - Germinate seeds in petri dishes lined with moist filter papers by incubating at 30°C for 48h.
   - Sow two pre-germinated seeds per hole on the styrofoam.
   - Suspend the styrofoam seedling float on the tray filled with tap water.
   - After 3 days, replace the tap water with salinized nutrient solution.
   - Set initial salinity at EC=6dS/m
   - After 3 days, increase salinity to EC=16dS/m by adding NaCl to the nutrient solution.
   - Renew the solution every 8 days and maintain pH at 5.0 daily using 1N HCl and 1N NaOH
6. Evaluation of salt stress symptoms
   - Rate visually the test entries for salt injury at 10 and 16 days after initial salinization using the modified standard evaluation score for visual symptoms of salt toxicity (Table 11.18).

Table 11.17. Sequential step in rapid saline mass screen using Yoshida nutrient solution.

Days after Seeding (DAS)	Days from Pre-conditioning (DFP)	Days from Salinization (DFS)	Activity
0			Sowing of pre-germinated seeds
1			
2			
3	0		Preconditioning, initial salinization of nutrient solution, EC=6dS/m
4	1		
5	2		
6	3	0	Increase salinity of initial nutrient solution, EC=16dS/m
7	4	1	
8	5	2	
9	6	3	
10	7	4	
11	8	5	Salinization of new nutrient solution, EC=16dS/
12	9	6	
13	10	7	First scoring for salt stress injury
14	11	8	
15	12	9	
16	13	10	
17	14	11	
18	15	12	
19	16	13	Final scoring for salt stress injury

Table 11.18. Modified standard evaluation score (SES) of visual salt injury at seedling stage.

Score	Observation	Tolerance
1	Normal growth no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled, only few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Screening for Zinc Deficiency Tolerance

Corsenie A. Mabayag†

Zinc (Zn) deficiency is one of the common limiting factors in calcareous, sodic, peat and sandy soils, and near neutral to alkaline wetland soils. In many lowland rice areas, Zn is the third most limiting nutrient in irrigated lowland rice next to nitrogen (N) and phosphorus (P). However, the incidence of Zn deficiency would likely become more widespread and severity would be increased due to either or all of the following factors: (1) removal of large amounts of zinc by growing the high yielding varieties; (2) replacement of the acid fertilizer ammonium sulfate by urea; (3) increase use of phosphate fertilizer, (4) application of high amount of organic matter and crop residues in wet soils, (5) double- and triple-cropping of wetland rice, and (6) planting of rice varieties not efficient in Zn uptake.

The use of tolerant variety has been considered as one of the most effective strategies in solving the problem. Thus, various rice varietal improvement institutions endeavored to continuously breed and select rice lines that carry tolerant genes for Zn deficiency. Efficiency of rice varieties in acquiring this nutrient under conditions of low soil Zn availability is probably due to efficient ionic Zn uptake system; better root architecture, i.e., long and fine roots with architecture favoring use of Zn from larger soil volume; and higher synthesis and release of Zn-mobilizing phytosiderophore by the roots and uptake of Zn-phytosiderophore complex (Singh et al., 2005).

Hence, this screening protocol was developed to guide researchers or rice varietal performance evaluators in setting-up experiments on determining the tolerance of different rice lines to Zn-deficiency. This is to ensure that before a Zn-tolerant variety is released, it has passed a rigorous screening and appropriate testing methodology.

Methodology

A. Field Set-up

Earlier generations can be screened in the field with Zn-deficient soils. Entries should only be evaluated during the wet season (e.g., December-April cropping season under Agusan del Norte condition).

Soil characterization
The soil of the proposed experimental field must first be characterized physically and chemically to determine the Zn level and =other factors causing Zn deficiency in the area.

For physicochemical characterization, follow standard procedure in the collection of soil samples for laboratory analysis. Collect the following data: Zn content, pH, organic matter content, Ca, Mg, P, and other elements that would affect the availability of Zn.

Dobermann and Fairhurst (2000) provided the critical soil levels for the occurrence of Zn deficiency based on the methods used:

- 0.6mg Zn/kg : 1N NH_4 – acetate, pH 4.8
- 0.8mg Zn/kg : DTPA methods
- 0.2mg Zn/kg : 0.05N HCl
- 1.5mg Zn/kg : EDTA methods

Minus-one Element Technique is also recommended to determine the actual availability of Zn nutrient for plant uptake. This is to countercheck the availability of Zn for plant uptake.

Area needed
The screening of 200 - 400 rice entries needs a in area of 1,250 - 2,500m^2 for at least 60 hills per entry replicated three times.

Specific-site selection
Select the zinc deficient low-lying homogenously flat area of the field situated at the lowest base slope of the rice paddy, usually near irrigation canals or flowing water, which is continuously waterlogged with deep mud of >1ft.

The field must not be previously used in fertilizer trial, particularly of any Zn-containing fertilizer.

Field preparation
The field must be thoroughly prepared by 3 - 4 passes of the machine and must be well-leveled. There should be high spots so that water depth can be maintained at uniform level.

Do not allow the field to dry or get aerated even during land preparation.

Seedling age and planting distance
Seedlings must be transplanted within 18 - 21 DAS. Over mature seedlings (>25 days old) must not be used. Plant each entry at a distance of 20cm between rows and 15cm between hills (20cm x 15cm), in three-row plot with at least 20 hills per line, for a total of at least 60 hills per entry.

Orient the planting direction of different rice lines in perpendicular to the water flow or current (Figure 11.14).

Figure 11.14. Planting distance and orientation of the planting direction perpendicular to the flow of water source.

Water management
Do not allow the soil to dry up or get aerated. Maintain at least 2 - 5cm water depth throughout the growing season.

Fertilizer management
Apply inorganic fertilizer at the rate of 90-60-60kg/ha NPK. Use urea as source of N fertilizer; do not use ammonium sulfate. Nitrogen and K fertilizer will be applied in two splits; 50% within 7 DAT and another 50% at 30-35 DAT. All P should only be applied only once at 14 DAT.

Visual Observations and Scoring

In rice, seedling is the most susceptible stage to limiting Zn levels in the soil. Zn plays vital role in cell elongation and expansion being an essential element in the synthesis of auxin, maintenance of cell integrity, and in maintaining high chlorophyll and carotenoid ratio. Zn deficiency symptoms are commonly observed 2 - 4 weeks after transplanting. The appearance and the degree of Zn deficiency symptoms would vary depending on the susceptibility of the variety. The susceptible or Zn-inefficient variety would clearly show the symptoms as early as two weeks after transplanting (Figure 11.15). If symptom is not severe, the plant would just recover after 6 - 8 weeks even without intervention (Figure 11.16); however, maturity is already delayed and yield of susceptible varieties are reduced. Observe the Zn-deficiency symptoms within 2 - 4 weeks after transplanting (WAT). Repeat the observation of the recovering capacity of the rice plants within 4 - 6 WAT until early flowering stage.

B. Screenhouse or under Controlled Condition

Selected rice lines showing tolerance to Zn deficiency under field condition must further be evaluated under controlled condition, preferably in the screenhouse.

Rice plant characters that confer tolerance to Zn deficiency can also be determined from this set-up.

Collection of soils as growing media
Soil samples to be used as growing media must be collected from Zn deficient areas, which are usually waterlogged with deep mud (>1.0ft.), high in organic C content (>3%) and pH (> 6.8).

Do not allow the soil samples to get dry or get aerated during the collection. Place them in plastic pail (or any container with no holes) or large cellophane bag and put them in sack. Keep the soils always wet by adding water from the area where the samples are collected.

Figure 11.15. Rice crops 21 days after transplanting (DAT) without $ZnSO_4$.

Figure 11.16. Rice crops 21 days after transplanting (DAT) applied with $ZnSO_4$.

Immediately transfer soil samples in the growing chamber in the screenhouse. Ensure that concrete growing chamber has no water outlet to maintain waterlogged or anaerobic soil condition.

Seedling age and planting distance
Seedling must be transplanted within 16 - 18 DAS. Plant each entry at a distance of 10cm x 10cm, in two lines with at least five hills per line, or may vary depending on the space within the growing chamber and the number of entries to be screened or tested.

Water management
Do not allow the soil to dry up or get aerated. Maintain at least 2 - 5cm water depth throughout the growing season.

Fertilizer management
Apply inorganic fertilizer at the rate of 90-60-60kg/ha NPK. Use urea as source of N fertilizer; do not use ammonium sulfate. Nitrogen and K fertilizer will be applied in two splits; 50% within 7 DAT and another 50% at 30 - 35 DAT. All P should be applied only once at 14 DAT.

Other crop management
Follow other management practices for rice using *PalayCheck* system except those specifically mentioned above.

Data to be gathered

Observe Zn deficiency symptoms and score data within 2 - 4 WAT. Observe the recovering capacity of the rice plants or plant tolerance in 4 - 6 WAT until early flowering stage.

- Data to be gathered for characterization:
- Plant height (cm)
- Nodal interval
- Root length (volume)
- Total biomass
- Tissue analysis. For further analysis, Dobermann and Fairhurst (2000) provided the following ranges of Zn deficiency in the whole shoot during vegetative growth (tillering) of rice crop:
  - <10mg/kg: Zn deficient
  - 10 - 15mg/kg: Zn deficiency very likely
  - 15 - 20mg/kg: Zn deficiency like
  - >20mg/kg: Zn sufficient

Evaluation Guide

Degree of deficiency symptoms (refer to PhilRice Techno Bulletin #32 for more photos):

- Very slight deficiency. The growth and tillering are nearly normal but basal leaves are slightly discolored.
- Slight deficiency. Growth is slightly stunted, tillering is decreased, some lower leaves are brown or yellow, and some brown spots are observed from older leaves.
- Moderate deficiency. The growth and tillering of the plant is severely retarded; 50% of all leaves become narrow and appear rusty or yellowish.
- Severe deficiency. Plant growth and tillering stops; most leaves become narrow, rusty, and yellowish with brown spots. Leaf blades also become narrow.
- Very severe deficiency. Almost all plants are dead or dying.

Table 11.19. Zn deficiency rating.

	Scale	
At growth stage 2 - 4 *(seedling to stem elongation stage)*	1	Growth and tillering nearly normal
	2	Growth and tillering nearly normal, basal leaves are slightly discolored
	3	Slightly stunted, decreased tillering, some basal leaves are brown or yellow
	5	Growth and tillering are severely retarded, about half of all leaves are brown or yellow
	7	Growth and tillering ceases, most leaves are brown or yellow
	9	Almost all plant dead or drying

Source: INGER Genetic Resources Center-IRRI.1996. Standard Evaluation System for Rice (SES). 4th ed. IRRI, Los Baños, Laguna, Philippines.

CHAPTER 12

Screening for Grain Quality

Marissa V. Romero, Angelina dR. Felix, Evelyn H. Bandonill, and Emily C. Arocena

Rice breeding targets for various desired traits. Other than developing varieties with high yield potential and strong resistance against pest and diseases, breeders also work on improving grain quality due to the discriminating taste of a significant portion of consumers who consider rice as staple food for daily energy requirement. Breeding for quality traits is a challenging task because rice farmers and consumers differ in their preferences. A specific rice variety may be high quality for one but is considered low quality by another. Therefore, rice breeding programs must ensure the availability of good quality rice (Figure 12.1) based on consumer acceptability and preferences. The development of modern rice varieties; thus, provides more choices for farmers, traders, millers, food processors, and household consumers.

Figure 12.1. Commercial rice in the market.

Adding to the intricacies of breeding for quality is the range of different parameters under the general term grain quality. These include milling recovery, physical attributes, physicochemical properties, cooking parameters, and sensory characteristics. Rice breeders decide on the specific trait to consider depending on the target clienteles. In general, high milling and head rice recoveries, long and slender grains, soft cooked rice texture, and good eating quality are prioritized. Breeding for special purpose rice with unique quality traits such as aromatic, japonica-type, glutinous, and pigmented rices are also becoming more mainstreamed.

Rice varietal development is a sequential process consists of:

- Identification of genetic donors for the desired trait,
- Development of segregating populations and selection of plants or lines possessing the desirable traits, from F_1 to F_6 to observational nursery (ON), Preliminary Yield Trial (PYT) and Advance/General Yield Trial (A/GYT), and
- Multi-location trial through the NCT.

Screening for grain quality at different phases, including early stages of rice breeding, is essential because it helps prevent the advancement of individual plants with poor grain quality. The quality assessment of parentals and those in the NCT should include all parameters based on standard protocols as contained in the NCT Manual (Rice Technical Working Group, 1997).

Grain quality evaluation of materials in the second step of the breeding process is dependent on the number of materials for testing, the amount of sample available, and the utility of the results for decision making. Quality assessment in the F_1 - F_3 generations is focused on kernel

visual inspection. Measurement of the amylose content (AC) and the gelatinization temperature (GT) using standard laboratory procedures is recommended for F_4 and F_5 generations to have an idea of the quality type of the breeding materials. Subsequent generations prior to yield trial are also assessed in terms of AC and GT, and additional determination of gel consistency (GC) among high-AC rices. Rice entries in the yield trials are assessed in terms of milling recovery, physical attributes, and physicochemical properties using standard laboratory protocols. Figure 12.2 summarizes the grain quality parameters and methods for each phase of the breeding process.

Additional tests are needed for specialty rices. Aromatic rice entries should be tested for its scent through

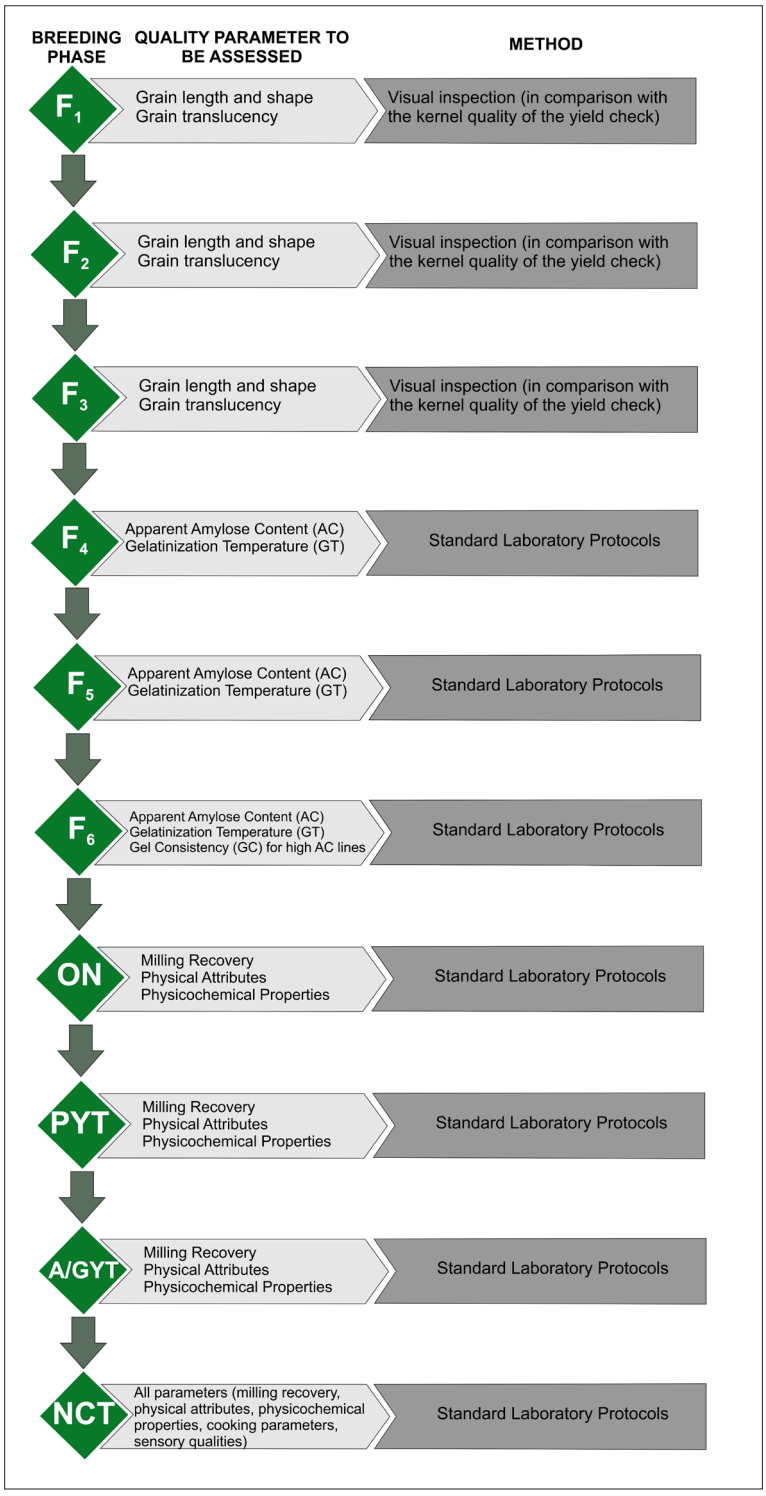

Figure 12.2. Summary of the grain quality evaluation at each breeding phase.

potassium hydroxide (KOH) test starting from F_3 generations up to ON. Aroma of rice sample is also assessed using the beaker method for entries in the yield trials prior to the NCT. Similarly, if one of the parentals for ordinary variety is aromatic, KOH test is also done at F_3 to ensure trait retention. Glutinous rice entries should possess opaque grains and an AC of less than 2% while Japonicas should be translucent, short and bold, and have low AC, low GT.

Grain quality evaluation is indeed a key element in assessing rice selections based on standards that are beneficial to the stakeholders. This chapter describes the screening for grain quality in rice breeding including the following specific parameters:

- Milling recovery (% brown rice, % total milled rice, % head rice)
- Physical attributes (grain length, grain shape, % chalky grains, % immature grains)
- Physicochemical properties (% apparent amylose, gelatinization temperature, % crude protein, gel consistency, Instron cooked rice hardness)
- Cooking parameters (optimum cooking water and time, % weight and height increase)
- Sensory characteristics (descriptive characteristics for raw and cooked rice, % acceptability, and rating score)

I. RICE SAMPLES

A. Sample Source and Amount

The specific grain quality parameters to be evaluated depend on the amounts of samples available for analysis under the particular stage of the rice breeding process. Under F_2 - F_5, the usual sample available is only about 25g; thus, grain quality evaluation is limited to grain length and shape determination and visual examination of translucency or chalkiness, specifically for F_2 - F_3; GT for F_3 - F_4; and AC for F_4 - F_5. Under F_6 - F_8, 400g of samples is available for determining AC and GT. Sufficient amount is available for milling recovery of ON and PYT samples; cooked rice hardness for PYT - AYT; and crude protein, cooking parameters, and sensory characteristics for AYT - Multi-adaptation Trials.

