Ministry of External Affairs sponsored Workshop for Capacity building in Agriculture under MGC Plan of Action (2019-2022

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Conservation of rice germplasm and productivity enhancement through mechanization

(27th January – 10th February 2024)

Training Manual

Division of Genetics
ICAR-Indian Agricultural Research Institute
New Delhi – 110012

Division of Agricultural Engineering
ICAR-Indian Agricultural Research Institute
New Delhi – 110012

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Conservation of rice germplasm and productivity enhancement through mechanization

A Training Manual

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Preface

Genetic resources found in crops are a significant part of human legacy. Therefore, they need to be preserved and made accessible to researchers worldwide. Rice is the principal crop of India, where a considerable range of diversity exists. The germplasm collection has also unfolded the occurrence of large number of rice landraces. In rice, around 5,00,000 accessions are conserved in the gene banks across the world, which also includes duplicates in different gene banks. Around 70.0% of the germplasm accessions of rice are conserved in six gene banks located in Asia, with half of the collections maintained in the gene banks at International Rice Research Institute (IRRI), Philippines and ICAR-National Bureau of Plant Genetic Resources (NBPGR), India.

In India, the ICAR-NBPGR has been coordinating the exploration and collection of rice germplasm from all over the country and is also supporting operational facilities for the explorations. During the maintenance, germplasm may lose their identify because of random and non-random processes of sampling. Therefore, streamline and systematize the conservation of rice germplasm is very essential. At the same time, they need to be characterized. Further evaluation and utilization has to be followed up for the purpose of crop improvement.

The objective of the course is to impart training on preservation of rice germplasm and application of mechanization so as to develop personnel capable of carrying out conservation and sustainable use of it. It further envisages providing meeting ground for the trainees and subject-experts for effective discussion and updating with latest information in the field of conservation. The course has been designed to give the trainees complete exposure to the basic principle of conservation of rice germplasms, pre-breeding and sustainable use towards crop improvement and their practical applications. To provide hands-on training, more time has been allocated for practical classes. Participants will also be taken to experimental field and molecular biological laboratories for wider exposure and experience. Provision has also been kept for Interim Review and Group Discussion to assess progress. Thus, the training is expected to develop a contingent of trained personnel capable of carrying out germplasm conservation activities with great confidence.

27th January 2024

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CHAPTER 1

Conservation of wild species of Oryza

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Introduction

Wild rice has survived in nature for millions of years and therefore has accumulated large repertoire of genes for resistance to various diseases and insect pests during its evolution. The wild rice is also adapted to extreme habitats viz. flood prone, drought prone, saline and acidic soil conditions and must therefore also have genes conferring **t**o these extreme environmental conditions. India is a Centre of Diversity where large number of wild rices grow in their natural habitats. A total of 1031 wild rice samples have been collected from 12 of the 15 agroclimatic zones of India during the National Professor B.P. Pal chair project and screening of these under controlled conditions has identified several accessions of wild rice with high levels of abiotic stress tolerance. The reference genome of rice has been annotated to have 37,544 protein-coding genes (IRGSP 2005). A number of agronomically useful genes have now been cloned and validated using functional genomics tools up to genetic transformation level. Thus, genes for high grain number (*CKX1*), salt tolerance (*SKC1*), grain quality (*GBSS1, GS3, BADH2*), submergence tolerance (*Sub1A*), BLB resistance (*Xa21, xa13*), blast disease resistance (*Pi1* and *Pi54*) etc. have been validated by functional complementation through genetic transformation. Each of these genes must have several alternative forms (alleles) due to accumulation of mutations, whose functional implications are not known as yet. The cultivated varieties of rice have only a small proportion of the total genetic variability as compared to the landraces and wild rice species due to breeding and domestication bottlenecks. We need to mine superior alleles of these genes for use in rice improvement programs. Unfortunately, much less attention has been paid to collection, evaluation and utilization of the wild rice genetic resources. Thus, out of over 100,000 rice accessions in the NBPGR Gene Bank, only about four hundred represent wild rice, theremaining are cultivated rice varieties and landraces from different agroecological regions of India and of exotic origin. Landraces of rice adapted to extreme stress environments are also valuable source of superior alleles of agronomically useful genes.

Due to population pressure, increasing urbanization and developmental activities our wild rice resources and traditional rice landraces are depleting at an alarming rate and genes that

have evolved through millions of years of evolution are getting lost forever. Utilizationof these wild rices and landraces for mining superior alleles of agronomically useful genes using cutting tools of genomics will promote a culture of basic research in the NARES that has relevance to enhancing rice productivity. Therefore, the National Professor BP Pal Chair project was formulated with the following broad objectives:

Molecular characterization, training of stakeholders for wild rice management and in situ conservation of wild rice in their prominent wetland habitats for natural evolution and future use.

1. Identification of novel QTLs and underlying genes for drought, salinity, anaerobic germination and submergence tolerance in Indian wild rice accessions and landraces of rice using modern genomic tools.
2. Introgression of useful QTLs for abiotic stress tolerance traits into high yielding rice varieties by genomics-assisted breeding.

Collection, multiplication and characterization of the wild rice germplasm

Rice is a staple food crop of global significance cultivated in diverse agroclimatic zones. However, in the process of domestication many beneficial alleles have been left out from the gene pool of cultivated rice that has eventually made the modern high-yielding rice varieties vulnerable to various environmental stresses. In contrast, the wild rice despite being agronomically inferior must have has inherited a distinct potential for survival in a range of geographical habitats and environmental stresses. These adaptations have enriched them with adaptability traits that are of immense agronomic value. A total of 802 accessions of wild rice have already been collected from of 11 agroclimatic zones of India under National Professor Project before until last year. A set of 418 accessions from this have been screened for tolerance to different abiotic and biotic stresses, including drought, salinity, flooding, sheath blight, BLB, blast, cereal cyst nematode, leap spot and morphological traits, identifying promising accessions which are currently being utilized for mapping and traitintrogression.

During Kharif 2022, 260 wild rice accessions including over hundred new collections were multiplied and evaluated in the field at New Delhi **(Fig. 1)**. These were planted in a plot size of 60 cm x 60 cm with plot-to-plot spacing of 50 cm. Till now, total 1031 accessions of wild rice collected from different state of India have been multiplied and characterized in this way, and a database web portal has been developed (nksingh.nationalprof.in:8080/iwrdb). The passport data on each of the accession including, village, block, district, state, longitude and latitude of the collected accession has been recorded and stored in the wild rice database.

Figure 1: Multiplication and field evaluation of wild rice accession during Kharif 2021

Wild rice accessions deposited in the National Gene Bank at NBPGR

Last year, a total 354 accessions were deposited in the national gene bank and obtained unique accession numbers. During 2022-23, a total 376 wild rice accessions collected from the states of Assam, Bihar, Chhattisgarh, Goa, Gujarat, Himachal Pradesh, Odisha, Gujrat, Madhya Pradesh, Jharkhand, Karnataka, Uttar Pradesh and Uttarakhand were deposited in the NBPGR National Gene Bank. This takes the total tally to 730 accessions already deposited in the National Gene bank.

Mapping of QTLs for anaerobic germination in wild rice accessions NKSWR 70 and NKSWR 349

For the success of the direct seeded rice, it is important to develop rice varieties with potential to tolerate flooding during germination, also known as anaerobic germination (AG). We used the wild rice germplasm resources available with us to identify accessions with promising anaerobic germination potential. Two of the accessions, namely NKSWR70 and NKSWR397 were used to generate mapping population. Total 180 $F_{2:3}$ lines derived from Pusa 1509 x NKSWR70 were phenotyped for germination under water **(Fig. 2A)**. Using the genotyping and phenotyping data, three QTLs were identified for increased tolerance to anaerobic germination: two overlapping QTLs on chromosome 7 and one on chromosome 3 with LOD scores ranging from 5 to 7.5. The major QTL on chromosome 7 was contribute by wild rice and explained about 27% of the phenotypic variation **(Fig.2B)**.

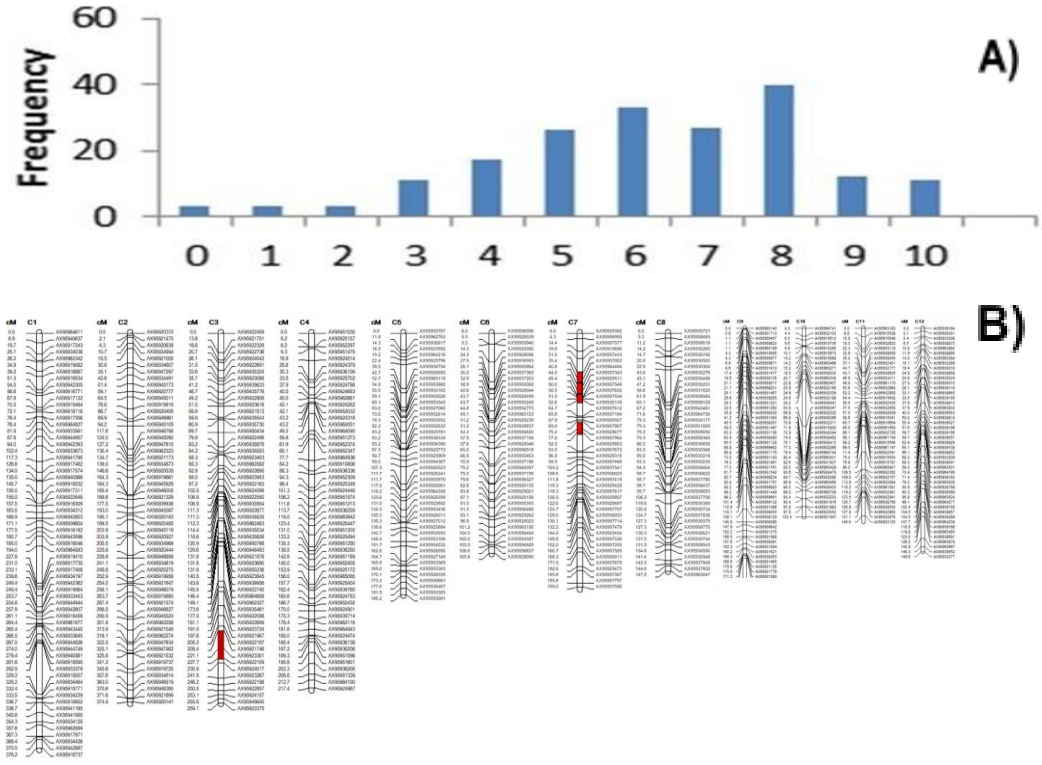

Fig. 2 (A) Phenotypic distribution of 184 $F_{2:3}$ progenies screened for anaerobic germination tolerance. **(B)** Location of anaerobic germination QTLs identified in the F_2 population. The position is shaded in the red color.

Evaluation of Sheath Blight resistance in BC1F2:3 mapping population.

A BC1F2:3 mapping population segregating for sheath blight resistance was evaluated earlier in years 2020 and 2021. Four phenotypic traits related to sheath blight tolerance viz., maximum lesion length, average lesion length, maximum disease rating and average disease rating were recorded **(Fig. 3a)**. Based on the IRRI standard evaluation system the phenotypic scoring was done for which the phenotypic distribution is presented in (**Fig. 3b)**. The genotyping and phenotyping has already being done and the QTL mapping is in process. A representative picture of DNA isolation from segregating population is provided in (**Fig. 3c)**.

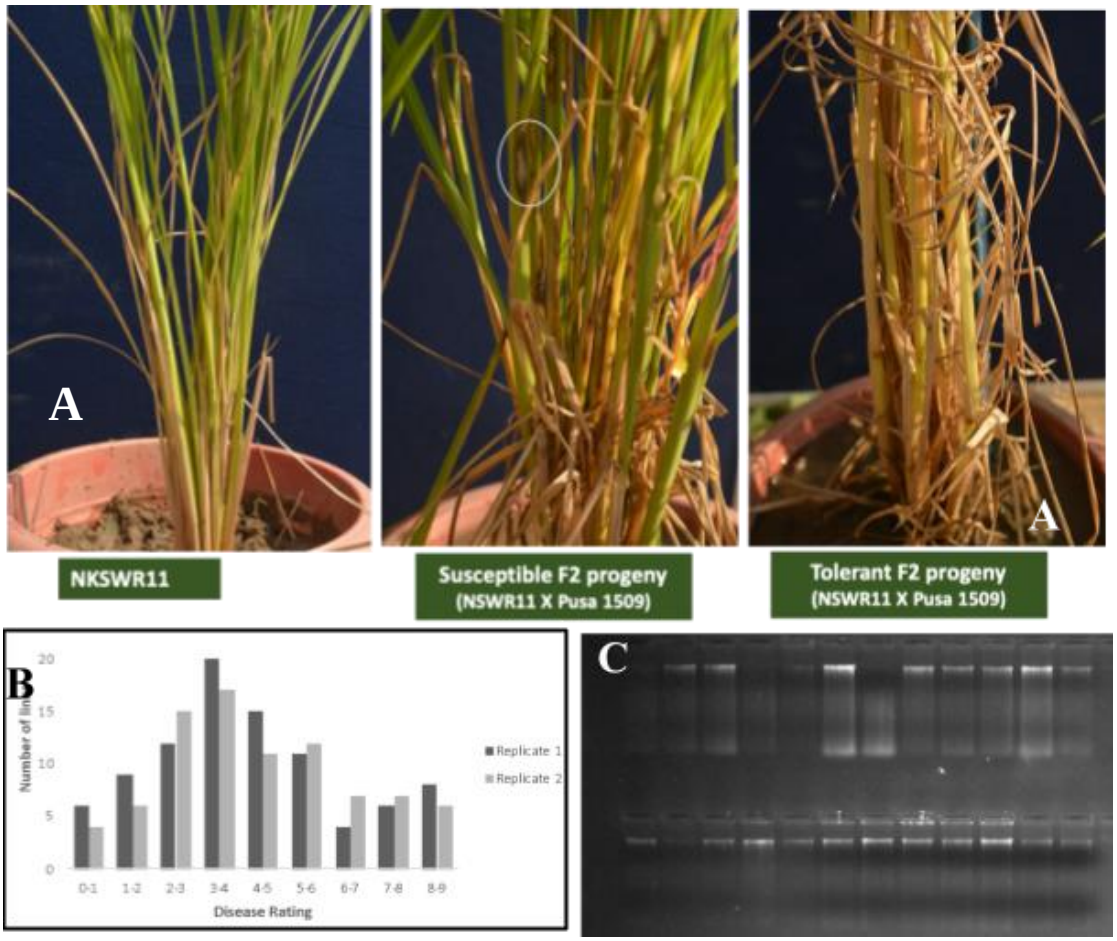

Fig. 3- Evaluation of mapping population segregating for Sheath Blighttolerance phenotype. **(A)** Disease phenotype in tolerant donor parent, and tolerant and susceptible individuals of the population; **(B)** Distribution of disease rating observed in replicates; **(C)** Isolation of high-quality DNA from individuals of mapping population.

Evaluation of NKSWR 173/IR 64 BC1F2 population for submergence tolerance

Submergence tolerance screening of $BC_1F_{2:3}$ mapping population was done in the outdoor tanks at NIPB phenomics facility. Total 73 RILs together with positive (SMSub-1 and IR64-Sub1) and negative (IR64) checks we transplanted in water tank and after 14 days plants were completely submerged with a water depth of approximately 1 meter **(Fig. 4a)**. The IR64 susceptible check was assessed on daily basis and upon 90-100% damage of the IR64 plants water was drained out of the tank. Plant survival was scored at 21 days after de-submergence, and percent survival was computed for QTL analysis **(Fig 4 b-d)**. The QTL analysis is underway and based on our phenotypic evaluation we expect to find a submergence tolerance source with immense breeding implications.

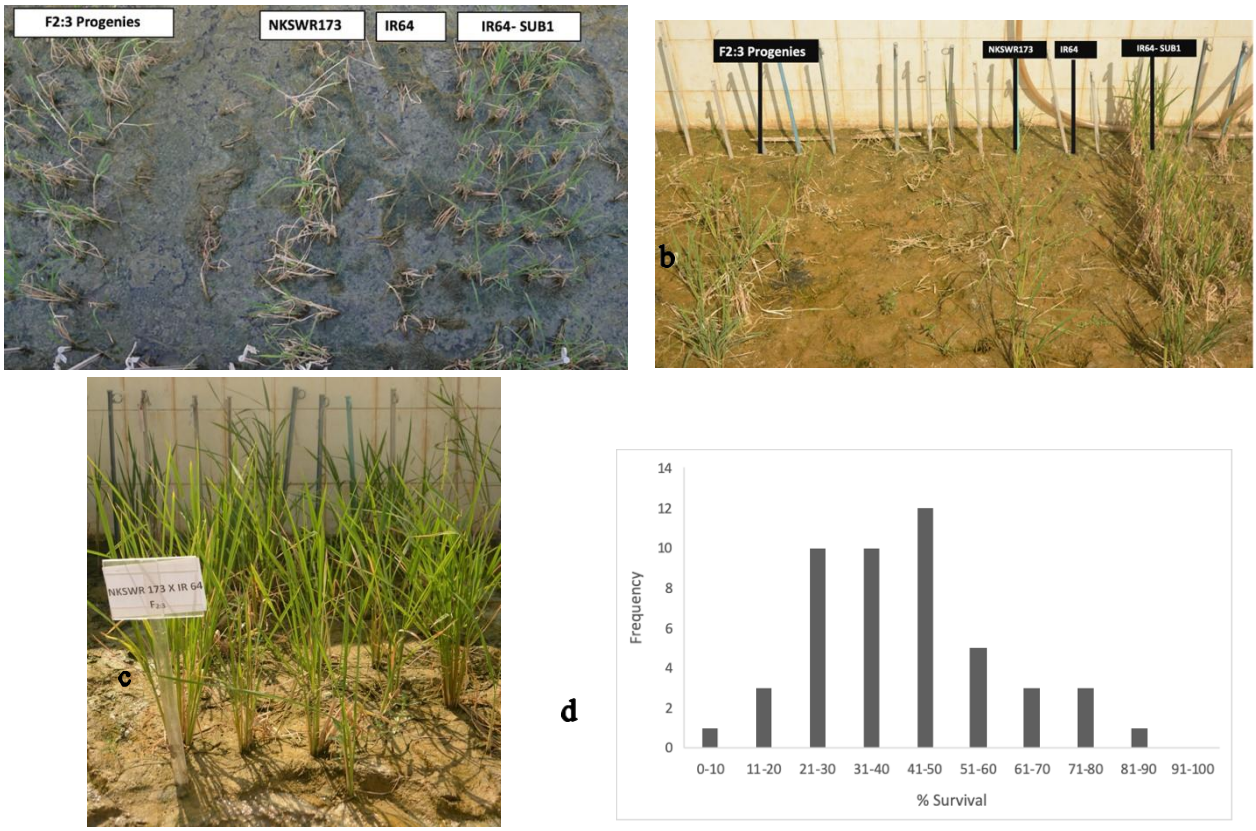

Fig. 4: Phenotypic evaluation of mapping population under submergence stress. **(a)** Immediately after draining the water from the tank; **(b)** Recovery after 21 days; **(c)** completely recovery after one month; **(d)** Phenotypic distribution of the population

Combining drought and submergence tolerance with high-yielding rice varieties

Earlier we have developed and released 12 different near-isogenic lines (NILs) of high-yielding rice varieties by introgression of quantitative trait loci (QTLs) for tolerance to drought, submergence and salinity under now completed DBT-funded project 'From QTL to Variety'. During 2022, F2 segregating populations were grown from crosses made for stacking multiple QTLs for abiotic stress tolerance in the genetic background of high-yielding mega varieties of rice, namely Pusa 44, Samba Mahsuri and Sarjoo 52. Lines were identified combining multiple QTLs using marker-assisted foreground and background selection. These include; (i) Pusa 44 with qSaltol1 and qGN4.1 for combining high yield with salinity tolerance, (ii) Samba Mahsuri with qSUB1, qDTY2, qDTY 3.2 and qGN4.1 for combining submergence and drought tolerance with high grain yield, and (iii) Sarjoo 52 with qSUB1 and qGN4.1 for combining submergence tolerance with high grain yield. For example, qGN4.1 in the background of Pusa 44 not only showed 15% higher grain yield but also mature about a weak earlier that will save the precious irrigation water (**Fig. 5**)

Fig. 5. Field performance of Pusa 44- *qGN4.1* QTL-NIL (right) in comparison with the recipient variety Pusa 44 (left) at IARI New Delhi during 2022

Introgression of major genes for salt tolerance from wild rice NKSWR 173

We have identified a wild rice accession 'NKSWR173' collected from Himachal Pradesh with very high level of salt tolerance and mapped five QTLs for salt tolerance contributed by the wild rice using IR64//NKSWR173 BC_1F_1/F_2 population. During 2022, we investigated the presence of these wild rice QTLs into BC_1F_6 inbred lines (BILs) so that additional backcrosses could be made for their introgression into IR 64. KASP markers were designed based on the QTL peak SNP markers and used for the screening of 41 BILs (**Fig. 6**). Total 5 BILs (one homozygote) were identified with QTL *qPHT8.2* for plant height, and 12 BILs (4 homozygotes) were identified for the presence of QTL *qSIS3.2*. Crosses have been made between IR64 and the selected QTL positive BILs for seedling and reproductive stage salt tolerance for introgression of the salt tolerance QTLs into IR64. Further backcrosses and phenotypic evaluation of the IR64/ NKSWR 173 BC_1F_8 BILs will be done for introgression and validation of the wild rice salt tolerance QTLs using KASP markers in Kharif 2023.

S.No	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	C	C	C	C	C	C	C	C	T/C	C
B	-	T/C	C	C	C	C	C	C	C	C	C	-
C	C	C	C	C	C	T	C	C	C	C	T/C	T/C
D	C	C	-	C	C	C	T					
						IR 64	**NKSWR173**					

S.No	1	2	3	4	5	6	7	8	9	10	11	12
A	T	T	T	T	T	T	T	T/G	T	T	T/G	T
B	G	T/G	T	T	T	T	T	T/G	T	T/G	T	-
C	G	G	T/G	T	T	T/G	T/G	T	T	T	T	T
D	T	T	-	T	G	T	G					
						IR 64	**NKSWR173**					

Fig. 6. Segregation of the BC_1F_6 backcross inbred lines for KASP markers linked to QTL for salt tolerance from wild rice accession NKSWR173. **Top**- Marker M3 for reproductive stage QTL *qPHT8.2* (SNP Id. AX-95930579). **Bottom**- Marker M4 for seedling stage QTL *qSIS3.2* (SNP Id. AX-95962416).

Introgression of a major QTL for anaerobic germination from wild rice

Anaerobic germination (AG), the ability of seed germination and heterotrophic growth under complete submergence, is an important trait for direct-seeded rice. The available rice cultivars show variable ability of AG. A major QTL for anaerobic germination (qAG9.2) has been mapped on rice chromosome 9 and *OsTPP7* coding for trehalose phosphate phosphatase has been identified as the causal gene underlying this QTL (Kretzschmar et al. 2015). However, this QTL enables only about 30 percent germination under water. In contrast, wild rice is highly adapted for germination under water and we have identified a wild rice accession 'NKSWR70' that shows 90 percent germination under water. We mapped a major QTL for anaerobic germination (qAG7.1) contributed by 'NKSWR70' on chromosome 7 using 95 BC_1F_1/F_2 populations and have continued introgression of this QTL in to popular Basmati rice variety Pusa1509 by additional backcrossing. During Kharif 2022, BC_1F_4 plants segregating for the tolerant wild rice alleles were grown and screened for the presence of the donor allele. KASP assays were designed for four different SNP markers linked to *qAG7.1* on chromosome 7 between 31.0 to 50.6 cM. These are: (M1) AX-95957443 (31.0 cM), (M2) AX-95937652 (50.5 cM), (M3) AX-95957534 (51.5 cM), and (M4) AX-95930135 (52.6 cM). Progenies of plants heterozygotes (A/C) for the donor alleles will be grown during 2023 to select homozygous QTL introgression lines (**Fig. 7**).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	A	A	A	A	A	-	A	A	A	A
B	A	A	A	A	A	A	A	A	A	A	A	A
C	A/C	A	A	A	A	A	A	A	A	A	A	A
D	A	A/C	A	A	A	A	A	A/C	A	A	A	A
E	A/C	A	A	A	A	A	A/C	A	A	A	A	A
F	A	A	A	A	A	A	A	A	A	A	A	A
G	A	A	A	A	A	A	A	A	A	A	A	A
H	A	A	A	A	A	A	A	A	A	C	NTC	NTC

Fig 7. Pusa 1509*4//NKSWR70 BC_4F_1 plants. **Top**- Uniform like Pusa 1509, **Bottom**- Segregating for qAG7.1 linked KASP markers AX-95930135 (M4). H9- PB 1509, H10- NKSWR70. C1, D2, D8, E1 & E7 are heterozygous (positive for wild rice QTL allele). NTC- no template control.

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CHAPTER 2

Ex-Situ Conservation of Plant Genetic Resources in National Genebank of India

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Introduction

Genetic Resources are all the living materials that include genes of present and potential value for humans. Plant Genetic Resources (PGRs) are defined as the entire generative and vegetative reproductive material of species with economical and/or social value [1] . In the State of the World's Plant Genetic Resources for Food and Agriculture (1998), the FAO defined Plant Genetic Resources for Food and Agriculture (PGRFA) as the diversity of genetic material contained in traditional varieties and modern cultivars as well as crop wild relatives and other wild plant species that can be used now or in the future for food and agriculture.[2] "Germplasm" refers to the reproductive or vegetative propagating material of plants, such as, seeds, tissues, cells, pollen, DNA molecule etc. containing the functional unit of heredity that can be utilised in crop improvement programme. PGRs are highly valuable raw materials in crop improvement, food and nutritional security and must be preserved for posterity. Conservation of PGRs refers to maintaining the diversity of the full range of genetic variation within a particular species or taxa. PGRs can be conserved both in-situ and ex-situ. In-situ conservation is the method of conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties. In situ conservation involves the location, designation, management and monitoring of species at the location where they grow naturally. Biosphere reserves, natural parks, gene sanctuary and on-farm are some examples of In-situ Conservation. Ex-situ conservation refers to the conservation of components of biological diversity outside their natural habitats. Ex-situ Conservation entails exploration, sampling, transfer and storage of the propagules of the species in an artificially created environment. In 1920, a Russian scientist, Nicholai Ivanovich Vavilov, an avid explorer realised the need to conserve genetic resources. He established the first Genetic Resource Center (GRC) of the world at Leningrad where he brought together his worldwide collections of seeds and propagules of a large number of cultivated crops and their wild relatives. Farming communities all over the world are to

be credited with the conservation and utilization of biological diversity. We will focus on the Ex-situ conservation of PGRFA in this lecture.

There are various techniques of Ex-situ Conservation such as Seed banks, In-vitro Genebanks and Cryobanks, Field Genebanks etc. The choice of the particular techniques of conservation is primarily determined by the longevity of the propagule of the species during conservation. The primary techniques of Ex-situ conservation for plant genetic resources include:

i) Seed Banks

Seeds of many plant species can be dried, up to 3-7 % moisture content and stored at low temperatures (-18°to -200 C) for long periods without significant loss of viability. Opting for a lower moisture content and higher storage temperature is a practical approach to reduce refrigeration costs in the seed bank.

ii) Field Genebanks

Living plants are grown and maintained in field conditions. Useful for conserving species that do not produce seeds, produce recalcitrant seeds or clonally propagated plants.

iii) Botanic Gardens and Arboreta

Botanic gardens and arboreta are institutions that cultivate collections of living plants primarily for scientific, educational, and recreational purposes. They play a pivotal role in ex-situ conservation, especially for ornamental, medicinal, and rare plant species.

iv) In Vitro Storage or Tissue Culture

Plant cells, tissues, or organs are grown and maintained in sterile containers with a nutrient-rich medium. Useful for species that are difficult to conserve as seeds or whole plants, and allows for the rapid multiplication of plants.

v) Cryopreservation

This involves the storage of plant tissues (shoot tips, gametes, embryos, embryonic axes or whole seeds) at ultra-low temperatures, typically in liquid nitrogen (-196°C). Under these conditions, all metabolic processes halt, enabling potential indefinite storage of some species. We will focus on the Seed Genebanks in this lecture.

Seed Genebank

The term ‘Genebank’ is used to describe an organizational unit which has the objective of conserving and managing PGR collections and facilitating their use. The National Genebank (NGB) of India, located at the ICAR-National Bureau of Plant Genetic Resources is the cornerstone of the Indian National Plant Genetic Resources System (INPGRS). Seeds are the easiest and the most convenient form for long-term conservation and hence the seed genebank forms the major component of a Genebank.

Advantages of seed genebanks:

- Handling of germplasm is easy

- A large number of germplasm samples or entire variability can be conserved in a very small space
- Germplasm is conserved under pathogen and insect free environment

Disadvantages of seed genebanks:

- Seeds of recalcitrant species cannot be stored in seed banks
- Failure of the power supply may lead to loss of viability and thereby loss of germplasm.
- It requires a periodical evaluation of seed viability. After some time, regeneration/ multiplication is essential to get new or fresh seeds for storage

The number of genebanks globally has risen to more than 1750 by 2019. Most of the genebanks are relatively small with around 10000 accessions. However, there are only four genebanks with germplasm accession exceeding 0.1 million. According to the international gene bank databases available in public domain, the four largest national level gene banks are:

(1) National Centre for Genetic Resources Preservation (NCGRP) in the United States of America

(2) National Bureau of Plant Genetic Resources (ICAR-NBPGR) in India

(3) Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences (ICGR-CAAS) in China

(4) NI Vavilov All Russian Scientific Research Institute of Plant Industry (VIR) at Russian Federation.

Let us look at some definitions before proceeding further:

Seed Viability: It is the ability to germinate under favourable condition. This includes the dormancy breaking before germination.

Seed Dormancy: It is the inability of a viable seed to germinate under favourable environmental conditions.

Seed Longevity: It is the ability to maintain vigour and viability during storage. Major factors affecting seed longevity are: 1. Moisture; 2. Temperature 3. Oxygen Seed type based on physiology

Orthodox Seeds: The seeds which can be dried to 2-5% without any damage are called as orthodox seeds. eg. Rice, Wheat, Maize etc.

Recalcitrant Seeds: The seeds which cannot survive desiccation below a comparatively high moisture content (12-31%) are called as recalcitrant seeds eg. Mango, Rubber, Bulbacious seeds etc.

 Intermediate type of seeds show desiccation tolerance of 7-10% to 20% eg. Coffee, Papaya, Salix

The methodology that can be adopted for determining the seed type is depicted in fig 1.

The conventional seedbanks are capable of storing only Orthodox seeds. More than 90% of the crop species have orthodox type of seeds and hence can be easily stored in seedbanks.

Active and base collections:

Two types of seed stores are used for the conservation of genetic resources: those holding seed samples for long-term storage referred to as base collections, and those holding seed samples for immediate use referred to as active collections. The temperature, relative humidity (RH), seed moisture content, containers and distribution arrangements of these stores vary.

a) Base collections- A base collection is a set of accessions in which each is distinct and as close as possible to the original sample in terms of genetic integrity. Normally, seeds are not distributed from base collections directly to users. Seeds for base collections are conserved at

-180C and have a maximum moisture of 7%.

b) Active collections- Active collections, according to FAO, also referred to as working collections, comprise accessions readily available for distribution. These accessions can be employed for various purposes such as research and breeding. The active collection undergoes regular evaluation, characterization, and multiplication to sustain their viability, ensuring a consistent supply of plant genetic resources. Accessions within the active collection are frequently accessed and are maintained with the objective of retaining at least 65% viability over a span of 10 to 20 years.

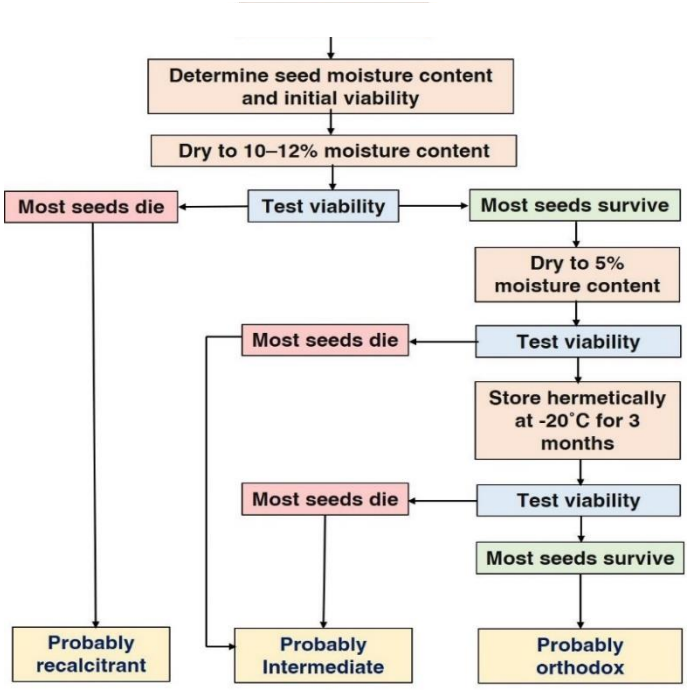

Fig 1. Determining seed type before conservation

The storage period, temperature, Relative Humidity (RH) and types of storage containersfrequently used for base and active collections are listed in the table 1

Table 1: Types of facilities for different durations of seed storage

Condition	Short Term or breeder's collections	Medium Term storage (for Active collections)	Long Term storage (for base collection)
Storage period	12 to 18 months	5–10 Years	50–100 Years
Temperature	+18 to +20 °C	0 to +10 °C	-18 to –20 °C
RH	45–50 %	35–40 %	No control
Storage container type	Cloth bags, paperbag, glass bottles	Cloth bag, metal can, glass bottle and plastic jars	specially designed tri-layered aluminum foil packets.

Processing for long-term storage in Seed Genebank:

Each of the steps in seed conservation in long term storage modules is depicted in Flowchart (fig 2) and explained in detail below:

1) **Germplasm acquisition**- the first step of PGR conservation is germplasm acquisition. Thelong-term conservation of orthodox seeds in the genebank begins with the receipt of freshly harvested, physically pure seed. The basic parameters that are checked up for conservation are:

   i. **Uniqueness of the accession**- The samples are carefully checked for uniqueness to avoid duplication of accessions in the genebank. Duplication in the collections is best identified by comparing relevant fields in database stored digitally.

   ii. **Availability of passport information**- The utilization of the conserved germplasm canbe facilitated only if all relevant passport information is available in the database. Passport information is provided by the explorers and the breeders. Parameters like biological status and collection details for explored germplasm and information on unique trait and pedigree details if it's a breeding line/genetic stock or cultivar, are required. Seed of released varieties and

parental lines of the hybrids are received alongdetails of the variety including pedigree, Institute where developed, whether recommended by state or central govt etc.

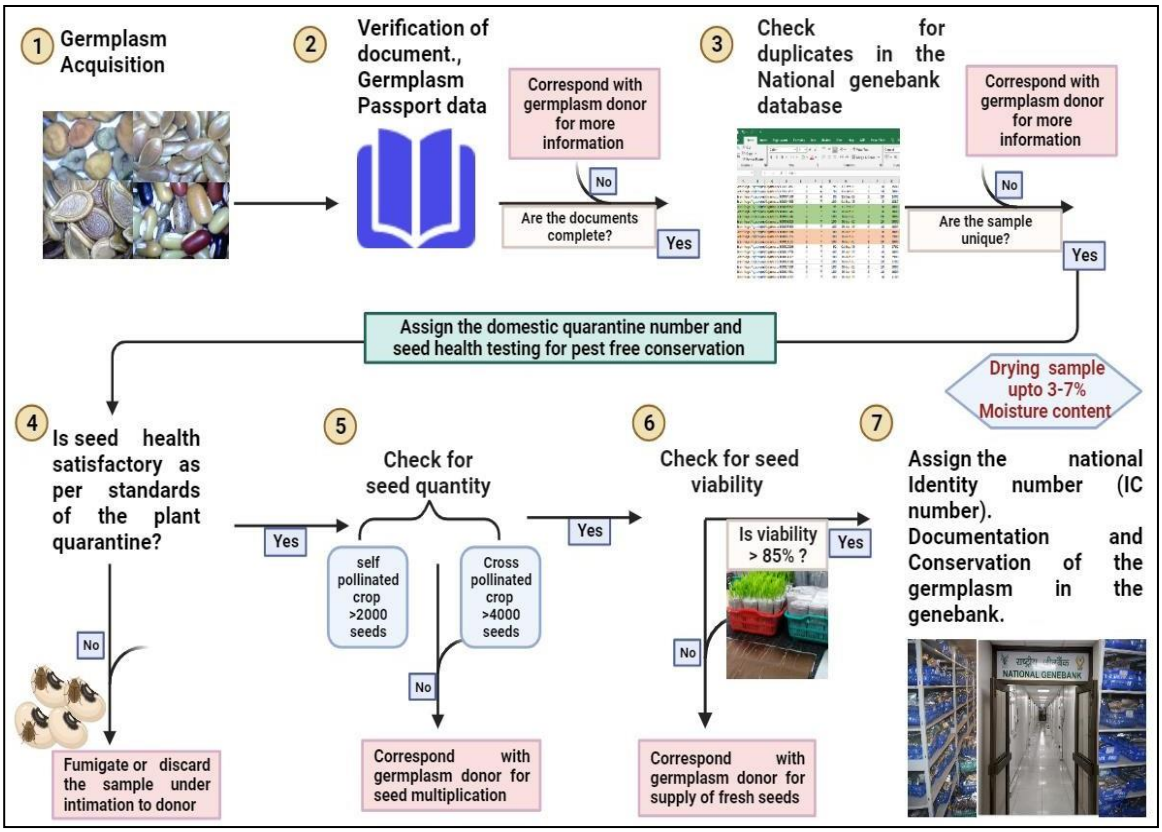

Fig 2: Flowchart for steps in seed conservation in seed bank

1) **Seed quantity**- The global gene bank standards recommend a minimum of 2000 seeds inself-pollinated crops and 4000 seeds in cross-pollinated crops. In wild germplasm accessions, the minimum number has been relaxed to 500 seeds.
2) **Seed health-** Pest-free conservation has to be ensured in seedbank. All accessions are tested for any form of insect or pest infestation before their processing
3) **Seed viability tests**- Seeds with an initially high viability are more likely to endure for anextended period in storage. The decline in seed viability occurs gradually initially but accelerates as the seeds age. Excessive deterioration could result in the irreversible loss of genetic material. It is essential to assess the viability of accessions: i) before seeds are packaged and deposited in the genebank; ii) at regular intervals throughout the storage period, termed as monitoring. Various methods exist for assessing seed viability, but the germination test stands out as the

most precise and reliable approach.

4) **Seed Germination test:** The purpose of a germination test is to determine the percentage of seeds within an accession that will germinate under favourable conditions, giving rise to healthy seedlings with essential structures viz., roots, shoots, and ample food reserves capable of progressing into fully developed, reproductive mature plants. Germination testis carried out for each species under specific controlled conditions by following standards given by the International Seed Testing Association (ISTA, 2019). On top of paper method: The small-seeded species are germinated on top of one or more layers of paper which are placed into petri dishes. Between paper (BP): the large-seeded are germinated

between two layers of paper. This may be achieved by loosely covering the seeds with anadditional layer of filter paper.

2) Reducing Seed moisture content

The moisture content is "the amount of unbound water molecules in the seed" and is usually expressed as a percentage. Even small changes in moisture content have a large effect on the storage life of seeds. The moisture content should be brought down to the range of 3-7% for most orthodox seeds. The FAO/IPGRI Genebank Standards recommendthe use of 15±5% RH and 15±5°C temperature for drying seeds. In genebank, the most common and safe methods used for drying are dehumidified drying and silica gel drying. Determination of the moisture content is required after final drying, but before packing them in containers and placing them in seed storage is required. Seed moisture content canbe determined by various methods; oven drying method prescribed by the International Seed Testing Association (ISTA, 2019) is frequently used for seedbank storage. Table 2 depicts species whose moisture content should be determined by ISTA methods.

3) **Packaging-** Once the desirable moisture is achieved depending on the species, the seeds are packed immediately in a container for storage. This is done to prevent moisture absorption from the air, averting any mix-up of individual accessions, and safeguard against contamination by insects and diseases. Various types of containers are available forpackaging, and the selection depends on both storage conditions and the specific species involved. Commonly utilized containers in genebanks

include glass bottles, aluminium cans, aluminium foil packets, and plastic bottles. Among these container options, aluminium foil packets hold an advantage due to their ease of sealing and resealing, as well as their space-saving characteristics.

4) Documentation

The accurate and reliable recording of data, along with its comprehensive documentation and efficient information transfer, holds equal significance to the proper handling of germplasm. The effective documentation of plant genetic resources not only facilitates optimal utilization of data but also ensures easy retrieval and applicability. In the National Genebank (NGB) of India, the documentation of genebank holdings involves two types of information files: passport data descriptors and Genebank management descriptors.

Passport data descriptors encompass details like the Name of the Crop, Taxonomic Code,Cultivar Name, National Number, Collector No, Other_ID, and Location in the Gene bank. On the other hand, Genebank management descriptors include information such as seed quantity, seed germination percentage, seed moisture percentage, source of material, and date of storage.

International Genebank standards

Ex-situ conservation in genebanks is recognized as the safest and most reliable method for long-term preservation of PGRFA. The most up-to-date scientific and technical practices in conservation need to be followed for safe, efficient as well as cost-effective management of seed genebanks. Recognizing this need, based on the recommendation of the Commission on Plant Genetic Resources of the United Nations Food and Agriculture Organization (later expanded as Commission on Genetic Resources for Food and Agriculture), 'Genebank Standards' were developed and published in 1994 in collaboration with the erstwhile International Plant Genetic Resources Institute (reorganized as International Board of Plant Genetic Resources and subsequently as Bioversity International). The standards for conservation of Orthodox seeds are depicted in table 3 below:

Table 2 - Species whose moisture content should be determined by ISTA methods

Low constant temperature oven method		**Low constant temperature oven method**	
Grinding is required	**Grinding not required**	**Grinding is required**	**Grinding not required**
Ricinus, Gossypium, Arachis, Glycine	Capsicum, Solanum, Camelina, Linum, Allium, Raphanus, Sesamum	Lupinus, Zea, Avena, Pisum, Oryza, Secale, Sorghum, Hordeum, Phaseolus, Fagopyrum, Cicer, Vicia, Vigna, Triticum	Alopecurus, Lactuca, Panicum, Petroselinum, Chloris, Lolium, Onobrychis, Ornithopus, Cucurbita, Melilotus, Arrhenatheru, Medicago, Asparagus Beta, Agrostis, Cynodon, Scorzonera, Phalaris, Carum, Daucus, Anthriscus, Cichorium, Trifolium, Dactylis, Lepidium, Cynosurus, Cucumis, Cuminum, Paspalum, Festuca, Lycopersicon, Lotus, Deschampsia, Holcus, Citrullus, Phleum

Table 3. Genebank standards for conservation of 'orthodox seeds' in seed genebanks (FAO, 2014)

Activity	Genebank standards
Acquisition of germplasm	• All seed samples added to the genebank collection have been acquired legally with relevant technical documentation. • Seed collecting should be made as close as possible to the time of maturation and prior to natural seed dispersal, avoiding potential genetic contamination, to ensure maximum seed quality. • To maximize seed quality, the period between seed collecting and transfer to a controlled drying environment should be within 3 to 5 days or as short as possible, bearing in mind that seeds should not be exposed to high temperatures and intense light and that some species may have immature seeds that require time after harvest to achieve embryo maturation. • All seed samples should be accompanied by at least a minimum of associated data as detailed in the FAO/Bioversity multi-crop passport descriptors. • The minimum number of plants from which seeds should be collected is between 30-60 plants, depending on the breeding system of the target species.
Drying and storage	• All seed samples should be dried to equilibrium in a controlled environment of 5-20 °C and 10-25 percent of relative humidity, depending upon species. • After drying, all seed samples need to be sealed in a suitable airtight container for long-term storage; in some instances where collections

	that need frequent access to seeds or likely to be depleted well before the predicted time for loss in viability, it is then possible to store seedsin non-airtight containers. • Most-original-samples and safety duplicate samples should be stored under long-term conditions (base collections) at a temperature of –18 ± 3 °C and relative humidity of 15 ± 3 percent. • For medium-term conditions (active collection), samples should be stored under refrigeration at 5-10 °C and relative humidity of 15 ± 3 percent.
Seed viability monitoring	• The initial seed viability test should be conducted after cleaning and drying the accession or at the latest within 12 months after receipt of the sample at the genebank. • The initial germination value should exceed 85 percent for most seeds of cultivated crop species. For some specific accessions and wild and forest species that do not normally reach high levels of germination, a lower percentage could be accepted. • Viability monitoring test intervals should be set at one-third of the time predicted for viability to fall to 85 percent of initial viability or lower depending on the species or specific accessions, but no longer than 40 years. If this deterioration period cannot be estimated and accessions are being held in long-term storage at –18°C in hermetically closed containers, the interval should be ten years for species expected to be long-lived and five years or less for species expected to be short-lived. • The viability threshold for regeneration or other management decision such as recollection should be 85 percent or lower depending on the species or specific accessions of initial viability.
Regeneration	• Regeneration should be carried when the viability drops below 85 percent of the initial viability or when the remaining seed quantity is less than what is required for three sowings of a representative population of the accession. The most-original-sample should be used to regenerate those accessions.

	• The regeneration should be carried out in such a manner that the genetic integrity of a given accession is maintained. Species-specific regeneration measures should be taken to prevent admixtures or genetic contamination arising from pollen geneflow that originated from other accessions of the same species or from other species around the regeneration fields. • If possible, at least 50 seeds of the original and the subsequent most-original-samples should be archived in long-term storage for reference purposes.
Characterization	• Around 60 percent of accessions should be characterized within five to seven years of acquisition or during the first regeneration cycle. • Characterization should be based on standardized and calibrated measuring formats and characterization data follow internationally agreed descriptor lists and are made publicly available.
Evaluation	• Evaluation data on genebank accessions should be obtained for traits that are included in internationally agreed crop descriptor lists. They should conform to standardized and calibrated measuring formats. • Evaluation data should be obtained for as many accessions as practically possible, through laboratory, greenhouse and/or field analysis as may be applicable. • Evaluation trials should be carried out in at least three environmentally diverse locations and data collected over at least three years.
Documentation	• Passport data of 100 percent of the accessions should be documented using FAO/Bioversity multi-crop passport descriptors. • All data and information generated in the genebank relating to all aspects of conservation and use of the material should be recorded ina suitably designed database.
Distribution and exchange	• Seeds should be distributed in compliance with national laws and relevant international treaties and conventions. • Seed samples should be provided with all relevant documents required by recipient country.

	• The time span between receipt of a request for seeds and the dispatch of the seeds should be kept to a minimum. • For most species, a sample of a minimum of 30-50 viable seeds should be supplied for accessions with sufficient seeds in stock. For accessions with too little seed at the time of request and in the absence of a suitable alternative accession, samples should be supplied after regeneration/multiplication, based on a renewed request. For some species and some research uses, smaller numbers of seeds should be an acceptable distribution sample size.
Safety duplication	• A safety duplicate sample for every original accession should be stored in a geographically distant area, under the same or better conditions than those in the original genebank. • Each safety duplicate sample should be accompanied by relevant associated information.
Security and personnel	• A genebank should have a risk management strategy in place that includes inter alia measures against power cut, fire, flooding and earthquakes. • A genebank should follow the local occupational safety and health requirements and protocols where applicable. • A genebank should employ the requisite staff to fulfil all the routine responsibilities to ensure that the genebank can acquire, conserve and distribute germplasm according to the standards.

Regeneration of the conserved germplasm

It is the process of restoration/rejuvenation of germplasm accessions either intended for long- term conservation in base collection or already conserved in the base collection with low seedviability and/or low seed quantity by raising a specific population of plants from the original seeds or vegetative propagules and harvesting the seeds or plant materials to reconstitute the original population makeup of the accession as closely as possible. Thus, it requires crop- specific knowledge and expertise Regeneration being a resource intensive and time-consuming process, should be carried out only when essential to save resources and to avoid unnecessary exposure to risks. Usually, it is undertaken when the seed viability falls below 85% of the initialviability during periodic

monitoring of viability or when the number of viable seeds in an accession is <1,500 for populations and <250 for inbred lines/purelines. Low viability accessions should be prioritized over low quantity accessions for regeneration.

Suggested reading:

1. Cromarty AS, Ellis RH, Roberts EH (1982) The Design of Seed Storage Facilities for Genetic Conservation, Revised 1985 and 1990. International Board for Plant Genetic Resources, Rome, Italy
2. Hong TD, Linington S, Ellis RH (1996) Seed Storage Behavior: A Compendium. International Plant Genetic Resources Institute, Rome, Italy
3. Dulloo ME, Thormann I, Jorge MA, Hanson J (eds) (2008) Crop Specific RegenerationGuidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme (SGRP), Rome, Italy
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5. ISTA. *International Rules for Seed Testing*; International Seed Testing Association: Bassersdorf, Switzerland, 2019; p. 276. http://doi.org/10.15258/istarules.
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7. FAO (2022) Practical guide for the application of the Genebank Standards for Plant Genetic Resources for Food and Agriculture: Conservation of orthodox seeds in seed genebanks. FAO, Rome, Italy
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CHAPTER 3

Hybrid Rice Breeding Technology: Tools and Techniques

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Introduction

Rice is considered as a staple food crop which provide food for half of the global population. Therefore, a consistent steady production of rice is required in globally to make a balance between demand and supply. Among all the various genetic approaches possible to break the yield plateau, hybrid rice technology is the most feasible and readily adoptable one, as was successfully demonstrated in China. Currently more than 60% of the rice areas in China are under the hybrid rice, contributing for more than 67% increase in total rice production, whereas in India, hybrid rice cultivation area was less than six percent of the 44-million-hectare area under rice. The attempts for hybrids rice breeding in India were made in early 1970s, these were mostly academic rather than product oriented.

Hybrid rice

Hybrid rice is the commercial rice crop grown from F_1 seeds of a cross between two genetically dissimilar parents. Good rice hybrids have the potential of yielding 15-20% more than the best inbred variety grown under similar conditions. To exploit the benefits of hybrid rice, farmers have to buy fresh seeds every cropping season.

Development of hybrid rice

Rice is self-pollinated crop. Therefore for the commercial production of hybrid seed, a stable pollination control mechanism is needed. Male sterility is one of the key tools for the successful commercial exploitation of heterosis in any crop. In rice, Cytoplasmic Genetic Male Sterility (CGMS) has been most widely exploited for hybrid seed production. When a nuclear fertility restorer gene (*Rf*) is identified for a CMS system, it is referred as CGMS. The system consists of three lines: a CMS line (A-line), a maintainer line (B-line) and restorer line (R-line). CMS (A-line) and B lines are iso-genic in nature. The R line has homozygous dominant nuclear restorer gene(s) for restoring fertility of CMS line. The hybrid (A x R) thus obtained is fully fertile. In addition, the R-line also has diverse genetic background resulting in heterotic hybrid, in combination with CMS line.

In male sterility system the pollen grains remains unviable and such rice spikelet's are incapable of setting seeds through selfing. Thus, a male sterile line can be used as female parent

of a hybrid. When a pollen parent is grown in surrounding the male sterile line in an isolated plot, can produce a bulk quantity of hybrid seed due to cross pollination with the adjoining fertile pollen parent. The seed set on male sterile plants is the hybrid seed which is used for growing the commercial hybrid crop. The harvested seeds from the male sterile plants are the hybrid seed which is used for growing the commercial hybrid.

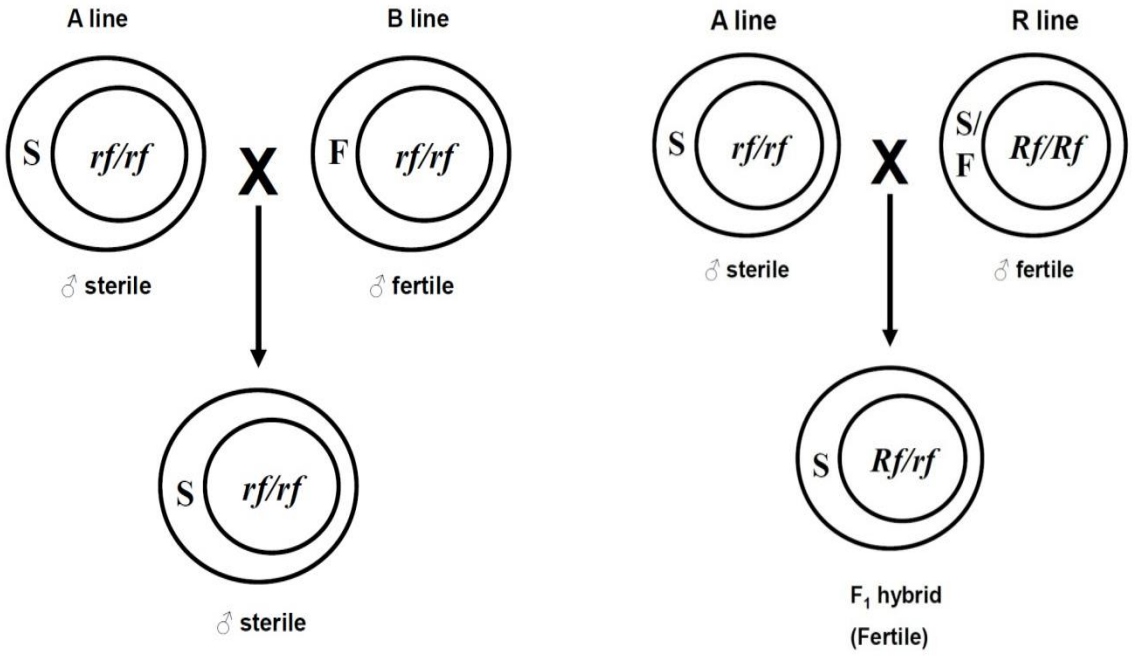

Fig. 1: Schematic description of cytoplasmic genetic male sterility system

The first practically useable CMS system in rice was discovered by Prof. Yuan Long Ping and his team in Hainan island of China in 1970 in *Oryza sativa* f *spontanea*. This system was designated as Wild Abortive (WA) system of male sterility. There are several other male sterility systems reported in rice but WA system is the most exploited and stable system in China and in other countries.

Table 1: List of the different sources of male sterility inducing cytoplasm in rice.

Designation	**Cytoplasmic Source**
CMS-WA	Wild rice with abortive pollen
CMS-DA	Dwarf wild rice with abortive pollen
CMS-IP	Indonesian Paddy
CMS-DI	Dissi type
CMS-HL	Hong Lian
CMS-KR	*O. rufipogon*
CMS-BT	Chinsurah boro II
CMS-TN	TN 1

CMS-GAM	Gambiaca
CMS-ARC	Assam Rice Collection IRRI Acc. 13829
CMS-*O. perennis*	*O. perennis*, Acc.104823

Transfer of CMS source into the elite rice lines

To transfer the CMS source into the elite lines, first we need to test-crossed the elite line with the CMS line of a desired cyto-sterility source to test their maintaining ability. Those elite lines which are identified as maintainers are repeatedly backcrossed up to six generations for complete transfer of cyto-sterility source.

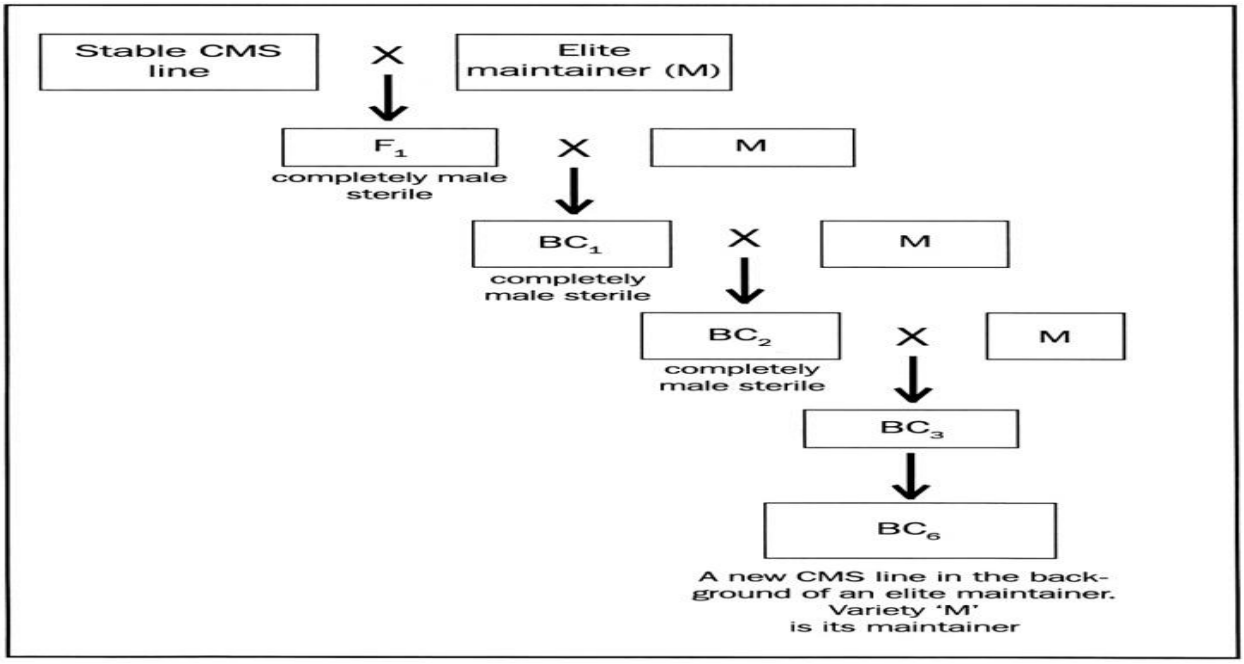

Fig. 2: Procedure of transferring a CMS source into an elite maintainer line.

Environment Sensitive Genic Male Sterility (EGMS)

In this male sterility system the sterility expression is conditioned by environment. There are two type EGMS.

Photoperiod sensitive Genic Male Sterility (PGMS): Most of the PGMS lines remain male sterile under a long-day (>13.75h) conditions and revert back to fertility under short-day (< 13.75h) conditions.

Thermo-sensitive Genic Male Sterile (TGMS): Most of the TGMS lines remain male sterile at a high temperature (maximum >30°C) and they revert back to partial fertility at a lower temperature (maximum <30°C). The critical sterility/fertility points vary from genotype to genotype.

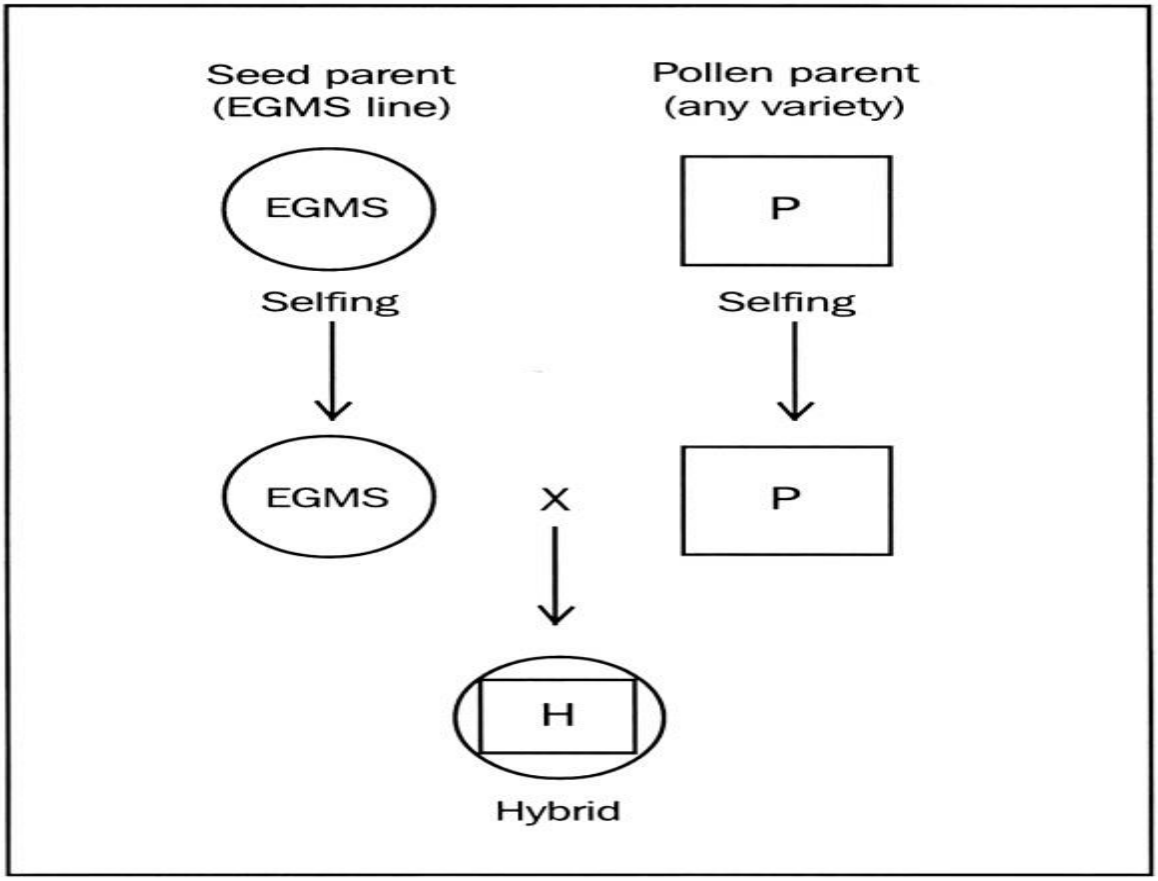

Fig. 3: Use of EGMS lines for developing two-line hybrids.

Hybrid rice seed production

Seed production package is a pre-requisite for economic feasibility and commercial viability of the hybrids rice technology. Being a self-pollinated crop, maintenance of purity in rice verities is much easier than rice hybrids. For producing best quality F_1 seeds, higher genetic and physical purity of parental lines is imperative. Adoption rate of this innovative technology primarily depends on the magnitude of heterosis realized at field level and availability of pure seeds at reasonable cost. Several technical intricacies involves in hybrid rice seed production which have to be manage well to obtain a seed yield of 1.5-2.0 t/ha. Some of the aspects which need to be standardized for different combinations and locations are as follows (Mao, Virmani et al. 1998):

1) Identification of suitable female line
2) Obtaining proper synchronization of flowering between A and R lines
3) Determining the optimal female to male ratio
4) Effect of date of sowing on flowering synchrony
5) Effect of age of seedling on flowering synchrony
6) Identifying the critical out pollination promoting factors
7) Determination of appropriate dosage and stage for application of GA_3
8) Finding out frequency and precise timing for supplementary pollination

Table 2: A generalized optimum package for rice hybrid seed production

Activity	Particulars	
Seed rate	Seed parent (A line)	15 kg/ha
	Pollen parent (B & R line)	5 kg/ha
Nursery	Sparse seedling (20 g/m^2) to ensure multi tillered (4-5) seedlings in 25 days	
Row ratio	2B:6A, for CMS multiplication	
	2R:8A, for hybrid seed production	
Number of seedlings/hill	2seedlings/hill for seed parent; and 3 seedling/hill for male parent	
Spacing	B/R to B/R	30 cm
	B/R to A	20 cm
	A to A	15 cm
	Plant to plant	15 cm or 10 cm
GA_3 application	60-90 g/ha in 500 liters of water at 5-10% heading in two split doses on consecutive days (60% on first day & 40 % on the second day)	
Supplementary pollination	Four to Five times a day at peak anthesis with 30 minutes interval during flowering phase (10-12 day period)	
Rouging	At vegetative phase - Based on morphological characters of leaf and plant type	
	At flowering - Based on panicle/anther characteristics	
	At maturity - Based on grain characteristics and percent seed set	
Seed yield	1.5-2.0 t/ha	

The success of hybrid rice cultivation depends on the success of the hybrid rice seed production programme which enables seed producers to produce high quality seed at an economical price. Hybrid rice seed production requires specialized techniques which must be fully understood by the production staff.

Hybrid rice seed production using the CMS system, i.e. the three-line system: A line (female), B line (maintainer) and R line (restorer) involves three steps:

a) A line: It is cytoplasmic male sterile line which is used as female parent in hybrid seed production. It is maintained by crossing with the B line (maintainer line). Both these lines are iso-genic having homozygous recessive nuclear genes conferring male sterility, differing only in cytoplasm which is sterile (S) in A line and fertile (N) in its maintainer

b) B line: It is iso-genic to A line and is used as pollen parent to maintain male sterility in A line. This line is maintained by growing in isolation, atleast 5 m away from any rice variety.
c) R line: This is also called as fertility restorer or pollinator line. This is used in hybrid seed production by growing along-with A line in a standard row ratio. It is also maintained by growing in isolation, at least 5 m away from any rice variety

In order to obtain the best quality F_1 seed in the hybrid seed production programme, high genetic and physical purity of the parental lines is a prerequisite. Impure parental lines lead to variation in plant type, duration, plant height and grain size, and ultimately the quality of the F_1 hybrid is affected. It is therefore essential to adopt methods to ensure quality seed production. Quality seed should have the following characteristics:

- true-to-type genetic purity
- no contamination or admixture in the seed
- high germination capacity
- free from disease
- free from weeds, soil particles, sand and stones
- no broken seeds

Fig. 4: Different operations in hybrid seed production plots. a. Spraying of GA_3; b. Sticking for supplementary pollination; c. Rope pulling for supplementary pollination; d. Collection of 'A' line panicles for nucleus seed production

Fig. 5: Hybrid rice seed production plot

Selection of land

The land selected for seed production should be fertile, preferably light-textured, with adequate irrigation and a proper drainage system. The field should be free from weeds and volunteer plants from the previous paddy crop. In order to achieve synchronous flowering, a homogenous plot with an even topography is required. The field should not be infested with serious pests and diseases. Hybrid rice seed production fields should be isolated as rice pollen can travel longer distances with the wind; negligence leads to impurity of F_1 seed. When selecting for isolation of land, the following points must be considered:

- **Space isolation:** Space isolation of at least 100 m from seed production plots to other rice varieties is normally satisfactory for quality hybrid seed production. It is safer to have an isolation distance of up to 200 m for male sterile (A line) multiplication, while for B and R line multiplications in varieties, an isolation distance of 3 to 5 m is sufficient.

- **Time isolation:** When space isolation is not possible, time isolation of about 30 days is satisfactory. This means that the flowering stage of the parental lines in the seed production field should be at least 30 days earlier or later than that of other varieties grown within the area to avoid contamination by pollen.

- **Barrier isolation:** Tall and compact trees or bushes or some tall crops (e.g. sorghum, pigeon pea and sugar cane) with 30-40 m distance can serve as barrier isolation.

Seeding time

Seeding of the parental lines should be planned in such a way that flowering coincides with the most favourable climatic conditions listed below:

- daily mean temperature of 24°-30°C
- relative humidity of 70-80%
- differences between day and night temperature of 8°-10°C
- sufficient sunshine with moderate wind velocity

Nursery bed preparation and sowing

Given the high cost of seed, it is essential to raise the nursery in a well-managed field if healthy and robust seedlings are to be obtained. Optimum seed rate should be applied and every seed must be utilized by adopting good nursery management practices. A sparse well-managed nursery gives healthy seedlings for the main field. The normal recommendation is 1 kg of parental line seed in an area of 40 m^2. For 1 ha of main field, 12.5 kg of A line seed and 5 kg of R line seed are required.

Staggered sowing of parental lines for flowering synchrony

Hybrid seed set on the female line depends primarily on its flowering synchronization with the R line; the sowing of male and female lines must therefore be planned to achieve this. For example, if the duration of the male line is 10 days more than that of the female line, the male line is sown in 2-3 staggered sowings so as to ensure a continuous pollen supply. In such cases, 3 sowings of the R line (i.e. 13, 9 and 5 days ahead of the female line) are carried out. However, in countries such as China where the technology has been perfected, only 1 or 2 sowings of the male line are necessary.

Transplanting

Conventional high-yielding varieties may be transplanted once the nursery crop is 25-30 days old; but in hybrid seed production plots transplanting may commence (depending on the difference in duration of the A and R lines) when 21-35 days old. Timely transplanting ensures good picking of parental lines. Transplanting of too young or very old seedlings may either delay or accelerate flowering and affect tiller number.

While pulling out the nursery and during transplanting, special care should be taken to avoid mixing seedlings of male and female parents. It is also important to avoid mixing seedlings of different ages of the male parent, which could affect the uniform distribution and availability of pollen. The long- duration parental line must be transplanted first in order to obtain good synchronization at flowering.

Transplanting R lines

- Paired rows with 15 cm spacing between plants.
- Seedlings of different ages transplanted in a sequential order (e.g. I, II, III, then again I, II, III).
- Single seedlings per hill with row-to-row spacing of 15 or 30 cm (as per recommendation) in the main field.

Transplanting A lines

- Six rows with 15 cm spacing between the paired rows of R line seedlings is the normal recommendation in many Asian countries.
- One seedling per hill with a spacing of 15 x 15 cm.
- In India, spacing of 30 cm between A line and R line rows to facilitate bumper male growth and supplementary pollination. In China, A: R row ratio varies from 2: 8 to 2: 14.

Row ratio, spacing and direction

Years' experience in hybrid rice seed production indicates that row ratio and spacing play a major role. Seed parents and pollen parents planted in a specific row ratio and with specific spacing have a marked effect on seed yields. A row ratio of 6: 2 seed parent to pollen parent has proven very effective. Row direction perpendicular to the prevailing wind direction at flowering stage allows easy pollen dispersal on the seed parent.

Optimum fertilizer management

Application of farmyard manure (FYM) at a rate of least 10 t/ha and a fertilizer dose of 120: 60: 40 kg/ha NPK is recommended in the main field. In order to achieve optimum fertilizer-use efficiency, it is recommended that the fertilizer be placed in the root zone with split application: one basal and two top dressing, at tillering and at panicle initiation. In general, split application of N will prolong the pollen supply of the male line and increase tillering capacity. One heavy N application results in a leafy crop, whereas controlled N levels during middle and late crop growth stages prevent excessive growth of flag leaf and provide good aeration and sunshine, which is good for pollen spread.

Water management

Following transplanting, the main field should be irrigated or drained based on the growth stage of the crop:

- Up to the third stage of panicle development: shallow (2-3 cm).
- From the third stage of panicle development to heading: about 5 cm.
- From heading to grain filling: no shortage of water.
- One week before harvesting: water drained out.

Ideal synchronization

For optimum synchronization of flowering, the female parent should flower 2-3 days earlier than the male parent.

- If A and R lines have the same growth duration, the A line should flower 1-2 days earlier than the R line in all panicle developmental stages.
- If the A line has shorter duration than the R line, the R line should be one stage earlier than the A line during the first three panicle development stages.
- If the A line is longer than the R line in growth duration, the A line should be 2-3 days earlier than the R line during the first three panicle development stages.

Adjustment of flowering

If the difference in predicted flowering is more than 3 days between the parental lines, measures should be taken to synchronize flowering. The application of quick-releasing N fertilizers on an early-developing parent in the early panicle development stages tends to delay flowering. Similarly, spraying phosphatic solution (1%) on the later-flowering parent tends to enhance flowering by 2-3 days. If the pollen parent (R line) reveals a tendency towards heading earlier than the seed parent (A line) after the third stage of panicle initiation, root zone placement of N fertilizer is helpful in delaying panicle development.

Leaf clipping

Leaf clipping of A and R lines is helpful for better out-pollination and seed set. Long and erect flag leaf may obstruct pollen dispersal from the R to the A line and affects the outcrossing rate. Flag leaves should be clipped off in such cases, when the main culms are still in the boot leaf stage. Flag leaf clipping gives uniform distribution of the pollen over A line plants. However, it is not advisable to perform leaf clipping in areas where diseases such as bacterial leaf blight, sheath blight and bacterial leaf streak prevail, as they may spread further and reduce seed yield.

Use of GA_3

GA_3 is used to enhance panicle exertion. Female lines with WA cytoplasm have poor or incomplete panicle exertion. Spraying GA_3 not only helps exert the panicle but also increases the duration of floret opening, improves stigma exertion and stigma receptivity, and widens the flag leaf angle. Spraying GA_3 increases plant height by 10-15 cm and it can also be used to adjust the plant height, in particular of R line in relation to A line. In India, a dose of 50 g GA_3/ha has been found to be optimum (30 g GA_3 sprayed at 5-10% heading and another 20 g GA_3 1 day later, i.e. a 1-day gap between the two sprayings). If the male line is no higher than the female line, it is advisable to give one extra dose of GA_3 to the R line to increase its plant height. GA_3 should preferably be sprayed in the evening (15.00-18.00 hours) and on sunny days.

Supplementary pollination

Rice is basically a self-pollinated crop. Supplementary pollination serves to enhance the out crossing rate in order to increase seed set. Supplementary pollination should be done by shaking the pollen parent with the help of ropes or sticks so that the pollen is shed effectively on A line plants. Supplementary pollination needs to be done 3-4 times at 20- to 30-minute intervals and should be continued for 10-12 days during flowering. With improved management of parents and effective supplementary pollination, hybrid seed yield can be increased significantly.

Roguing

Purity of the hybrid seed is top priority for the production of quality seed. Roguing of off-types and voluntary plants at several stages is essential for obtaining physical and genetic purity. Roguing is the removal of undesirable rice plants from both parents (male and female). Undesirable plants include off-types (eg. maintainer or B-type plants in A line). Off-type plants can be identified by their morphological characters (eg. height, leaf size, leaf shape and colour, panicle shape, panicle size and pigmentation) in the late vegetative/early flowering period. B line plants with similar morphological features to A line plants can be identified by their

plumpy anthers, completely exerted panicle and 3-4 days earlier flowering compared to the A line. These plants in the A line row must be uprooted as soon as they are identified. Roguing at an appropriate time (flowering initiation) ensures good seed quality. Roguing is normally done from the vegetative to the flowering stage.

Harvesting operations

In order to maintain high purity, extreme care should be taken at harvesting and threshing. Just before harvesting, check female rows for left-over pollen shedders (i.e. maintainer plants), off-types and male parent plants (restorers). After confirmation that the field does not contain unwanted plants, the male rows should be harvested first and all panicles carefully removed. Move the harvested male plants to a separate threshing floor where only male parents should be threshed. The female rows which are ready for harvest should be carefully rechecked for left-over plants or panicles of male (restorer) parents. Female plants should then be harvested and threshed on a separate threshing floor. The threshing floor should be free from seed of the previous crop and must be very clean. Threshing can be done by a harvest combiner, thresher, tractor or bullocks. With the harvest combiner or thresher, extreme care must be taken to clean the machine thoroughly before use, so as to avoid admixture with other varieties of paddy.

Conclusion

Hybrid rice technology has shown the potential to meet the ever increasing demand of food in its commercial success in China and it is considered as one of the challenging practices which break the yield ceiling in rice cultivation. The potentiality of this technology efforts were made to adopt this technique in as early as 1970s but systematized and intensified in 1989 with launching of a mission oriented project. The hybrid rice variety was released with a short period of five years. Since then many other hybrids with improved traits like disease resistance, grain quality and higher level of heterosis have been developed. Also, seed production technology has been optimized to obtain constant yields of 1.5 to 2.0 t/ha. Production packages and practices for hybrid rice cultivation in specific regions have been optimized. The future of large scale adoption of this technology in India appears glorious.

CHAPTER 4

Polymerase Chain Reaction (PCR): Principle and Applications

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Introduction

Polymerase Chain Reaction (PCR) is one among the most powerful laboratory techniques developed so far. It has a unique combination of sensitivity coupled with flexibility. It is a technique used to amplify a specific DNA sequence into high copy number enough to analyze by other laboratory techniques. PCR is ubiquitous in all fields of science including clinical, forensic, and diagnostic fields. Several variants of PCR are now available for varied applications.