B. Moisture Content of Rough Rice

Moisture content (MC), expressed in percent, is the quantity of free water in rough rice or paddy. It is determined using a standard moisture meter (Figure 12.3) calibrated for rough rice using the air oven method (1h at 130°C). To eliminate the need for moisture correction, samples for analysis must have 12 - 14% MC. Rough rice with MC outside the recommended range tends to break easily resulting in low head rice recovery.

II. MILLING RECOVERY

Milling recovery gives an indication of the quantities of brown rice (BR), total milled rice (TMR), and head rice (HR) that can be produced from a unit of rough rice. BR is the unpolished form of rice where only the hull is removed. TMR is composed of head rice and broken grains. HR consists of whole grains and those with not less than 3/4 the length of the whole grain. Milling recovery is generally expressed in percent. The major steps involved in the determination are depicted in Figure 12.4.

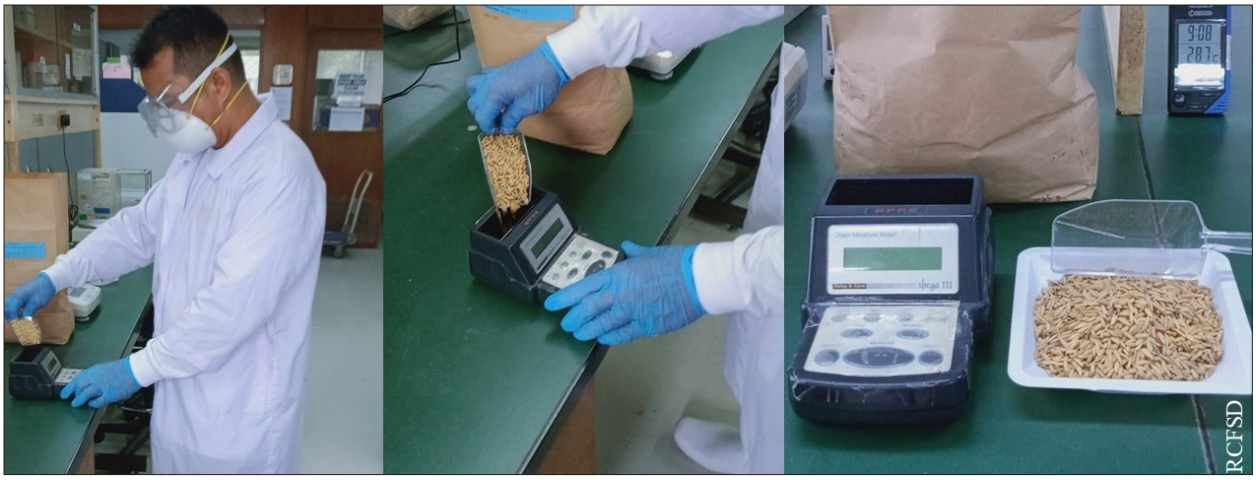

Figure 12.3. Moisture content determination using a standard moisture meter.

Figure 12.4. Determination of milling recovery (A) dehulling, (B) polishing, and (C) grading.

A. Percent Brown Rice (%BR)

- Prepare replicate samples of appropriate amount of rough rice depending on the capacity of the machine (e.g., 125g)
- Pass through a dehulling device (SATAKE testing husker or equivalent equipment) to remove the hull
- Weigh BR (dehulled rice)
- Calculate % BR as follows:

$$\%BR = \frac{\text{weight of brown rice (g)}}{\text{weight of rough rice (g)}} \times 100$$

- Determine the average of replicates

B. Percent Total Milled Rice (%TMR)

- Polish BR to remove the bran and embryo using appropriate equipment (e.g. McGill or Grainman) for 30sec as initial milling time. Adjust the time accordingly to ensure complete removal of the bran and embryo.
- Weigh TMR
- Calculate %TMR as follows:

$$\%TMR = \frac{\text{weight of total milled rice (g)}}{\text{weight of rough rice (g)}} \times 100$$

- Determine the average of replicates

C. Percent Head Rice (%HR)

- Separate HR from broken grains using a cylindrical mechanical grader (e.g., Satake)
- Weigh HR
- Calculate %HR as follows:

$$\%HR = \frac{\text{weight of head rice (g)}}{\text{weight of rough rice (g)}} \times 100$$

- Determine the average of replicates

Classify the samples (Table 12.1). For each parameter, a recommended value/classification is used as reference for varietal recommendation. Recommended BR recovery is 75% and above. For TMR, test entries should obtain at least 65% while head rice should be 48% and above.

Table 12.1. Classification and recommended value for parameters of milling recovery.

Milling Recovery	Classification	Recommended Value
% Brown Rice		
≥ 80%	Good (G)	75% and above (Fair - Good)
75.0 – 79.9%	Fair (F)	
< 75%	Poor (P)	
% Total Milled Rice		
≥ 70.1%	Premium (Pr)	65.1% and above (Grade 1 - Premium)
65.1% – 70.0%	Grade 1 (G1)	
60.1% – 65.0%	Grade 2 (G2)	
55.1% – 60.0%	Grade 3 (G3)	
% Head Rice		
≥ 57.0%	Premium (Pr)	48% and above (Grade 1 - Premium)
48.0% – 56.9%	Grade 1 (G1)	
39.0% – 47.9%	Grade 2 (G2)	
30.0% – 38.9%	Grade 3 (G3)	

III. PHYSICAL ATTRIBUTES

Physical attributes consist of four parameters: grain length, grain shape, % chalky grains, and % immature grains. These properties are important grain quality indicators because they determine the market quality of rice (Juliano, 1993). Consumer preference studies of more than 30 years showed that Filipinos, including traders and millers, prefer long and slender grains (Rice Technical Working Group, 1997). However, these are more prone to breakage than the shorter type; thus, have lower milling recovery. Meanwhile, chalkiness of rice grain is of great importance because it also influences milling quality. Consumers have a general preference for rice that is translucent.

For plant breeders, these parameters are indispensable considerations in their selection of early generation lines because they are greatly influenced by the environment as well as crop- and post-production management. As physical properties are easier to assess than other grain quality parameters, breeders have established a rapid way (visual kernel evaluation) of determining these properties for early generation rice selections.

A. Visual Kernel Evaluation

- Dehull all the sample seeds of the selected plants per population/line and place in a coin envelope.
- Conduct kernel evaluation by classifying the dehulled samples according to kernel type [non-glutinous (NG), glutinous (G), pigmented (P), japonica (J)], translucency, chalkiness, opaqueness, pigmentation, discoloration, broken grains, grain size and shape (GSS) (Table 12.2).
- After evaluation of all the selected plants from each of the population/line, thresh the panicles.
- Break seed dormancy by placing the seeds in the oven at 50°C for 3 days. The selected plants will constitute the family lines of the corresponding population and will be planted in the pedigree nursery.

Table 12.2. Rating used in the visual kernel evaluation.

Rating	Symbol	Description
Excellent	1	Kernels are whole, translucent for NG/opaque for G, pigmented for P, uniform GSS
Good	3	With some broken grains, translucent, opaque, pigmented, uniform GSS
Fair	5	With some broken grains, with some chalky grains for NG, with some NG grains for G, uniform GSS
Poor	7	Broken grains, chalky for NG, NG for G, no pigment or with non-pigmented kernels for P, not uniform GSS
Very poor	9	Mostly broken grains, mostly chalky, mostly NG for G, no pigment or with non-pigmented kernels for P, not uniform GSS

B. Grain Length and Shape Determination

Grain length is the length in millimeters of rice while grain shape is the ratio of its length and width (Figure 12.5).

- Prepare duplicate sets of 10 whole milled grains.
- Measure the length and width under a photo-enlarger or using a caliper.
- Classify the sample based on the average length and length to width ratio (Table 12.3).

Figure 12.5. Determining grain length and shape.

Table 12.3. Classification of milled grain length and shape.

Grain Length (in mm)	Classification	Grain Shape (length/width)	Classification
≥ 7.5	Extra long EL)	> 3.0	Slender (S)
6.6 – 7.4	Long (L)	2.1 – 3.0	Intermediate (I)
5.5 – 6.5	Medium (M)	≤ 2.0	Bold (B)
≤ 5.4	Short (S)		

C. Chalky and Immature Grains Determination

Chalky grains are whole or broken grains, one half or more of which is white like the color of chalk and is brittle. This is due to the starch granules in the grain not tightly packed together. High degree of chalkiness, which is caused by interruption during the final stages of grain filling, downgrades its quality and reduces milling recovery. On the other hand, immature grains are light green, thinner than mature grains, and chalky with soft texture, which is affected by time of harvest (Figure 12.6).

- Prepare duplicate 30g TMR samples (use 3 replicates of 10g for pre-NCT samples).
- Separate all chalky and immature grains manually and weigh separately.
- Calculate % chalky and immature grains as follows:

$$\%\ Chalky\ Grains = \frac{weight\ of\ chalky\ grains\ (g)}{30g} \times 100$$

$$\%\ Immature\ Grains = \frac{weight\ of\ immature\ grains\ (g)}{30g} \times 100$$

- Classify based on Table 12.4. Recommended values for chalky and immature grains are 5% and 2%, respectively.

IV. PHYSICOCHEMICAL PROPERTIES

The physicochemical properties of the rice grains are important determinants of cooking and eating qualities. They include GT, crude protein, apparent AC, GC, and cooked rice hardness.

Sample Preparation

At least 10g of TMR is needed for the entire physicochemical analyses. Sample is ground to obtain the flour needed for the analyses (Figure 12.7).

A. Gelatinization Temperature (GT)

GT is the range of temperature within which starch granules start to swell irreversibly in hot water with accompanied loss of birefringence and crystallinity. Low GT implies shorter cooking time while high GT indicates longer cooking time.

It is estimated by the extent of alkali spreading of raw milled rice soaked in 1.70% anhydrous potassium hydroxide (KOH) for 23h at 30°C or at room temperature (Little et al, 1958). Reaction of the grains is recorded based on the rating scale for GT classification (Table 12.5). The average alkali spreading value (ASV) and GT types is reported in order of decreasing frequency or as mixed reaction.

Rice with low GT disintegrates completely while rice with high GT remains largely unaffected in the alkali

Table 12.4. Classification and recommended values for % chalky and immature grains.

Chalky Grains		Immature Grains	
%	**Classification**	**%**	**Classification**
< 2.0%	Premium (Pr)	< 2.0%	Premium (Pr)
2.0-5.0%	Grade 1 (G1)	2.0-5.0%	Grade 1 (G1)
5.1-10.0%	Grade 2 (G2)	5.1-10.0%	Grade 2 (G2)
10.1-15.0%	Grade 3 (G3)	10.1-15.0%	Grade 3 (G3)

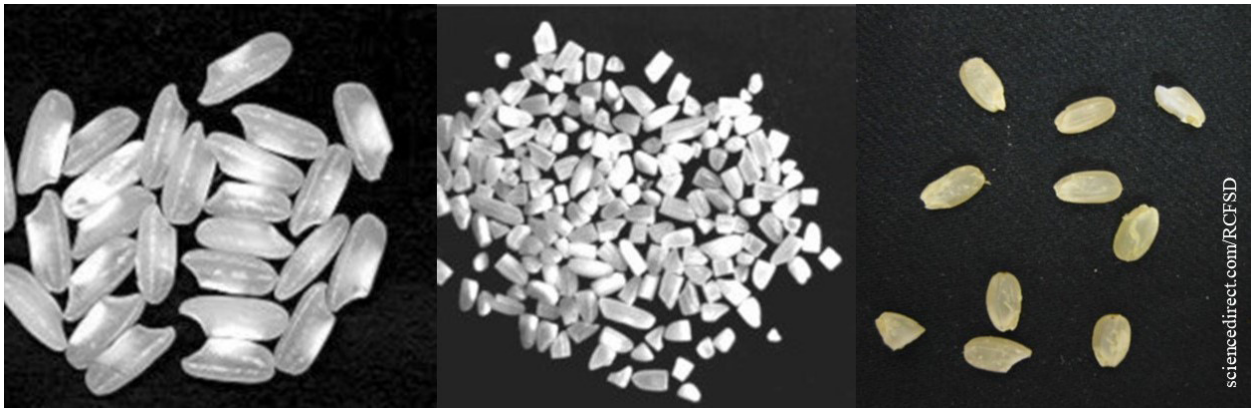

Figure 12.6. Determining chalky and immature grains.

Figure 12.7. Preparation of rice flour.

Table 12.5. Rating scale for GT type classification.

Alkali Spreading Value	Description	GT Type
1	Grain not affected	High (74.5 - 80°C)
2	Grain swollen	
3	Grain swollen, collar incomplete and narrow	High-intermediate
4	Grain swollen, collar complete and wide	Intermediate (70 - 74°C)
5	Grain split or segmented collar complete and wide	
6	Grain dispersed merging with collar	
7	Grain completely dispersed and intermingled	Low (<70°C)

solution (Figure 12.8). In general, samples grown in mid elevation show low GT reading. Refer to the National Cooperative Testing Manual for Rice for the detailed procedure of GT determination.

B. Crude Protein (CP)

Kjeldahl is the prescribed method for crude protein determination using the protein factor of 5.95 (Figure 12.9). Alternately, a near infrared spectroscopy (NIRS) machine calibrated with the same method can be used provided, approximately 50g milled rice or 25g flour sample is available. This analysis is to be conducted only once for each selection. As detailed in the NCT Manual, ground sample is digested with Kjeltab and concentrated sulfuric acid under a fumehood, distilled, and titrated using an autoanalyzer. Percent CP is computed using the formula:

$$\%\ CP = \frac{[Vol\ H_2SO_4\ (spl) - Vol\ H_2SO_4\ (blk)] \times N\ H_2SO_4}{weight\ of\ sample\ (g)} \times 0.014 \times 5.95 \times 100$$

C. Apparent Amylose Content (AC)

Apparent amylose content (AC) is determined based on the pH 9.2 iodine colorimetric method (Figure 12.10) of Juliano et al (2012). The term apparent is used because true amylose does not exceed 18 - 21% (Juliano, 1992). In determining apparent amylose content, milled rice checks and samples are soaked overnight and amylose standards and standard curves of non-waxy (Table 12.6) and waxy milled rice (Table 12.7) are prepared before determining apparent amylose content. The entry is classified as to apparent amylose type (Table 12.8). Step by step procedure of the assay is detailed in the NCT Manual.

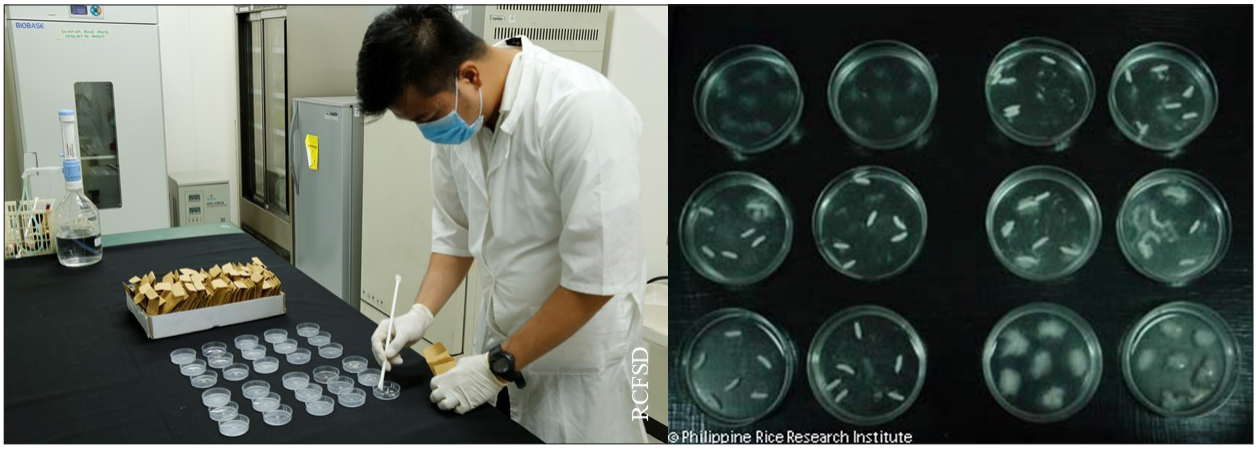

Figure 12.8. Gelatinization set-up.

Figure 12.9. Crude protein content determination.

The AC obtained for waxy rices is mainly due to amylopectin-iodine complexing in excess iodine. The complex formation is reduced by lowering the iodine concentration to about 1/7th of the amount used for non-waxy rices. AC values obtained are <1% and are true AC values as iodine preferentially binds with amylose over amylopectin.

Table 12.6. Standard solutions for AC determination of non-waxy milled rice sample.

Working Standards (% Amylose)	Amylose (mL)	0.09N NaOH (mL)
0	0	100
10	10	90
20	20	80
30	30	70

Table 12.7. Standard solutions for AC determination of waxy milled rice sample.

Working Standards (% Amylose)	Amylose (mL)	0.09N NaOH (mL)
0	0	100
5	5	95
10	10	90

Table 12.8. Classification of apparent amylose type.