History

1953: The discovery of DNA double helix structure

1967: Thomas Brock reports on the isolation of extremophilic bacterium *Thermophilus aquaticus*

1971: Kleppe and co-workers first describe a method using an enzymatic assay to replicate a short DNA template with primers *invitro*

1976: Isolation of Taq Polymerase

1977: Development of dideoxy chain termination method of DNA sequencing called Sanger sequencing by Frederik Sanger and colleagues

1983: Kary Mullis discovered the technique of PCR at Cetus Corporation

1985: PCR technique was published in Science

1988: Patent for Taq DNA Polymerase was filed by Mullis et al.

The first automated Thermal Cycler was introduced into the market by the joint venture of Cetus Corporation and Perkin Elmer

1989: Science magazine names Taq Polymerase as its first "Molecule of the year"

1993: Nobel Prize awarded to Kary Mullis

Comparison of PCR with *invivo* DNA replication

Process	Replication	PCR
Purpose	Copies whole genomic DNA	Generate multiple copies of a short segment of DNA
Separation of double stranded DNA	Helicase enzyme separates the duplex DNA	Denaturation of duplex DNA is achieved by heating DNA at high temperature of 96^{o}C
Priming	RNA primers synthesized by Primase enzyme	Artificially synthesized short oligonucleotides
Enzyme	DNA Polymerase	Thermostable Taq DNA polymerase

Components of PCR

Target DNA: The phrase "garbage in-garbage out" is relevant for target DNA**.** The quality and quantity of target DNA is very crucial for a successful PCR reaction. Care should be taken to avoid contamination from non-targeted DNA source. The quantity of the target DNA depends on the source and method. If the target is plasmid DNA which is small and enriched for specific target, smaller quantities of target DNA would be sufficient. When genomic DNA is used target, larger quantity of it is required as genomic DNA contains only one copy of target sequence per genome equivalent.

Water**:** Water provides the liquid environment for the reaction to take place and acts as a matrix in which other reactants interact with each other. Sterile, de-ionized nuclease free water is used to avoid potential contaminants.

PCR Buffer: The purpose of PCR reaction buffer is to provide an optimal pH and monovalent salt environment for the final reaction volume. The buffer is commonly supplied along with the commercial polymerases and most often as a 10x concentrate.

$MgCl_2$: $MgCl_2$ supplies the divalent Mg^{++} cations that act as a cofactor for Type II enzymes, including polymerases and restriction endonucleases used in PCR. Most of the commercially available PCR reaction buffers contain $MgCl_2$ at a standard concentration of 1.5mM used in PCR. However, sometimes it is required to change this concentration for optimizing the PCR reaction conditions. In such cases, it is preferred to procure buffer without MgCl2 and add it separately.

dNTPs: deoxy Nucleotide triphosphates (dNTPs) are the building blocks for the synthesis of new DNA strand in PCR reaction. The β and γ phosphates of individual dNTPs also acts as a source of energy required for the PCR reaction. Unlike reaction buffer and $MgCl_2$, dNTPs cannot withstand repeated freezing and thawing. Hence it is recommended to prepare aliquots of smaller working volumes from the standard 10mM dNTP mix available commercially.

Polymerase: Polymerase enzyme takes up the dNTPs and incorporates them opposite to the complementary base in the template strand. There is a large variety of DNA polymerase enzymes available and it is critical to select the right enzyme depending on the experiment.

Since it was first isolated from the bacteria *Thermus aquaticus*, Taq DNA polymerase has become the standard reagent for the PCR reaction. In addition, a number of other thermal-stable DNA polymerases, isolated from other thermophilic species, have become available. Among these are enzymes from *Pyrococcus furiosus* (Pfu polymerase), *Thermus thermophilus* (Tth polymerase), *Thermus flavus* (Tfl polymerase), *Thermococcus litoralis* (Tli polymerase aka Vent polymerase), and *Pyrococcus species* GB-D (Deep Vent polymerase).

Primers: When all the above-mentioned PCR components are taken care of, the success of a PCR reaction will ultimately depend upon the primers and the reaction conditions. Primers specify the address of the target sequence to be amplified. The length and the actual sequence of the primer are two very crucial aspects of primers. The length of the primer must be sufficient to guarantee that it will occur in the background target DNA less than once by chance alone. Longer the primer, lesser will be the probability of its complementary sequence present more than once in the target genome. Primers of length 20-25bp will have a good chance of being unique in the genome. Two such primers (forward and reverse) are required to target amplification of a sequence of specified length.

Primer Designing Guidelines:

- Melting temperature (Tm) between 55 and 65°C
- Primer pairs should possess 40−60% GC content
- Primers should lack significant secondary structure
- Primers should not be complementary to themselves or partner primers, particularly at the 3’ end
- Avoid 3’ clamping (examine the 5 bases of the 3’ and accept 3 of these as A or T and 2 as G or C)
- Avoid runs of the same nucleotide that are longer than 4 repeats or palindromic regions

Procedure:

A typical PCR consists of following steps:

Initial Denaturation: The reaction temperature is increased to 95 °C and the reaction is incubated for 2−5 min (up to 10 min depending on enzyme characteristics and template complexity) to ensure that all complex, double-stranded DNA (dsDNA) molecules are separated into single strands for amplification.

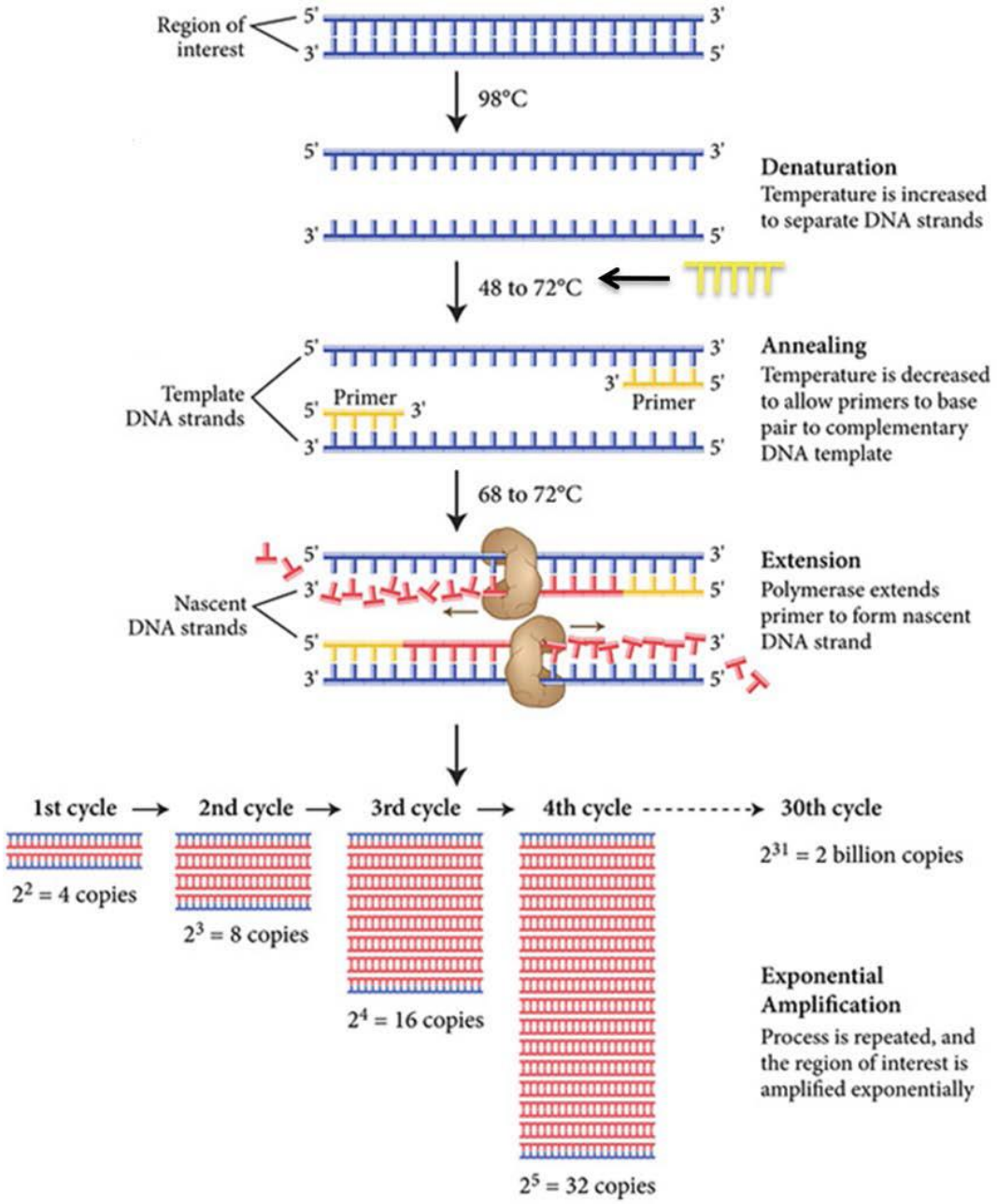
Cycling:

1. Denaturation: The reaction temperature is increased to 95 °C, which melts (disrupts the hydrogen bonds between complementary bases) all dsDNA into single stranded DNA (ssDNA).

2. Annealing: The temperature is lowered to approximately 5 °C below the melting temperature (Tm) of the primers (often 45−60 °C) to promote primer binding to the template.

3. Extension: The temperature is increased to 72 °C, which is optimum for DNA polymerase activity to allow the hybridized primers to be extended.

Repeat: Steps 1−3 are performed in a cyclical manner, resulting in exponential amplification of the amplicon



Adopted from S, M. (2014, October 3). Principle of PCR and applications. Retrieved from http://www.slideshare.net/MetheeSri/principle-of-pc

Variants of PCR

RT-PCR and qRT PCR

Nested PCR

Multiplex PCR

Inverse PCR

Hotstart PCR

Touchdown PCR

Applications

1. Genotyping-to detect sequence variations in alleles
2. Assessment of variation in gene expression
3. DNA fingerprinting
4. DNA sequencing
5. Cloning DNA fragments of interest
6. In site-directed mutagenesis- PCR primers are designed to introduce base substitutions, deletions, and insertions
7. Diagnosis of infectious diseases
8. Evolutionary studies
9. Identification and Classification of organisms
10. Genetic engineering and detection of genetically modified organisms

Further reading:

Methods in Molecular Biology, Vol 226: PCR Protocols, Second Edition. Edited by JMS Bartlett and D Stirling © Humana Press Inc., Totava NJ

CHAPTER 5

Analysis of grain quality parameters in rice

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Analysis of grain dimension

Materials: grain shape tester or dial micrometer

Procedure:

- After dehusking the resultant brown rice or after milling the polished rice is taken for computing the grain shape and size.
- A minimum of 10 full grains with intact tips are taken and evaluated using grain shape tester or dial micro meter.
- Average of length and breadth are taken in mm. and length/breadth ratio is calculated according to classification given by Ramaiah, 1969.

Systematic classification.

Long slender(LS)	Length 6mm and above, length/ breadth ratio 3and above
Short slender (SS)	Length <6mm, length/ breadth ratio 3and above
Medium slender (MS)	Length <6mm, length/ breadth ratio 2.5 to 3
Long Bold (LB)	Length 6mm and above, length/ breadth ratio <3
Short Bold (SB)	Length <6mm, , length/ breadth ratio <2.5

Grain size, shape and appearance

On the basis of average length of kernels brown / milled rice is classified into following International classification SES/INGER, IRRI 1996 is given below

Scale	Size	Length (mm)
1	Extra-long	>7.50
3	Long	6.61-7.50
5	Medium	5.51-6.60
7	Short	5.50 or less

Grain shape is estimated by length /breadth ratio of kernels.

Scale	Size shape	L/B ratio (mm)
1	Slender	Over 3.0
3	Medium	2.1 to 3.0
5	Bold	1.1-2.0
9	Round	1.0 or less

Chalkiness of endosperm

Materials: (1) Magnifying lens; (2) Enlarger

Procedure:

Degree of chalkiness describing the milled sample rices with respect to (a) White belly, (b) White centre, (c) White back

	Kernel area (Extent)
Absent (A)	None
Very Occasionally present (VOC)	Small (less than 10%) kernel
Occasionally present (OC)	Medium (11% to 20%)
Present (P)	Long (more than 20%)

- Chalky white spots often appear in the starchy endosperm. Soft textured, white spots occurring in the middle part on the ventral side (side on which the embryo lies) are called abdominal white or white belly.
- A white chalky region extending to the edge of the ventral side and towards the centre of the endosperm is called a white core. This is present in Basmati 370 only. Basmati rices do not have abdominal white when the breadth of the grain is more than 2.2 only abdominal white appears.
- A long white streak on the dorsal side is called the white back.

Chalk Index determination

Materials: (1) Light box; (2) Fluorescent light

Procedure:

The chalkiness of grain sample may be determined as follows:

- Select whole grains from a sample and place approximately ten grains of whole grains on a light box.
- Visually identify grains with 50% or more chalk content

Weigh chalky grains and calculate the percent chalkiness of sample

Chemical Analysis

1. **Estimation of Amylose content**

Amylose is a polysaccharide made of a-D-glucose units, bonded to each other through a (1-4) glycosidic bond.it is one of the two components of starch, making up approximately 20-30% .

Principle: the iodine is absorbed within the helical coil of amylase to produce a blue colored complex which is measured colorimetrically.

a) Reagent

- 1.0 N sodium hydroxide:40gm of anhydrous NaoH was dissolved in one litre of distilled water
- 1.0N acetic acid : 57.75ml of glacial acetic acid was dissolved in one litre of distilled water
- Iodine solution : (0.2% I2 in 2% KI) 2 gm of iodine and 20 gm of KI dissolved in one litre water.
- Absolute alcohol: >95% alcohol (rectified spirit).
- Standard amylose solution: take 40 mg of amylose add 1ml of absolute alcohol and 1.0n Sodium Hydroxide. Shake well and boil over water bath for 15 min and make the solution to 100 ml volumetric flask

Procedure of amylose estimation for standard protocol.

- 40 mg amylose potato
- Add 1 ml ethanol 99.9%
- Add 9 ml 1N- NaOH
- Vortex and then put in water bath at 100° C for 15 min.
- Make up the volume upto 100 ml.
- Take out 1ml, 2ml, 3ml, 4ml & 5ml of the standard amylose into volumetric flask in three replicates.
- For 1 ml of standard amylose solution, add 0.2 ml of acetic acid and 2ml Iodine+KI
- For 2 ml of standard amylose solution, add 0.4 ml of acetic acid and 2ml Iodine+KI
- For 3 ml of standard amylose solution, add 0.6 ml of acetic acid and 2ml Iodine+KI
- For 4 ml of standard amylose solution, add 0.8 ml of acetic acid and 2ml Iodine+KI
- For 5 ml of standard amylose solution, add 1 ml of acetic acid and 2ml Iodine+KI
- After adding Iodine+KI solution make up the solution to 100 ml and cover the flasks with a black cloth.
- After 20 min take reading in spectrophotometer at 620nm.

Procedure of amylose estimation for rice sample

- Take 100 mg of sample and add 1 ml of absolute ethanol
- Add 9 ml 1N NaOH, vortex and then put in water bath at 100° C for 15 min.
- Rinse and make up the volume upto 100 ml.
- Draw 5 ml of sample in 100ml volumetric flasks in three replications
- Add 1ml of 1N glacial acetic acid and add 2ml of iodine solution
- Make up the volume upto 100 ml with distilled water.
- Leave for 20 min in dark and record observation at 620 nm.

Calculation:

- Draw standard graph by taking amylose concentration on x axis and net optical density (Absorbance) on y axis (see following figure).
- Equation in graph can be used to determine the concentration of amylose in the samples.

Multiplication factor (MF) is developed the graph as explained below

- Get X/Y value (A) around midpoint of the graph.
- Multiply A with dilution factor (20) to get B (MF).
- Multiply net optical density of biological sample with B to get AC%.

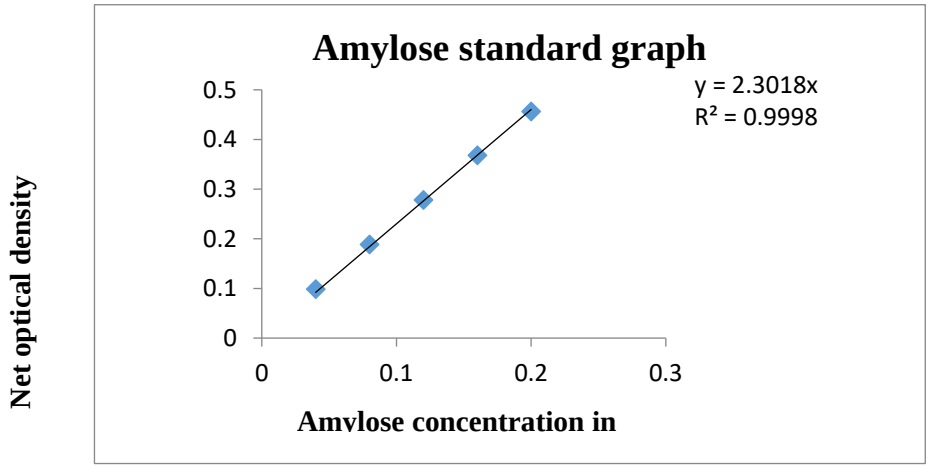

Fig 1: Different concentration of standard amylose

2. Alkali Spreading and Clearing test

A test for estimating the gelatinization temperature of starch utilizing a seven point scale of the degree of spreading of milled rice grain in potassium hydroxide solution.

Methods: (1) Plastic boxes (5.0 x 5.0 x 1.9 cm); (2) Auto pipettes; (3) Incubator;

Reagent: Potassium Hydroxide (KOH): Dissolve 19.54 gm KOH pellets (85%) in 1000ml of distilled water, store at least 24 hours and filter it before use.

Procedure:

- Select duplicate sets of six whole milled kernels without cracks and put them in plastic boxes. Broken kenels can also use if whole grains are not available.
- Add 10 ml of 1.7 % Potassium Hydroxide to the sample. Provide enough space between kernels to allow spreading.
- Keep the sample undisturbed in an incubator at 27- 30°C for 23 hours
- A standard variety is used as a check and all samples are evaluated at least in two replications. The spreading and clearing of kernels noted on a 7 point scale is expressed as average of six values

Scoring is done as follows:

Spreading Scale	**Clearing Scale**
Kernel not affected	Kernel chalky
Kernel swollen.	Kernel chalky, collar powdery
Kernel swollen, collar incomplete and narrow	Kernel chalky, collar cottony or cloudy.
Kernel swollen, collar complete and wide	Centre cottony, collar cloudy
Kernel split or segmented, collar complete and wide	Centre cottony, collar clearing
Kernel dispersed, merging with collar	Centre cottony, collar
All kernel dispersed and inter mingled	cleared Centre and collar cleared.

Classification	Alkali spreading value (ASV)	Gelatinization temperature (GT)
1-2	Low	High >74°C
3	Low, Intermediate	High, intermediate
4-5	Intermediate	Intermediate (70°c-74°c)
6-7	High	Low (55°c-69°c)

3. Gel Consistency Test

Gel consistency measures the tendency of the cooked rice to harden after cooling. Within the same amylose group, varieties with a softer gel consistency are preferred and the cooked rice has a higher degree of tenderness. Harden gel consistency is associated with harder cooked rice and this feature is particularly evident in high- amylose rice. Hard cooked rice also tends to be less sticky. Gel consistency is determined by heating a small quantity of rice in a dilute alkali **Materials:** (1) Cyclone mixture; (2) Water bath; (3) Tripod; (4) Low temperature bath; (5) Gas burner; (6) Test tube holder; (7) Analytical balance; (8) Glass marbles (1.27 mm); (9) Wig-L-Bug Amalgamator; (10) Culture tubes (13 x 100 mm, Pyrex No: 9820); (11) Graph paper; (12) Auto pipettes (1 & 2 ml); (13) Ethanol (95%) with 0.025% thymol blue; (14) 0.2 N Potassium Hydroxide (KOH)

Reagents:

(1) 0.2 N KOH - 2.8 gm KOH in 250 ml distilled water;
(2) Thymol blue: 0.025 gm in 100 ml absolute alcohol.

Procedure :

Make sure that all samples have been stored in the same room for at least 2 days so that the moisture content is equalized.

- Place 10 milled grains in the Wig-L-Bug amalgamator. Grind these for 55 seconds to obtain a fine flour (100 mesh).
- Take 100 mg of flour quadriplicates in culture tubes.
- Add 0.2 ml of ethanol containing 0.25% thymol blue.
- Add 2.0 ml of 0.2 N Potassium Hydroxide.
- Mix the solution on a cyclone mixer.
- Keep the test tubes in water bath at 90-100 °C for 8 minutes after putting one glass tube marble on each test tube.
- After removing the culture tubes from water bath cool them for 5 minutes.
- Mix the solution on a cyclone mixer.
- Keep the culture tube in the low temperature bath at 0-2°C for 20 minutes.
- The culture tubes are removed from ice bath are laid horizontally for one hour over ruled or graph paper.
- Length of the blue coloured gel from the inside bottom of the test tube to the gel front was then measured as gel consistency of the sample.

A) 26-40 mm Hard gel consistency
B) 41-60 mm Medium gel consistency
C) 61-100 mm Soft gel consistency

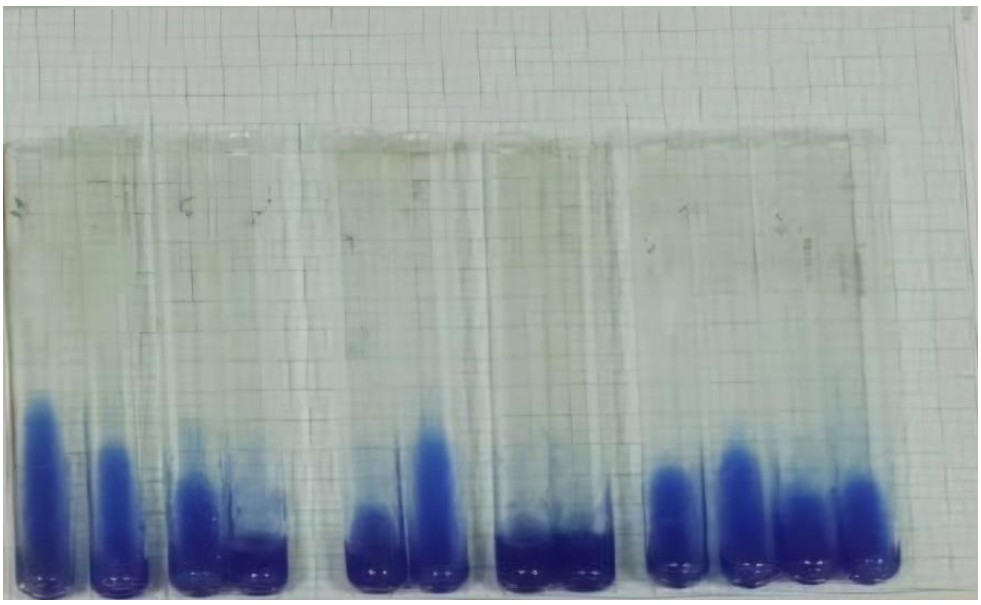

Fig 2: Gel consistency of rice samples

CHAPTER 6

Procedures and protocols for isolation of genomic DNA, quantification, polymerase chain reaction and gel electrophoresis

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A. Isolation of plant genomic DNA from rice germplasm

Reagents required for DNA extraction

1. DNA Extraction Buffer: Following chemicals are required for preparation of DNA extraction buffer

Name of the chemical	Quantity in grams
Cetyltrimethylammonium bromide (CTAB)	20g
Sodium chloride (NaCl)	81.8g
Ethylenediaminetetraacetic acid (EDTA)	7.4g
Tris base	12.1g

All these chemicals were added and dissolved in small quantity of distilled water and pH adjusted to 8.0. Finally, the volume is made up to 1 litre.

2. Chloroform Isoamyl : Alcohol (24:1) - Chloroform (480ml) was mixed with 20ml of Isoamyl alcohol and stored in bottle.
3. Ice cold Isopropanol
4. 70 % Ethanol: 70ml of absolute ethanol was mixed with 30 ml of sterile water.
5. RNase: Ready to use stock of RNase 10mg/ml for removing RNA from the DNA sample.

Step wise protocol for DNA extraction:

1. The fresh leaf samples placed in 2ml micro-centrifuge tubes were immersed in liquid nitrogen for few minutes and ground to powder using tissue-lyzer machine.
2. In 100ml of pre-warmed DNA extraction buffer, 200 µl of β-mercaptoethanol was added. 900 µl of extraction buffer was added to each 2ml micro centrifuge tube containing leaf powder.
3. The samples were incubated for 1 hour in a water bath at 65°C. Throughout the incubation, the samples were intermittently inverted upside down.
4. The samples were allowed to cool down to room temperature and 500µl of chloroform: isoamyl alcohol (24:1) was added to each tube

5. The samples were inverted and centrifuged at 10,000 rpm for 10 min at 4°C. A supernatant of 500 µl was transferred to new 1.5ml micro centrifuge tubes.
6. To the supernatant, equal volume of ice-cold isopropanol was added along with 100 µl of 3M sodium acetate. The tubes were properly inverted and incubated for one hour at -20°C.
7. The tubes were centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was discarded, and 70% ethanol was added. The ethanol was discarded after centrifugation at 10,000 rpm for 5 minutes and the DNA pellet was allowed to dry at room temperature.
8. After drying, the pellet was re-suspended in 100µl of TE buffer (pH 8.0). Also, 2 µl of RNase (10mg/ml) enzyme was added and incubated at 37°C for half an hour.
9. The DNA sample is stored in freezer for its further use.

B. <u>DNA quantification on 0.8% agarose gel</u>

Preparation of 50X TAE for agarose gel preparation

Name of the chemical	**Quantity for 1 litre buffer**
Tris Buffer	242.0 grams
EDTA	19.0 grams
Glacial acetic acid	57.1 ml

All these chemicals were added and dissolved in small quantity of distilled water and pH adjusted to 8.3. Finally, the volume is made up to 1 litre.

1. Weigh 4.0 grams of agarose for 500ml gel preparation.
2. Take 490 ml of double distilled water in a measuring cylinder and add 10 ml of 50 X TAE to it.
3. In a conical flask add the prepared 500 ml solution with the weighed 17.5 grams Agarose and mix well.
4. Boil the solution for 10 minutes in microwave oven until agarose powder is dissolved.
5. The solution was left to cool down for 2-3 minutes and 12.5 µl of Ethidium bromide (EtBr) was added and slowly mixed.
6. The casting trays with combs and rubber stoppers were placed properly and the gel solution was poured into the casting tray.
7. Let the gel solidify for 10 minutes.
8. Put the solidified gel into electrophoresis tank containing 1 X TAE and remove the combs from the gel.
9. 1µl of loading dye is added to the 2 µl of DNA samples
10. Load the samples along with known quantities of λ DNA for reference and run it for half an hour at 90 V.

11. Visualize the gel under UV light/ gel documentation system and quantify the DNA based on the intensity of band as compared to known quantity of λ DNA.

C. Polymerase chain reaction (PCR)

The PCR was performed in thermal cycler using a programme for the SSR marker. Following programme was used for amplification as mentioned below.

Components required for PCR reaction

Reagents	Quantity for 1 well
Genomic DNA	1 µl
Master Mix	5 µl
Molecular grade water	3 µl
Primer (Forward and reverse)	0.5 µl + 0.5 µl

1. PCR reaction mixture was prepared by adding 5µl of master mix, 0.5 µl each of forward and reverse primer and 3 µl of molecular grade water for each sample. Accordingly, PCR reaction mixture is prepared based on number of samples.
2. The 1 µl of 20ng genomic DNA was added to the PCR plate.
3. PCR reaction mixture was dispensed into the wells of PCR plate containing DNA
4. The plate is sealed and placed in thermal cycler for carrying out PCR reaction using following program.

Details of PCR cycles and program for SSR

Step	Temp. °C	Duration	Function	Cycles
1	95	5 min	Initial denaturation	1
2	95	30 Sec	Denaturation	35
3	55	30 Sec	Annealing	
4	72	1 min	Extension	
5	72	10 min	Final extension	1

Agarose gel electrophoresis for visualization of PCR product

To visualize the results of PCR a 3.5% Agarose gel was prepared as described earlier. However, 17.5g of agarose is mixed in 500ml of 1x TAE buffer for 3 hours at 150 V. The results documented using gel documentation system.

CHAPTER 7

Germplasm Evaluation and Utilization for Disease Resistance in Rice

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Introduction

Rice (*Oryza sativa* L.) stands as a crucial staple food crop globally, with almost half of the world's population relying on it. It plays a vital role in the human diet and feeds more than 50% of the world's population (Rathna Priya et al. 2019). By 2050, global demand for rice is projected to rise more than 40% to feed the rapidly growing world population (Milovanovic and Smutka 2017). Despite impressive global increases in production from 289 million tons in 1968 to 782 million tons in 2018, this quantum jump still must keep pace with demand for rice from the rising population (FAOSTAT 2020). While playing a vital role in global food security, rice faces susceptibility to various diseases instigated by plant pathogens like fungi, bacteria, viruses, and nematodes. At present, rice cultivation throughout South Asia and in ASEAN countries is facing significant threats because of a few major biotic stresses (Yugander et al. 2017). Approximately 52% of the global productivity of rice grain yield is severely damaged by biotic factors (Park et al. 2008). Notable economically impactful diseases include rice blast, bacterial leaf blight, brown leaf spot, sheath blight, sheath rot, stem rot, false smut, rice tungro virus, and rice root-knot nematode, leading to substantial economic losses. Recent shifts in global temperatures and climatic conditions have elevated the significance of previously minor diseases, such as false smut of rice and rice root-knot nematode. Effectively managing rice diseases involves employing diverse strategies, including the utilization of resistant varieties, adoption of cultural practices, and the application of biological and chemical control measures. These methods exhibit varying degrees of success in the overall management of rice diseases.

Table 1: Rice diseases and yield loss

Disease	Pathogen	Yield loss	Reference
Blast	*Magnaporthe oryzae (Mo)*	70–80%	Jamaloddin et al. (2020)
Bacterial blight (BB)	*Xanthomonas oryzae* pv. *oryzae (Xoo)*	Up to 50%	Liu et al. (2014)
Sheath blight	*Rhizoctonia solani*	20–60%	Molla et al. (2020)
False smut	*Villosiclava virens (Anamorph:Ustilaginoidea virens)*	85–100%.	Huang et al. (2019)
Bakanae	*Fusarium fujikuroi*	40%	Ou, 1980
Sheath rot	*Sarocladium oryzae*	20–85%	Peeters et al. (2020)

Blast: **Causal organism-*Magnaporthe oryzae***

It is also known as leaf blast, nodal blast, panicle blast, or neck blast. Blast lesions on the leaves are spindle-shaped with brownish margins, ashy centers, and pointed ends. When nodes are infected, they become black and culm may break. At the flower emergence, dark necrotic lesions are formed at the panicle base due to neck blast and cause the panicle to fall off. In early neck infection, grain filling is partial or do not occur. Infected plants often display white, partly or completely unfilled panicles.

leaf blast

nodal blast **Panicle blast**

Brown spot: causal organism-*Cochliobolus miyabeanus*/ (*Bipolaris oryzae*)

Brown spot is a fungal disease that can infect both seedlings and mature plants. Infected seedlings have small, circular or oval, brown lesions, which may girdle the coleoptile and cause distortion of the primary and secondary leaves (seedling blight). Infected seedlings become stunted or die. Black discoloration of the roots cause distorted seedlings. On mature plants, typical spots on the leaves are circular to oval with gray to light brown center and reddish-brown margin. Black to dark brown spots may also appear on the glumes and when infection is severe, the entire panicle can turn brown.

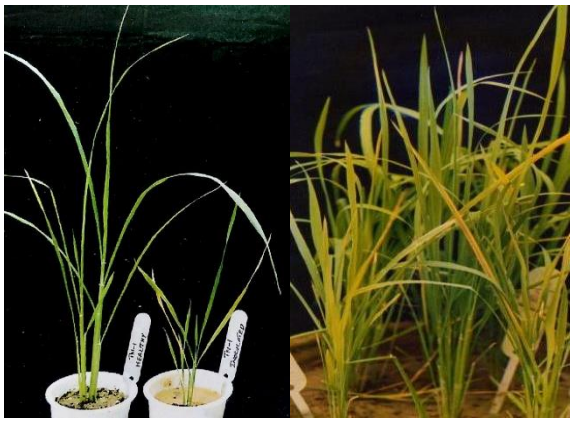
Spots on leaves

Glume blight

Tungro: Causal organism: *Rice tungro baciliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV)

Symptoms: Leaf discoloration to yellow-orange color. Discoloration begins from leaf tip and extends

down to the blade or the lower leaf portion. Infected leaves may also show mottled or striped appearance – stunting, and reduced tillering. Delayed flowering, which may delay maturity – panicles small and not completely exerted.



Stunting **Leaf discolouration**

Sheath rot: Causal organism*: Sarocladium oryzae* (Sawada) W. Gams & D. Hawksw
Symptoms: The typical sheath rot lesion starts at the uppermost leaf sheath enclosing the young panicles. It appears oblong or as irregular spot with dark reddish, brown margins, and gray center or brownish gray throughout. Infected panicles are discolored, sterile, shriveled, or with partially filled grain.

Dark brown lesions on sheaths **Lesions at maturity stage**

Bakanae: Causal organism: *Fusarium fujikuroi* (Nirenberg) [*Gibberella fujikuroi* Sawada Wollenworth]
Symptoms: Affected seedlings are pale yellowish green, thin and elongate abnormally. They are scattered in seedbed and do not appear in definite patches. Infected plants are taller than normal, have pale tillers and usually die before producing grains. partially filled grains, sterile, or empty grains for surviving plant at maturity


Elongated pale green


lanky tillers Rotting of infected tillers

False Smut: Causal organism: *Ustilaginoidea virens* (Cooke) Takah [*Claviceps oryzae sativae* (Hashioka)]

Symptoms: Symptoms are seen only in maturing panicles. Usually few, occasionally several grains are affected in each panicle. Affected grains are transformed into masses of spores that are greenish outside and eventually turn dark. These bodies are twice the size of normal grain.

A 
 B

Spore balls on infected grains: Orange colour (A) and Black colour (B)

Bacterial leaf blight: Causal organism*:* *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings et al.
Symptoms: In **kresek phase** yellowing of seedlings after transplanting is evident. The **leaf blight phase** appears in maximum tillering phase to maturity phase. The symptom appears water-soaked lesion on leaf blade starting at leaf tips, which later becomes yellowish and spreads downwards with a wavy margin. Lesions gradually turn necrotic and later dry quickly.

Lesions with wavy margin **Field view of disease**

Bacterial panicle blight: Causal organism: *Burkholderia glumae* (Kurita & Tabei) Urakami *et al.*
Symptoms: BPB is destructive on emerging panicles and leaf sheaths. Diseased panicle bears light to dark brown partially or fully discolored glumes. Under severe conditions, grain filling in the diseased panicles is affected resulting in chaffy grains. Sheath and leaf lesions appear as irregular elongated brown patches.

Dark brown discolored grains partially discolored grains

Evaluation of Rice Genotypes for Disease Resistance

Phenotype: the physical parts that we can see outwardly.
Phenotyping: is the process of predicting physical appearance.
Phenotyping for disease resistance: is the process of predicting genotypes/cultivars/varietal reaction to pathogen/disease.

a) Inoculation of plant pathogens
b) Quantifcation of disease/plant disease assessment

a. Inoculation: Inoculation is transfer of inoculum to Petriplate/ slant with medium or onto the host plant by using different kinds of techniques. Mode of inoculation can be seed, soil and foliar

(i) Fungus Inoculation Methods

- Natural exposure to inoculum
- Direct application to the host surface
- Inoculation through soil or roots
- Stem injection
- Seedling dip method

Sand witch method

(ii) Inoculation of Bacterial Pathogens

Seed inoculation

- *Burkholderia glumae* (bacterial grain rot)
- *Xanthomonas oryzae* pv. *oryzae* (bacterial leaf blight)

Leaf tip clipping

Xanthomonas oryzae pv. *oryzae*

(iii) Inoculation for viruses

Mechanical inoculation: *Tobacco mosaic virus, Cucumber mild mosaic virus, Potato virus X*

Inoculation through insect vectors

Aphid & whitefly: *Beet yellow virus, Citrus tristeza virus,* Clostero viruses

Rice tungro virus – **leaf hopper** (semipersistent)

Plant disease assessment

Disease is measured in term of **intensity** and Disease intensity can be expressed either as disease incidence or disease severity. The choice between evaluation of disease according to its **incidence** or **severity** depends largely on the type of disease and on the objectives.

Disease severity is the proportion of area or amount of plant tissue that is diseased.

b. Measuring disease severity

Direct estimation

I. Direct, with aid of disease diagrams/Standard area diagram
II. Descriptive keys
    a. Use of disease scales
    b. Use of ordinal rating scales

III. Remote sensing and electronic methods

Image analysis, etc.

Indirect methods

Predict mean severity from incidence

Standard Area Diagram: It allows to estimate intermediate level of disease severity by comparing with diagram showing both diseases. Pictorial representation of host plant with known and graded amount of disease is compared with disease leaves to estimate disease severity. Standard area diagram. eg: leaf rust of wheat provides 1%, 5%, 10%,20%,50 % leaf area infected.

Descriptive keys

- **Nominal or descriptive scale**

Descriptive terms are used: Slight Moderate, Severe

- **Ordinal scale**

A 0 -3 scale has been used to rate clubroot disease of crucifers

- **Interval or category scale**

Category	Severity
0	Free from disease
1	1% or less plants having stag head symptom

Standard Evaluation System for Rice (SES)

- Identifying promising rice germplasm with useful traits is an important activity in rice improvement. The genetic potential of breeding materials, whether developed by conventional breeding or genetic engineering, is evaluated based on phenotypic expressions in target environments with the stress of interest.
- Thus, an accurate and precise yet rapid and practical assessment method should be utilized.

Growth Stages of Rice Plants

- When reporting results for specific characters, use this code to identify the stage of plant growth at which the observation was recorded.
- Specific applications might be sequential data on disease reaction for a season's record of epidemic buildup (e.g. blast notes at growth stages 2, 3, 4, 5, 6, 7, 8, 9).