Apparent Amylose Content	Apparent Amylose Type
0 - 2.0%	Waxy/glutinous (W)
10.0%	Very low (VL)
10.1 - 17.0%	Low (L)
17.1 - 22.0%	Intermediate (I)
>22.0%	High (H)

D. Gel Consistency (GC)

Gel consistency is a measure of the flow characteristics of milled rice gel (Figure 12.11). High apparent amylose rices may be differentiated in terms of tenderness of cooked rice by the GC test. Within the high apparent amylose group, varieties with soft/medium GC are more tender when cooked than those with hard GC. Thus, this analysis is conducted only for entries with high apparent AC. The procedure based on the method of Cagampang et al. (1973) involves the gelatinization of 100 mesh-sieved flour added with thymol blue. The mixture is gelatinized in covered GC tubes using boiling water bath and subsequently cooled down to flow. The distance (mm) travelled by the gel from the bottom of the tube to the gel front is measured prior to GC classification (Table 12.9).

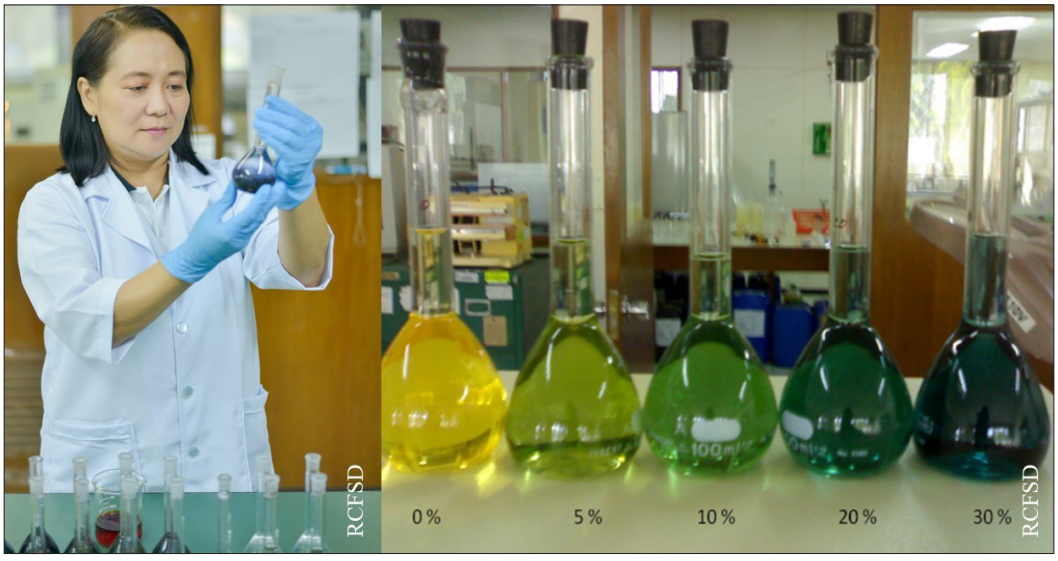

Figure 12.10. Amylose content determination and iodine blue color of 0, 10, 20, and 30% amylose standards.

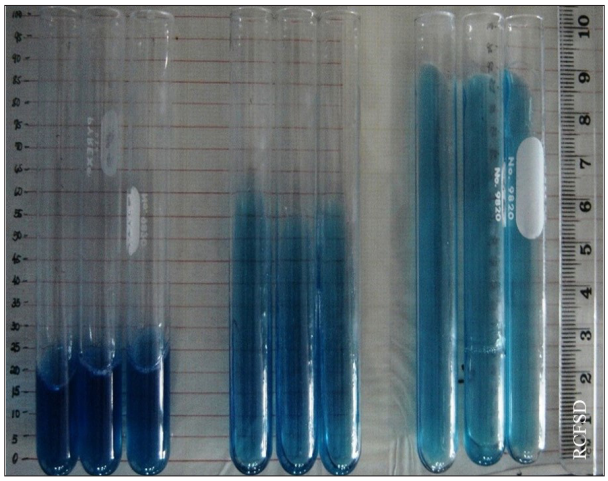

Figure 12.11. Gel consistency set-up.

Table 12.9. Gel consistency classification.

Length of Gel (mm)	Gel Consistency
26 – 40	Hard (H)
41 – 60	Medium (M)
61 – 100	Soft (S)

E. Cooked Rice Hardness

Cooked rice hardness is measured with an Instron Model 3342 or 3343 Food Tester (Figure 12.12). Hardness can be quantitatively determined using two methods of cooking the rice prior to measurement. For the beaker method, 20g of head rice is cooked in a beaker covered with foil containing 24ml distilled water to make a rice to water ratio of 1:1.2. Cooking takes about 20min inside a rice cooker, then kept for 10min and cooled at room temperature for 1h prior to Instron measurement.

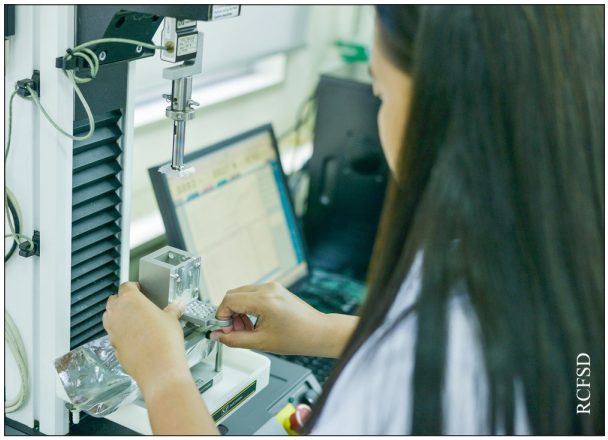

Figure 12.12. Cooked rice hardness determination using Instron 3342.

For rice cooker method, the procedure used in cooking samples for sensory evaluation is adopted. However, an additional waiting time of 40min is required prior to reading in the Instron machine. Measurement is done by pressing 17g cooked rice evenly with a 2.2cm x 2.5cm plunger and extruding cooked rice into the Instron machine using 0-50kg load cell.

Using the different AC-GT combinations, rice samples are grouped based on cooked rice hardness adopted from the clustering of Romero et al., 2022:

- Cluster 1. Very soft. Waxy - high intermediate to low GT
- Cluster 2. Very soft, soft to Hard. Low to high AC – high intermediate to intermediate GT
- Cluster 3. Soft to hard. Intermediate to high AC – low GT
- Cluster 4. Very soft to soft. Low AC – intermediate to low GT

Typical Instron cooked rice hardness (kg/cm^2) and RVA consistency for the clusters are shown in Table 12.10. AC, cooked rice hardness, and RVA consistency measure complementary aspects of cooked rice texture and are positively significantly correlated.

V. COOKING PARAMETERS

Cooking and eating qualities are equally important parameters considered by rice consumers, which determine the final acceptability of the rice line be it high or low yielding. Cooking and eating qualities are known to be directly related to some physicochemical properties such as amylose and protein contents and pasting viscosity. Milling and cooking procedures also affect the quality of milled raw and cooked rice grains. Standardized methods to evaluate the cooking parameters and sensory attributes are therefore necessary.

Sample Preparation

- Divide samples into 1kg portions or depending on the capacity of the milling machine.
- Dehull samples in a Satake rice dehuller then mill in the McGill Miller #3. Combine all 1kg portions of milled samples according to type.
- Remove broken grains using a cylindrical mechanical grader. Other foreign matter such as chaff, stones, rough rice, and seeds not completely removed during the previous steps must be picked by hand.
- Place recovered and cleaned head rice in plastic bags, then seal and label with assigned 3-digit code number on top of the selection name.

Table 12.10. Instron cooked rice hardness (kg/cm²) and RVA consistency of various AC-GT combinations.

AC Type	Low GT				Intermediate - High GT			
	Cooked Rice Hardness		RVA Consistency (RVU)		Cooked Rice Hardness		RVA Consistency (RVU)	
	No.	Range	No.	Range	No.	Range	No.	Range
Waxy	29	0.5 - 1.0	17	10 - 50	9	0.7 - 1.4	15	10 - 50
Low	62	0.9 - 1.8	69	70 - 170	19	1.3 - 2.5	24	75 - 185
Intermediate	80	1.4 - 2.8	86	90 - 200	271	1.2 - 2.6	135	60 - 175
High	39	2.3 - 3.7	17	100 - 250	64	1.6 - 3.4	33	100 - 180

Determination of Cooking Parameters for Non-glutinous Rices

Cooking quality evaluation starts with the determination of the optimum cooking water followed by the measurements of the height increase and cooking time (Figure 12.13). The optimum amount of water for cooking different rice lines to similar doneness - neither too dry nor too soft - is based on preliminary cooking trials.

A. Optimum Cooking Water for Non-glutinous Rices

Using an electric automatic rice cooker with a capacity of 2 cups, proceed as follows:

- Measure 80g head rice into the inner pan (of known weight); add 120mL tap water. Swirl the pan three times to wash the rice.
- Decant and measure the wash water. Replace the wash water with the same amount of tap water.
- Place the inner pan on the cooker and cover. To ensure uniform cooking, rice cookers can be plugged to an outlet connected to a variable transformer that maintains a uniform flow of current (220V).
- Switch the rice cooker and wait for the audible switch off. Leave undisturbed for 15min.
- Mix the cooked rice and get representative sample for evaluation of laboratory staff trained to determine doneness of cooked rice. At least three evaluators are required.
- Let the laboratory evaluators taste and press a few cooked grains between the forefinger and thumb for doneness. Based on the evaluation, the water is reduced or increased with 10mL increment until the optimum amount of water is achieved for each rice selection.

Figure 12.13. Cooking parameters determination.

Height Increase and Cooking Time

- Measure the height or depth of 80g uncooked rice in the inner pan at three points (i.e., middle, right middle, and left middle sides). Use a sliding steel tape calibrated in millimeters.
- Cook each selection using the optimum amount of cooking water. Record the exact cooking time in minutes and in seconds using a stopwatch. Simultaneously switch on and off rice cooker and stopwatch. Convert time into minutes.
- Measure the height or depth of the cooked rice at the same points.
- Calculate % height increase by getting the difference in height of cooked and uncooked rice, divided by the height of uncooked rice and multiplied by 100.

Cooking of Glutinous Rice

Glutinous or waxy rice selections are cooked as *suman inantala* (Figure 12.14). This way of preparation is preferred for two reasons. First, the ingredients for *suman inantala* are evenly absorbed during cooking. Customarily eaten as prepared, it eliminates the concern of controlling the amount of other ingredients like sugar and grated coconut normally served alongside other *suman* preparations. Second, *suman inantala* is ideal for evaluating tenderness and cohesiveness. It is eaten after standing overnight at room temperature, simulating evaluation of tenderness and cohesiveness after cooling.

Prepare *suman inantala* as detailed:

- *Preparation of banana leaves for wrapping.* Separate banana leaves from the stalk. Roll and dip in boiling water to wilt. Unroll and dry with a clean, lint-free piece of cloth. Cut into 8-in wide pieces.
- *Preparation of coconut milk.* Add one cup warm water into 3½ cups grated mature coconut. Extract the milk with bare hands or with cheesecloth. Repeat extraction using ½ cup water. Combine extracts and set aside.
- *Pre-cooking of glutinous rice.* Place 200g glutinous rice into the inner pan of a rice cooker. Add 300mL tap water. Wash once by swirling gently for five times. Decant wash water immediately to prevent soaking and drain the rice on a strainer for 2 - 3min. Return the drained rice into the inner pan. Add 1 1/3cups coconut milk and one teaspoon (approximately 3g) rock salt. Mix well with a wooden spoon. Place the inner pan with the sample on the cooker and cover. Plug the rice cooker to an outlet connected to a variable transformer to maintain uniform flow of current. Cook for exactly 15min from switching on to off. Uncover the cooker and stir cooked rice. Return cover and leave undisturbed for 10min.
- *Steaming of suman.* Evenly spread three tablespoons of the precooked glutinous rice-coconut milk mixture on the non-glossy side of the wilted banana leaf. Wrap evenly to ensure an even thickness through a 4-in length. Tie two pieces with the end folds facing each other. Arrange in a single layer inside a steamer and steam for 30min. Let stand overnight or 10 - 12h prior to sensory evaluation.

Figure 12.14. *Suman inantala.*

VI. SENSORY CHARACTERISTICS

Sensory evaluation is used to interpret sensations perceived by the human senses of sight, smell, taste, touch, and hearing. The complex sensation that results from the interaction of senses is used to measure food quality in activities dealing with quality control and product development. Inasmuch as sensory test is valuable in predicting consumer reactions, it is one of the tools used to screen rice grain quality. Sensory qualities are determined for rice selections entered in the NCT except for aromatic rice entries. Sensory evaluation of aromatic entries starts in the early generation (F_3) stage.

Sensory Evaluation of Aromatic Rice Entries

The aroma of raw rice selections having aromatic parents in the early generation (F_3) and yield trial stages is assessed using KOH test. Follow the procedure:

- Prepare 1.7% (0.3035N) KOH solution.
- Select 10 dehulled grains from each of the selected test materials derived from aromatic crosses that passed the kernel evaluation.
- Powder the grains using mortar and pestle and transfer to a vial.
- Add 5mL of 1.7% KOH solution, cover, and leave for 1h.
- Smell for the presence of aroma.
- Rate the aroma as follows:
  1 – strong aroma
  2 – slight aroma
  3 – no-aroma

Furthermore, the presence of aroma in these entries are confirmed in the yield trial stages by cooking 20g head rice using the beaker method (refer to procedure of sample preparation for cooked rice hardness determination) and evaluated by sniffing the samples from the covered beaker and rated by the laboratory evaluators as extremely aromatic (5), very aromatic

(4), aromatic (3), slightly aromatic (2), or non-aromatic (1). When entered in the NCT, the standard protocol for sensory evaluation is followed.

Sensory Evaluation of Rice Entries in the NCT

For rice entries in the NCT, laboratory and consumer panel evaluations are conducted. Laboratory panel assessment is done in the laboratory employing trained panelists. Sensory characteristics of rice entries in the NCT Phase I are identified using laboratory panel, while consumer panel testings (in areas that simulate the agro-ecological environment where the rice entries are grown) are done for rice entries in the NCT Phase II or Multi-location Adaptation Trial (MAT). Simultaneous conduct of sensory assessment using both the laboratory and consumer panels are also implemented, as warranted. The procedures for sensory evaluation of the different groups of rice entries are detailed in the NCT Manual.

A. Panel of Judges.

Two types of panels are involved in rice sensory evaluation. The **laboratory panel** consists of 5 - 10 trained panelists who are selected from a large group for their acuity and consistency in recognizing differences (Figure 12.15). Rather than compare samples, it describes the characteristics of each rice attribute such as aroma, color, gloss, tenderness, cohesiveness, and taste of cooked rice as well as aroma, color, gloss, translucency, and hardness of raw rice.

A computerized system (Rice ICT-Based Sensory System or RISS) for conducting laboratory evaluation was recently developed, which aids in the more efficient and faster determination of rice sensory description (Figure 12.16).

Figure 12.15. Laboratory panel evaluation.

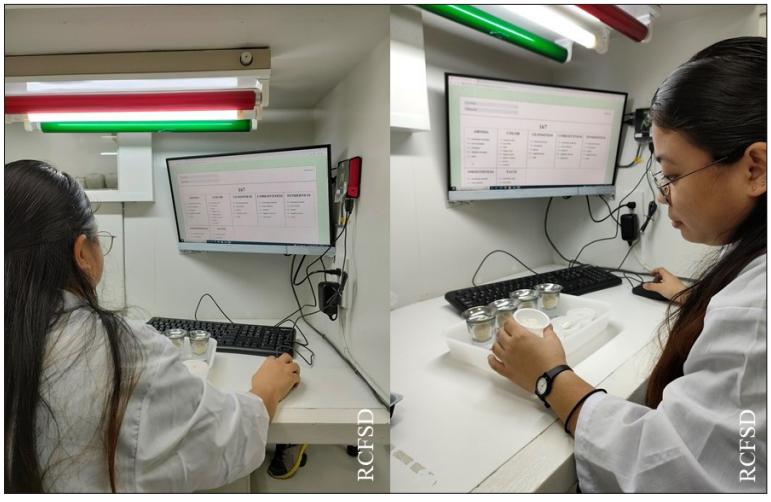

Figure 12.16. Computerized laboratory panel evaluation.

On the other hand, **consumer panel** is composed of a larger number of untrained members, usually from 30 to several hundreds of persons, as the case warrants based on the statistical design (Figure 12.17). They measure acceptability (likes or dislikes) and rating and consider the general characteristics of the sample. The ability of its members to discriminate based on the degree for specific attributes is not considered. It is enough that they represent consumers and are willing to participate.

Absence of colds or other illness that reduces sensitivity of the senses is considered in choosing participants for sensory evaluation. Eating and smoking time is also considered. At least 1h interval between eating and/or smoking and time of evaluation is observed particularly for cooked milled rice. Drinking liquor before sensory evaluation is unacceptable.

B. Randomization of Samples

Samples are randomized for presentation during laboratory panel. A minimum of two and maximum of four entries are evaluated at any one time. A quality check is always evaluated with the test entries.

C. Sensory Room and Sample Preparation

The taste test area should be comfortable, spacious, well ventilated and lighted, free of distractions, and accessible. An atmosphere of comfort and relaxation is a must to encourage panel members to focus on evaluation. Samples for sensory testing should be prepared in a way that no foreign taste or odor is imparted. Sample size, container, and temperature must be similar for all. Coded samples are given at random to avoid time or position error (Figure 12.18).

Figure 12.17. Consumer panel evaluation.

Figure 12.18. Set-up in tray of rice samples for sensory evaluation.

D. Coding the Rice Samples

Three digits are assigned as code number for each of the entries. To code each rice entry for the sensory evaluation, a table of random numbers from any statistics book is used.

E. Sample Presentation for Sensory Evaluation

Before distributing the samples for sensory evaluation, background information sheet is distributed and accomplished by each participant. Scorecards must be fully explained to the participants to minimize time and error in answering the evaluation forms.

1. *Cooked non-glutinous samples.* Warm cooked rice samples are dispensed in wine glasses covered with aluminum dish (Figure 12.18). The code for each sample is placed at the bottom of the wine glasses and on the cover. Sample containers are arranged on trays according to the experimental design (consumer panel) or randomized plan (laboratory panel). A glass of water and a teaspoon are provided.
2. *Cooked glutinous samples or suman.* Following the order of the randomized plan, uniform portions of *suman* samples (4cm x 3cm x 1cm) are arranged in a tray labeled with the respective code number for each sample. A fork and glass of water are provided.
3. *Milled raw samples.* Head rice is used specifically for sensory assessment of raw rice to ensure uniformity of the samples presented to each panelist. One teaspoon (5 - 7g) of head rice sample is placed inside the compartment of the miniature rice bin that has been labeled with code numbers arranged according to the experimental design or randomized plan. Milled raw rice samples are evaluated after cooked samples.