Table 2: Growth stages of rice plants

Code	Description
1	Germination
2	Seedling
3	Tillering

4	Stem elongation
5	Booting
6	Heading
7	Milk stage
8	Dough stage
9	Mature grain

1. Screening for Leaf Blast Resistance

Inoculum: It is necessary to sow the nursery during blast favourable weather conditions. To create severe blast incidence additional inoculum may be provided. For this collect diseased leaves, chop them into pieces of 3-6 cm long and scatter them over the plot. Infected plants can also be transplanted between boarder rows. This operation may be carried during prolonged wet weather to facilitate infections and polycyclic development of the disease.

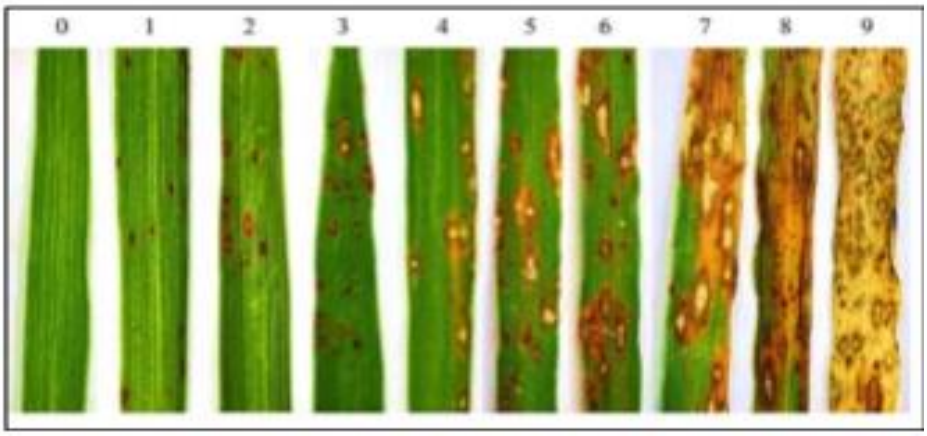
Observations to be recorded: The test entries are to be scored based on leaf blast severity following SES scale. At least two readings on blast severity in entries are to be taken at 10 days intervals from 25 to 30 DAS.

Uniform Blast Nursery for Blast trials at ICAR-IARI, New Delhi

Table 3: SES scale for leaf blast severity

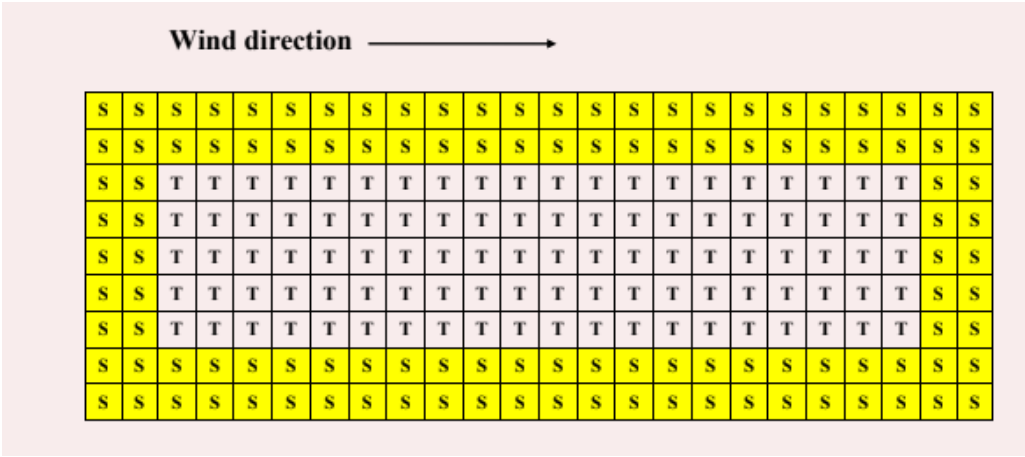
0-9 scale	**Disease severity**	**Host response or reaction**
0	No lesion observed	Highly Resistant
1	Small brown specks of pin point size	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2mm in diameter, with a distinct brown margin.Lesions are mostly found on the lower leaves	Moderately Resistant

3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Moderately resistant
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area	Moderately Susceptible
5	Typical susceptible blast lesions, 3 mm or longer infecting 4-10% of leaf area	Moderately Susceptible
6	Typical susceptible blast lesions, 3 mm or longer infecting 11-25% of leaf area	Susceptible
7	Typical susceptible blast lesions, 3 mm or longer infecting 26-50% of leaf area	Susceptible
8	Typical susceptible blast lesions, 3 mm or longer infecting 51-75% of leaf area many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesions, 3 mm or longer infecting more than 75% of leaf area	Highly Susceptible



Layout of uniform blast nursery for screening against leaf blast at seedling stage

Layout should contain two rows of susceptible lines as a Border: 20 rows of test entries: Two rows of susceptible entries: 20 rows of test entries.



Length of nursery bed depends on land availability and topography of the area.

T = Each test entry in a single row of 50 cm long and 10 cm apart.

S = Highly susceptible blast varieties flanked alround the test nursery.

Screening for Neck Blast Resistance Layout: The test entries included in the nursery vary with respect to maturity duration (from 70 to 150 days). Hence, local susceptible (neck blast) check may be staggered planted for 68 times at 10-day intervals, starting from the date of planting of test nursery. The purpose is to evaluate the neck blast resistance of test entries in relation to the susceptible check. The staggered planting of the susceptible check may be done in a space left in the plot after planting test entries. However, the first planting of check variety must be done along with the test nursery planting after every 50 test entries. Each test entry may be planted preferably in two rows each of 1m length, adopting a spacing of 20 x 10 cm.

Observations to be recorded: The first recording on neck blast incidence should be done when heading is complete in test entries and the second between milk and dough stages. The scoring should be done following the SES scale

SCALE BASED ON SYMPTOMS	
0	No visible lesion or observed lesions on only a few pedicels
1	Lesions on several pedicels or secondary branches
3	Lesions on a few primary branches or the middle part of panicle axis
5	Lesion partially around the base (node) or the uppermost internode or the lower part of panicle axis near the base
7	Lesion completely around panicle base or uppermost Internode or panicle axis near base with more than 30% of filled grains
9	Lesion completely around panicle base or uppermost internode or the panicle axis near the base with less than 30% of filled grains.

Table 4: At growth stage: 8 (20-25 days after heading)

ıle (Incidence of severely infected panicles)	
0	No incidence
1	Less than 5%
3	5-10%
5	11-25%
7	26-50%
9	More than 50%

Based on the number of panicles with each scale, compute panicle blast severity (PBS) as follows:

$$PBS = \frac{(10xN\ 1)+(20 \times N\ 3)+(40x\ 5)+\ (70xN7)+(100xN9)}{\text{Total no. of panicles observed}}$$

where N 1-N9 are the number of panicles with score 1-9. At growth stage: 8 (20-25 days after heading).

2. Screening for Sheath Blight Resistance

Layout: Transplant 25 days old seedlings. Each test entry should be planted in two rows, each of two-meter length adopting a spacing of 20 x 15 cm.

Inoculum and inoculation: Multiply pure culture of the pathogen on autoclaved typha bits or corn or rice culm bits (5-7 cm) or rice: hull (1:3) medium. Inoculate test entries at tillering stage by placing the inoculum between the tillers just above the water line. Alternatively, fresh sheath blight infected material with active lesions can also be used as inoculum.

Observations to be recorded: Take the first reading 15-20 days after inoculation of sheath blight pathogen or first appearance of the disease. A second observation should be made at flowering stage adopting SES scale

Scoring Scale given by IRRI (2014)

Scale	Rating	Disease symptoms
0	Highly Resistant	No infection (Immune reaction)
1	Resistant	Lesions limited to lower 20% of he plant height
3	Moderately Resistant	20-30
5	Susceptible	31-45
7	Highly Susceptible	45-65
9	Highly Resistant	>65

3. Screening for Bacterial Blight Resistance

- **Layout**: Plant each entry in two rows each of 2-meter length, adopting a spacing of 20 x 15 cm. The nursery is flanked by 3 to 4 border rows of TN 1 or any other susceptible variety. Include local susceptible and resistant checks after every 100 test entries.

- **Inoculum and inoculation:** The pathogen is multiplied on peptone sucrose agar and 48-hour old pure culture of the pathogen is brought into suspension by adding 10 ml of water per slant to give a concentration of bacterial cells of about 10^8 to 10^9/ml. In the absence of pure culture, the bacterial suspension may be prepared by cutting the fresh diseased leaves into small pieces of about 2 mm and soak them in water for 10 to 20 minutes so that the suspension has bacterial cell concentration of 10^8 to 10^9 /ml. Inoculate the plants between maximum tillering and booting stages. Dip the scissors in the bacterial suspension and cutoff top 2 to 3 cm of leaves.

- **Observations to be recorded:** Score the entries 15 days after inoculation adopting the SES scale

SES scale (2014) for bacterial leaf blight	
Score	Description (affected lesion area)
1	1-5%
3	6-12%
5	13-25%
7	26-50%
9	51-100%

4. Screening for Brown Spot Resistance

Layout: The nursery can be screened either at seedling stage in nursery adopting UBN or in a transplanted field. In either situation 50 kg N/ha and other fertilizers may be applied as per the recommended practice.

Observations to be recorded: The entries should be evaluated based on brown spot disease severity following the SES scale

Scale	Affected leaf area
1	No incidence
2	Less than 1%
3	1-3%
4	4-5%
5	11-15%
6	16-25%
7	26-50%
8	51-75%
9	76-100%

5. Screening for False smut resistance: At growth stage 9

Scale	**Infected florets**
0	No incidence
1	Less than 1%
3	1-5%
5	6-25%
7	26-50%
9	51-100%

6. Screening for Grain Discoloration (Gd): At growth stage 8-9

Severity of grain discoloration can be estimated by counting grains with more than 25% of glume surface affected

SES Scale

Scale	**Grains with severely discolored glumes**
0	No incidence
1	Less than 1%
3	1-5%
5	6-25%
7	26-50%
9	51-100%

7. Screening for Resistance to Rice Tungro Disease

- **Layout**: Each entry has to be planted in two rows, each of 2m length with 20 x 15 cm spacing. Each entry is alternated with a row of TN1.
- **Inoculation**: Where natural infection is absent inoculate test entries 20 days after planting. For this, release 2 to 3 viruliferous leafhoppers per hill and cage them for 24 hours for effective transmission. Insecticides should not be used in the nursery.
- **Observations to be recorded**: Score the entries 50 days after inoculation for tungro incidence based on the SES scale

SES scale (2014) for rice tungro disease	
Score	Description
1	No symptoms
3	1-10% plant height reduction with no distinct leaf discolouration
5	11-30% plant height reduction with no leaf discolouration
7	31-50% plant height reduction and yellow to orange leaf discolouration
9	More than 50% plant height reduction and yellow to orange leaf discolouration

8. Screening for resistance to sheath rot disease: At growth stage: 7-9 Panicle

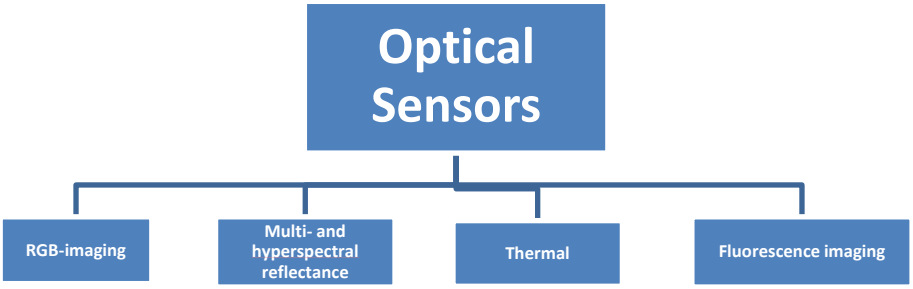
Scale (Incidence of severely affected tiller)	
0	No incidence
1	Less than 1%

3	1-5%
5	6-25%
7	26-50%
9	51-100%

10. Screening of germplasm for resistance to bakanae disease in glasshouse: At growth stage: 3-6

Evaluation of rice genotypes for bakanae disease resistance

Optical Sensors for Plant Disease Detection



These sensors are used in plant phenotyping on different scales from single cells to entire ecosystems. Depending on the scale, different platforms can be operated and consequentially different plant parameters can be observed (Oerke et al. 2014).

Table: Diagnosis of fungal diseases of rice using imaging techniques in high-throughput plant phenotyping platforms

Technology	Sensors	Raw data	Parameters	Applications
Visible light imaging	Visible light camera	Gray or colour value images (RGB channels)	Whole organs or organ parts, time series (minutes to days)	Morphologic traits, digital biomass, height, etc.
Fluorescence imaging	Fluorescence cameras	Pixel-based map of emitted fluorescence in the red and far-red region	Multiple chlorophyll fluorescence parameters and multi-spectral fluorescence parameters	Photosynthetic status/quantum yield/seedling structure/leaf disease, etc.

Infrared imaging	Thermal imaging, Near-infrared cameras	Pixel-based map of surface temperature in the infrared region	Leaf area index, surface temperature, canopy and leaf water status, seed composition, time series (minutes to days)	Measurements of leaf and canopy transpiration, heat dissipation, stomatal conductance differences etc.
Spectral imaging	Spectrometers, hyperspectral cameras	Continuous or discrete spectra	Water content, seed composition, etc. indoor time series experiment	Disease severity assessment/leaf and canopy growth potential.
3D imaging	Stereo camera/TOF camera systems	RGB/IR/Depth images	Plant or organ morphology, structure, and color parameters,	Shoot structure, leaf angle, canopy structure, etc.
Laser scanning	Laser scanning instruments	Depth maps, 3D point clouds	Plant or organ morphology, structure parameters, time series at various resolutions	Shoot structure, leaf angle, canopy structure, etc.
MRI	Magnetic resonance imagers	Water (^{1}H) mapping	Water content, morphology parameters (200-500 µm), 1-600 s	Morphometric parameters/water content.
PET	Positron emission detectors	Radiotracer mapping and co-registration with positron emission signals	Transport partitioning, sectorality, flow velocity, 1–2 mm, 10 s–20 min	Visualize the metabolic distribution and transport of radionuclides.
CT	X-ray tomography	Voxels/tissue slices	Morphometric parameters in 3D (1–100 µm),	Tissue density, tiller number, seed quality, and tissue 3D reconstruction.

Table: Comparison of various approaches used in rice diseases diagnosis

Technique Used	Disease Identified	Accuracy	Merits	Demerits	References
Neuro-Fuzzy expert system	LBD, BSD, BLB	74.21%.	Recognized the diseases at their early stages.	Issues in tackling the noises and other lighting problems due to external forces	Kahar et al., 2015
PCA, Colour Grid-centered Moment and GLCM for feature extortion and SVM for classification	Leaf blast, leaf streak, BLB, leaf brown spot	90%	Attained Highest accuracy	This methodology was not applicable for categorization of crop diseases	Khaing at al., 2018
Deep CNN centered classification	pests and diseases in rice plants were recognized	95%	Accurately and timely detect the diseases	Deep learning methodology contained several layers for classification. So it took	Chowdhury et al., 2018

				more time to spot the diseases contrasted with others	
SVM classifier	rice blast diseases, narrow brown spot, BLB, brown spot	70%	Efficiently classified 4 kinds of diseases in rice	Lowest accuracy when contrasted with others	Suman T., Dhruva kumar, 2015
ESforRPD2 application, Unified Modelling Language and Waterfall Paradigm	8 sorts of diseases and 48 symptoms of the rice plants were recognized	87.5%	Showed Good Reliability	Performance of this method was low compared with other expert systems	Fahrul et al., 2009
Radial basis function network (RBFN) model	Sheath Blight, Panicle Blast, Brown spot, Leaf Blast	95.5%	Good recognition efficiency and generalization	some of the diseases were not identified accurately	Toran Verma and Sipi Dubey, 2017
PCA and NN	Rice Blast	95.83%	Identify the disease quickly and efficiently	Limitations exist in the recognition of lesions with similar morphology and color	Maohua Xiao et al., 2018
PSO- centered incremental classifier	Rice blast, Sheath Rot, Leaf brown spot, BB	84.02%	Reduces the computational time	the system may demand a self-adapted parameter setting scheme	Shampa Sengupta, Asit K. Das, 2017
Hyperspectral data	RLF damage in rice	82%	More accurate than others because of reflectance	Applicable only to specific fields due to the variation of spectra	Jianrong Huang et al., 2012
A visual method based on PdNPscatalyzed TMB/H2O2 system	Blast fungus,*Magnaporthe grisea*	85%	Sensitive, and cost-effectual methodology	Cannot find Other sorts of diseases in rice.	WeiJuan Yang et al., 2013

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CHAPTER 8

Germplasm Evaluation and Utilization for insect resistance in rice

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Introduction

Rice (*Oryza sativa* L.) is the world's most important staple food crop. More than three billion people worldwide consume rice as their major food source. Rice is grown in more than 115 countries in the world. China (145.59 million metric tonnes) stands first in the production of milled rice followed by India (135.76 mMT) and Bangladesh (36.35 mMT). The modern agricultural practices intensify the risk of significant crop losses due to diseases and insect pests. Cultivating multiple rice crops in irrigated regions significantly increases the likelihood of disease epidemics or major outbreaks of destructive insect pests. Changes in climate patterns may alter the distribution and behavior of insect pests, making them more adaptable and capable of thriving in new regions. Also, increased use of fertilizers and irrigation water favors the multiplication of disease organisms and insect pests along with the luxuriant plant growth that results from intensive cultural practices.

Rice is known to be infested by more than 200 species of insects, out of which 30 are reported to inflict economic damage. The yield loss due to insects can reach about 25%. Among insect pests, yellow stem borer (YSB), leaf folder, brown plant hopper (BPH), white backed plant hopper (WBPH), gall midge, gundhi bug, case worm, swarming caterpillar, mealybug, and whorl maggot are the major problems across different geographical regions. Stored grain pests also are gaining significant importance in causing post-harvest losses. Farmers predominantly resort to pesticide application as the primary method of pest management. However, this practice contributes to environmental pollution and poses health risks by contaminating the food chain. Host plant resistance emerges as a promising alternative for efficient, cost-effective, and environmentally friendly pest management in rice. Rice plants can exhibit resistance to biotic stresses through inheritance (resistance from a donor plant) or by inducing resistance through the application of elicitors, which activate the plant defense mechanisms. Mechanism of plant resistance against insects can be assured by biochemical analysis of the plant for its resistance imparting contents such as sugar, silica, phenol etc. as well as antixenosis, antibiosis and tolerance studies. Molecular characterization of identified resistant donors is necessary to look for the presence of resistance genes using reported markers or identification of new genes, if any.

Rapid change in the virulence characteristics of plant pathogen/ insect populations pose continuous threat to existing popular rice varieties as well as for development of a virulent pathotype or biotype. In late 1970s, it has been realized that breeding programs with a wide genetic base can

help prevent widespread plant diseases and insect pest outbreaks, ensuring sustainable crop yields. To address growing varietal improvement requirements, it is crucial to gather, assess, and preserve the entire existing germplasm for more successful breeding initiatives. Rice germplasm collections, varying in size from a few hundred to several thousand accessions, are maintained in various countries by national research programs and by International Agricultural Research Centers of the Consultative Group on International Agricultural Research.

Types of insect resistance

There are types of apparent resistance that are not heritable, and also been known as "pseudoresistance" which are explained as *host evasion* (The host plant passes through the susceptible stage quickly or when insect populations are low); *escape* (A particular host plant is neither infested nor injured despite the local presence of the pest insect) and *induced resistance* (Some environmental conditions, temporarily increase the level of resistance). Mechanisms of resistance have been grouped as non-preference (antixenosis), antibiosis and tolerance. Non-preference and antibiosis refer to the response of the insect to the plant; tolerance refers to the response of the plant to the insect.

Non-preference (antixenosis): Nonpreferred plants lack the characteristics of hosts for insect feeding, oviposition, or shelter. Antixenosis means the plant is considered a bad host.

Antibiosis: Antibiosis is an adverse effect on the biology (survival, development, or reproduction) of the insect.

Tolerance: A tolerant plant can produce good yield even while it supports an insect population that would severely damage and decrease the yield of a nontolerant plant.

Need for germplasm evaluation

The pivotal factor for the success of resistance breeding in the development of pest-resistant varieties lies in identifying resilient sources or donors. Given the growing challenges posed by pests and the crucial role played by resistant donors in pest control, the primary aim should be to identify sources with resistance to insect pests within the extensive gene pool. This can be accomplished by assessing a large number of genotypes using established screening techniques, supplemented by an in-depth investigation into their resistance.

Using the evaluation database on the world collection of rice*,* conserved at different international and national institutes different sampling strategies can be executed for choosing germplasm for evaluation. There is a rapid increase in the in the numbers of germplasm conserved which has led to increased emphasis on how to handle the large numbers of samples in these collections for evaluation and utilization. The basis for choosing germplasm for evaluation depends on a number of factors; the trait to be evaluated or similar ones; the quality of available information on the trait and is it directly or only indirectly relevant to choosing germplasm; the cost and resources required for seed preparation and evaluation.

Random, stratified, or sequential sampling methods can be selected for choosing the germplasm and analysed sets of germplasm can be chosen. A random sample is unbiased and provides equal opportunity for all accessions to be represented in the evaluation process. The assessment of germplasm has frequently aimed to systematically screen all available germplasm for morpho-agronomic traits or significant stresses, starting from the initial accession, includes sequential evaluation. The basis for stratification is only limited by the extent of information on the germplasm. Thus, germplasm can be stratified by country of origin, maturity group, morpho-agronomic characteristics, isozyme pattern, species, etc.

General procedures for Insect screening program

Seeds to be screened are obtained from the IRRI germplasm collection or directly from national programs. In the initial screening, entries are nonreplicated. In retesting, entries are replicated from 3 to 10 times depending on the insect species. If reactions are still not distinct after retesting, a second retest is conducted. Resistant entries are then given to the breeder for use in hybridization.

Development of screening methods

Requirements for an insect resistance screening program are

- *Adequate amount of test seed*: About 5 g of seed is required for screening against one insect species. The International Rice Research Institute (IRRI) holds in trust the world's largest collection of rice diversity, with more than 130,000 accessions of cultivated rice and wild species. Between 2012 and 2018, a total of 2174 requests for rice germplasm were received from more than 1000 unique requestors. IRRI distributed germplasm externally to requestors from universities (32%), national research programs (14%), private companies (9%), and individuals, including farmers (24%) as well as other CGIAR centers (3%) (Jamora and Ramaiah, 2022).
- *Test insects:* Major insect pests of rice
- *Method to evaluate the levels of resistance among test entries:* To establish a screening method, a comprehensive understanding of the insect's biology and its potential impact on plants is essential. Development of a method for one species may be simple but for another, difficult. Developing an efficient rating method is extremely important. In rice insect resistance screening programs, assessments primarily rely on the extent of plant damage, although in some cases, insect numbers are also considered. The Standard Evaluation System for Rice (SES), introduced in 1980 by the (IRRI), serves as the Standard Evaluation System for Rice (SES). It encompasses most major rice insects and employs a rating scale ranging from 0 to 9, where 0 indicates no damage and 9 signifies severe damage.

Table 1: Damage rating scale for rice insect pest (SES,1980).

Scale	Level of resistance	Economic loss
0	Immune	None
1	Highly resistant	None
3	resistant	None
5	Moderately resistant	Moderate

7	Moderately susceptible	Severe
9	Susceptible	Severe

Selecting a screening site

Screening can be conducted in a greenhouse, a screenhouse, or the field, depending on available facilities and the insect species. Greenhouse screening is generally the most productive. With an efficient insect-rearing program, greenhouse screening can be conducted throughout the year. The factors which affect the field rearing difficult are; insufficient insects, the presence of insect species other than the target pest, and the destruction of test plants by disease, other organisms and adverse weather. Where greenhouse screening of breeding lines is conducted, field screening should be done before a breeding line is released as a variety.

Sources of insects for screening

For greenhouse and screenhouse screening, insects are usually reared and plants artificially infested. Some difficult-to-rear insects can be collected in the field or at lights at night. Techniques for obtaining sufficient insects for field screening are selection of hot spot and proper planting date, planting border rows of susceptible variety, following cultural favouring pest build up and applying resurgence-inducing insecticides.

Germplasm evaluation for insect pests in rice

Rice yellow stem borer: The yellow stem borer *Scirpophaga incertulas* is the most important stem borer attacking rice and is widely distributed throughout South and Southeast Asia. It develops only on rice and feeds within the stem, causing dead hearts and whiteheads. The larvae of stem borer bore at the base of the plants. The larval damage to tillers during the vegetative stage results in drying up of the central shoot called ‘dead heart’ and during reproductive stage causes chaffy unfilled grain known as ‘white ear. The economic threshold level (ETL) is 5% dead hearts or 2% white ear.

Field screening:

Plot size	2 rows of 20 hills per entry with one skip row between entries
Planting dates	Two planting dates One normal planting and the second one 15 days after the normal planting (Accordingly the two sowing dates may be fixed to coincide with peak stem borer incidence of your area)
Methodology	Stem borer infestation may be augmented by pinning of the yellow stem borer egg mass (at black head stage) collected from greenhouse, at maximum tillering stage and at booting stage of crop growth. Count whiteheads before harvest (around 90 DT). This is done only when testing breeding lines because of maturity date differences when evaluating a germplasm collection.
Seedlings per hill	One, check variety should be used

Observations	• Immediately after transplanting, incidence of stem borer incidence count the number of hills that are affected and also for the recovery of the plants. • Count the total number of tillers and number of dead hearts (DH) on least 10 hills/entry at 30 DAT or 50 DAT. • Record total panicle bearing tillers and white ears separately from 10hills/entry at early flowering stage and prior to harvest. • Grain yield from 5 infested hills to be taken separately. • Stubbles – Count the no. of surviving larvae in three individual infested hills, separately.
	• Compute percentage of dead hearts and convert the corrected figure to a 0-9 scale as • Compute percentage of whiteheads: • % whiteheads = no. of whiteheads/total productive tillersx 100 • The test is considered valid when whiteheads in the susceptible check average at least 10%. • Scale Percent dead heart and whiteheads (D) 0 ------No damage 1------ 1-10 3------ 11-25 5------ 26-40 7------- 41-60 9 ------61-1 00

Antixenosis for oviposition in the green house:

Steps	**Key points**
Preparing test	Obtain seed of test varieties
Sowing seed	Sow seed of the test varieties in wooden seed-boxes
Transplanting	At 15 days after sowing (DAS), transplant 4 seedlings in each of 4 pots for every variety. One pot represents one replication. Label each pot properly with a garden stake. Arrange pots in a randomized complete block design in metal trays filled with water
Infesting the plants	Cage with nylon or fiberglass mesh the potted plants. One cage is one replication. Release 20 newly emerged adults per pot, at 1 female: 1 male, into each cage. If there are not enough moths to infest all replications at one time. stagger infestation by releasing 20 moths (2 females:1 male) in each replication every night for 10 consecutive nights.

Evaluation	Count egg masses 3 days after infestation (Dl) or 3 days after last moth infestation when infesting on a staggered basis. Compare the number of egg masses among varieties

Antibiosis on larval survival

Steps	**Key points**
Preparing test	• Transplant 15-day-old seedlings in 38-cm-diam clay pots at 5 seedlings/pot. Plant 10 pots for each test entry, with 1 pot representing 1 replication. Include a resistant and a susceptible check. • Arrange the pots in a randomized complete block • design in a water pan in the greenhouse
Infesting the plant	• Prune the tillers 45 DT so that the number of tillers in each pot is the same and the number of larvae used for infesting each pot is similar • Infest the plants with newly hatched larvae at one larva per tiller • Place the larva near the auricles of the youngest leaf using a fine camel hair brush • Put each pot in a nylon mesh cage to prevent the larvae from escaping or transferring from one plant to another. If cages are not available, trim the leaves so that the leaves of one variety do not touch the leaves of another. This will discourage larval transfer
Evaluation	• Dissect the plants 30 Dl and count the larvae and • Weigh the larvae and pupae separately per hill or pot and calculate percentage of survival • pot and express weight in milligrams. • % survival = (no of live larvae and pupae counted/no of initial larvae infested)*100 • Compare results of the various entries with those of the susceptible and the resistant checks

Leaf folder screening

The leaf folder is a major pest in almost all rice growing areas of the country. The adoption of high yielding varieties has increased the incidence of the insect. The moth is orange brown with many wavy lines and dark band on the margin of the wings. The larvae spin threads that form bands across the leaf, contracting as they dry and tie the margins together, causing the leaf to roll. Inside the rolled leaves, the larvae scrape the surface tissues, creating whitish stripes. Severely infested rice fields appear scorched due to the damage. The economic threshold level for leaf folder is 4% folded leaves.

Field screening of rice leaf folder

<table>
<tr><th>Steps</th><th>Key points</th></tr>
<tr><td>Planting dates</td><td>Sowing and planting dates should be adjusted so as to coincide with high leaf folder infestation</td></tr>
<tr><td>Methodology</td><td>release leaf folder adults. Collect adults from neighbouring fields or laboratory/glass house culture. Release adults two times, once at 40 DAT and second at 60 DAT @ 100 adults per release. In locations where the leaf folder adult population occurrence is delayed due to climatic variations or other factors, adults may be collected as and when available but preferably release before booting stage. If it gets delayed, releases may be discontinued. Dip cotton in 20% honey solution and place it with a pin inside the net as adult food. Let the adults remain inside the net to lay eggs for a week and then remove the net</td></tr>
<tr><td>Observations</td><td>Take observations twice, at 60 DAT and 80 DAT preferably. In case of delayed releases, observations are to be taken 20 days after release. In each entry, select 10 plants at random. Count the total number of leaves and damaged leaves (consider as damaged leaf only if onethird of the leaf area is damaged). Calculate per cent damaged leaves in each entry.</td></tr>
<tr><td></td><td>
<table>
<tr><td>Scale</td><td>Adjusted dmage rating</td></tr>
<tr><td>0</td><td>0</td></tr>
<tr><td>1</td><td>1-10</td></tr>
<tr><td>3</td><td>11-30</td></tr>
<tr><td>5</td><td>31-50</td></tr>
<tr><td>7</td><td>51-75</td></tr>
<tr><td>9</td><td>more than 75</td></tr>
</table>
</td></tr>
</table>

Brown planthopper

The BPH stands out as a highly destructive insect pest with an oligophagous nature, primarily feeding on the phloem sap of rice crops. BPH, characterized as a long-range migratory pest, has acquired the status of a regular threat to rice in northern India.Female lays about 150 eggs on the leaf sheath, which hatch in 6-7 days. The plant hoppers remain confined mainly to the basal portion of the plants. One generation of the insect is completed in 18-25 days. The insect is also a vector for viruses such a grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV). Damage symptom: The nymphs and adults both suck sap from stem and leaf sheath. The infested turn yellow and severe infestation causing drying of the plant and the symptom is commonly known as ‘hopper burn’. As the infestation increases the circular patches of "hopper burn" can be seen in the field. The hoppers excrete honey dew on which sooty mould develops. The economic threshold level of planthoppers is 5-10 hoppers /hill or plants.

Brown planthopper screening

Method	Metodology	Evaluation
General screening (seedling)		
Modified Seed Box method	Screening can be carried out using germplasm within an insect-proof cage utilising a modified seed box test (Heinrichs et al., 1985). The seeds are planted in plastic trays with the resistant check (PTB 33) in the central row and the susceptible check (TN1) around the edges of the rectangle tray. When seedlings reach the three-leaf stage, the seedlings are infested with three to five 2^{nd} - 3^{rd} instar nymphs per seedling. Once the TN1 has reached 90% dryness, the germplasm can be scored	The rice germplasm was graded on a 0-9 scale utilising the IRRI Standard Evaluation System (2014).
Antixenosis/non preference		
Settling behaviour of nymphs	The conventional seed box test (Heinrichs et al., 1985) was utilized for evaluation. Pregerminated seeds of the test germplasm are sown in trays. After ten days, seedlings were infested with 2-6 nymphs (2^{nd}-3^{rd} instar) per seedling.	Nymphal settlement on each seedling was counted after 1^{st} , 2^{nd}, and 3^{rd} days post-inoculation, with seedlings being disturbed after each count to enable hopper nymphs to reposition themselves
Feeding marks	A pair of newly emerged adults are starved for one hour and confine to a 30 days old caged plant of each test genotype. Feeding marks can be stained with 0.1% Rhodamine B Analytical Reagent dye for 15 minutes	Feeding marks can be counted
Honeydew excretion	For honeydew studies 'area method' is used Heinrichs et al. (1985). 0.1% of bromocresol green solution is used	
Fecundity	The number of eggs laid on plant tissue is calculated. On 30-day-old uninfested plants newly emerged adult male and female BPH are released for oviposition. The plants were secured with a mylar sheet cage.	The eggs are counted after five days
Antibiosis		

Nymphal emergence	The experiment is conducted on 30-day-old plants that are enclosed with a mylar sheet cage and contained newly emerging BPH nymphs.	Daily emergence nymphs are counted till emergence ceased.
Nymphal survival	The experiment is conducted on 30-day-old plants enclosed in a mylar sheet cage with 10 recently emerged BPH nymphs. The number of nymphs that survive and mature can be counted, and the percentage of nymphal survival is computed.	Percent nymphal survival = Number of adults emerged Number of nymphs released × 100
Nymphal development	The nymphal development phase is evaluated using the same nymphal survival test which would record the number of days it takes for nymphs to reach adulthood	Daily observations can be done the duration of nymphal development, including the process of ecdysis, until the insects reached the adult stage
Growth index	The growth index of each germplasm can be calculated	It is computed by dividing the percentage of nymphal survival by the time of nymphal development

Role of varietal resistance in Integrated Pest Management

During the evolution of agriculture, traditional varieties, which exhibited some level of tolerance to pests, have been displaced by higher-yielding but more susceptible counterparts. Insecticides have replaced the traditional pest controls and have helped in increased yields and thus there has been greater dependence on insecticides. Recognizing the transient effectiveness of single-method control programs, scientists have developed integrated pest management strategies that merge various tactics for managing insect pests. Varieties with only moderate levels of resistance, seamlessly integrate into such integrated control programs. These resistant varieties harmonize well with biological control agents such as predators, parasites, and pathogens, as they exert no direct adverse effects on them. Varieties demonstrating moderate resistance permit subeconomic pest populations to persist on plants, serving as hosts for natural enemies. Furthermore, incorporating insect resistance and a shortened growth duration into a variety proves more effective in pest control than either trait alone. For example, early maturing rice lines, with yields equivalent to intermediate-maturing varieties, have the added advantage of evading the third generation of Brown Planthopper (BPH), typically the one responsible for causing hopper burn.

Conclusion

The continuous threat of insect pests to rice crops necessitates the development of resilient and adaptive varieties. Through systematic germplasm evaluation, researchers can identify and harness

the genetic diversity present in rice varieties to enhance resistance against insect pests. The utilization of germplasm for insect resistance involves not only the identification of resistant traits but also their incorporation into breeding programs. This integration of resistant germplasm into breeding initiatives is crucial for developing rice varieties that can withstand pest pressures, reducing the reliance on chemical pesticides, and promoting environmentally friendly agricultural practices.

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CHAPTER 9

IARI-National Pusa Collection: India's oldest and largest Insect Collection for Agricultural Important Insects

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Introduction

Insects are the most diverse group of animals on Earth, making up more than half of all known living organisms. Understanding the diversity and distribution of insects is crucial for understanding the overall biodiversity of the planet. Insects play important roles in many ecosystems, such as pollination, seed dispersal, and nutrient cycling. Some insects are agricultural pests, vectors for diseases, they have potential industrial and medicinal uses and understanding their diversity and distribution can help in developing effective pest management strategies, aid in controlling vector-borne diseases, discovery of new compounds and the development of new biotechnological applications and insects can help us understand how different species are responding to changes in the environment, and how they may be impacted by climate change.

It is estimated that there are around 10 million species of insects on Earth, although the exact number is still unknown. This estimate is based on the number of known species and estimates of the total number of species that have yet to be discovered. The concept of a species is one of the fundamental units of biological classification, which is used to organize and understand the diversity of life on Earth. In biology, a species is a group of organisms that are similar in form and function and are able to interbreed and produce fertile offspring. Organisms that belong to the same species are considered to be more closely related to each other than to organisms from other species.

The description of new species offers systematic rank in the hierarchy tree. It is significant because their knowledge of a species' history, status, and importance within an ecosystem helps to conserve them in nature. Finding new species is a difficult and drawn-out procedure that can only be accomplished by biologists and taxonomists. Taxonomists are scientists who research the naming and classification of organisms. They seek to comprehend the connections between various species and create systems for naming and classifying them. The goal of taxonomy is to understand the diversity of life on Earth, and to provide a framework for organizing and communicating information about different organisms. Taxonomists use a

variety of techniques and approaches to study organisms, including observation, dissection, and analysis of DNA and other genetic information. They often specialize in a particular group of organisms, such as plants, insects, or fish. They are also responsible for describing new species and revising existing classifications as new information becomes available. Taxonomists work in a variety of settings, including academic institutions, museums, government agencies, and private research organizations. They also work in fields such as agriculture, forestry, medicine and conservation biology. The Global Taxonomy Initiative (GTI) is a UN Convention on Biological Diversity (CBD) cross-cutting issue that aims to address the lack of taxonomic knowledge and expertise in many regions of the world and, as a result, to improve decision-making in conservation, sustainable use, and equitable sharing of the benefits derived from genetic resources.

In agriculture, insects can cause significant damage to crops, which can lead to reduced yields and lower crop quality. Different types of insects can cause different types of damage, and some insects are considered to be major pests of specific crops. Insect identification is important in agriculture because it allows farmers and other agricultural professionals to effectively manage pest populations and protect crops from damage. By identifying the specific insects that are causing problems, farmers can select the most appropriate and effective control measures.

Here are a few instances of how identification of insects might be useful in agriculture:

- **Pest management:** By identifying the insects that are causing problems, farmers can select the most appropriate control measures, such as pesticides, biological control methods, or cultural practices.
- **Prophylactic crop protection:** Identifying insect pests early on can help farmers take action to protect their crops before significant damage is done. For example, if a farmer identifies a population of aphids on a crop, they can take steps to control the population before it causes significant damage.
- **Monitoring:** Insect identification can also be used to monitor pest populations over time. By tracking the presence and abundance of different insects, farmers can detect changes in pest populations and take action to prevent problems before they occur.
- **Biodiversity Conservation:** By identifying the insects present in an ecosystem, farmers can take steps to conserve beneficial insects, such as pollinators, which are important for maintaining the health of the ecosystem and supporting crop production.
- **Research**: Insect identification is also important for research in agriculture, as it allows scientists to study the biology and behaviour of different insects and understand how they interact with crops.

Overall, insect identification is a critical tool for managing pest populations and protecting crops in agriculture, and it helps farmers and other agricultural professionals make informed decisions to maintain crop health and productivity.

National Pusa Collection (NPC) is an integral section of the Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi. Division of Entomology is one

of the first five Divisions of the Indian Agricultural Research Institute established in 1905. The Division has pioneered investigations in insect systematics and economic entomology vis-a-vis important crop pests. Over the last 100 years, NPC has directly contributed to the discovery and description of more than 1500 arthropod species previously unknown to science. Several taxonomic treatises on agriculturally important insects belonging to orders Lepidoptera, Coleoptera, Hemiptera, Orthoptera, and Hymenoptera, and class Acarina have been published. NPC comprises historical collections, dating from the early-1900s.

Insect taxonomists at NPC, are providing biologists, extension workers, farmers, biosecurity agents, and quarantine authorities, accurate and timely pest identification. As a national service for pest diagnostics, every year, on average over 2000 specimens are identified by the NPC taxonomists. NPC, by providing names of arthropod specimens for the stakeholders, has been instrumental in responding to national and regional pest management needs. Also, as a part of the entomology curriculum we offer courses to masters and doctoral students in insect taxonomy. Museum specimens are used in classes to educate students on insect diversity and taxonomy.

BRIEF HISTORY OF NATIONAL PUSA COLLECTION (NPC)

Eminent entomologists like H.M. Lefroy, T.B. Fletcher and M.G.R. Menon laid strong foundation for insect systematics research in National Pusa Collection (NPC). Faunistic surveys led to the establishment of the National Pusa Collection, one of the largest collections of its kind in this part of the world. Now this collection houses more than half a million specimens of which 0.1 million are authentically identified, comprising about 20,000 species. Over the last 50 years, NPC has directly contributed to the discovery and description of more than 1500 arthropod species previously unknown to science.

The first mention of the insect collection at ICAR-IARI, then Imperial Agricultural Research Institute, was in its annual scientific report as follows “during the year 1907 to 1909 the third assistant to H. M. Lefroy was Mr. G. R. Dutt, has been in charge of economic records and collections, and has done original work on aculeate Hymenoptera. The assistant in charge of the collections, Mr. D. Nowrojee, did excellent work with the arrangement and upkeep of the general insect collections.” During this time H.M. Lefroy wrote a series of ground-breaking books, including Indian Insect Pests (1906) and Indian Insect Life: A manual of the insects of the plains (Tropical India) (1909), an 800-page guide with many hand-painted illustrations and still in print.

Established in 1905 at Pusa, Bihar
Shifted to New Delhi in 1936

Major contributors

H.M. Lefroy

T.B. Fletcher

M.G.R. Menon

Fig 1. Eminent Entomologists who are instrumental in developing insect collection

By 1916-17, steady progress was made in additions of specimens and arrangement of the collection. Rearrangement and compilation of specimens of Lepidoptera (including the Micro-lepidoptera), Coleoptera, Orthoptera and part of Rhynchota was completed and placed in series. The identification of the collection of Diptera was undertaken by Mr. Brunetti. All the specimens were identified by parceling them to the experts. This was identified and returned back. Some of the experts are listed below

• Mr. H. Andrewes - Carabidae

• Dr. G. A. K. Marshall - Curculionidae

• Dr. Karl Jordan- Anthribidae

• Mr. G. J. Arrow - Rutelidae

• Mr. G. J. Arrow. Partly - Melolonthidae

• Mr. C. J. Gahan - Cerambycidae

• Mr. G. Lewis - Histeridae

• Mr. Rowland E. Turner - Sphegidae

• Dr. C. M. Wheeler - Formicidae

• Mr. Rohwer - Tenthredinidae

• Mr. E. Meyrick - Microlepidoptera

Over the years great progress was made in identification of specimens in addition to the augmentation. By 1919 the collection had become large and important from systematic point of view. Specimens were started to be shifted from paraffin waxed box to cabinets. By this time the Microlepidoptera collection, contained in cabinets, was by far the largest. About thousands of specimens were received every year. Numerous collections of Indian insects were

received and named and returned as far as possible. These included collections sent by the Forest Research Institute, the Provincial Agricultural Departments, the Bombay Natural History Society, and by numerous correspondents collection in India. The collection by this time was a major source material for describing the Indian insect fauna and played a pivotal role in meeting the resolution of the Third Entomological Meeting, held at Pusa in February 1919, to catalogue of all described Indian Insects.

By 1920, Pusa collection had more than 7000 named species of Indian insects with Microlepidoptera inside cabinets comprising 700 named species. Orthoptera, Neuroptera, Ryncota were all in fair order and Odonata was revised by then. Dipteran collection in entomological and pathological entomology was amalgamated in to one. By 1921-22 it had around one million specimens. Good deal of work was done in sorting out and collection of Diptera.

During the years 1922-23 many catalogues and revision were done for example Major F. C. Frazer published many novelties on Odonata in Memoirs of IARI and Journal of Bombay Natural History Society, Mr. B.P. Uvarov on short horned grasshoppers,

second and third part of Catalogue on Culicidae and Bombyliidae by Mr. R. Senior White.

During 1923-24 the period major identification was done by the following workers:

• Lt. Col F.C. Fraser - Odonata

• Mr. Morgan Hebard – Dermaptera, Blattidae and Mantidae

• Mr. B.P. Uvarov - Acrididae

• Dr. H. Scott-Chrysomelidae

• Dr. Horn - Cicindelidae

• Dr. K. G. Blair-Meloidae

• Mr. G. Hermann Alexander Ochs- Gyrinidae

• Mr. P. Esben-Petersen - Neuroptera

• Mr. E. Meyrick - Micro-Lepidoptera

• Mr. W.S. Patton - Diptera

• Mr. R. Garcia Mercet- Hymenoptera.

Many card catalogues and fauna volumes were kept updated.

During 1921-60, NPC contributed significantly to "Catalogue of Indian Insects" series which is an inter-departmental publication, which is edited by a standing committee of entomologists appointed by the Entomological meetings held in India published by Indian Council of Agricultural Research, New Delhi. The catalogue is divided into 5 volumes and covers 29 parts. Volume 1 deals with Part l to Part IX. It covers Acrydidae, Culicidae, Bombyliidae, Trypetidae, Nitidulidae, Stapylinidae, Lasiocampidae, Amatidae and Zygaenidae. Volume 2 gives a detailed account of Part X to Part XVII. This volume covers Stephanidae, Brenthidae, Tabanidae, Cicindelidae, Palpicornia, Cecidomyidae, Cosmopterygidae and Yponomeutidae. Volume 3 is devoted to Part XVIII i.e., Carabidae. Volume 4 covers in detail Part XIX to Part XXV. This volume deals with Gyrinoidea, Alucitidae, Lycidae, Phalonidae and Chlidanotidae, Chalcidoidea, Evanidae and Thysanoptera. Volume 5 of the book describes the Part XXVI to Part XXIX. This volume deals with Serphoidea, lsoptera, Anthribidae and Asilidae.

This is the list of 29 parts of Catalogue of Indian Insects

Pt. 1. Acrydidae (Tettigidae) by T. Bainbrigge Fletcher, 1921

Pt. 2. Culicidae, by Ronald Senior-White, 1923

Pt. 3. Bombyliidae, by R. Senior-White

Pt. 4. Trypetidae (Trypaneidae) by R. Senior-White, 1924

Pt. 5. Nitidulidae, by S.N., Chatterjee, 1924

Pt. 6. Staphylinidae, by Malcolm Cameron, 1925

Pt. 7. Lasiocampidae, by T. Bainbrigge Fletcher, 1925

Pt. 8. Amatidae (Syntomidae) by T. Bainbrigge Fletcher, 1925

Pt. 9. Zygaenidae, by T. Bainbrigge Fletcher, 1925

Pt. 10. Stephanidae, by G.R. Dutt, 1926

Pt. 11. Brenthidae by R. Kleine, 1926

Pt. 12. Tabanidae by R. Senior-White, 1927

Pt. 13. Cicindelidae by M. Heynes-Wood & C. Dover, 1928

Pt. 14. Palpicornia by A. d'Orchymont, 1928

Pt. 15. Cecidomyidae by R. Senior-White, 1928

Pt. 16. Cosmopterygidae by T. Bainbrigge Fletcher, 1928

Pt. 17. Yponomeutidae by T. Bainbrigge Fletcher, 1928

Pt. 18. Carabidae By H.E. Andrews, 1930

Pt. 19. Gyrinoidea by G. Ochs, 1930.

Pt. 20. Alucitidae (Pterophoridae) by T. Bainbrigge Fletcher, 1931

Pt. 21. Lycidae by R. Kleine, 1931

Pt. 22. Phaloniadae & Chlidanotidae by T. Bainbrigge Fletcher, 1931

Pt. 23. Chalcidoidea by M.S. Mani, 1938

Pt. 24. Evanidae by M.S. Mani, 1939

Pt. 25. Thysanoptera by T.V. Ramakrishna & V. Margabandhu, 1940

Pt. 27. Isoptera by R. Lal & R.D. Menon, 1953

Pt. 28. Anthribidae (Coleoptera) by R.N. Mathur, 1957

Pt. 29. Asilidae: Diptera by R. Lal, 1960

From 1909 the collection increased from 2221 to 8815 named species by 1926 with majority being Lepidoptera (3606) followed by Coleoptera (2470). By this time Pusa collection was also getting request from foreign entomologists regarding supply of Indian Insects. For example, Idiocerus atkinsoni to Mr. Whitehead, Canada, Indian Honey bees to Mavromonstakis, Cyprus.

Following a devastating earth quake on 15th January 1934, Pusa Collection was shifted to New Delhi. Proper rearrangement and card cataloguing was undertaken. Large numbers of Insects occurring in Pusa were collected to fill the damage in collection which occurred due to the disaster. During this period the insect pest identification service was undertaken as a part of pest advisory. During 1936-37 card cataloging progressed with about 25000 specimen's of 2000 species being catalogued. Many new species received as donation were added to the collection.

After 1940 many taxonomists contributed to the NPC namely

• Dr. M.G.R. Menon - All insects, Psocoptera

• Dr. E.S. Narayanan- Hymenoptera

• Dr. B.R. Subbarao - Hymenoptera

• Dr. H.S. Pruthi - Hemiptera

• Dr. S.I. Farooqi - Hymenoptera

• Dr. S. Ghai - Acarina

• Dr. U. Ramakrishnan - Hemiptera

• Dr. S.L. Gupta - Lepidoptera

• Dr. R.K. Anand - Meloidae and Chrysomelidae

• Dr. V.V. Ramamurthy - Curculionidae and Scarabaeidae

• Dr. S. Joshi - Acarina

During 2005 to 2016, Network Project on Insect Biosystematics (NPIB) funded by Indian Council of Agricultural Sciences, New Delhi started in NPC. This project was headed by Dr. V.V. Ramamurthy and comprised of 13 different centers all over India. Modernization of NPC has been carried out through NPIB. NPC launched its own website http://npc.iari.res.in/ in 2019. More details about publications and who are working in different groups is available online.

MAIN OBJECTIVES AND ACTIVITIES OF NPC

1. **Research and explorations:** Collection, identification and preservation of reference collections of insect specimens. Description of new genera and species of insects in India.

2. **Consultation and outreach:** Providing national insect identification service to biologists, extension workers, farmers, biosecurity agents, and quarantine authorities.

3. **Teaching and human resource development in taxonomy:** As a part of the entomology curriculum, we offer courses to graduates, masters and doctoral students in insect taxonomy/diversity. We also conduct taxonomy training to different stake holders.

Preservation and maintenance of insects

Insects that are larger are usually mounted using non-corrosive entomological pins of various sizes, depending on the size of the insects. Once mounted, they are dried properly in an autoclave to remove moisture. Insects that are too small are double mounted on card points, card platforms, minute pins, etc., depending on the group. Immature stages like larvae, grubs, and other soft-bodied insects are preserved in 70%–95% alcohol. Small and soft-bodied insects (whiteflies, thrips, scales, aphids) and mites, taxonomically important body parts like wings, legs, mouthparts, genitalia, etc. that are preserved on permanent slides. Slides are maintained in separate slide cabinets.

All specimens are labelled with the correct name of locality, date of collection, collector's name, host or habitat records, collection method, parts of the sample, latitude, longitude, accession number generated, determiner's name with identification details, or any other relevant group information. Authentically determined specimens are provided with an accession number, and a register is being maintained with the details of accession numbers generated. Determined specimens and type specimens are grouped into different orders

Fig 1. Insect Museum is well maintained using centralized air-conditioning with fire proof mobile racking system

and kept separately in insect cabinets. Type specimens are labelled using red labels and

housed separately in fireproof type cabinets. A separate type register is also being maintained.

CHAPTER 10

Weed management in rice

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1. Introduction

Weeds are prolific breeder and seeder. They are ubiquitous and eternal pest, having efficient seed dispersal mechanisms (Das, 2008) and huge seed bank in soil. They are most underestimated crop pest in tropical agriculture although cause higher reductions in crop yields than other pests and diseases. Out of the total annual loss of agricultural produce from different pests in India, 33% yield loss is caused by weeds, 26% by diseases, 20% by insects, 7% by storage pests, 6% by rats and rodents, and 8% by others pests (Anon., 2021; Figure 1). Weeds decrease quantity as well as quality of produce/food, fibre, oil, forage/fodder, animal products (meat and milk) and cause health hazards for humans and animals. A variety of weeds owing to different rice cultures usually prevail in rice fields across the countries of the world. Both population and biomass distribution of weeds undergo continuous dynamics in crop-field ecosystem on temporal scale mainly due to changes brought about by humans in crop cultivation/ management practices. Transplanting of rice has certain bearing in weed control efficacy over direct-seeding. On yield front too, transplanted rice proves to be usually superior to direct-seeded one. Many weed control methods/options, e.g. manual & mechanical, biological, chemical, are advocated, but almost all these methods have inherent limitations. No single method of weed control can reach to the desired level of efficiency (Das, 2008). Herbicide is proven to be easier to apply, most efficient and cost-effective tool for weed management. It replaces or supplements traditional practice of manual weeding and, as a result, is helpful to economize production cost. Yet, it should not be considered as a fool-proof strategy for weed management since it leads to weed shift and resistance, and preponderance of perennial weeds.

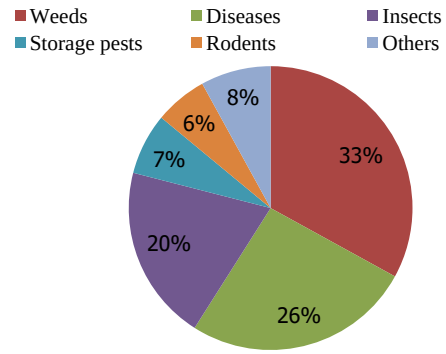


Fig. 1. Crop yield losses due to pests in India (Anon., 2021)

2. Rice cultures/cultivation niches/situations

Rice is grown in different methods/cultures/conditions as adopted by the farmers. Upland rice can be grown as dry directed-seeded or wet/puddled direct-seeded methods, irrigated or rainfed bunded conditions. Transplanted rice is generally grown under natural lowland water -stagnant rice cultures or irrigated upland rice (in some states of India). There are four low-land rice cultures cultivated in India: i) shallow (0-30 cm deep water), ii) intermediate (0-50 cm deep water), iii) semi–deep (0-100 cm deep water) and iv) deep water (>100 cm deep water). Therefore, weed species composition and diversity differ largely according to rice cultures/ growing situations.

3. Weed flora in rice

Weeds comprising of grassy, broad-leaved and sedge weeds are present in rice (Figure 2). Weed species composition and diversity differ largely due to rice cultures/ growing situations across the countries of the world. Over the years, weed species dynamics both in population and biomass distribution are observed in crop-field ecosystem, mainly due to changes brought about by humans in crop cultivation/ management practices. Weed flora (both upland and low land situations) across the countries of the world are given below.

3.1 Annual weeds

i) Annual grassy weeds

Echinochloa colona L., *Echinochloa crusgalli* (L.) Beauv., *Echinochloa glabrescens, Amischophacelus* (= *Cyanotis*) *axillaris/cuculata, Dactyloctenium aegyptium, Leptochloa chinensis* (L.) Nees, *Dinebra retroflexa, Acrachne racemosa* Heyne ex Rhoem, *Panicum sp, Paspalum distichum* L., *Eleusine indica, Brachiaria platyphylla, Setaria glauca/verticillata, Dicanthium annulatum, Digitaria sanguinalis/adscendens, Ischaemum rugosum, Leersia hexandra, Oryza sativa* var *fatua* (wild red rice).

			
Trianthema portulacastrum	*Digera arvensis*	*Amaranthus viridis*	*Commelina benghalensis*
			
Phyllanthus niruri	*Corchorus acutangulus*	*Echinochloa colona*	*Echinochloa crusgalli*
			
Acrachne racemosa	*Eleusine indica*	*Dactyloctenium aegyptium*	*Setaria viridis*
			
Dinebra retroflexa	*Leptochloa chinensis*	*Eclipta alba*	*Alternanthera philoxeroides*

			
Cyperus esculentus	*Cyperus rotundus*	*Cyperus difformis*	*Cyperus iria*

Fig.2. Some prominent weed species associated with rice crop

ii) Annual broad-leaved weeds

Trianthema portulacastrum/monogyna, Ammannia baccifera, Digera arvensis, Amaranthus viridis/ retroflexus, Parthenium hysterophorus, Physalis minima, Phyllanthus niruri, Tribulus terrestris, Ammania baccifera L., *Ageratum conyzoides, Bidens pilosa, Celosia argentea, Corchorus aestuans (=acutangulus), Centella asiatica, Cleome* (= *Gynandropsis*) *gynandra/viscosa, Datura stramonium, Eclipta alba* (Linn) Hask, *Commelina benghalensis/ nudiflora,Euphorbia hirta, Galinsoga parviflora, Ludwigia* (=*Jussiaca*) *parviflora/ perennis/ octovalvis, Marsilea quadrifolia/minuta, Monochoria vaginalis* (Burm.f.) Presl., *Scoparia dulcis, Solanum nigrum, Sphenoclea zeylanica* Gaertn., *Striga asiatica (lutea), Xanthium strumarium, Caesulia axillaries* Roxb.

iii) Annual sedge weeds

Cyperus iria/difformis/compressus/compactus, Fimbristylis miliacea (L.) Vahal., *Fimbristylis tenera/dichotoma, Scirpus supinus* var *lateriflorus, Eleocharis atropurpurea.*

3.2 Perennial weeds

i) Grassy weeds: *Cynodon dactylon*

ii) Broad-leaved weeds: *Ipomoea repens, Ipomoea aquatica* Forsk.

iii) Sedges: *Cyperus rotundus /esculentus, Scirpus supinus/maritimus, Scripus tuberosus* (Desf.), *Eleocharis dulcis, Fimbristylis littoralis/barbata*

3.3 Aquatic weeds

Some aquatic weeds are also encountered in certain areas of tropical flooded rice and they pose problem in rice, e.g. *Monochoria vaginalis, Eichhornia crassipes, Pistia stratiotes/ lanceolata, Salvinia molesta/ auriculata, Lemna minor, Sagittaria guayanensis* (H.B.K.).

4. Critical period of weed competition

Usually, early season weed competition is most detrimental to crop, therefore, early season weed control is indispensable, although weeds occurring at later stages of crop growth cause yield loss and other inconveniences. The critical period of weed competition (Table 1) may be defined as the short time span in the life cycle of a crop, when weed causes maximum reduction in yield, or when weed control measure if adopted may fetch near maximal or maximum acceptable crop yield. A thumb rule is that the first one-fourth ($1/4^{th}$) to one-third ($1/3^{rd}$) period

of the total growing duration of a crop, irrespective of crops, weed species, and environment (climate, soil) may be assumed as "the critical period for weed competition." The tall *indica* varieties are more competitive against weeds than dwarf *japonica* varieties and on the contrary, dwarf varieties are higher yielder than tall varieties. Therefore, there is more reliance to dwarf varieties by the farmers although they encounter heavy weed problem.

Table 1. The critical period of weed competition in crops

Crops	Critical period (DAS/DAP/DAT)*
Rice (upland) direct-seeded	15-45 days after sowing (DAS)
Rice (lowland transplanted)	30-60 days after transplanting (DAT)
Rice (lowland direct-seeded)	50-60 DAS

5. Rice yield reduction/loss

As per FAO report (2021), 20-40% of the potential yield of crop is lost due to pests and diseases of which weeds alone account for nearly one-third of the total losses due to various pests, which is the highest loss globally. In India, annual total economic loss was estimated to be 11 billion USD (appx.) due to weeds in 10 major field crops in 18 States of India (Gharde *et al*., 2018). Generally, due to weeds, 15-20% yield loss is observed in transplanted rice, 30-35% yield loss in direct-seeded puddled rice, and more than 50% yield loss in direct-seeded upland rice. Gharde et. al. (2018) also reported that around annual economic loss to the tune of 4420 million USD occurred in rice due to weeds. Among all pesticides currently used globally in agriculture, herbicides constitute the major proportion (44%) followed by fungicides (27%) and insecticides (22%). Contrary to the global pattern, in India the major use is of insecticides (44%) followed by herbicides (22%), fungicides (21%) and plant growth regulators (PGR)/ biostimulants/ seed treatment chemicals (13%).

6. Weed Management Options in Rice

6.1. Rice nursery

There are several options for weed control in rice depending on rice cultures and growing situations suggested below for rice nursery, direct-seeded and transplanted situation. Weed management in rice nursery is a pre-requisite to combat weed problem in the main/ transplanted field.

6.1.1 Cultural practices

In rice, both dry and wet nursery are prepared. Line sowing of rice may be adopted in both these nurseries, although majority farmers still adopt broadcasting of seeds. The area of nursery should be one-twentieth of the main field for transplanting and the required seed rate should be put in that area to get a uniform close and dense population of rice seedlings. Otherwise, if area increases, seedling population becomes thin per unit area and weeds get chance to proliferate.

6.1.2 Mechanical weeding

It cannot be carried out in broadcast sown rice nursery, but proves to be useful under line sowing. Mechanical weeding is not advisable under the close dense population of rice seedlings in broadcast-sown nursery. Manual weeding / hand pulling is less effective but may be advocated only at the early stage of seedling growth. It becomes less effective when grassy

weeds and sedges similar to rice seedlings grow in the nursery. Then, chemical weeding becomes the ultimate option for weed control in the nursery (Table 2).

6.1.3 Continuous standing water

Continuous standing water if maintained in nursery right from 2-3 days after germination of rice, weeds problem would be greatly reduced. This is normally practised in wet nursery, but difficult to maintain standing water under dry nursery. Uniform population, vigour, and closeness in growing of rice seedlings and shallow standing water have significant bearing in reduction of overall weed growth in nursery.

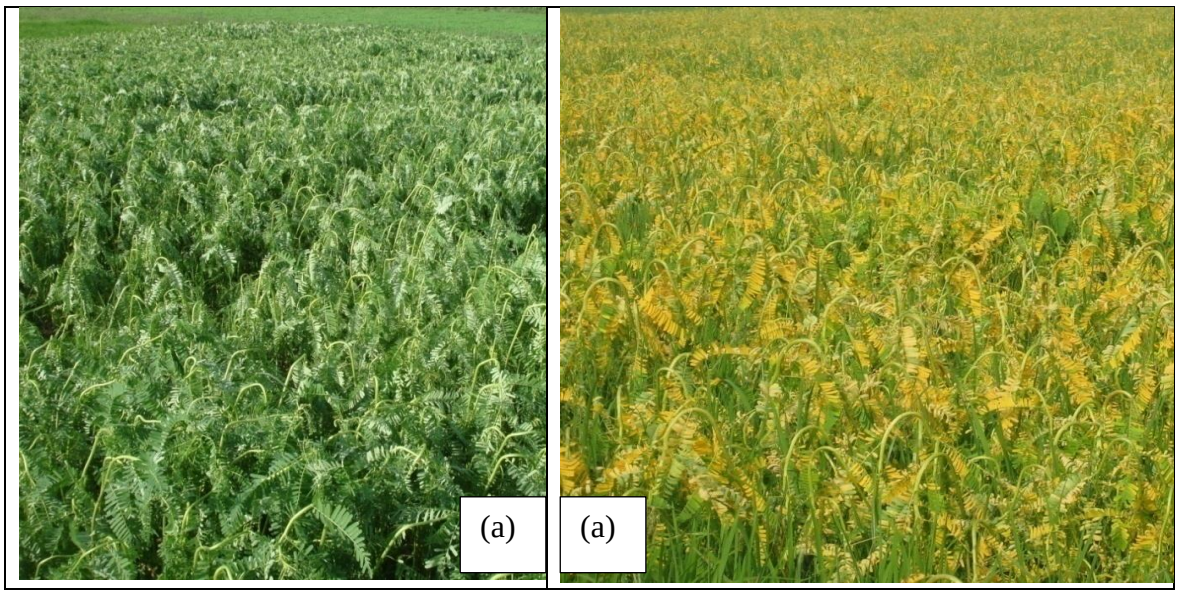
6.1.4 Chemical weed control

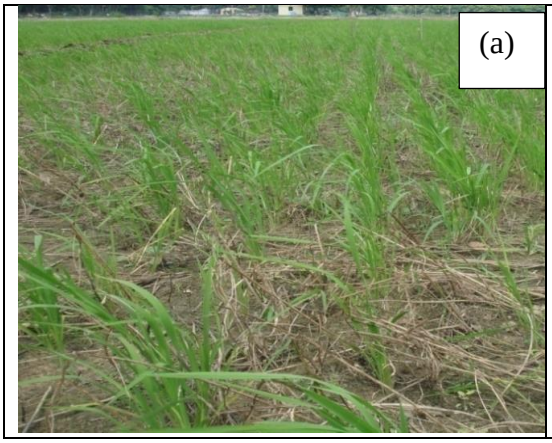
Herbicides recommended for weed control in rice nursey is given in Table 2.

6.2 Direct-seeded rice (rainfed and irrigated situations)

6.2.1. Brown manuring (live and dead mulch)

A promising option for suppressing weeds under direct-seeded rice is brown manuring (Figure 3). In this system, rice and *Sesbania* are sown together and allowed to grow for 25-30 days. Then, co-culture *Sesbania* crop is knocked down with 2,4-D at 500 g/ha or bispyribac-Na at 20 g/ha in rice. This could reduce 70-80% broad-leaved weeds and 20-30% grassy weeds compared with sole rice crop. Brown manuring also reduces the infestation of problematic *Cyperus rotundus* considerably. *Crotalaria juncea* can also be used as brown manure crop in rice to combat nematodes.





Brown manuring with *Crotalaria juncea* in direct-seeded rice

Fig.3. Brown manuring with *Sesbania aculeata* (a) and *Crotalaria juncea* (b) in rice

6.2.2 Mechanical weeding: It is difficult to be carried out in direct-seeded broad-cast upland rice. If it is a direct-seeded drilled/line-sown rice, mechanical weeding may be executed. Manual weeding also appears difficult to be carried out in direct-seeded broad-cast rice.

6.2.3 Beushening: It is a sort of blind tillage practised by the farmers in direct-seeded broad-cast upland rice, which controls weeds *vis-à-vis* makes the rice plants in rows.

6.2.4 Chemical weed control

Herbicides recommended for weed control in direct-seeded rice is given in Table 2.

6.3 Transplanted rice (low land flooded or irrigated conditions)

i) **Puddling:** For growing rice under transplanted situations, several pre-transplanting tillages are done for making soft muddy base (puddling) for easy transplanting and holding rice seedlings erect.

ii) **Mechanical/manual weeding**: A rotary paddy weeder or small implements like spade, khurpi, onion hoe may be used for weeding in transplanted rice. Manual hand weeding is useful but availability of labourers is a concern. Labourers sometimes leave/skip *Echinochloa colona/ crusgalli/ glabrescens* and wild rice (*Oryza sativa* var *fatua*) unweeded since these weeds form mimicry with rice plants at the early stages of growth.

iii) A continuous standing water maintained after transplanting may reduce weed competition to a large extent. Flooding of 10 cm has been found very effective.

iv) **Chemical weed control**

Herbicides recommended for weed control in direct-seeded rice is given in Table 2.

v) Wild rice (*Oryza sativa* var *fatua*) sometimes poses a big problem because of its mimicry with cultivated rice. One approach towards control of wild rice adopted since long is the cultivation of purple-leaved rice varieties, namely R 575, CP 1, P 502 etc.

vi) Alga control

Sometimes undesired alga may grow luxuriantly in low-land rice field, which cause suffocation due to more anaerobic condition and prevent root growth and tillering of rice.

Therefore, it needs to be removed manually or destroyed chemically. Copper sulphate ($CuSO_4$, $5H_2O$) or copper oxychloride @ 10 kg/ha may be recommended to control algal growth in rice.

Table 2. Herbicide recommendation for rice crop

Herbicides	Dose (kg/ha)	Time of application	Conditions/remarks
i) Rice nursery			
Pendimethalin	1.0-1.5	Pre-em (1-2 DAS)	Broad-spectrum control of weeds except sedges and *Digera arvensis*; do not spray on dry nursery soil; In dry nursery, first irrigate and then spray this herbicide on optimum moisture condition; moisture is a pre-requisite for its application, but not standing water; In wet nursery, drain out water and spray on saturated soil; irrigate after 1-2 days
Pyrazosulfuron-ethyl	0.020-0.025	Pre-em (1-2 DAS) or early post-em (10-15 DAS)	Broad-spectrum control of weeds except sedges and *Polygonum*; do not spray on dry soil; In wet nursery, drain out water and spray on saturated soil; irrigate after 1-2 days.
Pretilachlor (S)	0.75	Pre-em (3-5 DAS)	Broad-spectrum control of weeds except sedges; do not spray on dry soil; irrigation should follow immediately if moisture less; In wet nursery, drain out water and spray on saturated soil; irrigate after 1-2 days
Butachlor	1.0-1.5	Pre-em (1-2 DAS)	Broad-spectrum control of weeds except sedges; *Echinochloa colona* and *Ischaemum rugosum* reported not being controlled or resistant; do not spray on dry soil; In wet nursery, drain out water and spray on saturated soil; irrigate after 1-2 days
ii) Direct-seeded upland rice			
Pendimethalin	1.0-1.5	Pre-em (1-2 DAS)	Broad-spectrum control of weeds except sedges and *Digera arvensis*; moisture in soil is a pre-requisite for its application;
Pyrazosulfuron-ethyl	0.020-0.025	Pre-em (1-2 DAS) or early post-em (10-15 DAS)	Broad-spectrum control of weeds except sedges; moisture in soil is a pre-requisite for its application;
Pretilachlor (S)	0.75	Pre-em (3-5 DAS)	- do -

Butachlor	1.0-1.5	Pre-em (1-2 DAS)	- do -
Oxadiargyl	0.08	Pre-em (3-5 DAT) or early post-em (15-20 DAT)	Broad-spectrum control of grass, sedge and broad-leaved weeds; Do not spray if rains expected within 6 hours;
Bispyribac-Na	0.020-0.025	Post-em (20-30 DAS)	Broad-spectrum control of grass, sedge and broad-leaved weeds; moisture in soil is a pre-requisite for its application; Do not spray if rains expected within 6 hours; Do not mix with sulphur- or copper-containing pesticides
2,4-D (ester, Na/K or amine)	0.75-1.0	Post-em (25-30 DAS)	Good control of broad-leaved weeds and few sedges; Apply if field dominated by broad-leaved weeds
Ethoxysulfuron	0.018-0.020	Pre-em (3-5 DAT) or early post-em (15-20 DAT)	Broad-spectrum control of grass, sedge and broad-leaved weeds
Cyhalofop-butyl	0.100	Post-em (20-30 DAS)	Good control of grassy weeds; Apply if field dominated by grassy weeds
iii) Direct-seeded puddled and transplanted rice			
Pendimethalin	1.0-1.5	Pre-em (3-5 DAT)	Broad-spectrum control of weeds; Drain out water from the field before application, apply herbicide in saturated soil and refill the field with water after 2-3 days
Oxadiargyl	0.08	Pre-em (3-5 DAT) or early post-em (15-20 DAT)	Broad-spectrum control of grass, sedge and broad-leaved weeds; Do not spray if rains expected within 6 hours;
Pretilachlor (S)	0.75	Pre-em (3-5 DAT)	Broad-spectrum control of weeds; Drain out water from the field before application, apply herbicide in saturated soil and refill the field with water after 2-3 days
Pyrazosulfuron-ethyl	0.020-0.025	Pre-em (3-5 DAT) or early post-em (15-20 DAT)	- do -
Butachlor	1.0-1.5	Pre-em (3-5 DAT)	- do -

Bispyribac-Na	0.020-0.025	Post-em (20-30 DAT)	Broad-spectrum control of grass, sedge and broad-leaved weeds; Do not spray if rains expected within 6 hours; Do not mix with sulphur- or copper-containing pesticides; Drain out water from the field before application, apply herbicide in saturated soil and refill the field with water after 2-3 days
Ethoxysulfuron	0.018-0.020	Pre-em (3-5 DAT) or early post-em (15-20 DAT)	Broad-spectrum control of grass, sedge and broad-leaved weeds
2,4-D (ester, Na/K or amine)	0.75-1.0	Post-em (25-30 DAS)	Good control of broad-leaved weeds and few sedges; Apply if field dominated by broad-leaved weeds; Drain out water from the field before application, apply herbicide in saturated soil and refill the field after 2-3 days
Cyhalofop-butyl	0.100	Post-em (20-30 DAS)	Good control of grassy weeds; Apply if field dominated by grassy weeds

7. Herbicide-tolerant rice

Herbicide tolerant (HT) rice to ALS inhibitor herbicides may lead to efficient weed control in direct-seeded and transplanted rice and may economize cost for weed control and delay herbicide resistance in weeds. It may also reduce water use by 20-30% under direct-seeded rice. Several single or ready-mix ALS inhibitor herbicide formulations belonging to the imidazolinones (Group 2, WSSA) have been developed by multinational companies (MNCs) for weed control in HT rice. The HT rice may be an important component of the integrated weed management schedule in rice**.**

8. Integrated weed management (IWM)

A single isolated approach has inherent potential, but cannot be a sole and fool-proof strategy for weed management (Figure 4). It would be wise if they are integrated as components in an integrated weed management schedule as applicable under a situation, crop or cropping system. There is need to choose and hypothesize a set of IWM Schedules tested at the local, regional and state levels for recommendations. Several IWM modules are given below for direct-seeded or transplanted rice

8.1 IWM modules for direct-seeded upland rice

i) Good crop husbandry + pre-emergence herbicide + beushening/blind tillage.
ii) Good crop husbandry + pre-emergence herbicide + hand weeding at 30-35 DAS.
iii) Good crop husbandry + residue incorporation/green manuring + post-emergence herbicide or hand weeding or cultivation at 30-35 DAS

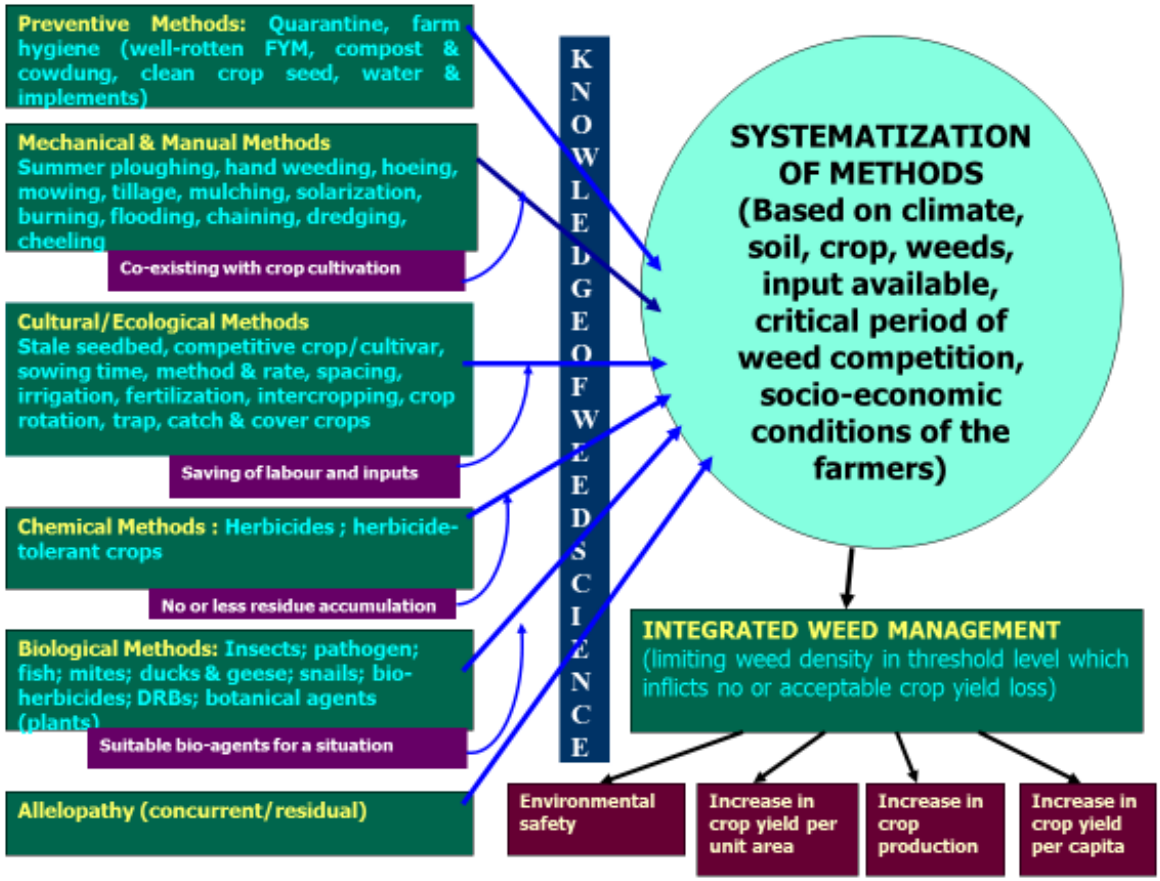


Fig.4. A schematic diagram/model of integrated weed management

8.2 IWM modules for transplanted lowland rice

i) Good crop husbandry + residue incorporation + clean seedling + pre-emergence herbicide + hand weeding at 30-35 DAT (days after transplanting).

ii) Good crop husbandry + green manuring + clean seedling + pre-emergence herbicide + hand weeding at 30-35 DAT. If *in situ* green manuring is practised concurrently with rice transplanted, pre-emergence herbicide should be selective to both rice and green manure crop. Otherwise, post-emergence herbicide may be adopted after incorporation of green manure crop into soil. Row transplanting necessarily becomes the pre-requisite for *in situ* green manuring in transplanted rice culture.

iii) Good crop husbandry + pre-emergence herbicide + hand weeding or rotavation at 30-35 DAT.

iv) Good crop husbandry + residue incorporation/green manuring + clean seedling + high-density planting or skip row planting + pre-emergence herbicide + fish cultivation (under lowland condition).

v) Good crop husbandry + hand weeding at 15-20 DAT + post-emergence herbicide at 30-35 DAT.

vi) Good crop husbandry + pre-emergence herbicide + shallow depth (5-10 cm) of standing water + hand weeding at 30-35 DAT.

vii) Good crop husbandry + shallow depth (5-10 cm) of standing water + post-emergence herbicide at 30-35 DAT. A few centimeters of standing water during the growing period of rice helps the weed control process in a large way.