F. The Score Cards

The scorecards for laboratory panel consist of score sheets for cooked and raw milled rice. The cooked rice attributes described or characterized are aroma, color, gloss, tenderness, cohesiveness and taste as well as raw rice attributes like aroma, color, gloss, translucency, and hardness of grains.

Four scorecards are used for the consumer panel to evaluate cooked milled non-glutinous rice, raw milled non-glutinous rice, *suman* or cooked glutinous rice, and *malagkit* or raw glutinous rice. Acceptability and rating for the selections are highlighted.

G. Rating Score and Percent Acceptability

Consumer panels evaluate the promising rice entries for their rating score and acceptability. Rating score is determined by getting the average rating of samples based on the number of sensory panelists evaluating the rice entry. Acceptability is indicated by a "yes" or a "no" response.

1. Rating Score

The ratings of the rice samples are averaged based on the number of panelists evaluating the rice entry. The equivalent ratings are 1 poor, 2 fair, 3 good, 4 very good, and 5 excellent. Rice entries with mean rating of at least 3 are considered.

2. Percent Acceptability

Acceptability is expressed in percent of the acceptable "yes" response. In a 30-member panel, for instance, 29 of them must have responded "yes" for the rice sample to be considered 97% acceptable (i.e., 29/30 x 100). A score of 75% is the threshold but 80% and above is desirable.

CHAPTER 13

Multi-location/Regional/National Testing

Thelma F. Padolina, Dindo Agustin A. Tabanao, Alex T. Rigor, and Nenita V. Desamero

Two phases of multi-location evaluation trials are conducted under irrigated lowland conditions. The initial phase, NCT I is composed of new entries nominated by breeders /breeding institutions in the Philippines and are established in strategic rice research stations. Promising test entries that passed the criteria of NCT I are elevated to Phase II, the multi-location adaptation trials (MAT), and the final stage for variety release. MAT trials are usually conducted in farmers' fields. Both trials are conducted under two methods of crop establishment, transplanting (TPR) and direct seeding (DSR) and are managed by researchers.

MAT is a crucial step in developing elite lines with potentials to be released as varieties in the National Seed Industry Council (NSIC) variety registration systems These trials generate accurate data under multi-environment conditions to identify lines with high level of performance for yield, optimum resistance to insect pest and diseases, and adaptability to environmental conditions. It also ensures the authenticity of the potential variety through its distinctness, uniformity, and stability (DUS) traits. Varieties are seed produced ensuring the availability of quality seeds for the breeding institutions, seed growers and farmers. For the other agroecological and adverse ecosystem types, only the NCT Phase I multi-location trials are conducted, which are typically researcher-managed and require more than two season-trials without the MAT.

Purpose

The main goal of multi-location yield trials is to identify elite lines with high and stable yields and wide adaptation by exploiting G, E, and GxE interactions. Superior materials adapted to one or more specific environment(s) and agro-ecologies are also identified. Breeders may find gaps and differences on trait performance and address the needed traits. Through multi-location trials, partnerships with the public, NGO, and private sectors on rice variety development are strengthened to achieve the targets.

Entries for testing and checks used

Advanced breeding lines developed by breeding institutions (PhilRice, IRRI, UPLB-CA, and private sector) are nominated. These are the selected lines that passed the breeding pipelines. It can include both inbred and hybrid varieties with widely adapted released inbred and hybrid varieties of comparable growth duration as national checks. The best performing NSIC released inbred or hybrid variety in a certain region and/or agro-climatic zone (ACZ) is used as local check.

Number and choice of sites

Test locations are rarely chosen at random, as trials are conducted in the area of coverage of collaborating institutions or interested villages and farmers. The inclusion of test sites such as breeding stations or experimental farms, in which selection work can easily be carried out, is useful in defining optimal selection environments. The sites, however, should encompass the major cropping areas and farming practices in the target region to reflect variations in climatic, soil, biotic, and crop management factors. Specifically, testing sites represent the key agro-ecological areas strategically located across the country and rice areas under the agro-climatic zones (ACZ) classification. General insect pest and disease occurrences in the test sites must also be identified.

Experimental design and number of replications

Experimental design is the process of choosing treatments, responses, and controls, defining experimental and sample units, and determining the physical arrangement, or layout, of experiment units. By following an experimental design, greater homogeneity is achieved for reducing experimental error. Multi-location yield trials are typically researcher-managed, comprise of 6 - 15 elite breeding lines, conducted in 10 - 20 locations, and are laid out in a RCBD with at least three replications. Plot size is preferably 3m x 4m with 15 rows made parallel to the length of the plot and 21 hills per row. A minimum of $5m^2$ harvest area for all sites may be an option in limited areas. In laying out a trial, a replication should be as square as possible. An 18-entry trial for example should have three replications with three sub-blocks for each replication. A sub-block is made up of 6-entry plots. A paddy does not have to contain all replications. It is important, however, that all plots in a replication be located in the same paddy. For the statistical analysis, analysis of variance, coefficient of variation and r^2 are determined using SAS or any statistical software with similar function or capability. Test entry is compared against standard checks by Dunnett's t-test.

More complex designs are sometimes adopted to undertake initial evaluation of a large set of germplasm accessions to select genotypes suitable for different aspects of crop breeding (Federrer, 1956, Shaner et al., 1982; Hildebrand and Poey, 1985). One of the examples is the Row-Column Design using only two replications (80 rows and 8 columns per replicate). This design is used to consider possible field variation.

Observations to be taken

The general reference for data collection is the Standard Evaluation System for Rice (SES, IRRI, 1996, 2014). The following agronomic data are collected:

- Sowing and transplanting dates
- 50% heading date
- Phenotypic acceptability rating at maturity of the test entry in comparison with the check varieties
- Insect pest and disease field reactions
- Per cent lodging incidence
- Any unusual events/circumstances affecting the trials
- Yield component analysis (optional)
- Eight random plants per plot at maturity are sampled for the following parameters:
  - » Plant height (cm) is measured from the base of the plant to the tip of the longest panicle, excluding awns if any.
  - » Number of productive tillers is taken by counting the number of panicle-bearing tillers.
  - » Plot yield (g) and moisture content (% MC) per plot is taken from a harvest area of 13 rows x 19 hills = 247 hills equivalent to $9.88m^2$, sundried and cleaned.
  - » In case of missing hills adjust the plot yield using the formula:

$$\textit{Corrected plot yield} = \frac{\textit{weight of harvest (g) from plot}}{\textit{number of hills harvested}} \times \textit{Total number of hills in harvest area}$$

or

$$\textit{Corrected plot yield} = \frac{\textit{weight of harvest (g) from plot}}{\textit{actual area harvested}} \times \textit{harvest area of normal plot } (m^2)$$

- Adjusted grain yield at 14% MC is obtained using the following formula:

$$\textit{Adjusted grain yield} = \textit{corrected plot yield (g)} \times \frac{\textit{100-MC}}{\textit{86}}$$

- Express yield in kg/ha at 14% MC by using the formula:

$$\textit{Grain yield (kg/ha)} = \frac{\textit{Adjusted grain yield (g)}}{\textit{Harvest area of normal plot } (m^2)} \times \frac{10{,}000m^2}{\textit{1 hectare}} \times \frac{\textit{1kg}}{\textit{1000g}}$$

As part of the regulatory varietal release process, the promising rice lines developed and selected by rice breeders from different agro-ecosystems and climatic conditions, undergo the last stage of selection in a nationwide selection/testing scheme through the NCT. It is the last stage of selection to test the adaptability of the elite lines that would potentially become new varieties. The overall process flow is shown in Figure 13.1. The achievement of the NCT project has resulted in quantitative and qualitative improvement of our rice varieties. The multi-location, interdisciplinary testing permitted the screening and evaluation of varieties that satisfy the diverse and stringent requirements of the different agro-ecological zones of our country. The scheme also enhanced yield stability, provided broad spectrum of pest resistance, catered to the whims of the now-sensitive taste palate of the Filipinos, maintained biodiversity and provided solutions to location-specific on-farm problems. It is implemented by the members of the Rice Technical Working Group RTWG) through the Rice Varietal Improvement Group (RVIG).

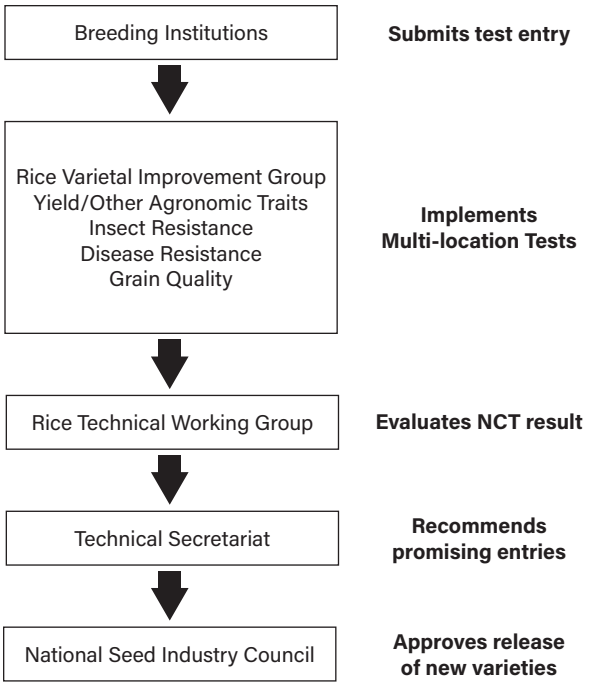

Figure 13.1. Overall process flow for rice of National Cooperative Testing.

Purpose

Breeders are generally concerned on the yield level and stability of the varieties about to be released. Two factors determine the suitability of any breeding line being tested. Yield level is a function of yield potential as determined by the traits incorporated during its development while stability depends on the capacity of the cultivar or line to react or respond to prevailing environment. Both traits can be measured in adaptation trials conducted in a wide range of environment. Lines that are stable in wide range of environment are considered widely adaptable, while those lines performing exceedingly high in specific highly favorable condition but very poor in inferior condition are narrowly adaptable lines. The non-adaptive lines are potentials for location-specific varietal releases while the highly adaptive lines are for national release. For stability, the amount of fluctuation or deviation in the expression of yield of a certain genotype can be measured by obtaining the line's yield performance in several locations and seasons.

The main purpose leads to the identification of the best breeding line for recommendation to the NSIC varietal release.

General Methodology

All NCT sites are generally among the major rice growing areas and from diverse agro-climatic types (Modified Corona Classification and GIS-based agro-climatic zones (Figure 13.2) and/or nature and extent of abiotic stresses and major river basins (Figure 13.3). Implementers follow the standard protocol stated in the third edition of the NCT Manual for Rice. It describes the important guidelines from the nomination of breeding line entries up to the recommendation to the Technical Secretariat, which provides complete information on how the tests were conducted including the criteria for variety recommendation and release. The group is

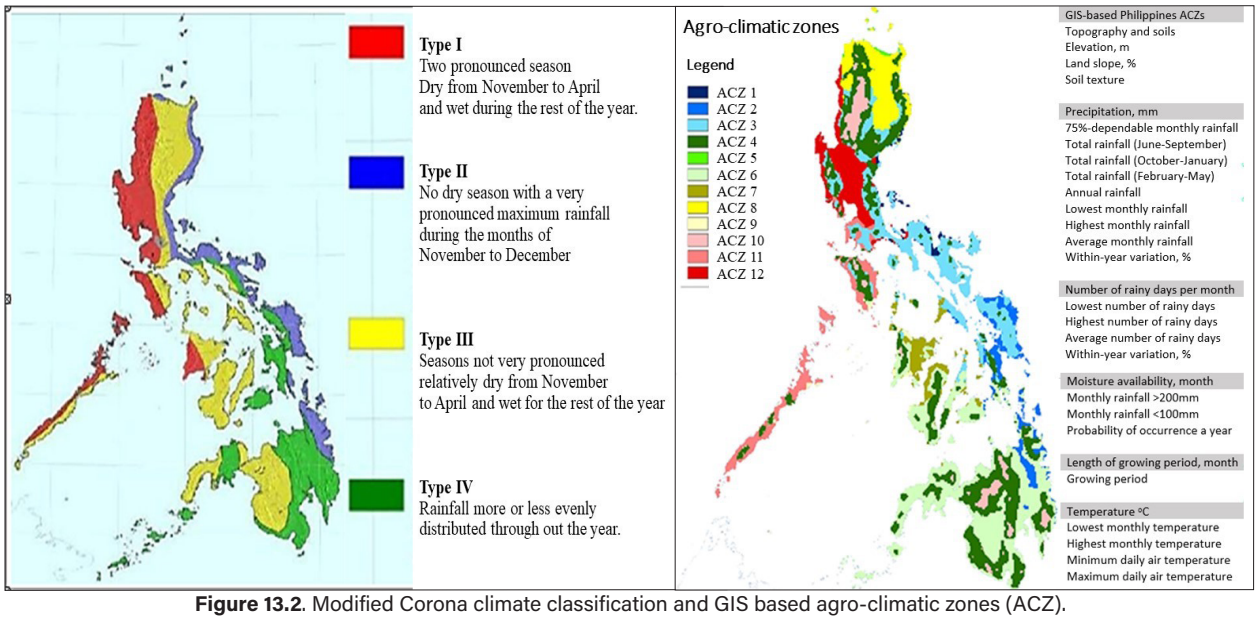

Figure 13.2. Modified Corona climate classification and GIS based agro-climatic zones (ACZ).

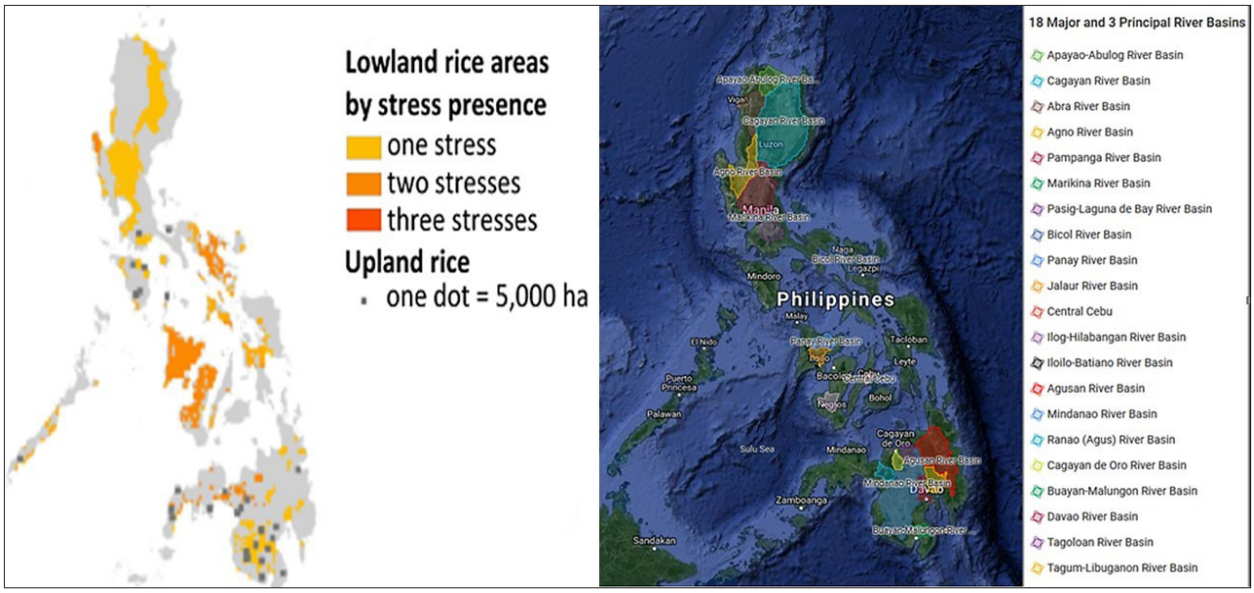

Figure 13.3. Philippine areas affected by abiotic stresses (salinity, drought, and submergence) and major river basins considered in the classification of the major rice-producing areas.

composed of breeders, agronomists, crop protectionists, economists, rice chemists, food scientists, students, farmers, and stakeholders from public and private institutes collectively known as the NCT Network.

Data to be gathered

All parameters stated in the NCT Manual for Rice are collected. These included the field performance tests including yield and yield components, other agronomic traits, reactions to biotic and abiotic stresses, and grain quality traits.

Updated standard criteria for variety release

Table 13.1 outlines the updated standard criteria for yield, biotic and abiotic stresses and grain quality of elite lines with potential for recommendation to the NSIC Technical Secretariat. Comparison of the performance of the test entries is based on the dynamic check in every ecosystem-based trials. The principle of "moving target" shall be applied, as better check varieties become available. During transition to new sets of checks, however, both the old and new checks will be used at the same for one wet and one dry season.

Table 13.1. Standard criteria for yield, biotic and abiotic stresses, and grain quality of elite lines with potential for commercialization.