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CHAPTER 11

Good agricultural practices for (GAPs) for diversified rice-based cropping systems

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Introduction

Crop production is an important agricultural activity providing employment and food security to millions of people in the country. However, growth in crop production has reached a plateau and is increasing at a slower rate. Adoption of modern tools and technologies, no doubt, has helped the farmers to increase the crop productivity, but has also generated some problems, as well. Increased cropping intensity and over use of chemicals has left the land barren in many areas of the country. Ground water has been over exploited in some states/ areas causing shortage of water for crop production. In addition, the resource use efficiency has also declined, causing a further burden on the farmers. The cost of crop production has increased several folds during the last three decades. But the prices of crop output (economic product) have not increased proportionately. This unbalanced increase in cost of production and price of crop output has diminished the profitability of farmers from crop production. Thus, it becomes imperative to increase the crop productivity and resource use efficiency so as to enable the farmers to get more profit in crop production.

Farmers adopt a variety of practices to produce the crops. Broadly speaking, such crop production practices can be divided into three classes, i.e. (i) modern, chemical or conventional farming, (ii) organic farming and (iii) integrated crop management. Some farmers grow crops by adopting the modern tools and technologies, such as, chemical fertilizers, new high-yielding crop varieties, and pesticides, etc. This kind of crop production is referred to as conventional farming. Large number of farmers in the country have switched over from conventional farming to organic farming. In organic farming, the use of synthetic chemicals and fertilizers is prohibited. Too much or no use of synthetic chemicals and fertilizers seems to be undesirable to sustain the crop production and hence the food security in the country. Therefore, people started thinking to find a middle path to avoid the excessive or no use of synthetic chemicals and fertilizers. This intermediate path is integrated crop management (ICM). The ICM can be thought of as a means of production which falls somewhere between conventional and organic production. In fact, practices falling under the ambit of integrated crop management are good agricultural practices (GAPs).

GAPs combine the best of traditional methods with appropriate modern technologies, balancing the economic production of crops with positive impact on the environment. The basic/ main components of GAPs are: adoption of crop rotations/ crop diversification, integrated pest

management (IPM), integrated disease management (IDM), integrated weed management (IWM), integrated nutrient management (INM), and so on (Kumar and Shivay, 2008; Kumar, 2010). These components largely focus on better crop production with minimum pollution of air, water and soil. Each component of these components is associated with GAPs. Through GAPs, farmers make better use of on-farm resources. GAPs include: the use of crop rotations/ crop diversification, appropriate cultivation techniques, careful choice of seed varieties, minimum reliance on artificial inputs such as fertilizers, pesticides and fossil fuels, maintenance of the landscape and enhancement of wildlife habitats. Adoption of crop diversification/ crop rotations is the most important practice for enhancing the crop productivity, profitability, resource-use efficiency and soil health with minimum adverse effect on the environment.

Major rice-based cropping systems in India

The major rice-based cropping systems being followed in India are given below:

Irrigated areas

Rice-Wheat; Rice-Wheat – Mung bean; Rice-Toria-Wheat; Rice-Wheat-Jute ;Rice-Sunflower; Rice-Vegetables; Rice-Potato-Mung bean; Rice-Rice; Rice-Rice-Rice;

Un-irrigated areas

Rice-Chickpea; Rice-Fieldpea; Rice-Lentil; Rice-Mustard;

East-India

Rice-Rice-Wheat; Jute-Rice-Wheat; Rice-Maize-Jute; Rice-Potato-Jute

The most important rice-based cropping systems adopted in India, particularly based upon the area occupied, are: rice-wheat, rice-rice, rice-fallow, rice-groundnut, rice-lathyrus and rice-vegetables, etc. (Table 1).

Table 1. Area under different cropping systems in India

Cropping system	**Area in million hectare**	**States**
Rice-Wheat	9.85	West Bengal, UP, Gujarat, Punjab, Haryana, MP, J & K, HP, Bihar, Maharashtra, Assam
Rice-Rice	5.89	AP, Kerala, Tamil Nadu, Assam, Orissa, Gujarat, and Karnataka
Rice-Fallow	4.42	Jharkhand, Karnataka, MP, J & K and Maharashtra
Fallow-Gram	2.40	MP, Rajasthan, Haryana, UP
Fallow- Wheat	2.08	MP, UP
Maize-Wheat	1.86	Punjab, J & K, Himachal, Haryana, Bihar , UP
Pearl millet-Wheat	2.26	Rajasthan, Haryana, UP, Maharashtra
Soybean-Wheat	2.23	MP, Rajasthan, Maharashtra
Soybean-Fallow	1.17	MP, Rajasthan, Maharashtra
Rice- Groundnut	1.02	Tamil Nadu, Maharashtra, AP, Orissa, Karnataka
Sugarcane/ratoon-Wheat	0.97	UP, Haryana, Punjab
Cotton-Wheat	1.09	Punjab, Rajasthan, Haryana
Cotton-Fallow	0.90	Gujarat, MP, Maharashtra, Karnataka

Fallow- Sorghum	1.43	Maharashtra, Karnataka
Fallow- Mustard	1.30	MP, Rajasthan, UP, Gujarat
Pearl millet-Mustard	0.94	Rajasthan, Haryana, Gujarat, MP
Rice- *Lathyrus*	0.95	MP, Bihar
Rice-Vegetables	1.24	Orissa, West Bengal, Gujarat, Maharashtra

Rice-wheat cropping system (RWCS) is most dominant one among the rice-based cropping system occupying about 10.0 million-hectare area in the country. At present, the IGP contributes nearly 42% to the total food grain production in the country with the rice-based cropping systems as the major cropping system. In India, the IGP covers about 20% of the total geographical area (329 Mha) and about 27% of the net cultivated area, and produce about 50% of the total food consumed in the country. RCWS system is the main source of food and income for millions of people in India. However, recent years have witnessed a significant slowdown in the yield growth rate of this system and the sustainability of this important cropping system is being questioned.

The growth in crop productivity of component crops is either stagnating (wheat) or declining (rice) despite the use of higher yielding cultivars. This raises major concerns over the long-term sustainability of current practices and the threat to future food security against a background of climate change. Key problems associated with the sustainability of this system include (1) decline in soil organic matter (SOM) due to reduced inputs of bioresources and lack of an adequate rotation; (2) negative macro and micro-nutrient balances leading to depletion of soil fertility and nutrient deficiencies; (3) deterioration in soil structure under continuously puddled soils in rice paddies; (4) overexploitation of groundwater resources leading to a decline in the groundwater table; (5) increased energy cost of pumping water, and deterioration of groundwater quality; (6) increasing salinity; (7) the development of herbicide resistance and a shift in weed flora and pest populations; and finally (8) poor management of crop residues, leading to their burning,

GOOD AGRICULTURAL PRACTICES (GAPs) FOR RICE-BASED CROPPING SYSTMES

Good agricultural practices (GAP) are considered to be environment-friendly and sustainable in nature. If followed judiciously and efficiently, such practices enhance sustainability of crop production under varied cropping systems. There is need to adopt the GAPs in rice-based cropping system as these cropping systems are considered as backbone of Indian food security. The important GAPs with respect to diversified rice-based cropping systems are described below:

CROP DIVERSIFICATION

Crop diversification can be adopted by two different strategies. Crop diversification in time (temporal) can be achieved by adopting crop rotations (also known as sequential cropping) and in time and space (spatial) by intercropping or mix cropping. A complementary longer-term strategy for preventative management involves redesigning the cropping system. The aim is to design cropping systems that can function effectively with fewer off-farm inputs. Since cropping systems

are site-specific, it follows that design changes must also be site-specific. Rotation of crops with varied planting dates, growth habits and fertility requirements will minimize the likelihood that pests will adapt to the cropping systems and proliferate. Crop rotation also enhances soil biological activity and fertility. Farmers rely on crop rotation to maintain cropping system function, including management of weeds and maintenance of soil fertility. The role of crop rotation in reducing problems of weeds, diseases and in enhancing soil nutrient status has been well documented.

Substitution of rice with maize

Among cereal crops available for replacement of rice, maize stands first. To meet the food demand of increasing population, we need to find third crop in future. Importance of maize as a potential source of human nutrition and health has been discussed by Shah *et al*. (2016); while Murdia *et al*. (2016) discussed the various uses of maize. It can be grown in different seasons with high productivity as it has C4-photosynthesis mechanism, has a higher yield potential than C3 crops such as wheat or rice. It can be successfully grown as a winter, spring or summer crop in the existing rice-wheat rotation, but will mostly replace the wheat crop during winter-spring. Maize production leads to considerable savings in water, particularly when it replaces irrigated rice in the rice-wheat sequence. There are some **c**onstraints for substitution of rice by maize crop. Due to sufficient availability of rice and wheat, maize is not used for consumption till date; hence incentives on maize cultivation are less as compared to rice. This can be realized from minimum support prices which is low in case of maize. Among other cereals, sorghum and pearl millet are other options available to replace rice.

Substitution of rice with legumes and oilseed crops

Soil fertility restoring capacity of legumes has been known since historic times even when their capacity to fix atmospheric N was not known. Legumes can fix 50-500 kg N/ha depending upon the crop and its growth period. It is estimated that in India, legumes fix 2.47 million tonnes (MT) N annually. In general, forage legumes fix more N. Legumes meet most of their N demands by atmospheric nitrogen fixation with the help of *Rhizobia* that grow on their root. Further, the deep rooted legumes or crops are capable of bringing plant nutrients from the lower layers of soil and leaving them as crop residues in the upper layers. Nutrients so fetched can be utilized by shallow rooted crops. Deep rooted crops also contribute to increased permeability of soil at lower depth to air and water. The other benefits of crop rotations are keeping soil under crop cover for most of the year, control of run-off, soil erosion and efficient use of fertilizers.

Green manuring is an age old practice and may be done with non-grain legumes such as *Sesbania* (dhanicha), *Crotolaria* (Sunnhemp), *Stylosanthes, Centrosema*, and *Desmodium* or with grain legumes such as mungbean, urdbean, cowpea, pigeonpea etc. In addition, loppings of perennial multi-purpose woody legumes, such as, *Gliricidia sepium, Cassia siamea* and *Leucaena leucocephala* (subabul) are widely added to rice fields in south India. One of the important findings

in recent years has been that a gap of 2-3 weeks between incorporation of GM and rice transplanting as recommended earlier is not required. On the other hand, transplanting of rice soon after incorporation of GM leads to better utilization of N released from GM.

The ameliorative effect of including legumes in continuous cereal cropping systems such as RWCS has long been known and explained by several researchers (Singh *et al*., 2011 and Stagnari *et al*., 2017). Over time, legume crops have generally declined in importance due to low yield potential of legumes, as compared to rice and wheat and their susceptibility to many stresses. The potential legume and oilseed crops which can be substitute rice in the IGPs are Pigeon-pea (*Cajanus cajan* (L.) Millsp.), Groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* (L.) Merr.); while the major legume and oilseed based cropping systems in western IGPs are pigeon pea-wheat, groundnut-wheat and soybean-wheat. Legumes and oilseeds crops like Black gram *(Vigna mungo* (L.) Hepper), Mung bean *(Vigna radiata* (L.) Wilczek) and Sunflower (*Helianthus annuus* L.) are mainly grown during spring/summer season and to a small extent during rainy (*Kharif*) season.

Substitution of rice with cash crops IGPs

Among cash crops, sugarcane and cotton are the available options for diversification of rice in RWCS. As both are cash crops, they give comparatively higher returns provided conditions are suitable for their cultivation. Cotton as a crop as well as a commodity plays an important role in the agrarian and industrial activities of India. Cotton-wheat cropping system (CWCS) is a long adopted crop production system of north-western plains of IGPs. While RWCS is a grain production system, CWCS is a grain plus cash cropping system which improves the profitability of farmers through cultivation of cotton as an industrial commodity and wheat as a component of food security. There are of course some constraints while substituting these crops with rice. Both crops are long duration in comparison to rice. Growth of cotton is indeterminate; this creates problems in harvesting. Cotton requires 3-4 picking which is again labour intensive practice. Weed problem is yet another issue, reducing the crop growth especially during early growth of both the crop due to wider spacing and slow growth during initial period.

Inclusion of forage and break crops

Forage crops can support additional enterprise of dairy and may become important part of farming system which involves dairy enterprise. Forage crop such as berseem can also act as a break crop in RWCS which help in reducing weed population in succeeding wheat crop. Introduction of break crop such as maize, pigeon pea, soybean and fodder sorghum in place of rice and berseem and mustard in place of wheat once in three-year cycle of rice-wheat is also followed to get rid of problems arising in RWCS. Along with diversification of either rice or wheat, intensification of system with inclusion of legumes and vegetables, such as green pea or green gram during lean period is also practiced on some part of IGPs.

Change in rice cultivation methods

Adverse effect of rice cultivation in non-traditional rice-growing area can be handled through changing cultivation methods from conventional puddle transplanted rice with flooded condition to other methods such as aerobic rice system (ARS) (Prasad 2011) and system of rice intensification (SRI) (Uphoff 1999; Doberman 2004). These changes in methods reduce the burden on natural resources such as water and increase water productivity of rice.

Nutrient management

Management of nutrients/ soil fertility under the diversified rice-based cropping systems would be governed by the following factors:

- Crops grown in cropping system (type, number, variety)
- Sequential/ intercropping
- Years of cropping system followed
- Sources/ nature of nutrients supplied/ available
- Irrigation facilities
- Soil type

The objective of optimizing nutrient management is to make the best use of soil and applied nutrients within the characteristics and demands of specific farming systems for optimal production with minimal depletion of soil nutrient status (Roy *et al*., 2006). Research results from many parts of the world show that high crop yields are sustainable through balanced and integrated nutrient management supported by suitable amendments to address problems such as excess acidity or alkalinity. A number of long-term field experiments were started in India in the early 1970s using high-intensity crop rotations involving 2–3 crops in succession per year under irrigated conditions. On the whole, these experiments have shown that high levels of crop productivity (8–12 tonnes grain/ha/year) can be sustained by integrating optimal and balanced fertilizer application rates with 10–15 tonnes FYM/ha/year. These experiments have established that fertilizer is the key input for increasing crop productivity, but also that the integrated use of fertilizers and FYM or lime, where needed, give higher and more sustainable yields as it could also correct some micronutrient deficiencies and improve soil physical and biological properties (Roy *et al*., 2006).

Under optimal management of rice-wheat cropping system, grain yields of 8–12 tonnes/ha/year can be harvested. Optimizing nutrient management in this system includes the application of NPK and other required nutrients such as S and Zn. The wheat crop must receive its optimal rate of P application while rice can benefit to a considerable extent from the residual effect of P applied to wheat. On highly P-deficient soils, P must be applied to both the crops. Incorporation of green gram residues after picking the pods before planting rice is an effective green manuring practice in this system. In general, research recommendations provide for application of the full recommended rates of fertilizer to the wheat crop, while 25–50 percent of the recommended

fertilizer to rice can be saved through the use of 10 tonnes/ha FYM, *Sesbania* green manure and crop residues. Information is also becoming available on INM in this highly intensive system. In many rice-growing areas, wherever the climate permits, 2–3 rice crops can be raised in succession within a year. For example, in India, rice–rice annual rotation is practiced on almost 6 million ha. Supply of N through BGA and *Azolla/Anabaena* symbiotic systems has some promise and could potentially replace a portion of N fertilizer. Examples of INM packages and their comparison with fertilizer recommendations for rice–wheat cropping in different agroclimate regions of India are given in Table 2.

Table 2. Examples of INM packages and their comparison with fertilizer recommendations for rice–wheat cropping in different agroclimate regions of India

Region	**Mineral fertilizer recommendation (kg/ha)**	**Integrated nutrient management recommendation (kg/ha)**
Trans Gangetic Plain	Rice: 120 N + 60 P_2O_5 + 60 K_2O + 20 zinc sulphate	Rice: 60 N + 30 K_2O + 10 tonnes/ha FYM or poultry manure
	Wheat: 180 N + 60 P_2O_5 + 30 K_2O	Wheat: 150 N + 30 P_2O_5 (through SSP) + 30 K_2O + *Azotobacter* or *Azospirillum* + PSB
Upper Gangetic Plain	Rice: 120 N + 60 P_2O_5 + 40 K_2O + 20 zinc sulphate	Rice: 90 N + 30 K_2O + 10 tonnes/ha FYM or green manuring with *Sesbania/Leucaena* lopping
	Wheat: 120 N + 60 P_2O_5 + 40 K_2O + 40 S	Wheat: 90 N + 60 P_2O_5 (through SSP) + 30 K_2O
Middle Gangetic Plain	Rice: 100 N + 60 P_2O_5 + 40 K_2O	Rice: 50 N + 30 P_2O_5 + 20 K_2O + green manure (green gram stover) + 20 zinc sulphate in calcareous soils
	Wheat: 120 N + 80 P_2O_5 + 40 K_2O	Wheat: 90 N + 60 P_2O_5 + 30 K_2O + 10 tonnes/ha FYM or
		Rice: 75 N + 45 P_2O_5 + 30 K_2O + 15 kg/ha BGA +10 tonnes/ha FYM + 20 zinc sulphate in calcareous soils

		Wheat: 100 N + 65 P_2O_5 + 30 K_2O
Lower Gangetic Plain	Rice: 80 N + 60 P_2O_5 + 40 K_2O	Rice: 40 N + 45 P_2O_5 + 30 K_2O + 10 tonnes/ha FYM or green manure + 10 tonnes/ha *Azolla* or 10 kg/ha BGA + 20 zinc sulphate
	Wheat: 120 N + 60 P_2O_5 + 60 K_2O	Wheat: 90 N + 45 P_2O_5 (through SSP) + 45 K_2O

(Sharma and Biswas, 2004)

Multi-location trials under AICRP on cropping systems of ICAR have shown that 50% substitution of N is possible by green manuring in rice (*kharif*) in the rice-wheat (Hegde, 1998) and rice-rice cropping systems (Pal *et al.*, 2008). In the same series of trials, application of 50% N though GM and 50% NPK to *kharif* rice and 100% of RDF (recommended dose of fertilizer NPK) to *rabi* rice produced 3.5-11.8% more grain than 100% RDF to both the rice crops, showing that FYM saves not only N but P and K also (Gill *et al.*, 2008) (Table 3).

Table 3. Effect of 50% substitution of N by FYM/green manure/crop residue on the productivity. (t/ha) of rice-rice cropping system at 4 research centres (Results averaged over 15-21 years)

Treatment	Bhubaneswar	Maruteru	Karjat	Karamana
100% RDF* through fertilizer in *kharif* and *rabi*	9.0	10.7	8.2	7.6
50% N through FYM or compost in *kharif* +50% RDF in *kharif* + 100% RDF through fertilizer in *rabi*	9.8	10.5	7.7	8.1
50% N through crop residue in *kharif* + 50% RDF in *kharif* + 100% RDF through fertilizer in *rabi*	9.4	10.4	7.9	7.9
50% N through green manure/green leaf manure + 50% RDF in *kharif* + 100% RDF through fertilizer in *rabi*	9.7	10.7	8.5	8.5

Source: Gill *et al.* (2008) ** RDF: Recommended dose of fertilizer (NPK)*

An FAO-sponsored workshop "Expert Consultation on Fertilizer Use Under Multiple Cropping Systems" held at New Delhi in 1982 made the following fertilizer scheduling recommendations (FAO, 1983):

Rice-based cropping systems

a. Irrigated rice

(1) Rice-wheat sequential system: for alluvial soils in the Indian subcontinent, N to be applied to both crops, P to be applied to wheat, and K and Zn to be applied to rice.
(2) Rice-rice-mungbean or soybean sequential system: N to be applied to both the rice crops, while P to be applied only to one (preferably the second, dry season) rice crop together with K, S, and Zn on the basis of soil tests.
(3) Rice-jute sequential system: N to be applied to both crops; P, K, S, and Zn, if needed, to be applied to jute.

b. Rainfed rice
(1) Rice-chickpea, rice-lentil, rice-horsegram, rice-niger, rice-mustard, rice-linseed, rice-groundnut, and rice-soybean sequential systems: N, P, and other nutrients, as required, to be applied to rice crop, only 20 kg P2O5/ha to be applied to the sequential legume crop if moisture conditions are favorable.
(2) Rice + pigeonpea, rice + maize, rice + cassava, rice + *Leucaena leucocephala*, and rice + kenaf intercropping systems: N, P, and K to be applied to the rice crop only; Zn and Fe to be applied to rice when needed (iron as foliar spray).

Crop residue management

Continuous removal or burning of crop residues can lead to net losses of nutrients under standard fertilization practices. This would eventually lead to a higher nutrient cost input in the short term and reduction of soil productivity in long term. Recycling or retaining the crop residues on soil surface or incorporating them in soil would improve nutrient cycling and, eventually, soil and environmental quality can be improved. Adopting the good agricultural practices would improve system productivity of rice-wheat cropping system and overall resource-use efficiency. This may result in higher profitability. At the current level of crop production, it is estimated that approximately 500-550 Mt of crop residues are produced per year in the country. On a cropping system level, an estimated 250 Mt (constituting 36% of the total) of residues is produced annually in rice-wheat cropping system (RWCS) in the Indo-Gangetic plains. However, in many areas of the country, the crop residues produced in rice-based cropping systems have been considered a nuisance by farmers and disposed through burning in fields.

Despite the availability of huge amount of crop residues available for addition in soil, a significant amount is diverted for other purposes. These crop residues are used for animal feeding, soil mulching, biomanure making, thatching for rural homes and for fuel production, etc. Further, large chunk of crop residues is burnt by farmers into flames, particularly in Indo-gangetic plains where RWCS is prevalent. This burning of crop residues has several consequences including air pollution. The problem of on-farm burning of crop residues is intensifying in recent years due to shortage of human labour, high cost of removing the crop residues by conventional methods and use of combines for harvesting of crops. The residues of rice, wheat, cotton, maize, millet, sugarcane, jute, rapeseed-mustard and groundnut are typically burnt on-farm across different states of the country. The problem is more severe in the irrigated agriculture, particularly in the

mechanized rice-wheat system of the northwest India. It is a paradox that burning of crop residues and scarcity of fodder coexists in this country, leading to significant increase in prices of fodder in recent years. Industrial demand for crop residues is also increasing.

Due to shortage of labour and time, the rice-wheat cropping system (RWCS) has been mechanized, particularly in Indo-gangetic plains (IGPs). As crop residues interfere with tillage and seeding operation for the next crop, farmers in NW India as well as in many areas of eastern and southern India often prefer to burn surplus residues on-farm after grain harvest to establish the next crop (wheat). Of the 89 million tonnes (Mt) of surplus cereal residues, rice and wheat constitute nearly 85% which are burned on-farm annually. The problems of open field burning straw include air pollution (particulates, greenhouse gases) and nutrient loss. Residue burning impacts human and animal health both medically, and by traumatic road accidents due to restricted visibility in NW India.

Emission of greenhouse gases namely carbon dioxide, methane and nitrous oxide, causing global warming is yet another consequence of residue burning. The loss of plant nutrients like N, P, K and S is another issue. The burning of crop residues is wastage of valuable resources which could be a source of carbon, bio-active compounds, feed and energy for rural households and small industries or otherwise ploughed back into the soil. Heat generated from the burning of crop residues elevates soil temperature causing death of active beneficial microbes, though the effect is temporary, as the microbes regenerate after a few days. But repeated burnings in a field, however, diminishes the microbial population permanently. Long-term burning reduces total N and C, and potentially mineralizable N in the upper soil layer. The burning of crop residues leads to significant emissions of chemically and radiatively important trace gases such as methane (CH4), carbon monoxide (CO), nitrous oxide (N2O), oxides of nitrogen (NOX) and sulphur (SOX) and other hydrocarbons to the atmosphere.

Good agricultural practices for management of crop residues

Crop residues could be utilized effectively in the background of conventional cultivation or conservation agriculture (CA) of RWCS. Crop residues can be used for improving soil health, increasing crop productivity, reducing pollution and enhancing the sustainability and resilience of agriculture. The resource conserving technologies (RCTs) involving no or minimum tillage, direct seeding, bed planting and crop diversification are the possible alternatives to the conventional energy and input-intensive agriculture.

Management of rice straw, rather than wheat straw is a serious problem, because there is very little turn-around time between rice harvest and wheat sowing and due to the lack of proper technology for recycling. Among options available to farmers for the crop residue management (including burning), important are baling/removal for use as feed and bedding for animals, in-situ incorporation in the soil with tillage, and complete/partial retention on the surface as mulch using no or reduced tillage systems. After baling crop residues can also be used for paper and ethanol production, bioconversion, and engineering applications. Following practices are suggested for efficient use of crop residues available in RWCS.

1. *In-situ* incorporation of crop residues
2. Inclusion of green manuring and dual purpose summer legumes in RWCS
3. *In-situ* mulching of crop residues
4. Crop residue management in Conservation Agriculture
5. Use of crop residues for composting
6. Crop residues and bioenergy options
7. Fertilizer nutrient management practices on surface retained crop residues
8. Crop residues as biochar
9. baling/removal for use as feed and bedding for animals

Advantages of addition of crop residues in soil

Addition of crop residues in soils either by incorporation or as surface mulch results in following advantages to crop, soil and atmosphere.

- Enhanced crop yields and water productivity:
- Addition of plant nutrients and enhancement of soil health
- Reduced soil erosion and moderation of soil temperature
- Reduced pollution of atmosphere

Enhanced crop yields and water productivity:

Crop residues (CRs) incorporated or retained on the soil surface provide soil and water conservation benefits, and increase crop yield subsequently. Water saving and increase in water productivity (WP, defined as amount of grains produced per unit of water consumed) by mulches is achieved due to reduction in evaporation, suppression of weed growth and increase in soil water storage. When wheat is sown with Turbo Happy Seeder after rice harvest in the residual soil moisture eliminates the need for pre-sowing irrigation. Sowing wheat in the residual soil moisture without pre-sowing irrigation will save about 20% in irrigation water.

Addition of plant nutrients and enhancement of soil health

Crop residues are good sources of plant nutrients and are the primary source of organic matter (as C constitutes over 40% of the total dry biomass) added to the soil, and constitute important component for the stability of agricultural ecosystems. Typical amounts of nutrients in rice straw at harvest are 5–8 kg N, 0.7–1.2 kg P, 15–25 kg K, 0.5–1 kg S, 3–4 kg Ca and 1–3 kg/tonne of straw on a dry weight basis. Rice straw contains 50-100% higher concentration of K than in wheat straw. Besides NPK, one tonne of rice and wheat residues contain about 9-11 kg S, 100 g Zn, 777 g Fe and 745g Mn. Thus, long-term straw application will build soil organic matter level and N reserves, and also increase the availability of macro- and micro- nutrients, and enhance the microbial population and activity in the soil and subsequent nutrient transformations. Thus, indiscriminate removal of crop residues can adversely impact soil properties, soil organic matter (SOM) dynamics, water and wind erosion and crop production.

Retention of residues on soil surface improves soil physical (e.g., structure, infiltration rate, plant available water capacity), chemical (e.g., nutrient cycling, cation exchange capacity, soil reaction), and biological (e.g., SOC sequestration, microbial biomass C, activity and species diversity of soil biota) quality. Hydraulic conductivity and infiltration rate (final infiltration and the total infiltration) are higher in residue retention compared to conventional tillage due to the larger macropore conductivity as a result of the increased number of biopores that is commonly observed. The retention of rice residue in wheat may help reduce the adverse effects of hard pan in the RW system and benefit the wheat crop. The increased levels of carbon in soil (carbon sequestration) would: (1) help mitigate greenhouse gas emissions contributing to global warming and (2) increase soil productivity and avoid further environmental damage from the residue burning and unsustainable use of intensive tillage systems. Carbon credit trading will provide an economic opportunity for farmers to adopt these ecologically based approaches to farming.

Reduced soil erosion and moderation of soil temperature

Crop residue as mulch in no tillage (NT) systems provides multiple benefits, including soil moisture conservation, suppression of weeds, improvement in soil quality and reduction in greenhouse gas emissions. Surface mulch moderates soil temperature and can reduce maximum soil temperature by as much as 10^{o}C at 5-cm depth during summer months. Furthermore, straw mulch lowered canopy temperature by about 2.9 ^{o}C at the grain filling stage to mitigate the terminal heat effects in wheat. The beneficial effect of residue mulch on soil moisture and temperature changes can affect seed germination, seedling emergence, root growth, N fixation, etc., which ultimately determine growth and yield of crops. The magnitude of the beneficial effects associated with returning CRs to fields depends on the quantity and quality of the residue, the subsequent crop to be grown, edaphic factors, topography, climate and soil management. The suppression of weeds with straw mulch might help reduce herbicide requirements.

Incorporation of crop residues also has positive impacts on soil health. On decomposition in soil, crop residues add humus material in soil. This humus binds soil particles and improves soil structure. Such soils are less liable to be eroded than soils where residues are not added. Further, incorporation of crop residues in soils increases the water retention capacity of soil, which results in reduced runoff losses of water. The reduction in runoff loss of water reduces soil erosion. Thus incorporation of crop residues in soil conserves soil and reduces water losses.

Reduced pollution of the atmosphere

Short-term effects of cereal residues (wheat straw) incorporation into paddy field include stimulation of CH_4 emissions, immobilisation of available N, suppression of rice growth, and accumulation of phytotoxic materials. Incorporation of cereal residues into paddy fields at optimum time before rice transplanting can help in minimizing the adverse effect on rice growth and CH_4 emissions. The incorporation of wheat straw before transplanting of rice may reduce N_2O emission due to immobilization of mineral N by high C/N ratio of the straw incorporated. However, an increase in N_2O emission from fields with mulch compared to those with incorporated

residue has been observed in rice-based cropping systems. This mulch effect is the result of higher water content under mulch, which leads to more anaerobic conditions, promoting denitrification. Manipulating timing of residue in such way so that the N becomes available when needed by the upland crop should minimizeN_2O emission as compared with residue return at the beginning of the preseason fallow. Crop residues are unlikely to have significant overall effects on CH_4 emission in upland crops like wheat. For CH_4 production there must be anaerobic microsites for the activity of methanogenic bacteria. Any action that causes residue to decompose before becoming anaerobic will lessen the risk of CH_4 emission.

Conclusion

For sustainability of diversified rice-based cropping systems, it is quite important first to diversify these cropping systems by including the legumes and suitable cereal crops or oilseeds. Integrated and balanced nutrition is must to enhance use efficiency of nutrients that would lead to the reduced environmental pollution and increased farm profits. Efficient crop residue management is the need of the hour to replace the practices of burning them in rice-based cropping systems. This practice is not good for soil, crop or the atmosphere. A great proportion of plant nutrients is lost on burning. It also leads to the pollution of atmosphere. Thus, efficient management of crop residues is necessary to sustain the productivity of rice-based cropping systems. The best option available is to return back these residues, as far as possible, to the soil. Certain good agricultural practices (GAPs) needs to be adopted. These include *in-situ* incorporation of crop residues; inclusion of green manuring and dual purpose summer legume; *in-situ* mulching of crop residues; use of crop residues for composting and efficient fertilizer nutrient management practices when crop residues are retained on soil surface, etc.

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CHAPTER 12

Rice residue management through rapid composting

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Introduction

With the burgeoning human population there is rapid depletion of energy resources warranting the exploration of alternate energy sources. Cellulose is the most abundant and renewable biopolymer in the biosphere with an estimated synthesis rate of 1010 tonnes per year. India being an agrarian country produces around 600 million tonnes of cellulose rich agricultural residues. Cellulose-rich plant biomass is one of the foreseeable and sustainable sources of fuel, animal feed and feedstock for chemical synthesis. The utilization of cellulosic biomass is a subject of worldwide interest in view of fast depletion of our oil reserves and food shortage. The conversion of cellulosic mass to fermentable sugars through biocatalyst cellulase derived from cellulolytic microorganisms has been suggested as a feasible process and offers potential to reduce the use of fossil fuels and thereby reduce environmental pollution.

In intensive agriculture, crop residue management is a major challenge. The recycling of these wastes is not only an ecological necessity but, in a country like ours, an economic compulsion because of the depleting nutrient pools in soils.

But decomposition of these crop residues is a problem because of its lignocelluloses content and high C:N ratio (≈ 90:1). Apart from paddy straw, other agro-residues like legume stover, corn residues which contain approximately 40-50% cellulose, 20-30% hemicellulose and 10-15% lignin are also suitable substrates available for composting. Therefore, for rapid bioconversion of these agro-residues into mature compost, a consortium of lignocellulolytic fungi was developed. This consortium can be used for composting of paddy straw and other types of residues. The resulting compost can be incorporated in the soil as organic manure which can restore soil health by improving soil carbon pools and nutrient sequestration in soil.

Problems in natural composting

- High C:N ratio and recalcitrant nature of agro-residues results in slow decomposition
- Low P content of plant biomass results in nutrient poor compost
- Requires long time (120-150 days) to prepare quality compost
- Plant pathogens and weed seeds often survive during natural composting
- Incomplete degradation of plant parts results in phytotoxicity when applied

Pusa Compost Inoculant

A consortium of seven hyper cellulolytic fungal cultures has been developed on the basis of their lignocellulolytic enzyme production potential. This consortium can degrade diverse agricultural residues like paddy straw, soybean trash, pearl millet, maize residues and mustard stover quickly and effectively. Inoculum of these fungi for large-scale bioconversion of agro-residues may be prepared in sorghum/ millet grains supplemented with $CaSO_4$ (2%) and $CaCO_3$ (4%).The inoculant titled "**Pusa Decomposer**" is being sold at a price of Rs. 50/- per kit. A kit of four capsules is sufficient to decompose one ton of agro-residues within 90 days. The liquid inoculant is also applied in field after harvesting where 10litre culture is mixed in 200litres of water and sprayed on the residue soon after harvesting the crop. The material is mixed with machine like Rotavator, MB plough or any machine of choice. A slight irrigation is required for decomposition of residue.

In case of *ex-situ* decomposition, following steps are taken care

- The C:N ratio of crop residues is adjusted to 50 :1 by the addition of poultry droppings/cowdung by mixing in 8:1 ratio.
- One percent rock phosphate is also added as source of insoluble P. Moisture is maintained at 65% by sprinkling water at regular intervals.
- Application of consortium of fungi is recommended at the rate of 5 litres /tonne of crop residues. Contents are mixed properly in the pits and turning is advisable at fortnightly intervals.
- The compost prepared by traditional method contains only 0.5% N & 0.3 % P, while nutrient enrich compost prepared with Pusa Compost Inoculant contains higher N (1-1.5%) and P (0.3-0.5%).
- This compost can be used at the rate of 5 tonnes/ha along with half of the recommended dose of NPK in different crops.

Method of Composting

The following methods can be employed to prepare nutrient enriched compost by using compost inoculant.

Pit method: For pit method, the site should be selected near the cattle shed and water source. The site should be located at high level so that no rain water can seep in during the monsoon season. The pit should be at least 8 m long, 2 m wide and 1 m deep. The material for composting such as straw, crop residues, vegetable residues or any organic waste, is spread

evenly in the pit (3-4 layers). Then a water slurry of FYM/cow dung/poultry droppings can be sprayed above this layer. A second layer of organic residues can be spread in a similar manner and the process can be repeated until the pit is filled completely.

Rock phosphate can be added at the rate of 1%. Enough water (nearly 90%) is sprinkled over the material and compacting of material should be avoided. *To test the moisture content, take some agro-residue in hand and squeeze it. In ideal situation, water should not drip out of hand.* The material should be turned at least 3 times during the whole period of composting - i.e.at an intervals of 15 days up to one month and at 30 days duration thereafter. It takes about 3 months to decompose all types of organic matter.

Perforated Tank method

This is the modified method of composting. To set up this unit, a perforated rectangular brick tank about 3m x 2m x 1m needs to be constructed. The tank is filled up with layers of agro-waste poultry droppings/FYM/dung one above the other until the tank is full. The ground should be cemented to avoid the seepage of nutrients. This tank can also be filled in similar manner as described in pit method. Sprinkle water to keep the unit moist. Two turnings are desirable due to perforations in the brick wall. Within 60-90 days, the compost will be ready for application in field.

Windrow Method

This method is suitable for large scale composting and raw material is laid in windrows of any length in the form of piles as described in pit method. The windrows are prepared using tractor operated turner-cum- mixer together with the application of culture and water. The height of windrow should not exceed 1 meter. The material is turned fortnightly for proper aeration with the help of tractor turner or manually

Application of nutrient enriched compost in agriculture

The application of nutrient enriched compost in soil leads to a significant increase in the soil fertility status, in terms of enhanced microbial biomass, N and P availability, thereby leading to the improvement of overall physical, chemical, and biological health of soil. Application of 10 tonnes of compost provides 120-150 kg N, 40-50 kg P_2O_5 and 30-50 kg K_2O, which additionally leads to savings of chemical fertilizers. Mechanization of composting process could lead to reduction of labour and time and would be a rewarding option for community level composting.

Benefits of nutrient enriched compost

- The application of nutrient enriched compost in soil leads to an improvement in soil health.
- Application of compost leads to saving of chemical fertilizers.

- The mature compost did not show any phytotoxicity and led to reduced termite infestation in IARI farms.
- The organically grown products have better nutritional quality and always fetch higher price (25% more than the conventional produce).
- Composts can be further enriched with other minerals and microbial inoculants (N fixers, PSB and K solubilizing microbes) to enhance the overall quality of compost.

To prepare one tonne nutrient enriched compost the following materials are needed.

Material	Amount (in kg)	Approx. cost (in Rs.)
Crop residue	1100	Nil
FYM/ poultry droplings/ cow dung	100	200
Rock phosphate	100	100
Mixture of microorganisms (Compost inoculant)	0.5	50
	Total cost	1,250

To prepare nutrient enriched compost, FYM/ poultry droppings / cow dung (10-15%) can be added along with 1% rock phosphate. If FYM/ poultry droppings / cow dung is not available pyrite (10%) or urea (1%) can be added into the raw material. The main purpose of supplementation is to narrow down the C:N ratio of raw material in the desirable range.

Highlights of the technology

Nutrient enriched compost may be prepared within 70-90 days using Pusa Compost Inoculant by pit or windrow methods.
The windrow method of composting which involves layering the material in the form of piles and subsequent mixing using tractor operated turner-cum- mixer together with the application of culture and moisture is suitable for mass production of good quality compost at faster rate with minimum labour.

Standards of compost as described in Fertilizer Control Order (1985)

Parameter	Compost
Moisture percent by weight	15.0-25.0
Colour	Dark brown to black
Odour	Absence of foul odour
Particle size	Minimum 90% material should pass

	through 4.0mm IS sieve
Bulk density (g/cm^3)	<1.0
Total organic carbon, percent by weight, minimum	16.0
Total nitrogen (as N), percent by weight, minimum	0.5
Total phosphates (as P_2O_5), percent by weight, minimum	0.5
Total potash (as K2O), percent by weight, minimum	1.0
C:N ratio	20:1 or less
pH	6.5-7.5
Conductivity (as dsm^{-1}), not more than	4.0
Pathogens	Nil
Arsenic (as As_2O_3)	10.0
Cadmium (as Cd)	5.0
Chromium (as Cr)	50.0
Copper (as Cu)	300.0
Mercury (as Hg)	0.15
Nickel (as Ni)	50.0
Lead (as Pb)	100.0
Zinc (as Zn)	1000.0

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CHAPTER 13

Status and Prospects of Farm Mechanization in India

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INTRODUCTION

Agriculture in India is often looked as an industry rather than an activity for sustenance of which mechanization is an important component. Farm mechanization and crop productivity has a direct correlation; it not only saves time and labor, reduces drudgery, cut down production cost in the long run, reduces postharvest losses and boosts crop output and farm income but also results in increase productivity up to 30 per cent, reduced the cost of cultivation up to 20 per cent and savings in seeds and fertilizers. Cropping intensity also increases with the use of fam machines. Thus, mechanization not only helps in various advantages as above but is also seen as important source of livelihood to farmers; manufacturers, retailers and wholesalers, importers, and service providers. The fundamental requirement for a sustainable sub-sector is a strong linkage between these different parties and that all of them must be able to make a livelihood from their businesses. With 21 tractor units, 7 power tiller units, 250 Medium to Large Scale Units, 2,500 Small Scale Industries, 15,000 Tiny Industries and 1,00,000 Village level Artisans it is a rich source of employment and income generation. As estimated, farm mechanization is more than INR 300,000 million industry.

The major difference between large and small farms are land holding, use of inputs and use of manual labour on the farms. Recent trend indicates that many technologies are equally used by large and small farms; difference being in ownership; Small farms do not own but hire machines mainly for critical operations such as seedbed preparation, sowing, harvesting, threshing etc. Use of manual labour including women, per unit area is high on small farms and hence, there is need for gender friendly equipment. The cost of inputs is also low of small farms as compared to large farms. Small farms have limited resources for skilled worker which shows the need for capacity building.

FARM MECHANIZATION:

Farm mechanization is the application of engineering and technology in agricultural operations, to do a job in a better way to improve productivity. This includes development application and management of all mechanical aids for field production, water control, material handling, storing and processing. Mechanical aids include hand tools, animal drawn equipment, power tillers, tractors, engines, electric motors, processing and hauling equipment.

SCOPE OF FARM MECHANIZATION:

There is a good scope of farm mechanization in India due to the following factors:

1) Improved irrigation facility in the country.

2) Introduction of high yielding varieties of seeds.

3) Introduction of high dose of fertilizers and pesticides for different crops.

4) Introduction of new crops in different parts of the country.

5) Multiple cropping system and intensive cultivation followed in different parts of the country.

SOME OTHER FACTORS WHICH ARE RESPONSIBLE TO ENCOURAGE FARM MECHANIZATION ARE:

i) Population of the country is increasing at the rate of about 2.2% per year. Steps have to be taken to arrange food and fibre for such large population by adopting intensive farming in the country. Intensive farming requires machines on the farm.

ii) In multiple cropping programme, where high yielding variety of seeds are used, all farm operations are required to be completed in limited time with economy and efficiency. This is possible with the help of mechanization.

iii) Farm mechanization removes drudgery of labour to a great extent. A farmer has to walk about 66 km on foot while ploughing 1 ha land once by bullocks with a country plough having 15 cm furrow width.

iv) A large number of females and children work on farm. So, with mechanization females can work at home and children go to school.

v) The proper utilization of basic inputs like water, seeds and fertilizers will be possible with proper equipment.

vi) There are certain operations which are rather difficult to be performed by animal power or human labour such as:

a) Deep ploughing in case of deep rooted crops.
b) Killing the pernicious weeds by deep tillage operations.
c) Levelling of uneven land.
d) Land reclamation.
e) Application of insecticides during epidemic seasons. These operations need heavy mechanical equipment.

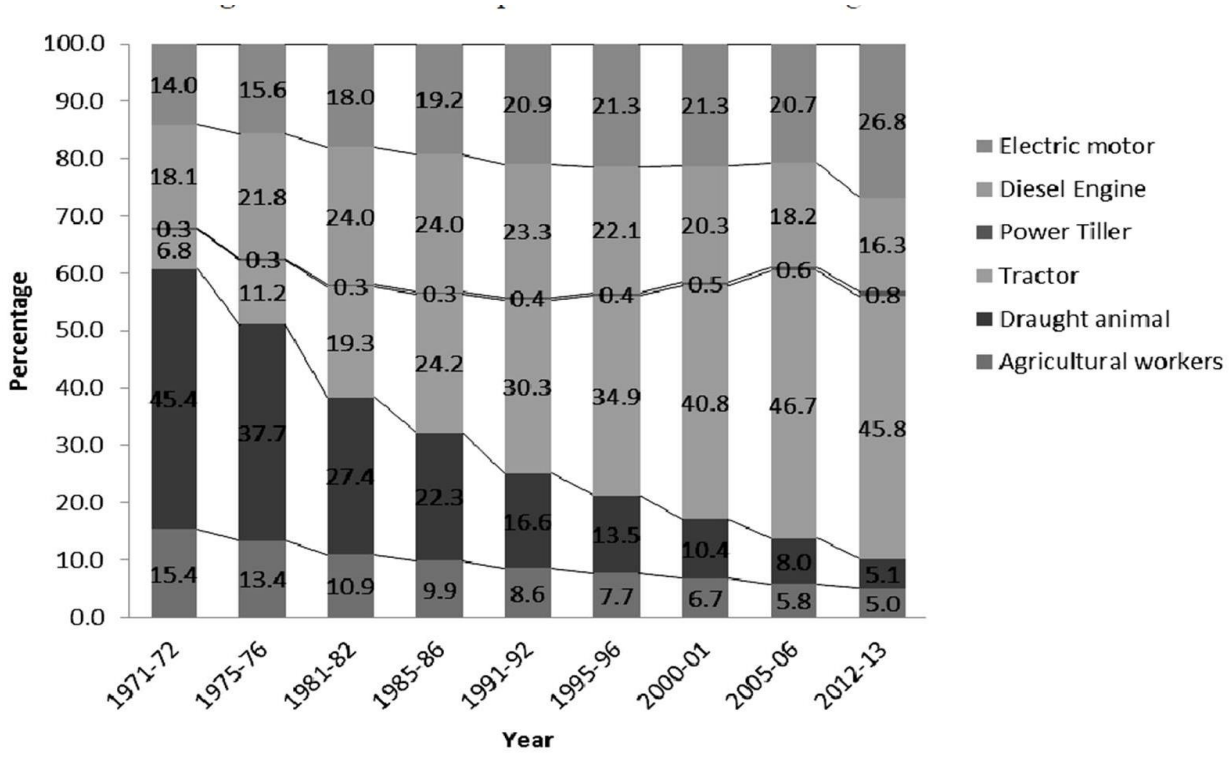
BENEFITS OF FARM MECHANIZATION:

There are various benefits of farm mechanization:

1) Timeliness of operation
2) Precision of operation
3) Improvement of work environment
4) Enhancement of safety
5) Reduction of drudgery of labour
6) Reduction of loss of crops and food products
7) Increased productivity of land
8) Increased economic return to farmers
9) Improved dignity of farmers
10) Progress and prosperity in rural areas

Status of Farm mechanization in India

India witnessed unprecedented growth in agriculture that helped the country to graduate from import dependence to self-sufficiency in food grains by increasing the food grain productivity from 0.64 MT/ha in year 1965-66 to 2.07MT/ha in 2015-16 resulting in export. This growth was mainly attributed to adoption of the agricultural technology during green revolution supported by positive governmental policies, liberal public funding for agricultural research and development and untiring work of farmers and manufacturers of agricultural machinery.



Source: Trends of Agricultural Mechanisation in India CSAM Policy Brief, June 2014

Fig 1. Relative share of different sources to power availability at Indian farms

The different sources of power available on the Indian farm for doing various mobile and stationary operations are mobile power viz. human, draught animals, tractors, power tillers and self-propelled machines (combines, dozers, reapers, sprayers etc.) and stationary power i.e. diesel/oil engines (for pump sets, threshers, sprayers and other stationary operations) and electric motors (for pump sets, threshers, sprayers and other stationary operations). The time series data (1960-61 to 2013-14) of population of farm power sources and power availability from various farm power sources shows that animate power is being replaced by inanimate power (Fig 1). This is an indicative of the fact that slowly and steadily India has been progressing towards higher farm mechanization.

Status of Farm Implement Manufacturing in India

The adoption of mechanization technology depends upon the local manufacture and after sales-services besides credit and financial incentive provided by the government. The manufacture of agricultural machinery in India is quite complex comprising from village artisans, tiny units, small scale industries to State Agro Industrial Development Corporations and organized tractor, engine, and processing equipment industries. As per Agricultural Machinery Manufacturers Association (AMMA) of India, there are approximately 250 medium to large scale units, 2,500 small scale industries, 15,000 tiny industries and 100,000 village level artisans (Table

1). Major farm machinery used in India includes tractors, threshers and power tillers. Among these, the biggest market in terms of annual sales is that of tractors (around 6 lakh units annually), threshers (around 1 lakh units annually) and power tillers (around 56,000 units annually) (Table 2). Among farm machinery, tractors are most widely used by the domestic farmers with the total market size estimated at around ` 34,000 crores annually.

Table 1 Status of Farm Mechanization Industry in India

Equipment manufacturers	**Number of units**
Agricultural tractors	22
Power Tillers	5
Irrigation Pumps	600
Plant Protection Equipment	300
Combine Harvesters	48
Reapers	60
Threshers	6,000
Seed Drills and Planters	2,500
Diesel Oil Engines	200
Plough, Cultivators, Harrows	5,000
Chaff Cutters	50
Rural Artisans	>1 Mn

Source: Trends of Agriculture Mechanization in India, CSAM Policy Brief, June 2014

Today, India is recognized as a leading country in the world for the development and manufacture of agricultural implements and equipments. India is exporting increasing volumes of these to various countries including USA, Africa and Asia.

Table 2 Major Farm Machinery Used in India

Name of Machinery	**Market Size Annually (units)**	**Annual Industry Size (` crores)**
Tractors	6,00,000	34,200
Power Tillers	56,000	706
Combined Harvesters	4,000 - 5,000	770
Threshers	1,00,000	1,230
Rotavators	60,000 - 80,000	693
Rice Transplanters	1,500 -1,600	62
Self-propelled Reapers	4000 - 5,000	45
Zero Till Seed Drills	25,000 - 30,000	132
Multi-Crop Planters	1,000 - 2,000	8
Laser Land Levellers	3,000 - 4,000	129
Weeders	25,000	1,275

Source: Trends of Agriculture Mechanization in India, CSAM Policy Brief, June 2014

Role of Farm mechanization in agricultural production/productivity

Besides its paramount contribution to the multiple cropping and diversification of agriculture, mechanization also enables timeliness of operations, a very important aspect of agricultural production system. Farm mechanization is key for agricultural productivity as it has positive correlation with level of farm mechanization (Fig 2).

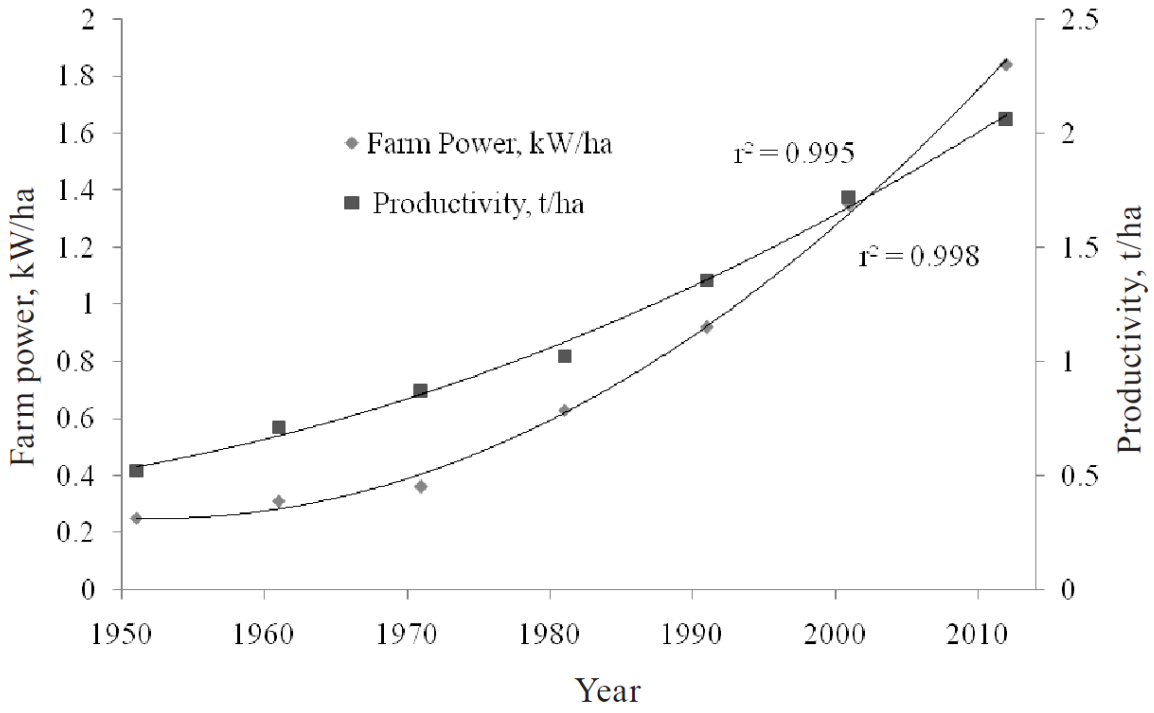


Fig 2 Relation between farm power availability and agricultural productivity

Cropping intensity also increased with an increase in per unit power availability (Mehta *et al*, 2014). It was 120% with power availability of 0.48 kW/ha during 1975-76 and increased to 139% with increase in power availability to 1.71 kW/ha in 2009-10 (ICAR, 2013).

Farm mechanization potential of Indian Agriculture

Farm Mechanization has immense potential in India.As a general trend, farm operations requiring high power inputs and low control (tillage, transport, water pumping, milling, threshing, etc.) are mechanized first and those requiring medium levels of power and control (seeding, spraying, intercultural operations, etc.) are mechanized next. The operations requiring high degree of control and low power inputs are mechanized last (transplanting, planting of vegetables, harvesting of fruits and vegetables, etc.). This happens so because any work, which is power intensive, can be done faster mechanically and at a lower cost. Whereas converting human knowledge into machine knowledge is difficult and costly. Growth of the mechanization in India has also followed the same general pattern found worldwide. As a result of this most of the precision requiring operations i.e planting and transplanting of vegetables and harvesting of fruits

and vegetables are yet to explore. There is a great scope to reduce the input cost and increase the productivity of farmer through mechanization of vegetable and horticultural crop.

Role of custom hiring in agricultural mechanization

Custom hiring plays an important role in mechanizing small farms as well as allows timeliness of operations in different farm holdings. Custom hiring helps in reducing the cost of operation by spreading variable costs over more number of acres. The custom hiring of agricultural machinery is supposed to emerge in the form of a big business model and/or employment source to in the future. Bansal and Mukesh(2014) studied the impact of custom hiring on farm mechanization in Haryana. Results revealed that the dissemination of improved technology in Haryana state through custom hiring has been found to be very effective and productive as far as mechanization is concerned. There was encouraging adoption of improved machinery which resulted in timely operation, increase in production and additional source of income generated. This ultimately benefited the farmers of Haryana State. The state of Haryana has benefited to the tune of Rs. 643 crores by adopting improved farm implements.

Key Drivers of Agricultural Mechanization in India

Labour shortage is being experienced at peak seasons due to the enactment of the National Rural Employment Guarantee Act and huge demand from the construction sector in cities. Labour is available at a higher cost per hectare and this would increase the demand for mechanization. It has been observed that the percentage of agricultural workers to total workers in India has been gradually declining from 59.1% in 1990-1991 to 54.6% in 2010-2011 which is further expected to decline to 25.7% by 2050 leading to severe farm labour shortage. As per the Vision 2030 document by Indian Council of Agricultural Research, domestic demand for food grains is expected to increase at around 2% CAGR in 2030 against that of year 2000. Food grains demand is expected to reach 355 MT in 2030 vis-à-vis 192 MT in year 2010. Fruits and Vegetables demand is expected to reach 290 MT in 2030 vis-à-vis 136 MT in year 2010. However, given the limitations in land use and in increasing cropping intensity over a certain period, increasing the yield from the same land is an urgent requirement to meet the needs of a growing domestic population. With limited land and increased demand for food, the cropping intensity needs to be increased through timeliness of operations. Also, increased participation of corporates through corporate farming has become very popular. Companies are entering into agreements with farmers through contract farming thereby requiring mechanization.

Government Initiatives in Promotion of Custom Hiring

Government of India has kept special provision for custom hiring in the project 'Submission of Mechanization on Agriculture' which is running during the current five year plan (2012-17). In addition different State governments have taken measures to scale up the custom hiring services in their respective states. In July 2014, Karnataka government appointed two private entities to run 178 CHCs around the state for six years. Shri Kshethra Dharmasthala Rural Development Project, popularly known as SKDRDP, a charitable trust runs 161 centres. The Indian Society of Agribusiness Professionals (ISAP), New Delhi has been assigned to run the remaining 17 centres. Under the PPP model, each centre has been given a budget for 50 lakhs in

the first year and 25 lakhs in the second year. Similar initiatives have been taken by the Government of Punjab, Haryana and Madya Pradesh to promote custom hiring of agricultural machinery in a big way.

Initiatives of Government of India to support Farm Mechanization

To support farm mechanization, Government of India has setup a Mission on Agricultural Mechanization (SMAM). It emphasizes the Promotion and strengthening of agricultural mechanization through training, testing and demonstration; Demonstration, training and distribution of post harvest technology and management (PHTM); Financial assistance for procurement of agriculture machinery and equipment - up to 40%. ; Establishment of farm machinery banks for custom hiring and custom hiring centres (CHCs); Establishment of HiTech, high productive equipment hub for custom hiring; Promotion of farm mechanization in selected villages and Financial assistance for promotion of mechanized operations/ hectare carried out through custom hiring centres.

Government of India has also set a target of doubling farmers' income (DFI) by 2022-23 from the base level of 2015-16 . One of the important recommendations of the committee is on setting up of custom hiring centers to facilitate extensive use of machines and equipment on farms in India. It recommends

- The DFI Committee recommended a target of at least one custom hiring centre (CHC) in every large village or Gram Panchayat (GP) in case of small villages. The custom hiring centre should typically house the low order machines needed to suit the crops and production systems in that village. The recommendation does not restrict more than one such centre in a village and multiple such centres can be welcomed.
- ii. An Agricultural Machinery Bank (AMB) should be established at District level. The Bank is expected to house cost intensive machines like combine harvesters etc as well as high level maintenance and repair facilities. The order of investment in these centres would be about Rs 75 lakh to Rs 100 lakh.
- As a third tier in the hierarchy of mechanization, Regional/State level Service Centres may be promoted in the private sector. These can service large geographies and cater to specialized and a package of services. They may imply an investment of about Rs 150 to Rs 200 lakh.

A large number of improved hand tools, bullock drawn tools , tractor and power tiller operated as well as self operated machines in various designs and capacities are being manufacture and are in use on farms of various sizes in India. Although use of tractors is increasing on farms in India, bullock continues to be extensively used in hilly areas of northern and north eastern hilly regions of the country. Bullock drawn indigenous plough of various designs, suiting to local soil and animal are in use in various parts of such areas. Tractor operated seedbed preparation machines include rotavator, disc harrow, cultivators and land levelers . Subsoiler, chisel plough, mould

board plough, disc plough and laser levelers are also in use for tillage operations. Ferti-seed drills including zero tillage drills, sprayers, harvesters, threshes and combine harvesters are being increasingly used on farms either through ownership or hiring. Use of unmanned aerial vehicle (UAV) or agricultural drone (Fig 3) is also being explored for timeliness in operation.

Fig 3: Application of drone (UAV) in agriculture

Precision Farming

With the increasing awareness of reduced use of costly inputs precision farming has also drawn attention of the planners, researchers and users. In Indian context, precision farming may be defined as an accurate application of agricultural inputs for crop growth considering relevant factors such as soil, weather and crop management practices. It is actually information and technology based farming system where inputs are managed and distributed on a site-specific basis for long term benefit. Precision farming helps farmers to realize maximum effectiveness of inputs. Some of the precision technologies in use on farmers field and research plots included laser levelers (Fig4), precision fert seed drills, experimental plot drills, plot combines (Fig5) etc.

Fig 4: Laser leveler in operation

Fig 5: Plot combine harvester

LIMITING FACTORS IN FARM MECHANIZATION:

There are various factors which limits adoption of farm mechanization:

1) Small and fragmented land holdings.
2) Less investing capacity of farmers.
3) Timely availability of agro-machines for different operations as merely availability of tractors is not mechanization
5) Lack of repair and servicing facilities for machines in rural areas.
6) Lack of skilled / trained man power for operating new machines.
7) Lack of co-ordination between different stakeholders.
8) High cost of machines along with higher cost of operation per unit time/area.
9) Inadequate quality control of farm machines.
10.) Limited accessibility of advance agro-machines for Precision farming, Conservation Agriculture, Hill Agriculture, sugarcane farming and Horticulture

CHAPTER 14

Innovative Sensor Based Agro-Machineries for Precise Input Application

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Introduction

Farmers all over the world are plagued by several issues. These problems indirectly and directly affect the farmer's life. Furthermore, farming practices and other aspects of agriculture can take up resources and time. Moreover, the climate change is a big challenge in agricultural production. The cost of operation and input are increasing rapidly. Therefore, agriculture requires innovative solutions to increase agriculture production and yield in a cost effective manner. It can be achieved by increasing the implementation of advance technology. Changes in farming and field management during the past few decades have been revolutionary. Water, fertilizer, pesticides, and other inputs are no longer applied "by eye" or uniformly across the field by large agricultural producers. The use of advanced agriculture technologies allows for the precise application of only what is required in each location, as well as the careful tailoring of treatment for each plant.

Technological progress in agriculture is intrinsically linked to the rise of urban centers and commercial exchange. New technological developments have always been prevalent in this field. Nonetheless, the technological model of agricultural production remained largely subsistence-based and characterized by poor productivity until the early 20th century. This era, known as "Agriculture 1.0," is marked by the invention of the plow and the widespread use of animal drafts. Agriculture 2.0 started towards the tail end of the 19th century with the introduction of mechanical machinery such as tractors. And later on, agricultural technology underwent a number of active development cycles as the pace of technological progress increased tremendously.

Precision or smart farming, also called Agriculture 3.0, evolved out of the need to track and more efficiently manage all inputs into crop production. The pursuit of precision agriculture and its associated agricultural technology has led to the development of new farming methods and tools. The Global Positioning Satellite System (GPS) was the breakthrough technology that made this age of farming possible. GPS helps find deviations within a given agricultural production space, allowing for more effective use of available resources. This was the main reason why the idea of sustainable agriculture and a number of automation options came about.

The leap from smart farming to connected farming is a good example of how fast production technology used in agriculture moved forward at the turn of the century. Technology like autonomous machines, sensor-equipped robots, augmented reality, the Internet of Things (IoT), drones, and satellites is all part of the new agricultural environment, named Agriculture 4.0. Decision-making in the agricultural sector is now based on data that is stored in the cloud and accessible via digital tools. With the help of this analyzed data, farmers and other major players in the agricultural industry can make better decisions. Agriculture 4.0 is being born in an era of ubiquitous automation and digital connectivity. All developments in agricultural technology are becoming more integrated and networked, with the goal of optimizing all stages of the production process and enhancing monitoring, management, and control of the business.

Agriculture technology 5.0, or simply put, "digital agriculture," refers to the next generation of farming methods and tools for maximizing crop yields and other agricultural outcomes. One such technology is 5G, which is currently undergoing rapid development and will improve the reach and accessibility of the latest agritech achievements around the world. Compared to prior farming methods, digital agriculture technology excels in the following aspects:

- Data collection efficiency: how much data can be collected in a given amount of time or space;
- Data accuracy: how close a measurement is to the truth;
- Timeliness: how quickly the data can be processed into practical information and reported to end users.

When it comes to weather, pests, and diseases, agricultural producers have little to no control. Yet, with the advent of digital technologies in agriculture, they may lessen the negative influence of these elements. Meanwhile, digital agricultural technologies give farmers the opportunity to greatly increase the efficiency of decision-making and the return on factors that they directly control. Some examples are:

- what types of crops to grow;
- how to rotate crops for the best results;
- when and how much water to use for precision irrigation;
- when, how much, and what kind of nutrients and plant protection products to apply;
- what kind of tillage works best with a given type of soil.

Innovative Sensor Based Agro-Machineries developed at the Division of Agricultural Engineering, Indian Institute of Agricultural Engineering for Precise Input Application –

- Pneumatic precision planter
- Robotic precision planter
- Variable Rate Technologies
- Pesticide applicator robot

- Amphibian robot
- Soil Sampler
- Electronic cultivator

Pneumatic precision Planter

This machine is developed for precision planting of vegetable seeds directly on the field (bed, ridge etc.) as timeliness of operations and judicious, efficient use of critical inputs is the key to achieve higher levels of quality and productivity. This machine is operated by a 45 hp tractor. The capacity of the machine is 0.2 to 0.3 ha/h.

Herbicide Applicator Robot

A Swath Herbicide Applicator robot is developed for spraying of weedicide in row crops. The robot can change its swath width during the operation to match the crop geometry on the field in real-time. This feature of the robot makes it more useful in comparison to conventional machines like tractor and power tiller. The robot uses the differential steering system for turning.

PUSA Electronic Seed Metering Retrofit Module (ESMM) for cultivators

The developed ESMM is a low-cost solution for farmers that can be retrofit with the commonly available secondary tillage implement (i.e. cultivator). The hopper and metering system can be stored in a small bag which makes it easy for transportation. The developed device can be powered with a tractor battery. These can be easily lent to other farmers for sowing/planting due to compact and easy design. The precision metering device consisted of a microcontroller, stepper motor, motor driver module, rotary Encoder. DC motor is used to rotate the metering plates. The individual metering module can be fitted with a retrofit mechanism that can be easily mounted on cultivator tyne.

Field performance measuring apparatus for farm implement

The field performance measuring apparatus is developed for farm implement. Currently, the Indian farmer use to pay the rent for hiring the service from the tractor owner on the basis of time taken to cover the land only. However, mechanical meter is also used in conventional seed drill to see the area coverage in the form of the number of turns taken by the ground wheel of the seed drill. The apparatus can measure the actual depth of working as well as actual and idle time. It also provides the information about the vibration on implement, number of turns, operating speed. This information can be used in many forms to predict the behavior of the implement during the operation. The information like area covered can be displayed on the mobile device in different units like Acre, hectare, Bigha, Guntha etc. suitable to the customer understanding. The information can be seen in different languages on the mobile device. The proposed invention is useful for custom hiring centers, implement testing centers and research organizations.

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CHAPTER 15

Engineering Interventions and Technologies for Rice Cultivation

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Introduction

Rice (Oryza sativa L.) stands as a foundation stone of global food security, serving as a primary dietary staple for more than half of the world's population, providing essential calories and sustenance. The worldwide rice cultivation spans an expanse of 165.25 million hectares, yielding an impressive 517.5 million tons in the year of 2022-23. India is the second largest rice producing country after China, occupy about 45.8 million hectares land under rice cultivation with annual production of 130.8 million tons in 2022-23. Farm machinery and equipment provide a package of technology to increase land productivity by improved timeliness of operations, reduced crop losses and improved quality of agro-produce. It also increases efficiency of inputs and labour productivity by using labour saving and drudgery reducing devices, and thus, reducing cost of cultivation.

Division of Agricultural Engineering, ICAR-IARI, New Delhi have developed technologies for rice cultivation and rice straw management and are briefly given below.

Machines for Rice Cultivation

Pre-germinated paddy seeder

The pre-germinated rice is sown in rows by the machine powered by 2-3 persons; capacity 0.5 ha/h. It helps use of machines for subsequent operations like weeding and inter-cultivation and reduces the cost of transplanting.

Precision Direct Seeded of Rice (DSR) Planter

Precision DSR planter is useful for precise planting of paddy seeds in nine rows. It is DSR Method where 2-3 seeds per heel can be planted at desired depth. It is provided with inclined plated metering mechanism for each planting row. Each seed box is fitted with slanted type metering plates for seed rate of 18-20 kg /ha. It can be operated by 45 hp tractor. The field capacity of the machine is 0.27 ha/hour.

Manual Paddy Thresher

It is pedal operated manual thresher for threshing of paddy. It facilitated detachment of paddy seeds with the help of rotating drum and its spikes. It can be operated by single person. Threshing capacity of the machine is 35-40 kg paddy per hour. Overall weight of the machine is 26kg

PUSA Basmati Paddy Thresher

Suitable for threshing of basmati paddy without internal / external injury, Operated by tractor PTO (Minimum 35 hp), capacity 1000-1500 kg/h.

Paddy Straw Collector cum Chopper:

The machine cuts the paddy straw stalks in combine harvested field at a height of 2-3 cm from ground level. The chopping action reduces the size of the paddy straw to a pre-selected level of 4-5 cm for rapid in situ degradation. The chopped straw then flows through trapezoidal shaped hopper and get collected or can be dropped in the field for in-situ degradation. To make the system more robust and effective, a 300-litre plastic tank with provision to vary the flow rate was installed below the trapezoidal shaped hopper. This tank contains the fungal inoculum at the pre-calculated dose for rapid in-situ degradation. The machine has a filed capacity of 0.4 to 0.6 ha /h and chopping Performance of 63.5 % of the paddy straw less than 5 cm

Pre-germinated paddy seeder

Precision DSR planter

Manual Paddy Thresher

PUSA Basmati Paddy Thresher

Before Operation

After Operation

Paddy Straw Collector cum Chopper

Machines for Accelerated Compost making

Compost Turner cum Mixer

Suitable for thorough mixing of cow dung, farm residues and biomass for compost preparation by pile method; Operated by 75 hp tractor; Capacity 1000 t/h, It saves 75 days per cycle in comparison to traditional pit method.

Compost Sieving Machine

Suitable for sieving of compost for separating the finer grade from coarse grade; powered by Three phase Electric motors (2 hp, 3 hp and 3 hp), capacity 5 tonnes per day. Separation in different sizes for value addition. Convert waste into the best.

Compost Loader

Suitable for lifting and carrying compost material up to a height of 3 m and loading the FYM in trucks and tractors; Operated by 55 hp tractor, capacity 12 t/h. The loader takes only 0.5 machine hour for loading a truck where as 5 man-hr is required for manual operation.

Compost Turner cum Mixer

Compost Sieving Machine

Compost Loader

Animal Feed Making Machines

Stationary Animal Feed Block Formation Machine

Useful for making animal feed blocks of size 20 cm x 20 cm by mixing crop residues with essential nutritional elements. The machine is powered by 25 hp electric motor and has the capacity of 250 kg/h. It is also available in 125 kg/h capacity with block size of 10 cm X 10 cm operated by 10 hp electric motor. The self-life of the feed blocks is more than one year, very economical to transport to distant places.

Mobile Feed Block Machine

Useful for making animal feed blocks of size 15 cm X 15cm, powered by 6 hp diesel engine (air cooled), capacity 100-125 kg/h. Easy to transport of fodder to any suitable place and useful to make block. The mobile unit is mounted on a four-wheel trolley of 3 m X 1.5 m size and can be transported to the place of availability of fodder.

Urea Molasses Mineral block Machine (UMMB)

Useful for making blocks of Size 22.5 cm × 4.5 cm using Urea, molasses, minerals and grains, powered by 1 hp electric motor, capacity 150 kg/h. Provides animal feed blocks of balanced

nutrients and minerals for balanced ration; feeding milking animals with 200-300 grams of this feed increases their milking production.

Powered Animal Feed Mixer

Useful for proper mixing of roughages, concentrates, minerals, vitamins and other ingredients before making blocks, powered by 3 hp electric motor, capacity 1000 kg/h. It facilitates making of blocks with uniformly distributed ingredients.

Animal Feed Crusher

Suitable for proper crushing of fodder, useful for pre-treatment, preservation and storage of crop residues, powered by 3 hp electric motor, capacity 1000 kg/h. It removes high moisture from crops residue by crushing the plant materials, thus enhance the rate of drying.

Stationary Animal Feed Block Formation Machine

Mobile Feed Block Machine

UMMB

Powered Animal Feed Mixer

Animal Feed Crusher

Commercially available Machines useful for Rice Cultivation

Reversible MB Plough

Rotavator

Disc Plough

Disc Harrow

Mulcher

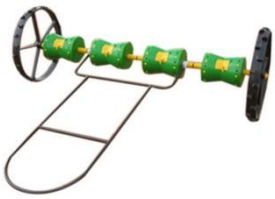
Laser Land Leveller

Puddling using Tractor : Cage wheel

Puddling using Power Tiller Cage wheel

Puddling using Rotavator

Self Propelled Paddy Transplanter



Drum Seeder

Cono weeder

Power Weeder

USG Applicator

Reaper

Combine harvester with Super Straw Management System (SMS)

Paddy Starw Chpper

In-situ Paddy Straw Management using Happy Seeder – Wheat Seeding in standing paddy stubbles

In-situ Paddy straw management using Super / Turbo Seeder – Incorporation of Straw into the soil

Straw Baler and Roatry Rake

CHAPTER 16

Tools/equipment for rice cultivation and their ergonomic assessment

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Introduction

Rice is a cereal grain that comes from the *Oryza sativa* plant, a member of the grass family of *Graminae*. It is native to the deltas of the great Asian rivers, the Ganges, the Chang (Yangtze), and the Tigris and Euphrates. It is a staple food for more than half of the world's population, especially in Asia. India is an important center for Rice cultivation and consumption. More than 1/4th of the land for cultivation in India is given to rice, according to data available on the governmental website Farmer.gov.in (2011-12). India stands in the second position after China in the production of Rice. In India, Rice is cultivated in the Rabi and Kharif seasons, and in some parts, it is cultivated three times yearly.

According to historians, the japonica variety of rice was domesticated from wild rice that was brought to India from southern China, whereas the indica variety originated in the region that stretches across Burma, Thailand, Laos, Vietnam, and the foothills of the Eastern Himalayas (north-eastern India). In Assam and Nepal, perennial wild rice is still growing. (https://farmer.gov.in/imagedefault/pestanddiseasescrops/rice.pdf).

Steps in the cultivation of rice

The steps in the cultivation of rice are given below:

Cultivation	Post Harvesting
Land Preparation	Drying
Seedbed Preparation	Milling
Seed selection and treatment	Storage
Nursery establishment	Transportation
Seed sowing (Direct seeding/ Transplanting)	Processing (If necessary)
Water management	Marketing
Weed control	Record keeping
Fertilization	
Pest and disease management	
Crop monitoring	
Harvesting and threshing	

Tools/Equipment/Machinery used in Rice Cultivation:

Various types of tools/equipment/machinery are used in the cultivation of rice. The details are given below:

1. **Land preparation:**
   It is the process of preparing the soil for planting by breaking up its surface, removing weeds, and leveling the field to create favorable conditions for the growth of the crop. It is the first and foremost step in the cultivation of rice. After harvesting of preceding crop, the residues left in the field can become useful for the upcoming plant by mixing them in the soil. The equipment for this purpose are:

a) Mouldboard plough: It is a primary tillage equipment used to cut, lift, invert and pulverize the soil for thorough mixing. It consists of share point, share, mouldboard, landside, frog, shank, frame and hitch system. The share point is of bar type and is made from high carbon steel or low alloy steel. The share is also made from high carbon steel or low alloy steel. Both are hardened and tempered to suitable hardness (about 45 ARC). The hydraulic system of the tractor controls the working of the plough. The working depth is up to 15-20 cm. Different types of mouldboards are used for different types of soil conditions. Generally, two or three bottom mouldboard ploughs are used in small fields. Reversible mouldboard ploughs make the tillage easier and proper manner by throwing the soil on only one side.