No.	Ecosystem/ Evaluation Component	Grain Yield Criteria	Specific Criteria
1	IL-TPR	GY ≥5% against best performing inbred checks with mean yield of 5.4t/ha and above	
2	IL-DSR		
3	MAT		
4	Hybrid	GY ≥5% against best performing hybrid check with mean yield of 6.1t/ha and above	
5	Specialty rice	GY ≥5% against best performing inbred checks; desired quality at least comparable than corresponding check variety	
	Aromatic		
	Glutinous		
	Pigmented		
	Japonica type		
	Basmati type		
	Micronutrient-dense		
	Rice for industrial purposes		
6	Abiotic stress	GY ≥5% against best performing inbred checks	
	Salinity		Salt injury assessment relative to tolerant and intolerant checks and level of stress imposed
	High temperature		% fertility under heat stress should be higher than N22
	Submergence: stagnant water		≥70% survival when tolerant check incurred highest survival rate under stress; ≥75% survival rate at maturity when tolerant check incurred lower survival rate than some entries
	Submergence: flashflood-prone		
	Low temperature		Higher levels of tolerance and better resistance to blast under stress
7	Biotech & EDL`s	GY and other traits of EDL ≥ relative to original variety; ≥95% similarity of EDL molecular marker profile to original variety	
8	TRVs (Upl, IL)	GY ≥5% against best performing inbred checks	
9	Upland		
10	Biotic stress	Evaluated based on appropriate corresponding stress and infection indices based on IRRI SES 4th edition (See guidelines)	
	Insect pest		
	Diseases		
11	Grain quality (milling recovery, physical attributes, physicochemical properties, cooking parameters, sensory quality)	Comprehensive evaluation of GQ aspects by RCFSD (e.g., Long and slender grains, ≥3 score for uncooked rice samples, ≥75% acceptability rating for cooked rice samples	

CHAPTER 14

Participatory Variety Selection

Norvie L. Manigbas, Teresita H. Borromeo, Danilo J. Lalican, and Nenita V. Desamero

A more farm-oriented plant breeding program can help improve efficiency in varietal development and often increases on-farm varietal diversity. Participatory varietal selection (PVS) is an informal approach in varietal improvement involving farmers in the evaluation and selection of rice genotypes suitable in their location. It is farmer-managed with less researcher-involvement; thus, portraying an actual farmer's field environment. Selection is based on farmer's preference. Farmers assess all the traits are important to them and test the adaptability of the breeding lines in their own farm. In this way, the best rice genotypes can be selected in location-specific environments.

Figure 14.1. Participatory variety selection in the farmers' field.

Conditions that warrant use

PVS is a simple and more direct way of using multiple traits to assess the value of a variety to farmers. PVS is limited to the testing of finished varieties and breeding lines whereby farmers assess all the traits they consider important. Before PVS, breeding lines should have undergone insect pests and diseases evaluation and other important tests so that farmers will only choose among the best entries. Farmers assess all of the traits inherent to the rice genotypes and compare the traits. PVS has been successfully applied in rice (Manzanilla et al., 2011; Dorward et al., 2007; Joshi and Whitcombe, 2002). This approach of participatory variety selection ensures farmers' participation of farmers in growing, testing, and selecting new rice breeding lines for location-specific recommendation (Figure 14.1).

All PVS employ two trials in the farmers' field. The main trials are conducted before the "baby" trials and are usually conducted by the researcher with the farmer(s). Farmer runs the trial for one or two test entries selected from the main trial. These tests have been greatly successful when used in marginal areas with low resource farmers (Whitcombe, 2005). Several data indicate that when the differences between selection environment and target environment are large, genotype by environment interaction effects are generated. Thus, the performing lines in the selection environment may perform poorly in the target environment and vice versa (Ceccarelli, 1989).

Farmer's and breeder's responsibility

PVS is a farmer-managed with minimal researcher or breeder involvement; thus, portraying an actual farmer's field environment. However, the farmer and the breeder are responsible of ensuring that the test entries or breeding materials are handled appropriately to maintain the purity of the genotypes being tested. Constant removal of off-types in the farmers' field is done throughout the growing period of the crop. Farmer

must monitor occurrences of insect pests and diseases and report these to the breeder.

Number and types of entries

Large number of entries is difficult to handle especially under farmers' field conditions. The minimum number of entries should be 15 and not more than 20 including 1 or 2 check varieties. The number of replications is the number of farmers handling the main and baby trial. The type of breeding lines entered in the PVS is dependent on the ecosystem under test. PVS is not only limited to stress environments but can be performed under transplanted or direct wet-seeded rice culture under irrigated lowland system.

Plot size

Plot size varies depending on the actual farm conditions but the minimum size is 10m x 10m using 20 x 20cm plant spacing at three seedlings per hill when transplanted. Direct wet-seeded rice system uses pre-germinated seeds with 25cm spacing between straight rows at 40-50kg per hectare seeding rate in straight rows.

Crop management

The crop management follows the farmers' practice of rice production in the area.

Observations

Before harvest, PVS is conducted through a farm walk or field day by the farmers, researchers, agriculture technicians, and municipal agriculture office in the community and neighboring village. PVS evaluation is based on preference analysis using the procedure developed by IRRI with modification. During the farm walk, the farmers are briefed about the PVS and are requested to evaluate the different breeding lines and varieties. The identity of the breeding lines and check varieties are not shown until the final tally. Each farmer is given three shipping tags with labels marked with '√' and 'x'. Larger ones (or blue if color coded) are for the males and smaller (or pink if color coded) for the women. Each of the tags with check label are dropped/voted in the paper bag attached to each entry (breeding line/variety). Farmer places '√' on his/her preferred breeding line and "x" for not preferred. The top three best and worst breeding lines are ranked after the tags are counted. Discussion and open forum among the breeders, farmers, and other participating stakeholders are conducted after ranking the genotypes, focusing on the reasons of selecting and rejecting the breeding lines or varieties. Other relevant topics which are of interest to the participants are also discussed. All information must be recorded in detail.

Field data are gathered by researchers at harvest. Two plots of 2m x 5m are taken as sample unit for yield. Tiller number and plant height are gathered from 10 random samples. Plant height is taken from the base of the plant up to the longest tip of the panicle. PVS can also be conducted after harvest to evaluate the breeding materials for milling recovery and eating quality. Two kilograms of *palay* are milled by the farmer and milling recovery is noted. Grain and eating quality are tested by a panel of farmers in the community. Statistical analysis is done after all the data are encoded. A preference rating can be generated for each breeding line by expressing the number of tags for that line as a proportion of the total number of tags placed by the farmers. It is expressed as:

$$PS = \frac{t}{N};$$

Where: PS is the preference score, t is the number of '√' or 'x' labels cast for the line/variety and N is the total number of '√' and 'x' labels cast.

CHAPTER 15

Plant Variety Protection and Plant Breeders' Rights

Teresita H. Borromeo, Jerry C. Serapion, and Alex T. Rigor

Plant variety protection (PVP) is granted in recognition of the intellectual property rights of breeders over the plant varieties they developed. The granting of PVP is in compliance with the country's obligation to the World Trade Organization on Trade Related Intellectual Property Rights or TRIPs. The PVP grants the breeders the exclusive rights to commercialize the protected variety for a specified period. With the enactment of Republic Act No. 9168 (Plant Variety Protection Act) in June 7, 2002, rice breeders can apply for protection over their varieties. It is a requirement that the variety should satisfy the criteria of newness, distinctness, uniformity, and stability. A PVP after being granted remains in effect for 20 years.

Need for variety protection

Normally, no one can retain an exclusive right over a product or service since it curbs free competition (Art. 186 of the Revised Penal Code of the Philippines, Republic Act 3815). However, as a reward for creativity and inventiveness, the state grants legal monopoly for a certain period to natural and juridical persons (e.g., private companies) in the form of patent, PVP, utility model, copyright, etc. in return for the full disclosure of the said intellectual property. In such manner, inventiveness and creativity is further encouraged; thereby, advancing society. After the extinction of the protection period, this property becomes part of the public domain.

Rice breeding is a long and arduous task. It usually takes four to seven years to develop a rice variety from initial hybridization. To encourage ingenuity in this arena, securing the rights of the breeders over their varieties not only for their career fulfillment but as an initiative to help the country attain food security.

Based on Section 36 of Republic Act (RA) No. 9168, a holder of a Certificate of Plant Variety Protection (CPVP) is entitled to the following acts: (1) production or reproduction; (2) conditioning for the purpose of propagation; (3) offering for sale; (4) selling or other marketing; (5) exporting; (6) importing; and (7) stocking for any purpose mentioned above.

Some breeders are hesitant to apply for PVP because they use public funds to come up with the current breed. The idea of applying for proprietary rights over their variety does not appeal to their conscience because they think it should be the public at large who should benefit. Seductive as the argument may seem, it defeats the reason it espouses when it emerged to the business world. As long as a variety has some commercial value, interested entities and parties would always take a leap to register it. They could always argue that they produce the variety independently, a provision that is allowed for in the PVP law (Section 20). Others could also use the variety to develop an essentially-derived variety then register it. Upon grant of CPVP, the holder could bar the original breeder from propagating the material commercially.

Had the original breeder applied for a PVP, he/she would have been in a better position to dispose the variety in a way that is beneficial for the greater public.

Benefits of PVP

- Brings recognition and personal satisfaction to the breeder
- Encourages research and ensures continuing effort in breeding
- Encourages investments and increase commercial activity

Application proper

PhilRice employee who wishes to apply for PVP certificate shall coordinate with the Institute's Intellectual Property Management Office (IPMO). IPMO is responsible for prosecuting any Intellectual Property applications for the institute.

Some steps and requirements for the PVP application of for rice are presented. For a more in-depth guide, please consult the Implementing Rules and Regulation of Republic Act (RA) No. 9168, and the "Guidelines for the Conduct of Tests for Distinctness, Uniformity, and Stability of Rice (*Oryza sativa* L.)" published by the Plant Variety Protection Office (PVPO).

Minimum Requirements for Application.

a) One copy of the completed application form signed by, or on behalf, of the applicant, together with all the required exhibits;

b) Official receipt to prove payment of the prescribed, non-refundable application fee. Application fee shall be paid in cash if filing is done personally; otherwise, it shall be paid in postal money order;

c) Samples of propagating materials, which are the subject of the application. The minimum seed quantity to be supplied by the applicant should be 500g. For hybrid varieties, an additional 500g of seeds of each component (parental lines, A, B & R) should be submitted, if requested. The seeds should meet the minimum requirements of at least 85% for germination and 1.1% analytical purity and health, and moisture content not greater than 14%. Should the seeds be stored, the germination capacity should be as high as possible and should be stated by the applicant. The seeds should not have undergone any treatment, which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

If requested by the competent authority, at least 50 panicles should also be submitted. The panicles should be well developed and not affected by any insect pest or disease. They should contain a sufficient number of viable seeds to establish a satisfactory row of plants for observation.

Contents of the Application Form. The application form for a CPVP shall contain the following information:

- Name of the applicant/breeder
- Contact details of the applicant/breeder in the Philippines, including address, telephone number, fax number, and electronic mail address
- Description of the variety and particulars of the variety bred, including particulars of its characteristics
- Variety denomination
- If the applicant is not the actual breeder, a statement of the basis of his/her right to file the application
- As applicable, a statement expressly claiming priority under an application for a CPVP previously filed by the applicant in another country, which by treaty, convention or law affords similar privileges to Filipino citizens [Sec. 24 and 25]
- Statement of newness

Exhibits. The application must include the following:

- Exhibit A - detailed origin and breeding history of the variety, including the source of the germplasm and the results of other plant variety tests or trials that have already been done on the Variety. [Sec. 28]
- Exhibit B - statement of distinctness, uniformity, and stability
- Exhibit C - statement of ownership
- Exhibit D - photographs, drawings or plant specimens and other additional information
- Such other exhibits as the Board may require from time to time.

Varieties and selection to include

The underlying assumption for PVP application is that we have a variety with some commercial value given that the application itself involves some financial investment. Therefore, the breeding objective is usually directed to a program that is geared towards satisfying a commercial goal. Selection usually targets the development of a variety with high yield potential and customer satisfaction. It could also address the adverse environments like upland, saline, and drought conditions.

Rice varieties are usually also cultivated because of their suitability for some industrial applications like rice-based products. In rice wine, for example, some varieties are preferred because it gives the rice wine a distinct taste or appearance.

Other proof of ownership other than PVP

These proofs of ownership are usually proprietary information that run the gamut from government certificate to intellectual property information. These include:

1. Certificate of NSIC Registration
   The National Seed Industry Council (NSIC) is established through RA No. 7308, which is an "Act to Promote and Develop the Seed Industry in the Philippines. NSIC is authorized to approve the release of new varieties.

2. Scientific publications
   The publication should describe the variety in sufficient detail that leaves no doubt as to the question of ownership. It only shows ownership of the breeder but it does not preclude others from registering similar variety on the claim that they developed it independently. To be legally enforceable, the breeder has to immediately apply for PVP.

3. Patent
   This is an exclusive right granted by the government to an inventor of a product or process which is new, non-obvious and industrially applicable. This monopoly is valid for 20 years. In plant breeding, DNA markers or sequences used for tagging important traits or identifying a variety could be patented. A plant breeding method that allows you to produce varieties with certain characteristics is also patentable. In principle and effect, a patent on the process grants ownership on the product, which is the variety in this case. Gadgets and methods used in plant breeding like a screening method could also be patented.

4. Utility model
   This is sometimes called petty patent because of its similarity to patent except that it does not satisfy the non-obviousness criteria. Therefore, patent applications that failed to pass due to lack of inventive step could be applied for utility model. This exclusive right is enforceable for seven years.

5. Copyright
   This refers to the rights of authors to control the reproduction, sale, dissemination, copying, and publishing of their works. It is literally "the right to copy" an original creation. This right is valid in the course of an author's lifetime and until 50 years after his death. Copyrighted and published description of varieties aid in the establishment of variety ownership. However, the legal right over the variety is only enforceable once a Certificate of Plant Variety Protection is granted (Figure 15.1).

Rights of breeders

- Entitlements of a Certificate of Plant Variety Protection (CPVP) over the protected variety
- Production or reproduction
- Selling or other means of marketing
- Exporting or importing
- Storing for any purpose mentioned above
- The holder may give authorization to another party to perform these acts specifying the conditions and limitations.

DUS (Distinctness, Uniformity and Stability) plot with a PVP examiner

Republic of the Philippines
Department of Agriculture
BUREAU OF PLANT INDUSTRY
Plant Variety Protection Office

PVPO

CERTIFICATE OF PLANT VARIETY PROTECTION

issued to

'IVC-31-1-10'

Developed by Philippine Rice Research Institute with

CPVP Number: PVPO 19-10/39-0273

has demonstrated in compliance with **Republic Act 9168** also known as the **"Philippine Plant Variety Protection Act of 2002"** and other pertinent Laws, Rules and Regulations of the Philippines.

Term of Protection: Twenty (20) years

Issued On: October 21, 2019
Expired On: October 21, 2039

GEORGE Y. EULASTE, PhD.
Director, Bureau of Plant Industry and
Acting Registrar, Plant Variety Protection Office

NV Desanero & RJD Buluran

Sample PVP (Plant Variety Protection) certificate

Figure 15.1. Plant Variety Protection for rice.

CHAPTER 16

Seed Production and Seed Classes

Errol V. Santiago†

Use of certified seed alone without any other intervention can contribute to 10% increase in grain yield. Farmers are more productive and inputs are more efficiently used with the high-quality seed of high yielding varieties. Varieties have been released for cultivation under irrigated, rainfed and upland growing environments. These varieties were bred and tested for yield performance, pest and disease resistance, tolerance to adverse environments, and grain quality. In the Philippines, only varieties approved by the Department of Agriculture (DA) are named and released as commercial varieties.

Basic seeds are produced by harvesting five panicles from true to type plants in the source population and growing them in separate progeny plots. Removal of volunteer crops is practiced at all stages of crop growth. Identical plots that are characteristic of the variety are harvested and processed as basic seed. Breeder seeds are used to raise the basic seed source population.

Seed Classes

DA recognizes four seed classes: breeder, foundation, registered, and certified. Each of the seed classes has clearly defined genetic and physical purity requirements for seed certification purposes (Table 16.2). Philippine rules and regulations provide that only varieties approved and released by the DA are eligible for certification. Seed production underwent field inspection and laboratory certification by the Bureau of Plant Industry (BPI) to ensure conformity with approved standards.

1. **Breeder seed**
   This is raised from basic seed stock and the source for the initial increase of seed of NSIC released varieties. It is the seed source for producing foundation seeds. Breeder seed is the purest among the recognized seed classes. With a white tag indicating its classification, this seed class is produced and controlled by the institutions responsible for breeding or developing the variety.

2. **Foundation Seed**
   Foundation seed is the progeny of breeder seed and is handled meticulously to maintain specific genetic purity and identity. This seed class is the base for production of registered seed used by seed growers. A red tag indicates its class.

3. **Registered Seed**
   Registered seed is produced from foundation seed. This green-tagged seed class is grown by accredited seed growers.

4. **Certified seed**
   Certified seed is produced from registered seed. Certified seeds are sold to farmers for commercial rice production. Its authenticity is guaranteed by a blue tag.

SEED PRODUCTION

A. Land Preparation

If the production area was planted to rice in the previous season, flash irrigate the field to allow drop seeds to germinate and to minimize volunteer plants or off types in the seed crop. Plow the field 3 - 4 weeks before the scheduled date of transplanting to allow weeds and stubbles to decompose. Plowing is followed by three harrowing (Figure 16.1). The last harrowing levelled the field and is done immediately before transplanting. Field for seed production must be uniformly fertile with available irrigation and drainage system. Avoid high and low spots to minimize uneven crop stand.

B. Seedbed preparation, seed soaking, incubation, and seeding

Seedbed should be located in an area free from dropped seeds and well protected from rodents and birds. Raised seed bed is used to grow seedlings for breeder seed production. Dry seeds are sown in shallow furrows and covered with fine dry soil or saw dust or coir dust. Beds are 80cm wide and the furrows 10cm apart. About 4 - 5g seed are sown in each furrow. Fertilizers are applied to the seed bed at the rate of 120-60-60kg NPK/ha during the dry season and 90-60-60 during wet season. Fertilizer is applied 10 days after seeding.

Soak seeds in clean water for 24 hours and incubate seeds for 24 - 36h in half-filled sacks placed in shaded area (Figure 16.2) (except for breeder seeds, which is soaked in panicle). For foundation, registered, and certified seed production, seeds are pre-germinated and sown in a wet bed (Figure 16.3) at the rate of 50g seed/m^2 (Figure 16.4). Seedlings are pulled and transplanted at 21 DAS.

Transplanting the Seed Production Plots

For breeder seed production, seedlings are transplanted at 20cm between hills and 25cm between rows during dry season and 25cm x 25cm during the wet season. Only one seedling is planted per hill (Figure 16.5). For foundation, registered, and certified seeds, planting distance is 20cm x 25cm in both dry and wet season. Missing hills are filled not later than 5 days for uniform maturity (Figure 16.6). Missing hills can cause uneven growth of the crop making rouging and detection of off types difficult. Planting should be in straight line both ways. Over fertilizing with nitrogen can cause lodging;

Figure 16.2. Seeds are soaked for 24h in container with clean water.

Figure 16.1. Harrowing of experimental fields after plowing.

Figure 16.3. Seedbed preparation for breeder seed.

Figure 16.4. Seeding.

Figure 16.5. Transplanting using the two straight methods.

Figure 16.6. Replanting should be done not later than 5 days after transplanting.

thus, rouging is not possible. Lodged crop also produce discolored seed with low germination.

Irrigation

Irrigate the field at 2 - 3cm depth 3 days after transplanting (Figure 16.7). Too much water after transplanting can cause missing hills or can delay seedling development. Water depth can be increased as the plants develop. Water should be withdrawn 2 weeks before harvest.

Fertilizer Management

High nitrogen application increases yield; however, it can also cause lodging in susceptible varieties. Nitrogen should be managed well to get optimum yield and seed quality. Lodging reduces yield and can cause grain deterioration. Detection of volunteer crops and off types is also difficult when the crop has lodged. Skilled worker should be assigned to fertilizer application to ensure uniform crop growth (Figure 16.8). The recommended rate for dry season is 120-60-60kg NPK/ha and 90-60-60kg NPK/ha for the wet season. Follow the recommended rate of split fertilizer application.

Rouging

Rouging is the removal of off-types or mixtures (Figure 16.9). This operation should be done any time after transplanting until maturity. Varietal mixtures should be removed as soon as these are detected. Off-types should be cut low at the base to prevent regrowth. This is one

Figure 16.7. Irrigate the field 3 days after transplanting.

Figure 16.8. Basal fertilizer application.

Figure 16.9. Removal of off-types or rouging.

of the most important operations in seed production to maintain varietal purity. Rouging can be based on the following agro-morphological differences: plant height; leaf color, width, length and angle; pigmentation; tillering habit; heading date; grain size, shape and color; and awning habit. Officials of the seed certifying agency may visit the seed production area at all crop stages.

Harvesting

Harvest when 80 - 85% of the grains in the panicle are mature (Figure 16.10). Cutting height should be appropriate to is the requirement of the thresher. Harvests are piled as they are cut then hauled to the threshing area. In breeder seed production, harvests are piled on top of plastic lining to avoid possible mixture. Thresher should be thoroughly cleaned off seeds to avoid mechanical mixture. Use of air blower is recommended for cleaning threshing machines (Figure 16.11). The first 5kg are discarded to further ensure removal of possible remaining mixtures sticking to the thresher. Only new sacks are used to avoid contamination (Figure 16.12). Proper labeling should be observed. Label should contain variety name, plot number, person responsible, seed class, and harvest date. Sacks should have labels inside and outside.

Drying

Drying could be done on concrete pavements lined with nylon nets (Figure 16.13) or artificial dryer (Figure 16.14). Inspect all corners and spaces of the drying facility to ensure cleanliness and avoid mixture. Dry the harvest one variety at a time to avoid mixing,

Figure 16.10. Harvesting.

Figure 16.12. Use new sacks during threshing.

Figure 16.11. Thorough cleaning of thresher.

Figure 16.13. Drying in pavements with nylon net.

Figure 16.14. Mechanical drying.

mishandling, and mislabeling. Dry seeds immediately after harvesting. Seeds are damaged when stored for one or two days without drying. Fresh seeds that are not immediately dried will discolor and have low viability and vigor. Seeds harvested with very high moisture content should be air dried first to 18% moisture content before gradually applying heat until 40°C is attained. Lower the seed moisture content to 12 - 14% moisture for safe packaging and storage.

Seed Cleaning and Bagging

Dried seeds could be cleaned using seed cleaners or blowers to separate empty, light, and diseased seeds; weed seeds; soil particles; and chaff. Breeder seeds are packed in 5kg plastic bags while foundation and registered seeds are packed in 10 and 20kg. Certified seeds are in 40kg bags. Labels should be prominently written in the seed bags (Figure 16.15).

Figure 16.15. New sacks are properly labelled with the variety name.

Seed Certification

Authorized personnel of the certifying agency must have an all-time access to the seeds for sampling that will be used in the laboratory testing and seed bag tagging.

Seed Storage

Seeds can be safely stored on wooden or plastic palettes (Figure 16.16). Seed storage rooms and palettes must be thoroughly cleaned before new seed lots are stored. Seed storage rooms must be well ventilated and free from birds, bats, rodents, and storage insect pests. Breeder seeds should be stored in a cold room. Licensed professional fumigators for warehouses and storage facilities could be employed to eliminate pest problems. A rule of thumb for seed is that its life is halved for every 1% increase in moisture content or a 5^0C increase in storage temperature.

Monitoring Germination on Stored Seeds

It is important to monitor viability and vigor of seeds in storage. Monitoring can start three months after harvest and monthly thereafter for seeds stored under ordinary room conditions. Seeds can remain viable until six months after which, germination declines rapidly.

Figure 16.16. Foundation seed lots are properly stored and placed in pallets.

VARIETIES FOR DISTRIBUTION

Rice varieties for distribution are based on the top five varieties in the technology demonstration trials per province, farmers preferred variety, and the newly-recommended varieties by NSIC.

The characteristics of a rice variety preferred by the seed growers and farmers include:

- Non-seasonal
- Early maturing
- Resistant to lodging
- Resistant to pests and diseases
- High yielding
- High milling recovery
- Good eating quality

Seed Standards in the Philippines

Table 16.1. Field inspection standards in the Philippines.

Factors	Tolerance and Requirements per 100 m²		
	Foundation	Registered	Certified
Other varieties	0	1	2
Red rice	0	0	1
Objectionable weeds	0	5	10

Table 16.2. Official laboratory standards for seed certification in the Philippines.

Factor	Breeder Seed	Foundation Seed	Registered Seed	Certified Seed
Pure seed (%)	99	98	98	98
Other varieties (grains per 500g)	0	2	5	20
Weed and other crop seed (%)	0	0	0.05	0.1
Inert matter (%)	1	2	2	2
Red rice (grains per 500g)	0	0	1	2
Germination (% minimum)	85	85	85	85
Moisture content (%)	14	14	14	14

CHAPTER 17

Other Methods of Creating or Adding Genetic Variability in Breeding Populations

Nenita V. Desamero, Victoria C. Lapitan, Tomas M. Masajo†, Antonio A. Alfonso, Thelma F. Padolina, and Teresita H. Borromeo

A. Induced mutation

Mutation induction has become a proven way of creating variation within a crop variety. It offers the possibility of inducing desired attributes that nature lacks or have been lost during evolution. Without gene for resistance or tolerance to stress available in the gene pool, plant breeders have no obvious alternative but to attempt mutation induction. Induced mutagenesis (either through physical or chemical means) have been proven useful for generating novel alleles for crop improvement and functional gene analysis. New alleles involved in the expression of agronomically important traits are of utmost importance in plant breeding. While a large number of alleles can be generated by spontaneous or experimental mutagenesis, positive identification of useful ones is the greater challenge because of the tedious selection process and the large number of mutant populations that have to be evaluated. Therefore, application of proper protocols for mutagenesis and mutant screening and confirmation is of paramount importance for the success of a mutation project (Figure 17.1).

I. Materials and methods

1. Selection of starting material

In a commercial breeding program, the ultimate objective is to develop new varieties that possess the desired characteristics, e.g,. high yield, excellent tolerance to abiotic and biotic stresses, and desirable quality parameters by carefully producing, evaluating, and advancing breeding materials. As a plant breeding tool, induced mutation can also be utilized as starting materials for mutagenic treatment of commercial varieties, elite or advanced breeding lines, and F_1 and F_2 materials to fulfill the sole objective of developing a new variety. However, if the interest is in identifying the underpinning gene(s) for the mutant traits, a pure and stable genetic line should be used as starting material for mutagenesis. This is because if heterogeneous (e.g., seeds harvested from commercial production fields) or heterozygous lines (e.g., advanced breeding lines) are used, it would be very difficult to determine whether the "mutant allele" has indeed originated from mutagenesis or it just appeared as a result of segregation. In most cases, mature seeds are used in irradiation although there have been few reports on the use of calli.

2. Selection of mutagen and dose

While it has been established that physical mutagens cause more drastic changes than chemical mutagens, which generally result in point mutations such as nucleotide substitutions, there is no general conclusion on which of the methods is more useful and effective in generating important mutations. Drastic changes caused by physical mutagens include deletions, insertions, and translocations of chromosomal fragments of various sizes.

The following general principles may be observed: (1) physical mutagens (e.g., gamma rays) have a higher tendency to produce knock-out and knock-

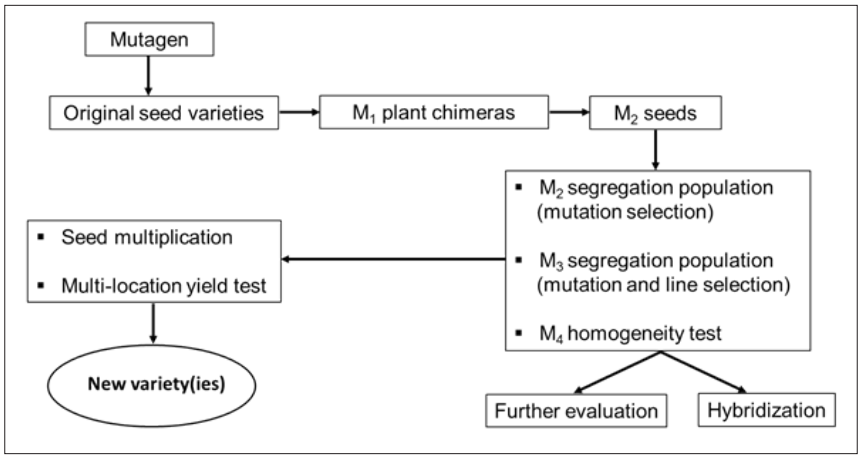

Figure 17.1. Simplified flow of materials in a mutation breeding project.

down mutations than chemical mutagens; and (2) chemical mutagens (e.g., EMS) is more useful in change-of-function mutation. In addition to the type of mutagen, the use of a proper dose is also important. Numerous experiments were already conducted on plant species that helped establish doses for optimal results. These doses result in high mutation rate without causing very high mortality. For such species using particular plant parts, there is no need to perform a dose determination study. However, the researcher may decide to treat seeds with 2 - 3 doses (recommended, 25% lower, and 25% higher than the recommended dose) because there are varietal differences and other factors (e.g., seed moisture, seed viability) that may affect sensitivity to mutagens. The mutated population to be used for large scale mutation screening may be determined at the maturity stage of M_1 plants: the dose that caused fertility (seed-set) reduction of around 50% or more.

3. Number of treated seeds

Seeds are the most commonly used material for mutagenic treatment because of convenience. As in handling other breeding populations, population size for irradiation is important. A small sample will reduce the chance of getting the desired mutation(s) while a sample larger than necessary would take up more resources. A general calculation can be made as a guide in deciding how much seeds to irradiate. For example, if the mutation frequency is 1 per 5,000 cells for the target gene, and if one M_1 seed has four effective cells that would eventually become the germline cell of a panicle, at least 1250 M_1 plants have to be grown into maturity. If the seed germination and field emergence rate is estimated to be 60%, then about 2,100 seeds have to be treated. This is the best scenario for a trait controlled by a recessive mutation. With the decrease of the mutation rate, the M_1 population size needs to be increased. In general, it is recommended to grow more than 1500 M_1 plants in rice mutation projects.

II. Growing and handling M_1 and M_2 populations

1. Growing of M_1 generation

There are two ways to handle M_1 populations in rice. One is to transplant rice seedlings individually and the other is to transplant 3 - 4 seedlings together. Rice seeds can also be broadcasted in the field (directly-seeded). As different tillers could be derived from different effective cells (germline cells) in a seed, these growing methods can produce similar population structures if rice seedlings are not transplanted too densely.

In M_1 generation, plants are expected to have reduced pollen viability; hence, the tendency of out-crossing increases significantly. It is important to protect M_1 plants from out-crossing with pollen of other varieties and from other M_1 mutants because only seeds produced through within-panicle pollination can be homozygous for the recessive mutant allele. Bagging of panicles and temporal isolation with other varieties in the area will help accomplish this target.

It is worthwhile to note that M_1 population should be grown in reasonably good fields to produce sufficient tillers and seeds.

2. Assessment of mutagenic effect

There should be no selection of 'mutants' in M_1 populations, except for the elimination of apparent off-type plants (mixtures, segregants from out-

crossed plants). However, a few dozens of plants should be assessed, particularly for seed set rate, to assure the significant effect of mutagenic treatment. If no significant reduction on seed set is observed, further examination of M_2 population at seedling stage is needed.

3. Harvesting of M_1 plants

Harvesting seeds from M_1 plants depends on how they will be screened for mutants:

- Harvesting of panicles of M_1 plants

About four panicles from each M_1 plant transplanted individually) or six panicles from each hill of rice (transplanted in hills) are harvested from paddy field. The seeds are kept attached to the panicle or they are threshed and stored in bags, keeping seeds from one panicle per bag. These seeds will be grown in panicle-rows in M_2 population, which is good for screening morphological and agronomic mutants.

- Bulk harvesting of M_1 plants

A few seeds are harvested from each panicle to develop a representative M_2 population. This can also be achieved by harvesting all seeds of M_1 plants and then taking a small portion for further growing into M_2 populations. This type is good for traits that will be evaluated in M_3 population based on M_2 plant lines, e.g., abiotic stress tolerance and quality traits.

4. Growing and assessment of M_2 population

M_2 plants should be planted individually to ensure purity of each mutant line. Based on the harvesting method of M_1 plants, M_2 plants are either grown in panicle rows or in bulks. At the seedling stage, it is important to estimate the frequency of chlorophyll deficiency mutants, which is often used as a reference for other traits.

If M_1 plants have quite normal seed set and their M_2 seedlings have very low chlorophyll deficiency mutation frequency, the mutagenic treatment might have been performed improperly; hence, the experiment should be stopped and redone, if necessary.

III. Screening and confirmation of induced mutants

1. When to start screening mutants

In most cases, mutant screening starts with M_2 population. This is good for most qualitatively controlled traits such as morphological characteristics. Putative mutants can also be identified for quantitatively controlled traits such as maturity, plant height and number of tillers. However, because quantitatively controlled traits could be significantly influenced by environmental effects, screening of these mutants often starts in M_3 generation using $M_{2\text{-}3}$ lines (seeds harvested from M_2 plants and grown in rows). $M_{2\text{-}3}$ lines are often used for screening mutants of enhanced tolerance to abiotic and biotic stresses, as well as quality and nutrition traits.

Once a plant or a line is selected for a mutated trait, further analysis should be performed using their progenies (e.g. M_3 or M_4 lines). Very often, a quantitative trait variant (putative mutant) could not be confirmed in their progeny because the observed difference of selected plant/line from the parental line were derived from micro-environmental differences in the field or controlled chambers.

2. Genetic confirmation of induced mutants

The best way to confirm a mutant is to study the genetic basis of the mutated trait. This can be performed by crossing the mutant with its parent and raising F_1 and F_2 populations. Distinct mutant plants should be recovered in F_2 populations because an induced mutant should have the almost identical genetic background to its parent line. Therefore, even quantitative mutation traits can be observed on individual plant basis and there should be distinct segregation of mutant and wild type plants in F_2 populations on the assumption that a single gene is involved. If there is no clear-cut segregation, the selected putative mutant might have emerged from mixture or out-crossing with other genotypes; hence, not a true mutant.

3. Molecular confirmation of induced mutants

A true mutant has almost identical genetic make-up to its parent line, except the selected mutation and some undetected background mutations. By using microsatellite markers, a true mutant can be easily distinguished from a contaminant arising from out-crossing or mixtures because the former often has identical SSR haplotype to its parent while a contaminant often has SSR patterns different from the wild type by 5% or more. Other available molecular markers such as SNPs (single nucleotide polymorphic markers) and techniques such as genotyping by sequencing, are other options to genetically characterize and confirm the mutants.

B. Anther culture

The anther culture (AC) technology is employed in rice breeding as a tool to expedite generation and development of stable breeding lines, resulting in the shortening of the breeding cycle, and rapid eventual release of new improved varieties.

A rice pollen grain within an anther contains one set of chromosomes (n=12), which is half dose (haploid) of the rice genome with diploid chromosome number 2n=24. The haploid chromosome set spontaneously doubles during AC through endomitosis, generating homozygous genotypes, termed as doubled haploids (nn), in just one generation. Fixing genotypes through culture of anthers from heterozygous sources such as the F1 progenies of the crosses between genetically diverse genotypes, is an innovative strategy of expediting and shortening the breeding cycle. The doubling of the haploid genome allows the early expression of recessive traits, which are suppressed, masked, or undetected during the early routine selection through classical plant breeding approach such as the pedigree method. The selection efficiency is likewise increased, especially when dominance variation is significant.

Integrating AC in the breeding program reduces the duration of generating fixed lines by at least half the time required for conventional hybridization and selection through pedigree method (Figure 17.2). It should be noted that the response to AC is highly dependent on the genotype. Thus, it is best to scan the breeding materials for AC response to achieve significant benefit from AC. In general, Japonicas are very responsive while *indicas* respond the least to AC. Genotypes with medium/intermediate sized grains were also reported to respond better to AC.

The rice variety NSIC Rc 130 (Tubigan 3), approved by the National Seed Industry Council (NSIC) for commercial cultivation on September 2, 2004, was the first variety developed by PhilRice from cultured anthers of the traditional variety Wagwag.

1. Material

Laboratory

- Healthy rice plants as source of anthers - F_1 and F_2 progenies of single, double, three-way cross and backcrosses
- Laminar-flow hood
- Muslin and cheese cloth
- 70% Ethanol
- Commercial bleach (2.5% w/v NaOCl)
- Scissors, stainless-steel, fine-tip, sterile
- Petri dishes
- Erlenmeyer flask (50mL)
- Test tube (32mm x 200mm)
- Double-distilled water (ddH_2O), sterile
- Forceps, stainless-steel, fine-tip, sterile
- Scalpels (No. 3883-B20) with No. 10 stainless steel blades (No. 3383-B35), sterile
- Macro and micro-elements, vitamins, and organic substances for MS (Murashige and Skoog, 1962) and for N6 (Chen et al, 1975) media

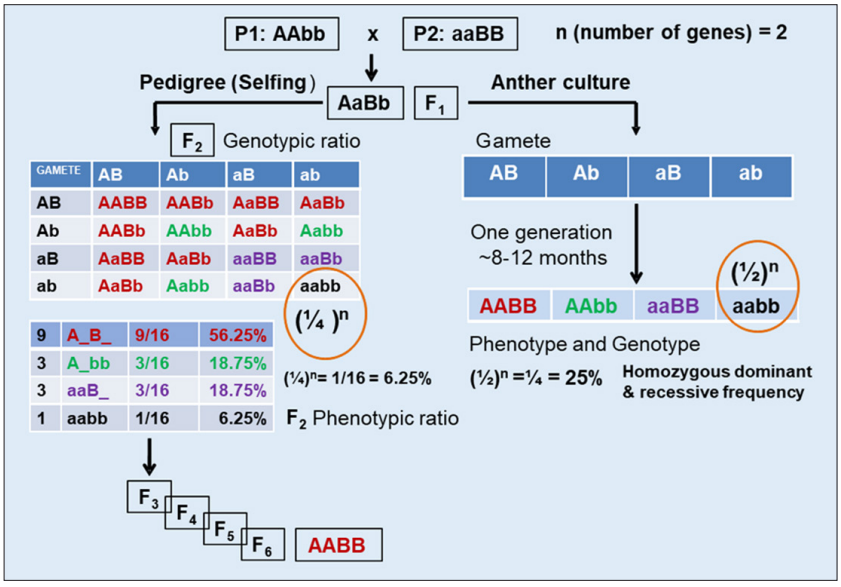

Figure 17.2. Genetics of anther culture compared with pedigree method. (Note: Consider two genes with two alleles each, each gene controlling a specific trait. With anther culture, homozygosity is achieved in just one generation, and at higher frequency, over pedigree method, which require at least six generations for genotype fixation).

Screenhouse

- Sterilized garden soil and carbonized rice hull or saw dust
- Plastic cups (12oz)

2. Culture Medium

The chemical composition of the culture medium is an important factor governing callus induction, plant regeneration and growth of regenerants from cultured anthers. The basal salt compositions for callus induction, plant regeneration and rooting medium are presented in Table 17.1. Stock solutions are prepared to save media preparation time because it reduces the number of repetitive operations and save storage space. The amount of macro- and micronutrients, chelating agents, vitamins, and organic substances for N6, M8, and MS media and the different plant growth regulators in the stock solutions are presented in Table 17.2.

Follow the "checklist method" in preparing solutions and culture media. In this technique, a checklist of all the components of a solution or culture medium and the corresponding amount or volume is prepared and used as guide. The checklist must also include preparation date, volume of medium to prepare, name of the person preparing the medium or solution, and remarks. Each item in the list is marked checked upon adding in

Table 17.1. Rice anther culture basal medium composition for callus induction and plant regeneration.

Component	Callus Induction		Regeneration
	N6 [a] (mg/L)	M8 [b] (mg/L)	MS [c] (mg/L)
Macro-elements			
NH_4NO_3	-	-	1650.0
$(NH_4)_2SO_4$	463.0	231.0	-
KNO_3	2830.0	2830.0	1900.0
$CaCl_2.2H_20$	166.0	166.0	440.0
$MgSO_4.7H_20$	185.0	370.0	370.0
KH_2PO_4	400.0	641.0	170.0
Micro-elements			
$MnSO_4.H_20$	3.335	8.485	16.9
H_3BO_3	1.60	6.2	6.2
$ZnSO_4.7H_20$	1.50	4.3	8.6
KI	0.83	0.83	0.83
$Na_2MoO_4.2H_20$	0.25*	0.25	0.25
$CuSO_4.5H_20$	0.025*	0.025	0.025
$CoCl_2.6H_20$	0.025*	0.025	0.025
Fe-EDTA chelating agent			
$FeSO_4.7H_20$	27.85	55.7	27.85
Na_2EDTA	37.26	74.5	37.26
Vitamins and Organic Substances			
Myo-inositol	100.0*	100.0*	100.0
Glycine	2.0	10.0	2.0
Nicotinic Acid	0.5	3.0	0.5
Pyridoxine.HCl	0.5	2.5	0.5
Thiamine.HCl	0.5*	5.0	0.1
DL-Alanine	-	10.0	-

*Modified at PhilRice tissue culture laboratory
[a] Chu et al, 1975
[b] Mei et al., 1988
[c] Murashige and Skoog, 1962

Table 17.2. Amount of macro and micronutrients, chelating agents, vitamins and organic substances in stock solution for N6, M8, and MS media.

Component	Amount/L ddH_2O) (g)[a]		
	N6	M8	MS
Macro-nutrients (10X)			
NH_4NO_3			16.5
$(NH_4)_2SO_4$	4.63	2.31	
KNO_3	28.30	28.3	19.0
$CaCl_2.2H_20$	1.66	1.66	4.4
$MgSO_4.7H_20$	1.85	3.7	3.7
KH_2PO_4	4.00	6.41	1.7
Micro-nutrients A (1,000X)			
$MnSO_4.H_20$	3.335	8.485	16.9
H_3BO_3	1.60	6.20	6.2
$ZnSO_4.7H_20$	1.50	4.30	8.6
KI	0.83	0.83	0.83
Micro-nutrients B (10,000X)			
$Na_2MoO_4.2H_20$	2.50	2.5	2.5
$CuSO_4.5H_20$	0.25	0.25	0.025
$CoCl_2.6H_20$	0.25	0.25	0.025
Fe-EDTA (100X)[b]			
$FeSO_4.7H_20$	2.785	5.57	2.785
Na_2EDTA	3.726	7.45	3.726
Vitamins and Organic Substances (100X) [c]			
Myo-inositol	10.0	10.0	10.0
Glycine	0.2	1.0	0.2
Nicotinic Acid	0.05	0.3	0.05
Pyridoxine.HCl	0.05	0.25	0.05
Thiamine.HCl	0.05	0.5	0.01
DL-Alanine		1.0	

[a] Multiply the amount of the chemical listed in Table 17.1 by the strength of the solution (i.e., 10X, 100X, 1000X) to be prepared and dissolve in 1L deionized distilled water.
[b] Mix the $FeSO_4.7H_2O$ and Na_2EDTA and heat gently until the solution turns orange. Store in an amber bottle or protect from light.
[c] Prepare separately at one liter each per component.

the container used to mix the components. File and consolidate the used checklist to serve as reference to the correctness of the prepared medium or solution and for monitoring the volume of the culture media prepared over time.

Callus induction medium (CIM)

N6 and M8 basal salts were reported to work well with indica rice anther culture. Based on our results, more indica rice varieties perform better with N6 than with M8 basal salts. For standard CIM, coded as MNK, we routinely use N6 basal salts, enriched with plant growth regulators

MNK preparation (Table 17.3)

- For a liter of medium, put 500mL deionized distilled water (ddH_2O) in a 1,000mL capacity beaker. Put a stir bar in the beaker, and place the beaker on a stir plate.
- Add appropriate amounts of the N6 macronutrients, micronutrients, Fe-EDTA, vitamins, and organic substances **(**Table 17.2**)**, stirring continuously.
- Add the required amount of plant growth regulators (Table 3). Stock solutions are prepared by dissolving 100mg growth hormones in 100mL of ddH_2O. IAA and 2,4-D are dissolved first in a small volume of 95% ethyl alcohol or KOH and then brought to volume with ddH_2O; NAA is dissolved in a small amount of 1N NaOH.
- Add carbon source: 50g/L maltose. Mix thoroughly.
- Adjust pH to 5.8 with 1N KOH.
- Fill the volume with ddH_2O to 1L.
- Add gelling agent: 2g/L phytagel and 3g/L agar for clearer semi-solid medium, or 7g/L agar
- Heat the mixture in a microwave until it turns clear. Cool down.
- Dispense 30mL of the medium in Gerber bottles (Sigma) or locally available wide mouth mayonnaise bottles or any suitable culture vessels.
- Cap the bottles with polypropylene caps for Gerber bottles from Sigma or with autoclavable plastic sheets available locally.
- Sterilize the medium-filled bottles by autoclaving at 115°C, 15psi for 15min.
- Cool the medium in the laminar flow hood. Store in clean area.
- Use the prepared medium within a week or two.

Plant regeneration medium (MSK_2)

The standard regeneration medium, coded as MSK_2, contains MS salts and other additives (Table 17.1), supplemented with 0.5mg/L NAA, 2mg/L kinetin, and 30g/L sucrose.

- Follow the protocol for CIM (MNK) preparation. Only this time, dispense 15mL of medium in culture tubes (150mm x 25mm test tubes).

Table 17.3. Composition of the callus induction (MNK), plant regeneration (MSK_2), and rooting (R33) media.

Component	Concentration of Stock Solution	Amount /L medium (ml)		
		MNK	MSK_2	R33
N6 macronutrients	10X	100.0		
N6 micronutrients A	1000X	1.0		
N6 micronutrients B	10000X	0.1		
MS macronutrients	10X		100.0	100.0
MS micronutrients A	1000X		1.0	1.0
MS micronutrients B	10000X		0.1	0.1
Fe-EDTA	100X	10.0	10.0	10.0
Myo-inositol	100X	10.0	10.0	10.0
Glycine	100X	10.0	10.0	10.0
Nicotinic Acid	100X	10.0	10.0	10.0
Pyridoxine.HCl	100X	10.0	10.0	10.0
Thiamine.HCl	100X	10.0	10.0	10.0
2,4-D	100mg/100ml	2.0		
NAA	100mg/100ml	10.0	0.5	
Kinetin	100mg/100ml	10.0	2.0	0.5
Picolinic acid	100mg/100ml			2.0

- Cover the tubes with polypropylene caps (Sigma) or cotton plugs wrapped with paper and tied with rubber bands.
- Sterilize filled-up culture tubes by autoclaving at 115°C, 15psi for 15min.
- Use the medium within a week or two.

Rooting medium (R33)

- For the rooting medium, use either full or half-strength MS salts with all the other additives **(**Table 17.1**)** for regeneration medium.
- Supplement the medium with 2mg/L picolinic acid (Sigma-Aldrich, St. Louis, Missouri, USA) and 0.2-0.5mg/L kinetin when shoot growth is poor.
- Supplement the medium with 2mg/L picolinic acid (Sigma) and 0.2mg/L kinetin.
- Follow medium preparation protocol for regeneration medium.

3. Anther Culture Procedure

Establishment of anther source

- Identify genotype(s) for anther culture.
- Break dormancy by incubating seeds at 50°C for four days. This may not be necessary for naked seeds of F_1 progenies.
- Pre-germinate seeds in petri dish lined with two layers of moist filter papers, incubate at 37°C for 24 to 48h.
- Sow pre-germinated seeds in seedboxes. Label properly and record the seeding date.
- Transplant the seedlings in the screenhouse plots, 18 - 21 days from sowing, at 15-20cm plant spacing. Record the transplanting date.
- Maintain and properly cultivate the plants through: (1) fertilizer application, 120-60-60kg/ha NPK and 90-30-30kg/ha NPK blanket application during dry and wet season, respectively; (2) proper water management; and (3) judicious use of pesticide.

Boot collection, processing, and cold pre-treatment

- Boots are ready for harvest when the distance between the base/node of the flag leaf and the subtending/penultimate leaf is 5 - 10cm, depending on the maturity of the genotype. Check if anthers are at the right stage for culture by opening random boot samples then examine the color and size of the spikelet and anthers. See section on anther selection and preparation for details.
- Collect boots between 6 a.m.- 9 a.m. during DS and 7 a.m. - 10 a.m. during WS by cutting at the plant base with a pair of scissors. Collect boots in a staggered fashion, 2 - 4 times for each genotype.
- Bunch boots of the same genotype, label properly, and dip in water to lessen stress.
- Record the boot collection date.
- Remove extra leaves from harvested boots.
- Pre-sterilize boots by individually wiping them with muslin cloth moistened with 70% isopropyl or ethyl alcohol.
- Bunch pre-sterilized boots by genotype, label properly with genotype and harvest date.
- Wrap boots with cheesecloth and place in plastic bag.
- Label each bag with boot collection date and expected date of anther plating.
- Keep boots at 8°C for 5 - 8 days.

Selection and preparation of anthers

- Dissect out the panicles from the boots and trim away very young and very old spikelets.
- Select spikelets containing anthers at the mid-uninucleate microspore stage. Anther stage can be judged empirically by the color and size of the spikelet and anthers. Morphologically the anthers should be 1/3 to 1/2 the size of the spikelet, and light yellow to creamy white in color. However, the precise stage has to be determined by cytological examination of the microspores.
- Sterilize the spikelets in 50% bleach (2.5% w/v NaOCL), with agitation, for 15min.
- Rinse spikelets with sterile distilled water at least three times.

3.4 Plating of anthers on callus induction medium

- Cut individual spikelet at the base with a pair of fine-pointed scissors.
- Collect spikelets in sterile petri dishes lined with sterile paper. Prevent the spikelets from drying out.
- Release the anthers (six anthers per spikelet) by holding the spikelet at the tip (apiculus) with a pair of fine-pointed forceps and tapping it gently on the rim of the culture vessel containing CIM. Follow rule of thumb: 1mL of medium for each spikelet. Plate anthers from 30 spikelets in each culture vessel containing 30mL of culture medium.
- Label each vessel with genotype, anther collection date, and plating.
- Number each vessel, count, and record the number of anthers plated per vessel for each genotype.

3.5 Callus induction

- Incubate anther cultures at 27±2°C in the dark.
- Observe callus formation at 25 - 40 days after incubation. Depending on the genotype, calli are formed in the microspores within 25 - 40 days from plating.
- Discard older calli as they are difficult to regenerate and if they do, most of the regenerants are albino.

3.6 Plant regeneration

- Transfer anther with callus (≤1mm in size or pin-head size) in culture tube containing 15mL of regeneration medium. For enhanced plant regeneration, desiccate calli by air drying under the laminar flow hood for 3 - 6h, targeting a moisture loss of ~50%.
- Label tubes with dates of boot collection, anther plating, transfer to regeneration medium, and tube number. Record the information in appropriate data sheet.
- Maintain the cultures on light benches, with 16h daily illumination from 500lx cool white fluorescent light tubes, at 25±2°C, for 4 - 6 weeks. Responding genotypes regenerate green plantlets within 4 - 6 weeks of incubation.
- Transfer regenerants without roots into rooting medium. Culture with poor shoot and root growth can be transferred into hormone-free regeneration medium with half- or quarter- strength MS basal salts.
- Record number of regenerated calli, green plant regenerated, and other relevant observations in the appropriate data sheets.

4. Handling and evaluation of doubled haploid lines (DHL)

4.1 Transfer of regenerants and establishment in the screenhouse

The best stage for transfer to the screenhouse is when the regenerated plant (R_1) has 4 - 6 leaves and already has good root system.

- Uncap the culture tubes, pour in 10 - 15mL distilled water.
- Pre-harden the cultures by keeping them in a place where there is ample diffused light for 3 - 4 days.
- Take the regenerants out of the tubes and separate individual plantlets.
- Plant each plantlet in a plastic cup containing a mixture of sterilized garden soil and carbonized rice hull or saw dust. The potting medium should be loose enough to facilitate root growth.
- Harden the plantlets by exposing them initially in the shade, then to sunlight gradually. If necessary, enclose the plantlets in a plastic chamber where spray water can be sprayed to regulate the relative humidity.
- Allow the plantlets to develop new roots and one or two leaves within a week.
- Transplant hardened plantlets into puddled soil in the screenhouse or in seedling boxes, with a spacing of 10cm x 10cm or 15cm x 15cm between and within rows or in pots. Label plants properly including the transplanting date.
- Fertilize the plants as recommended and apply proper water management for rice cultivation.

4.2 Screenhouse observation and harvesting

- At maturity, identify and record putative haploid (sterile, shorter, smaller leaves) and doubled haploid (fertile, taller, longer leaves) regenerants.
- Harvest the seeds (R_2) from the first selfing generation of each of the R_1 regenerants. Separate individual panicles from the tillers of each regenerant.
- Label each panicle properly with genotype code, plant number, and dates of transplanting and harvest.
- Clean the seeds carefully and sundry.
- Keep the seeds in a safe place until ready for evaluation.

4.3 Evaluation of doubled haploid lines (DHL)

- Establish the R_2 seeds, which are harvested from the R_1 plants, in the observational nursery for preliminary evaluation (uniformity and phenotypic acceptability) and R_3 seed increase.
- Evaluate the agro-morphological, field performance, pest resistance, abiotic stress (drought, salinity, submergence, cool, and high temperature) tolerance and other trait in the R_3 and succeeding advanced generations. When R_3 seeds are adequate, evaluate for grain quality traits, otherwise, the analysis is pursued after seed increase of R_4 seeds.

5. Expected Results

Anther culture response is highly dependent on genotype.

- Anther culture response includes callus formation, callus differentiation as manifested in the greening (chlorophyll synthesis) of the callus tissue, green and albino plant regeneration, rhizogenesis, tissue necrosis, and haploid and doubled haploid plant regeneration (Figure 17.3).
- The genetic control of callus formation is different from plant regeneration; thus, high degree of callusing is not a guarantee of high frequency in plant regeneration.
- Most of the indica rice varietieshave less than 1% green plant regeneration based on the total anthers plated.
- Both haploid and doubled haploid plants are regenerated (Figure 17.3). Increasing the proportion of the doubled haploid over the haploid plants and of the green over the albino plants are of utmost importance for efficient utilization of AC technology in rice breeding.

Wide Hybridization

There are two cultivated species and 22 wild species in the genus *Oryza* (Table 17.4). The cultivated species are *O. sativa*, the Asian rice, which is grown worldwide, and the African rice, *O. glaberrima*, which is mainly grown in West Africa. Both the *sativa* and *glaberrima* species are annual plants and have the AA genome. The wild rice species are listed in Table 17.1 with their genome designation, chromosome number, and geographical distribution. Six wild rices have the AA or *sativa* complex genome, which include four popular or commonly used species in the improvement of cultivated rice: *rufipogon, nivara, barthii*, and *longistaminata*.

Wild species are important sources of useful traits such as resistance to biotic and abiotic stresses. Wild rices have been used as a source of resistance to grassy stunt, yellow mottle disease, bacterial blight, and brown plant hopper, among other pests and diseases (Table 17.5). Wild rices could also be useful sources of traits for hybrid breeding. Examples are cytoplasmic male sterility, good outcrossing habit, longer duration of spikelet opening, and long and exserted stigma. However, there can be interspecific crossability barriers such as hybrid sterility, hybrid breakdown, and embryo degeneration, the degree of which largely depends on genome affinity.

Wild rice could also be used in pre-breeding work to broaden the genetic base of a breeding program without any reference to particular gene or trait. Sativa x wild rice crosses (and reciprocals if desired) can be performed and becomes the first filial generation (F_1) backcrossed to the *sativa* parent. Genetic recombination can be encouraged through intercrossing and keeping individuals in heterozygous conditions longer in the breeding process.

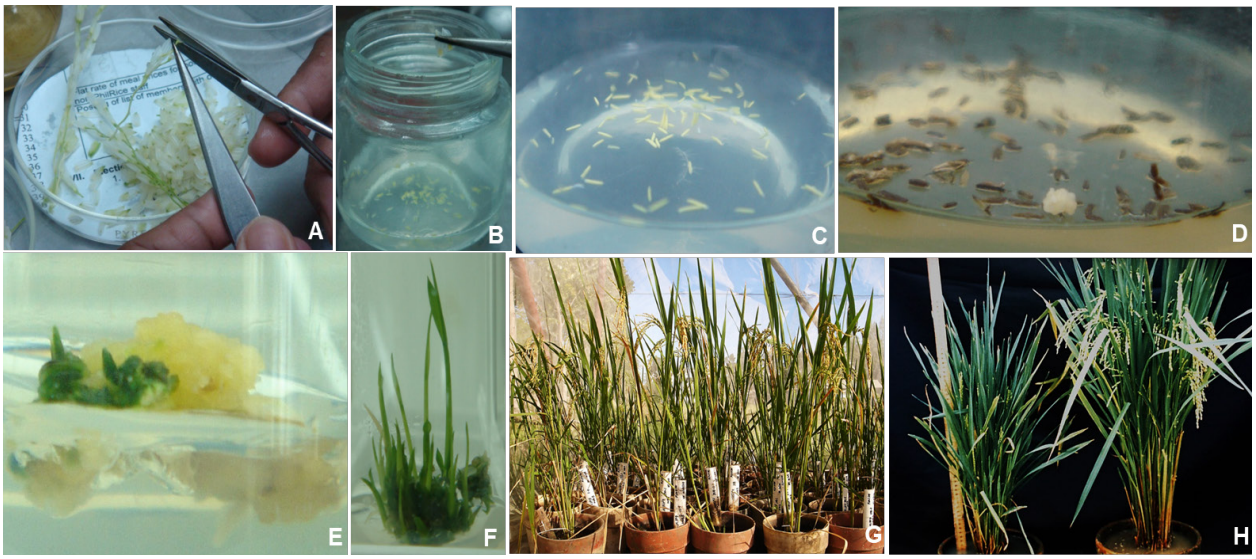

Figure 17.3. Rice anther culture: (**A)** spikelet clipping at the base and collected on sterile petri dish, (**B)** anthers released by tapping onto the rim of culture vessel containing callus induction medium, (**C)** culture incubation in the dark at 25±2^{0}C, (**D)** callus formation from microspores released from cultured anther, (**E)** green shoot development from callus, (**F)** multiple shoot regenerants, (**G)** transplanted regenerants, and (**H)** haploid and doubled haploid R_1 regenerants or pollen plants.

Table 17.4. Chromosome number, genome composition, and origin of wild species of *Oryza*.

Wild Species	Chromosome Number	Genome	Distribution
O. rufipogon Griff.	24	AA	Tropical Asia
O. nivara Sharma et Shastry	24	AA	Tropical Asia
O. longistaminata Chev. et Roehr	24	AA	Africa
O. barthii Chev. et Roehr	24	AA	Africa
O. meridionalis Ng	24	AA	Tropical Australia
O. glumaepatula Steud.	24	AA	South and Central America
O. punctata Kotschy ex Steud.	24,48	BB,BBCC	Africa
O. minuta J.S. Presl. ex C.B. Presl.	48	BBCC	Philippines and Papua New Guinea
O. officinalis Wall ex. Watt	24	CC	Tropical Asia
O. rhizomatis Vaughan	24	CC	Sri Lanka
O. eichingeri Peter	24	CC	South Asia and East Africa
O. latifolia Desv.	48	CCDD	South America
O. alta Swallen	48	CCDD	South America
O. grandiglumis Prod.	48	CCDD	South America
O. australiensis Domin.	24	EE	Tropical Australia
O. brachyantha Chev. et Roehr	24	FF	Africa
O. granulata Nees et Arn. ex. Watt	24	GG	Southeast Asia
O. meyeriana Baill	24	GG	Southeast Asia
O. longiglumis Jansen	48	HHJJ	Indonesia
O. ridleyi Hook	48	HHJJ	South Asia
O. schlechteri Pilger	48	HHKK	Papua New Guinea
O. coarctata Roxb.	48	HHKK	India

Table 17.5. Useful genes from wild *Oryza* species transferred into cultivated rice (Khush and Brar, 2003).

Trait	Donor *Oryza* Species		
	Wild Species	Genome	Accession Number
Grassy stunt resistance	*O. nivara*	AA	101508
Bacterial blight resistance	*O. longistaminata*	AA	-
	O. officinalis	CC	100896
	O. minuta	BBCC	101141
	O. latifolia	CCDD	100914
	O. australiensis	EE	100882
	O. brachyantha	FF	101232
Blast resistance	*O. minuta*	BBCC	101141
Brown planthopper resistance	*O. officinalis*	CC	100896
	O. minuta	BBCC	101141
	O. latifolia	CCDD	100914
	O. australiensis	EE	100882
White-backed planthopper resistance	*O. officinalis*	CC	100896
Cytoplasmic male sterility	*O. sativa, spontanea*	AA	-
	O. perennis	AA	104823
	O. glumaepatula	AA	100969
Tungro tolerance	*O. rufipogon*	AA	105908
	O. rufipogon	AA	105909

The main purpose for using wild species or wide hybridization is to introgress useful traits from a wild species genome into *sativa*. Introgressed materials are then channeled to mainstream breeding.

Wild rice species to use

Wild rices with the AA genome (*sativa* complex) are commonly used because these are crossed with *sativa* without the need for embryo rescue. Sterility in varying degrees is encountered depending on the wild rice parents used. F_1 seeds are readily produced in *sativa*/*rufipogon, sativa/barthii*, and *sativa/nivara* than in *sativa/longistaminata. O. longistaminata* is perennial wild rice with strong rhizomes. Figure 17.1 shows the wild rices having the AA genome.

Crossing *sativa* with other species outside the AA genome would involve embryo rescue to recover F_1 seeds. Other wild rice genome could be diploid (BB, CC, EE, FF, and GG), and allotetraploids (BBCC, CCDD, HHJJ, and HHKK).

Choice of *O. sativa* varieties to use in crosses

The *O. sativa* parents of improved plant type and donors of resistance or tolerance to stresses are the preferred female parents. Wide hybridization is essentially a pre-breeding activity, in which selected genotypes will also be used as parents in breeding. If *Sativa* is preferred as the female parent for extremely shattering wild species. However, wild x *sativa* is often crossed so that the progenies will have the wild rice cytoplasm.

Growing and management of wild rice species

Consider wild rices as potential weeds that should be handled inside a containment facility.

- Keep seeds in cold storage to conserve viability.
- Raise seedlings in plastic trays.
- If possible, plant wild rices in the wet season or June and July to catch up the short-day photoperiod for flowering.
- Pull and transplant seedlings 25 - 30 days from sowing or when at least 15cm tall.
- Plant seedlings in clay pots or plastic buckets. Growing wild species in plastic bags or directly in the soil is not recommended.
- At the end of the season, gather the soil and the plants from the buckets for disposal.
- Do not bring the progenies of *sativa*/wild rice out of containment until about BC_2F_3. This is to avoid the contamination of the field with genotypes that carry wild species traits like red pericarp, black hull grains, long-stiffed awn, high shattering, and high dormancy.
- Do not bring wide hybridization materials out of containment until the undesirable traits are eliminated.

If the materials are to be used for crosses, sow the seeds of wild species only once and the *sativa* parents 4 - 5 times at 10-day intervals.

In general, use *sativa* as the female parent and the wild rice species as the male parent. Oftentimes, wild species are so shattering that they shed seeds while still green, before maturity. For example, in making crosses with *longistaminata* as female, F_1 seeds would drop even before maturity. Grains of *barthii, rufipogon*, and *nivara* are also shattering, but using them as female parents in crosses with sativa is still possible.

Important considerations when growing wild rices

If not handled properly, wild species could escape containment and contaminate field canals and irrigation ditches as weeds.

They could be damaging as weeds but more importantly, they could also serve as alternate hosts to rice pests and diseases.

The seeds of wild rice species are generally shattering with long dormancy and can be considered noxious weeds that cannot be controlled by herbicides used in cultivated rice.

Some species are also perennial like *O. longistaminata*, which could invade not only irrigation canal, but also rice paddies. There are also species that have exceptionally good rooting ability at the nodes and develop into plants.

Production of interspecific hybrids

Observe the flowering habit of the wild rice parent, especially at the time of pollen dehiscence, to ensure pollen availability and stigma receptivity. Interspecific hybrids are produced following the emasculation and pollination procedures used in intervarietal crosses when the wild species with the AA genome are used. The *O. sativa* varieties are emasculated and used as the female parent. When a more distantly related species is used, special techniques are employed to ensure successful pollination and fertilization.

A combination of plant hormones may be sprayed on the pollinated florets shortly after pollination and on the successive days. The hormones induce pollen tube growth, enhance fertilization, and prevent embryo abortion. For cross combinations that produce abortive embryos, the embryo is excised at 14 days after pollination and transferred to a solid culture medium (1/4 MS) where it germinates into a seedling. The seedlings are transferred into a small pot and maintained in the screenhouse.

Handling the F_1, backcross, and F_2 populations

A major concern in *sativa* x wild or wild x *sativa* crosses is the possibility that the wild species or the weedy progenies could become weeds in cultivated rice fields. Special care should be exercised in handling species with rhizomes like *O. longistaminata* or other perennials with strong ability to regenerate from ratoons like *O. rufipogon*.

If a simple trait is to be transferred from the wild to the cultivated species, then recurrent backcrossing is used; however, if the purpose is for broadening the genetic base, care should be taken, especially for the highly heterozygous materials, because these might harbor negative traits that can only be seen in the homozygous state.

The handling of the parents and progenies depends on the wild species used and the breeding objective. If the objective is to transfer a desirable trait to *O. sativa*, the usual backcrossing can be employed. The number of backcrosses depends on the trait and other objectives of the plant breeder.

If the objective is to create or widen genetic diversity in breeding populations, individuals in the F_1, F_2, or backcrossed populations could be intercrossed to encourage heterozygosity and more genetic recombination.

Handling the F3 and later generations

F_3 selections are usually obtained after BC_2 or BC_3 depending on the wild rice used. Normally, F_1 and BC_1 progenies carry many wild rice traits, that populations are not advanced to F_3. The selection of F_3 lines from F_2 populations of BC_2 and BC_3 are grown in pedigree nurseries to isolate lines that are useful as parents in a rice breeding program. Lines for turn over to breeders are usually semi-improved with wild rice traits or genes introgressed into *sativa*. Some derived lines can also carry wild rice cytoplasm.

The products of wide hybridization could be evaluated for biotic (blast, bacterial blight, plant hoppers and leafhoppers, and stem borers) and abiotic stresses.

Containment facilities required

Wild species that are rhizomatous are more difficult to manage as these tend to have strong seed dormancy. The seeds are highly shattering and are easily dispersed and carried by flood or irrigation water. They may also stick on clothing, shoes, and tools. Therefore, it is necessary to grow the wild rices, interspecific F_1 hybrids, and early segregating generations (BC_1 to BC_2) in the screenhouse with proper containment such as double nets. A mesh screen is placed in the irrigation canal to prevent seeds from being carried away by rain or irrigation water. Burning and the use of a disposal pit are common ways of eliminating wild rice materials used in breeding work.

Managing wide crosses

In the field, undesirable plant types of segregating generations should be cut prior to heading. Undesirable plant types exhibit extremely droopy leaves, spreading panicles, long and thin culm, long awn, and black and thick hull. They are highly shattering and highly susceptible to pests and diseases. The discarded plants are placed in sacks and allowed to rot and decompose. The processing of selected plants should be done separately from regular breeding materials.

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GLOSSARY

Agro-morphological descriptors - refers to those characters observed and measured in the field. e.g., plant height, grain size, among others

Allele frequency - refers to how common or rare an allele is in a population

Amylose content - a term used to refer to the amount of starch fraction in milled rice. It is also used in determining eating and cooking quality. If the amylose content is high, the rice is dry and fluffy. If it is low, it is moist, glossy, and sticky.

Anthesis - refers to the shedding of pollen

Breeder seed - progeny of nucleus seed

Breeding nursery - an area for raising and selecting rice in its early development stages

Certified seed - the progeny of a foundation seed

Dominant allele - an allele that is always expressed

Endemic - means it is confined to a particular geographic area

Foundation seed - progeny of breeder seed

Gelatinization temperature - the temperature at which a significant portion of the starch have swelled irreversibly

Gene frequency - synonymous to allele frequency

Genotype - refers to the genetic constitution of an individual. In diploid, it refers to the pair of alleles.

Genotype frequency - the frequency of a genotype in a population

Germplasm - refers to seeds bearing genetic materials

Grain shape - refers to the length-width ratio of the grain

Harvest index - weight of filled grain over weight of all aboveground biomass

Headrow - also known as panicle to the row; seed from a panicle or head planted in a row

Heterozygosity - refers to the condition of having two different alleles in a locus

Homozygosity - refers to the presence of two identical alleles in a locus

Host - a plant that is invaded by a parasite from which the parasite obtains its nutrients

Hybrid rice - refers to the F_1 progeny of two genetically unrelated inbreds

Inbred rice - refers to the homozygous and homogenous rice lines

Inbreeding - mating of genetically related individuals

Infection - the establishment of a parasite within a host plant

Inoculum - the pathogen or its spores that can cause infection; that portion of individual pathogens that are brought into contact with the host

Inoculate - to introduce a pathogen into contact with a host plant or plant organ

Inoculation - the arrival or transfer of a pathogen into a host

Instron hardness - refers to the degree of resistance of a material from deformation as measured by the Instron instrument

Irrigated rice - rice grown and supported by irrigation water through artificial means such as gravity and water pumps

Isolation - this is the separation of a pathogen from its host in disease control

Locus - the location of a gene on a chromosome

Mass screening - the evaluation of large number of entries or genotypes for the presence of some trait, e.g., drought resistance

Milling recovery - estimated yield of head rice and total milled rice obtainable from rough rice

One locus model - deterministic model wherein natural selection acts on a single locus having dominant and recessive alleles

Participatory Variety Selection - selection carried out by the farmers in their target environments

Pathogen - an entity, usually a microorganism that can incite disease

Pedigree nursery - nursery for each segregating populations

Plant Variety Protection - refers to the right of breeders, granted under the law, over their new plant variety

Preference score - the ratio of favorable responses, e.g., tags cast (t) for a particular variety and the total number of tags cast (N); (PS = t/N).

Pure line selection - selections with the same genetic constitution

Pure culture - culture of a single form of microorganism

Quantitative trait loci - refers to those loci or chromosomal regions associated with the different genes that influence a particular complex trait, e.g., yield, drought tolerance

Rainfed rice - rice grown in areas, usually leveled bunded fields, that depend entirely on rain and/or run off or seepage water for its water supply

Rapid Generation Advance - a breeding tool to shorten plant generation time

Recessive allele - an allele that is not express in a heterozygote

Recombinant - a plant having a new recombination of genes not found together in either parent

Recombinant inbred line - inbred line that is a product of hybridization from two unrelated inbred lines, and continued selfing

Registered seed - progeny of foundation seed

Resistance - the ability of an organism/ plant to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factor

Resistant - possessing qualities that hinders the development of a given pathogen

Rouging - removal of off-type plants

Selective advantage - a trait that gives a plant a greater chance of reproduction over those of others

Selfing - refers to self-fertilization of the rice plant, in which the pollen from the anther of a flower is transferred to the stigma of the same or different flower

Single Seed Descent - a method of deriving plants, in which all of the progenies are advanced by single seeds until genetic purity is achieved leading to pure or inbred lines

Spreader rows - rows of susceptible crops are planted to effect, as much as possible, the uniformity of inoculum throughout the experimental area

Stem borer - an insect larva of the order *Lepidoptera* that bores on the stems of rice

Stock culture - a subculture from which the working culture are derived

Susceptible - any plant that can be attacked by a given pathogen; a host plant

Susceptibility - lacking the inherent ability to resist disease or attack by a given pathogen

Symptom - the external or internal reaction or alteration of a plant as a result of a disease

Teleomorph - the sexual or so-called perfect growth stage or phase in fungi. Also known as perfect stage.

Upland rice - rice grown under free draining aerobic soils regardless of elevation

Vector - an insect able to transmit a pathogen

Viruliferous - a vector carrying/ containing a virus and capable of transmitting it inside the body

Virus - a submicroscopic obligate parasite consisting of nucleic acid and protein

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