Specifications:

No. Of bottom	:	2 - 4
Length (mm)	:	1778-2392
Width (mm)	:	889-1194
Height (mm)	:	1092-1092
Weight (kg)	:	253-386
Capacity (ha/day)	:	1.5-2.0
Power requirement(hp/kW)	:	30-40 /22.5-30, tractor

b) Disc Plough: It is a tractor-mounted primary tillage implement that cuts and turns the soil with curved metal discs. It is used for deep ploughing and puddling in wet soils. The depth of ploughing varies from 20-30 cm. It has more weight than mouldboard plough. The disc angle ranges from 40-45° to obtain the desired width of cut and the tilt angle ranges from 15-25° for penetration.

Specifications:

Number of furrows 2-4	:	
Disc size (mm)	:	600-800
Length (mm)	:	1180-2362
Width (mm)	:	889-1194
Height (mm)	:	1092-1118
Width of cut per disc (mm)	:	200-300

Adjustable working width (mm)	:	600-1200
Working depth (mm)	:	up to 300
Weight (kg)	:	236-376
Power requirement (hp/kW)	:	25-50/17.25-37.5, tractor

c) Rotavator: It consists of a steel frame, a 3-point hitch system, a rotary shaft on which blades are mounted, a power transmission system and a gearbox. The blades are of L-shape, made from medium carbon steel or alloy steel, hardened and tempered to suitable hardness It uses the power from tractor PTO. Rotavator is used for both primary and secondary tillage operations. A good seedbed and pulverization of the soil is achieved in a single pass of the rotavator. It is used in both dryland and wetland conditions. It is also suitable for incorporating straw and manure in the field. The power requirement will vary depending on the width of the rotavator.

Specifications:

Weight (kg)	:	230-310
Working width (mm)	:	1200-1720
Working depth (mm)	:	80-100
Rotor speed (rpm)	:	210-240
Capacity (ha/hr)	:	0.38-0.5
Power Source	:	35-65 hp tractor (depending upon size)

d) Disc Harrow: A tractor-mounted implement that breaks and smooths the soil with a series of metal discs arranged in two or four rows. It is used for secondary tillage and puddling operations. The tractor-mounted disc harrow consists of two gangs of discs mounted one behind the other. The disc on the front gang throws soil outwards and the rear gang inward. Therefore, no soil remains uncut by the offset disc harrow. Disc harrow is a secondary tillage implement. It is used after the primary tillage. It is used to break the clods developed during primary tillage operation.

Specifications:

Length (mm)	:	1980-2260
Width (mm)	:	1150-1900
Height (mm)	:	1143-1350
Number of discs	:	10-16
Diameter of discs (mm)	:	457-660
Pitch of discs	:	228-280
Weight (kg)	:	330-490
Capacity (ha/day)	:	2.5
Power requirement (hp/kW)	:	20-60/15-45, tractor

e) Cage Wheels: It is a traction improvement device in the puddled fields. It is used as a puddling device in rice cultivation. It breaks the soil clods in waterlogged conditions.

f) Land levellers: It is used to level the land prior to sowing. It is used prior to sowing and after tillage. It ensures even distribution of irrigation to the field. Laser land levellers are advanced to raise or lower bucket using hydraulic action and commands from laser emitters.

(e) Cage Wheels

(f) Land Leveller

2. Seedbed preparation, sowing/transplanting:

Seedbed preparation is a crucial step in rice cultivation, as it sets the foundation for the healthy growth of rice plants. Properly prepared seedbeds provide an ideal environment for germination, early growth, and the development of robust seedlings, ultimately contributing to higher yields. By paying careful attention to these steps, farmers can establish an optimal seedbed for rice cultivation, setting the stage for a healthy and productive crop. Successful seedbed preparation contributes significantly to the overall success of the rice cultivation cycle. The equipment involved the preceding equipment along with the **cultivator.** It is an agricultural implement designed for seedbed preparation in crop cultivation. They are attached to the hitch of a tractor. Cultivators promote soil aeration, root penetration, and the incorporation of organic matter. They aid in creating a fine tilth for optimal seed germination and early plant growth. The most used configuration is 9-tyne cultivators.

Sowing/transplanting is a similar term used in rice cultivation. Direct-seeded rice uses the term sowing for placing the paddy seed in the field for direct germination in the field itself. Transplanting involves developing seedlings in the nursery and then planting the seedlings in the field. Sometimes, this nursery will be a part of a field. Seeds are closely spaced in the

field nurseries and then distributed in the field uniformly. Some equipment involved are: **Seed drill:** It is used for placing the seed in the sowing area in a fixed row spacing. It ensures the timely sowing of seeds and saves time. The seed metering mechanism helps in choosing the quantity of seed to be placed at each spacing and is driven mostly by the ground wheel. Types of seed drills include bullock-drawn seed drills, tractor-drawn seed drills, paddy drum seeders, etc. A Paddy drum seeder is manually drawn equipment that contains drums on a bar and a handle to pull it. The seeds will drop from the orifices of the drum according to the spacing. Desired seed spacing can be achieved by closing some of the orifices of the drum.

Bullock-drawn seed drill

Tractor-operated seed drill

Direct paddy drum seeder

- **Seed-cum-fertilizer drill:** It is used for both seed and fertilizer application. One hopper contains seeds and another hopper contains fertilizer. Both hoppers are connected to each tyne of drill through separate delivery pipes. It is driven by the ground wheel.

- **Transplanters:** It is used to transplant seedlings from nursery to field or from field plot to entire field. Transplanters reduce the fatigue of workers who transplant the seedlings in a bending posture. It reduces the high labour cost. Types of transplanters depend on the prime mover. Manual transplanters work on pulling force and a hand-operated rotating lever to place the seedlings. A small petrol-driven engine operates the rotating lever in some transplanters. Self-propelled transplanters have a prime mover

built in it such that no external power source is needed. Operator can usually sit on a seat while operation.

Manually operated transplanter

Engine-operated transplanter

Self-propelled transplanter

3. **Weed control:**
   Weed control is the activity of removing unwanted plants from the field. Weeds create problems by taking the nutrients and creating a deficit of nutrients in the crop. Removing weeds completely (by hand or by mechanical means) and use of chemicals to kill the weeds are the methods of weed control. Other techniques like flame weeding will be applied before the seeding of the crop.

- **ConoWeeder:** The ConoWeeder is an innovative tool designed for sustainable and efficient weed management in agriculture. Specifically developed for paddy fields, it features a conical drum with sharp blades that uproot weeds while sparing rice plants. Operating manually or with minimal power, the ConoWeeder provides an eco-friendly alternative to herbicides, reducing environmental impact. Its ergonomic design allows for easy maneuverability in flooded fields, ensuring thorough weed removal without damaging the crop. This low-cost and labor-efficient solution contributes to organic farming practices, promoting weed control while maintaining soil health. The ConoWeeder stands as a practical tool for enhancing productivity in rice cultivation.

- **Petrol-driven brush cutter with weeder attachment:** It is a backpack-type equipment driven by a petrol engine. It has a handle and a long shaft for power transmission to the

weeding attachment. It has a gearbox and a weeding tool. The weeding tool rotates about the horizontal axis. It ensures less weeding time. Carrying the equipment for longer duration results in backpain.

4. Fertilization:

It is the process of applying fertilizers to the crop to increase the availability of essential nutrients for plant growth. Manual spreading, mechanical spreading, etc., are some methods used for the application of fertilizers.

- A **fertilizer broadcaster** is an essential agricultural tool used for the even and efficient distribution of fertilizers across fields. This mechanical device, often attached to tractors, ensures precise spreading, promoting optimal nutrient distribution for crop growth. It enhances farming efficiency, reduces manual labor, and contributes to improved yields in various agricultural settings.

- An **organic manure fertilizer applicator** is a specialized agricultural implement designed for the precise and efficient application of organic fertilizers onto fields. This equipment ensures an even distribution of organic matter, enhancing soil fertility and promoting sustainable farming practices. It minimizes waste and optimizes nutrient utilization. Organic manure fertilizer applicators contribute to environmentally friendly farming, supporting the use of natural and renewable resources. They play a vital role in promoting soil health, crop nutrition, and overall agricultural sustainability while reducing reliance on synthetic inputs. Mostly, it will be applied prior to tillage for mixing with the soil.

5. **Pest and disease management:**
Pests are insects or microorganisms that will cause physical damage to the crop as well as reduce the quality of the produce. A deficiency of any nutrient in the crop causes a disease. The result can be identified by the physical appearance of the crop or by other methods like quality, chemical analysis, etc. Lack of care can be a cause for the invasion of pests and diseases. Proper management steps to be taken to eliminate pests and diseases

- A **manually operated knapsack sprayer** is a portable and versatile agricultural tool used for the application of pesticides, herbicides, and fertilizers. Worn as a backpack, it consists of a tank for holding the liquid solution, a pump for pressurizing the tank, and a nozzle for controlled spraying. Farmers or gardeners carry and operate it manually, allowing for precision in treating crops. This cost-effective and user-friendly device is particularly useful in smaller fields or areas where mechanized equipment is impractical.

- A **petrol-driven motorized sprayer** is a powerful and efficient agricultural tool designed for the precise application of pesticides, herbicides, and fertilizers in larger farming operations. This sprayer is equipped with a petrol engine that powers a pump, creating the necessary pressure for spraying. It typically consists of a tank for holding the liquid solution, hoses for directing the spray, and a nozzle for controlled application. The motorized sprayer allows farmers to cover large areas quickly, providing uniform and effective distribution of agrochemicals. This equipment is particularly valuable in commercial agriculture for optimizing crop protection and enhancing overall farm productivity.

- A **battery-operated sprayer** is a portable and eco-friendly agricultural tool powered by rechargeable batteries. This innovative device facilitates the precise application of pesticides, herbicides, or fertilizers, offering ease of use and reducing manual effort. Its versatility makes it suitable for various farming scales, ensuring efficient and controlled chemical application.

- A **tractor-operated boom sprayer** is an advanced agricultural implement designed for large-scale crop protection and fertilization. Attached to a tractor, it features an extended boom equipped with multiple nozzles, allowing for wide coverage. This

efficient system enables precise and uniform application of pesticides or fertilizers, reducing manual labor and ensuring optimal crop health. Tractor-operated boom sprayers are customizable, providing farmers with the flexibility to adjust spray patterns and rates. This technology enhances productivity in modern farming practices by offering a mechanized solution for accurate and timely chemical application across extensive fields.

- A **manually operated knapsack mist blower cum duster** is a versatile agricultural tool designed for efficient pesticide and fungicide application in orchards and crops. Worn as a backpack, it integrates mist-blowing and dusting capabilities for comprehensive coverage. Farmers manually operate it, delivering a fine mist or dust to target areas, ensuring effective pest and disease control. This portable device is particularly useful in areas where precision is crucial, offering flexibility and ease of use for integrated pest management strategies in smaller to medium-sized agricultural settings.

6. Harvesting and Threshing:

Harvesting is the activity of cutting the crop near the ground and collecting the whole mass. Threshing is the operation of separation of paddy seeds from straw. Both harvesting and threshing are interrelated to each other. Both operations can be done separately (manually) or at a time (machinery). Sickles, reaper conveyors, brush cutters, threshers, winnowers, combine harvesters, etc.

- A **reaper conveyor** is a vital component in modern harvesting equipment, specifically designed for efficient crop harvesting. The reaper conveyor is responsible for cutting and gathering standing crops, such as grains or cereals. As the conveyor moves, it transfers the harvested crop sideways. This automated system enhances harvesting speed and productivity. Reaper conveyors play a crucial role in mechanized farming, ensuring a seamless transition by automating the cutting, gathering, and conveying of crops during harvesting operations.

- **Threshers** are agricultural machines designed to separate grains or seeds from their husks and stalks after harvesting. They play a crucial role in automating the labor-intensive process of separating valuable grains from crop residues. Threshers work by beating or rubbing harvested crops, such as wheat, rice, or oats, to loosen the

seeds from the surrounding materials. The separated grains can then be collected for further processing or storage. Threshers come in various types, including **drum threshers,** rotary threshers, and axial flow threshers, each suitable for different crops and farming conditions. These machines significantly contribute to increased efficiency and productivity in modern agriculture.

Manually-operated paddy drum thresher

Tractor-operated paddy thresher

- **Winnowing** is an ancient agricultural practice used to separate grain from chaff by utilizing wind or air currents. It involves tossing harvested material into the air, allowing the lighter chaff to be carried away by the wind while the heavier grains fall back to the ground. This manual method of winnowing has been traditional in many cultures for centuries. **Manual winnowing** is a labor-intensive process and remains common in small-scale and traditional agricultural settings, especially where mechanized equipment is not readily available. An **electric-operated winnower** is a modern, motorized version of the traditional winnowing process. It consists of a machine with a motor that generates airflow. Grains and chaff are fed into the machine, where the electric fan or blower creates air currents, separating the lighter chaff from the heavier grains. This automated approach significantly increases efficiency, reduces labor, and is suitable for larger-scale agricultural operations. Electric-operated winnowers are widely used in commercial farming to streamline the grain separation process, improving overall productivity.

- **Self-propelled combine harvesters** are advanced agricultural machines revolutionizing the harvesting process. Combining multiple functions into a single unit, these harvesters efficiently cut, thresh, and separate grains from the crop, significantly enhancing productivity. They can navigate through fields, minimizing labour and time requirements. Equipped with advanced technologies such as GPS-guided systems, yield monitoring, and automatic height control, these harvesters optimize performance and accuracy. Self-propelled combine harvesters exemplify precision agriculture, providing farmers with real-time data for informed decision-making, ultimately contributing to increased yields and operational efficiency in large-scale farming operations.

- **Straw balers** are essential agricultural machines used to efficiently collect and compress crop residues, such as straw or hay, into compact and manageable bales. These machines aid in waste management, making it easier to store, transport, or utilize crop residues for various purposes. Straw balers contribute to sustainable farming practices by promoting resource utilization and reducing environmental impact. The term Ergonomics was coined by a Polish scientist B.W. Jastrzębowski in 1857 for a scientific discipline that includes all aspects of human activity, including labour, entertainment, reasoning and dedication. World War II brought this discipline into the limelight and was termed Human Engineering, Human Factors or Human Factors Engineering in the USA in the context of the design of military equipment. In 1949, K. F. H. Murrell again used the term Ergonomics for this discipline.

7. Ergonomics Assessment

Ergonomics is an amalgamation of two Greek words: εργον (ergon) means work and νομος (nomos) means law. Ergonomics is also known as Human Engineering, Human Factors or Human Ergology, and is the scientific study of the association between a person and his/her working environment. The term environment includes the tools, materials and method of work, ambient conditions and physical environment in which the job is carried out, and also the organizational factors. Thus, ergonomics focuses on the appropriate design of workplaces, systems, equipment, work processes, and environments to accommodate the workers with an aim to achieve compatibility between the needs of people, their limitations and the demands of their jobs. Ergonomics involves the application of the human biological sciences in conjunction with engineering sciences to achieve the optimum mutual adjustment of man and work, the

benefits being measured in terms of human efficiency and welfare. It promotes a holistic approach considering physical, cognitive, social, organizational, environmental and other relevant factors. Here, the worker is of prime importance and all the available knowledge is used to make him/her more productive, efficient, comfortable and safe.

Globally, agriculture is one of the most drudgerous occupations and a large number of agricultural workers suffer occupational injuries and ill health each year. It is also the largest sector for female employment in many countries, especially in Africa and Asia. Agriculture employs about one billion workers worldwide, or more than a third of the world's labour force. Of the 2.3 million fatal and 313 million non-fatal occupational injuries caused annually worldwide, the agriculture sector contributed to about 50% of the injuries (ILO, 2014). In India, studies indicate that farm equipment is significantly associated with rural injuries.

The ergonomic assessment can be done by measuring

Physiological assessment: Heart Rate and Oxygen consumption
Postural assessment: Overall discomfort and Body part discomfort score

Various research workers have given classifications of workload based on data obtained on heart rate and oxygen consumption.

Workload classification based on heart rate and oxygen consumption rate

Category	O_2 Consumption lit/min	Heart Rate	Energy kJ/min
Unduly heavy	>2.5	>175	>50
Very heavy	2.0-2.5	150-175	40-50
Heavy	1.5-2.0	125-150	30-40
Medium	1.0-1.5	100-125	20-30
Light	0.5-1.0	75-100	10-20
Very light	<0.5	<75	<10

The posture influences energy consumption, as given in the Table below.

Table: Percentage increase in energy consumption for different bodily postures

Posture	**Percentage increase in energy**
Sitting	3-5 %
Standing	8-10 %
Stopping	50-60 %
Kneeling	30-40 %

The assessment and design should be to minimize heart rate and energy consumption along with avoiding odd postures, which consume higher energy.

CHAPTER 17

Mechanical rice transplanter and USG applicator

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1. Introduction

Agriculture is one of the important sectors for employment in Asian countries such as India, Vietnam, Myanmar, Cambodia and others. There is paradigm shift in farming as it is changing from subsistence to surplus and commercialization. Increasing productivity in agriculture sector increased the economic activity but still the lowest expenditure categories get larger share of their income from agriculture.However, the role of agriculture in Gross domestic product declines gradually in the process of progress.

Rice is the staple food in Asia and the demand for rice is expected to grow as the population is expected to increase 35% by 2025 **(United Nations, 1999).** The demand of the food can be met through effective use of inputs (particularly nutrients, seed, and pesticide) in an environmentally sustainable fashion.In Asia, approximately 130 million hectares of agricultural land are dedicated to rice production, with India leading the pack with about 43 million hectares under rice cultivation, closely followed by China. However, the region faces significant challenges such as natural calamities like floods and droughts, as well as the timely availability of labor during critical operations such as transplanting, which pose major obstacles to successful rice cultivation.

Fertilizer use is one of the major factors for the continuous increase in rice production since the Green Revolution era. Efficient nutrient management in rice has assumed great importance because along with high production levels of rice, it ensures minimal leakage of applied nutrients to the environment. The different fertilizers used in paddy cultivation are urea, DAP, NP/NPK, SSP, MOP (in which Urea contributed about 65% of total fertilizer input). Estimated fertilizer use intensity in South Asia was 121 kg ha^{-1} and it may increase to 268 kg ha^{-1} by 2050. Judicious use of fertilizer keys for food security and environmental sustainability. Since main gradient protein is made of nitrogen therefore in food production the nitrogenous fertilizer

plays notable role. The rice plant uses only 15 to 35% of the total applied nitrogen (Kumar and Prasad, 2004).

Major challenge with N management is the poor nitrogen utilization efficiency (NUE), which varies from 20 to 40% in low land rice ecosystems **(Akter et al., 2018; Fei and PENG, 2017; Ganghua et al., 2018; Plett et al., 2020).** To overcome the losses due to leaching, excess N is applied. which not only results in negative impact on soil health and crop yield but also on the ecosystem, climate, and public health **(Boretti and Rosa, 2019; Dasgupta, 2021; Godfray et al., 2018)**. This leads to serious economic and environmental consequences in terms of groundwater contamination, global warming and the flow of reactive N from the rice ecosystem into the environment. When extra fertilizer is added to the crop, nutrients that cannot be absorbed by the soil matrix or microbial biomass may be discharged into the atmosphere (e.g., NH_3, N_2O, NOx, and N_2), as well as surface and/or below-ground water bodies (e.g., NO_3, HPO_4/H_2PO_4**(Fan et al., 2012; Weaver et al., 1988).**

The form and method of urea application significantly impact nitrogen use efficiency and environmental losses. Broadcasting prilled urea results in only 30 to 45 percent utilization by plants, with the rest lost to air or water, posing environmental concerns **(Dong et al., 2012).** To mitigate this, deep placement of urea has been suggested to reduce volatilization, with urea super granules (USG) emerging as an effective nitrogen management strategy in paddy fields **(Mohanty et al., 1998).**

Research indicates that deep placement of USG enhances nitrogen utilization efficiency by 31.7% compared to conventional urea application methods, leading to increased grain yields **(Jaiswal and Singh, 2014; Wani et al., 1999).** This method shields nitrogen against volatilization and nitrification-denitrification losses, resulting in higher nitrogen retrieval from deep-placed fertilizer compared to broadcasted prilled urea **(Gaudin, 2012; Fomba et al., 2020).**

Studies also show significant improvements in nitrogen use efficiency and yield when comparing deep placement of urea to broadcasted methods in paddy fields, with increases ranging from 30 to 40% **(Shah-Al-Emran et al., 2019).** This highlights the importance of adopting deep placement techniques, particularly using USG, to optimize nitrogen management and enhance crop productivity while minimizing environmental impacts.

In modern agriculture, the integration of technology has revolutionized traditional farming practices, offering innovative solutions to improve efficiency, optimize resource utilization, and enhance crop productivity. Among these advancements, the mechanical rice transplanter

coupled with a Urea Super Granule (USG) applicator stands out as a game-changer in rice cultivation.

2. The necessity of a mechanical transplanter in rice cultivation

Transplanting rice seedlings is a critical agricultural operation, and various methods, including manual, mechanical, and throwing, are employed for this purpose. In Asia, transplanting seedlings into heavy puddled soils is a common practice in rice cultivation. Studies have shown that even a one-month delay in transplanting can lead to a 25% reduction in yield, while a two-month delay can result in a staggering 70% yield reduction **(Rao and Pradhan, 1973).** Moreover, manual transplantation poses ergonomic challenges, with workers often experiencing musculoskeletal disorders (WMSDs) due to repetitive bending and squatting postures **(Hagberget et al., 1995).**

Mechanical rice transplanting has emerged as a promising solution to these challenges. It not only reduces labor requirements but also ensures timely transplanting and achieves optimal plant density, ultimately leading to higher productivity. Research indicates that plots transplanted mechanically yield significantly higher grain yields (9-14%) compared to hand-transplanted methods, thanks to the use of uniform seedlings and improved planting efficiency **(Islam et al., 2015).** Given these circumstances, there is an urgent need for a cost-effective and labor-saving method of rice transplanting that doesn't compromise yield **(Tripathi et al., 2004).** Therefore, adopting mechanical transplanters becomes essential to ensure timely and efficient operations in rice cultivation.

3. Types of rice transplanter

Rice transplanters can be categorized based on two main parameters, i.e., a. Based on type of nursery requirements and b. Based on type of prime mover. The available rice transplanter manufacturing companies in India is tabulated in Table 1.

3.1 Based on type of nursery Requirements

3.1.1 Washed Seedling Transplanter

This type of transplanter utilizes washed root seedlings placed on mats, typically with four to six leaves and a length of about 20 to 30 cm. In some cases, excessive root growth is pruned to facilitate easier transplanting, requiring approximately 175 people per hour per hectare.

3.1.2 Mat-type Seedling Transplanter

Seedlings for this type are raised on trays by spreading pre-germinated seeds on a soil layer of 1.5-2.0 cm thickness. The seedlings are then allowed to grow for 11-14 days in warm environments and 25-30 days in cold environments. It features a conveyor belt system that

accommodates seedlings pre-grown in mats, allowing for efficient and precise planting in the field. This technology minimizes labor requirements, increases planting speed, and promotes uniform spacing and depth, ultimately optimizing crop yield. The Mat-type Seedling Transplanter represents a significant advancement in agricultural machinery, offering farmers a more efficient and effective way to transplant seedlings, saving time and resources in large-scale farming operations.

Fig 1. Loading of nursery mat on the trays (Source: Kumar et al., 2012)

3.2 Based on type of Prime Mover

3.2.1 Manual Transplanter

A manual rice transplanter is a simple yet effective agricultural tool used for transplanting rice seedlings in small to medium-sized fields. It typically consists of a frame with handles, a seedling tray or compartment, and a planting mechanism.In operation, an operator manually pushes or pulls the transplanter across the field while simultaneously inserting individual seedlings into the planting mechanism. With each step, the mechanism deposits the seedlings into the soil at the desired spacing and depth.While manual rice transplanters are labor-intensive and relatively slow compared to mechanized alternatives, they are affordable and suitable for small-scale farmers with limited resources. They also offer greater flexibility in terms of terrain accessibility and can be used in areas where larger machines may not be practical.

Fig 2. Manual three row paddy transplanter (Source: Samal et al., 2020)

3.2.2 Self-propelled Transplanter

A self-propelled transplanter is a sophisticated agricultural machine equipped with an engine for propulsion and automated planting mechanisms. Unlike manual or human-operated transplanters, these machines can navigate fields autonomously, reducing the need for manual labor. They are commonly used in large-scale commercial farming operations where efficiency and productivity are paramount. With their ability to continuously transplant seedlings at high speeds and with precision, self-propelled transplanters significantly streamline the transplanting process, ultimately leading to increased yields and reduced labor costs.

3.2.2.1 Walking type transplanter

Walking-type transplanters, on the other hand, require an operator to walk behind the machine as it moves across the field. These transplanters are generally smaller and more maneuverable than riding-type machines, making them suitable for smaller fields or areas with difficult terrain where larger machines cannot access. While walking-type transplanters may not offer the same level of efficiency as riding-type or self-propelled machines, they are still more efficient than manual transplanting methods and can significantly reduce labor requirements, particularly for small to medium-sized farms.

Fig 3. Walking type Mechanical rice transplanter(Source: Kumar et al., 2012)

3.2.2.2 Riding type transplanter

Riding-type transplanters require an operator to sit on the machine and drive it across the field while controlling the transplanting process. These machines are typically larger and more mechanized than walking-type transplanters, featuring advanced planting mechanisms capable of handling multiple seedlings simultaneously. Riding-type transplanters are suitable for mid-sized to large fields where manual operation is feasible and cost-effective. They offer increased efficiency compared to manual transplanters, as they can cover more ground in less time while maintaining precise spacing and planting depth.

Fig 4. A view of field being transplanted by self-propelled rice transplanter(Source: Kumar et al., 2012)

Table 1: Rice transplanter manufacturing companies in India

S.No.	Make	Model	Name & Address of Manufacture
1	Mahindra	LE4 DB	M/s Mahindra & Mahindra Ltd. Machine Mohali, Punjab
2	Kubota	Kubota-NSPU-68C	M/s. Kubota Agril. Mech. India Pvt. Ltd.Level 2 altius, Olympia Tech. Park, No.1SIDCO Industrial

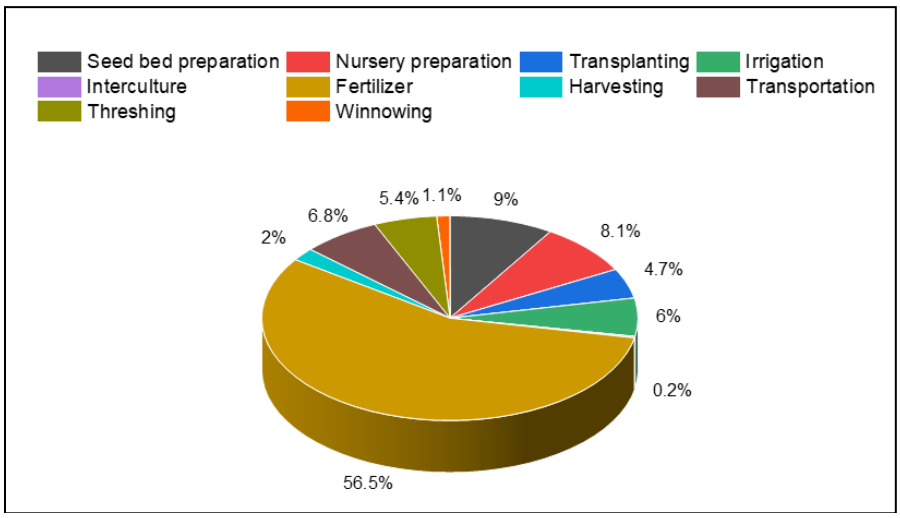
			Estate,Guindy, Chennai (T.N.)
3	Kubota	Kubota-NSP-4W	M/s. Kubota Agril. Mech. India Pvt. Ltd.Level 2altius, Olympia Tech. Park, No.1SIDCO Industrial Estate,Guindy, Chennai (T.N.)
4	Kubota	Kubota-NSD-8	M/s. Kubota Agril. Mech. India Pvt. Ltd.Level 2altius, Olympia Tech. Park, No.1SIDCO Industrial Estate,Guindy, Chennai (T.N.)
5	CLASS	PADDY PANTHER-14	M/s. Claas India Pvt. Ltd., Morinda By-Pass, NH-95, Village Marauli Kalan, Morinda-140101, Distt. Ropar
6	CLASS	PADDY PANTHER-26	M/s. Claas India Pvt. Ltd., Morinda By-Pass, NH-95, Village Marauli Kalan, Morinda-140101, Distt. Ropar
7	ASIA	Rice Transplanter (Walking type)	M/s. AIC Machinery Co Ltd, No.39, Hosur Road, Electronic City, Bangalore-560229
8	YANJI	Rice Transplanter (Riding type)	Ws. VST Tillers Tractors Ltd,PB 4801,Mahadevapura, Bangaiore560048
9	RI Agri	2Z 445 Rice transplanter	Ws. Greaves Cotton Ltd., Thoraipakkam, Chennal-600096
10	Yanmar	VP8D Rice transplanter (Riding Type)	Ws. Yanmar Co. Ltd., India Representative Office, 5th Floor, Sector-18, Noida-210 301 (U.P)
11	Mahindra	MP46 Rice transplanter (Walking type)	M/s. Yanmar Co. Ltd., India Representative Office, 5th Floor., Sector-18. Noida-210 301 (U.P)
12	Premier	GARUDA Rice Transplanter (Walk behind type)	M/s. Yanmar Co. Ltd., India Representative Office. 5th Floor, Sector-18, Noida-210 301 (U.P)
13	Fortune	FAI-2ZT-4 Rice Transplanter (Walk behind type)	M/s. Fortune Agro Impex, No.9, Mallathahalli, 1 Main Row:1.171 Layout, Bangalore (KK)-560 056
14	Yanmar	AP4 Rice Transplanter	M/s. Yanmar India Private Limited., K-4 Ocean Heights, 5th Floor, Sector-18, Noida – 201 301
15	VIJAY	VVRTR-01	M/s. Yanmar India Private Rice Limited., -4 Ocean

			Heights, K TransPlant 5th Floor, Sector-18 Noida-201301
16	Yanmar	VP8DN Rice transplanter	M/s. Yanmar India Pvt. Ltd., K-Machine- 4. Ocean height, 5th Floor, Sector 18, Noida-201 301, U.P., India.
17	Redlands	RP824 Self Propelled Riding Type Rice Transplanter	M/s. Yanmar India Pvt. Ltd., K-Machine- 4, Ocean height, 5 Floor, Sector 18, Noida-201301, U.P., India.
18	ACE	RTT 2Z-8238B-SL-Z-E-P Self Propelled Riding Type 8 Row Rice Transplanter	M/s. Action Construction Equipment Ltd., (Agri Equipment Division), Jajru Road, 25th Mile Stone, Mathura Road, Ballabgarh, Faridabad-121 004, Haryana
19	Rhino	RH 8 Self Propelled Riding Type Rice Transplanter	M/s. Assam Sail Motors Pvt. Ltd., 1A, Nanda Mallick Lane Kolkata-700 006, W.B,
20	VARUSHA PRIYA	Rgo 60 Sd Self Propelled Riding Type Rice Transplanter	M/s. Assam Sali Motors Pvt. Ltd., 1A, Nanda Mallick Lane, Kolkata-700 006, W.B.

4. Need of USG Applicator

For the application of Urea Super Granules (USGs), the placement of granules can be done either manually or mechanically using applicators. Currently, manual application of granular urea is akin to transplanting between rice seedlings in the field. Essentially, granular urea is dispersed in the center of four consecutive hills formed by two adjacent rows, at a depth of 60 to 70 mm beneath the soil. However, the manual placement of granular urea demands a considerable amount of labor and is a slow field operation, with a working pace ranging from 0.07 to 0.12 hectares per workday (Savant et al. 1991; Chatterjee et al. 2018). The high associated costs due to labor-intensive requirements and a shortage of labor during peak seasons are significant factors contributing to the limited popularity of USG applicators (Savant

and Stangel 1990). Figure 3 illustrates the energy consumption in various operations in paddy cultivation, revealing that fertilizer application consumes the highest energy share at 56.5%. This highlights the need for the development of a mechanically operated, cost-effective granular urea applicator to address the challenges associated with manual application and enhance the efficiency of the fertilization process in rice cultivation.



Energy consumption in different operations of paddy cultivation (Pradhan et al., 2015)

5. Performance Evaluation of Developed Deep Placement USG Applicator Attachment for Rice Transplanter

The field evaluation of the developed system with paddy transplanter as depicted in Table 2 , the paddy transplanter performed better as per the BIS standards.

Table 2: Transplanter parameters

Sl no	Parameters	Value
1	Hill spacing	15.2 cm
2	Number of seedlings per hill	2-3
3	Depth of transplanting	6 cm
4	Missing hill	10%
5	Floating hill	3.75%
6	Buried hill	2.5%
7	Damaged hill	0%

The evaluation of the attached USG applicator in terms of depth of placement, distance between USG in the field and application rate were observed as 4-6 cm, 32cm and 89-92 kg ha^{-1} , respectively(Table 3).

Table 3: Parameters of USG applicator attachment

Sl. No.	Parameter	Value
1	Depth of placement of USG	4-6 cm

2	Missing and multiple percentage	2.07 %
3	Distance between USG	32 cm
4	Application rate	89.2-92.13 kg ha^{-1}

Common parameters of both self-propelled rice transplanter and urea briquette applicator

Time taken to cover area

This was the main important parameter considered while computing the performance of transplanter. Time was noted at starting and ending point of transplanting for the self-propelled rice transplanting. So that actual time required for transplanting by transplanter was computed in terms of h/ha. The time required for one turn of transplanter, time taken to feed the trays to transplanter and time taken for adjustment were also noted to compute time losses in operation.

Fuel consumption

The fuel consumption has direct effect on economics of transplanter. The fuel consumption was measured using topping method. Initially the fuel tank of the transplanter was filled to its full capacity. The transplanter was run for 1 h, fuel was refilled in the tank up to the original level. The quantity of refilled fuel was measured by measuring cylinder. This observation was used for computation of fuel consumption in l/h and l/ha.

Effective field capacity

It is the actual rate of coverage of area by a machine. Effective field capacity was determined using the following relationship:

$$\text{Effective field capacity} = \frac{\text{Total area covered,ha}}{\text{Total time taken,h}} \times 100$$

The total time taken in above relationship includes time losses in turning, loading of trays and machine adjustment required during the operation.

Field efficiency

Field efficiency is the ratio effective field capacity and theoretical field capacity. It was determined by the formula given below:

$$\text{Field efficiency} = \frac{\text{Effective field capacity}}{\text{theoritical field capacity}}$$

Field machine index

It indicates the influence of field geometry on working capacity of machine. Field machine index was worked out by the following formula (Renoll, 1970):

FMI, % = $\frac{T_p + T_t}{T_t} \times 100$

Where,

FMI = Field machine index,

Tp = Total productive time, min

Tt = Total turning loss time, min

The field capacity of the mechanical transplanter with USG applicator and without USG applicator was compared (Table. 4). It was observed that fuel consumption and time requirement to a hectare land increased with attachment and field efficiency and effective field capacity reduced. But the reduction in efficiency and increase in time is not significant.

Table 4: Common parameters of transplanter and USG applicator

Sl. No.	Parameter	Transplanter without attachment	Transplanter with attachment
1	Time taken to cover area (1 ha)	4.27 h	5.23 h
2	Fuel consumption	1.66 l h^{-1}	1.81 l h^{-1}
3	Effective field capacity	0.234 ha h^{-1}	0.191 ha h^{-1}
4	Field efficiency	75.16 %	67.82. %
5	Field machine index	88.09 %	84.10 %

6. Conclusion

The application of USG coated with bentonite, neem oil, acacia oil and sulphur at different proportion and varying the curing period can delay the release of the nitrogen. Therefore, coated USG can be applied at the time of transplanting with the developed add on type unit with the mechanical transplanted. It has been widely reported that USG application not only save nitrogenous fertilizer but also reduces the losses & enhance nitrogen use efficiency. But the manual method of application involved human drudgery and more cost of cultivation. The developed add USG application system with mechanical transplanter would help the farmers in saving fertilizer as well as labour charges without compromising the yield of the rice .

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CHAPTER 18

Smart Device for Cutting Energy Measurement of Cereal Crops

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Introduction

Crop harvesting is a critical operation, which requires proper moisture content as well as a considerable amount of energy. Significant crop losses can also occur during the harvesting stage due to improper design or varying operational parameters. Losses induced during crop harvesting can be attenuated by designing suitable cutting units with respect to crop physical parameters. One of the key factors in crop harvesting is cutting force. Measuring and specifying the optimum required energy to cut the crop stalks can lead the technology to improve and can inform the development of more appropriate cutting tools. Integrating an under-standing of these factors results in more energy efficient crop cutting and increases the tools' potential applicability for different crops. To design energy efficient harvesting equipment, physical and mechanical parameters of a crop are crucial, and must be taken into consideration. Due to variation in crops mechanical properties, ideal energies for cutting are specific to each crop, and vary significantly. Furthermore, operational parameters of the cutting blade like bevel angle, blade orientation, and cutting speed have been shown to be vital in energy efficient crop cutting performance. Using the proper blade parameters also reduces the energy consumption during crop stem cutting. Therefore, to optimally design a harvesting system with a better understanding of cutting mechanics, a device was needed that can be used to study different parameters related to cutting energy. To address this challenge a sensor-based device was developed for on-field crop cutting energy measurement. The device contains an in-built load cell, a gyroscopic sensor and an ultrasonic sensor, and is capable of providing real time data on crop cutting energy, cutting angle and cutting speed. The design of this smart device was accomplished through studying stem properties and cutting energy requirements under laboratory conditions with varying parameters. The developed device was evaluated at field level for cutting energy measurement of wheat crop at different stem heights (10, 20 and 30 cm), cutting speeds (0.83, 1.16 and 1.5 m.s-1) and stalk size (one stalk, two stalk). The developed device will enable the design of the energy efficient crop cutting and harvesting systems, as well as testing and comparison between existing harvesting equipment designs.

Design of sensor-based crop cutting energy device

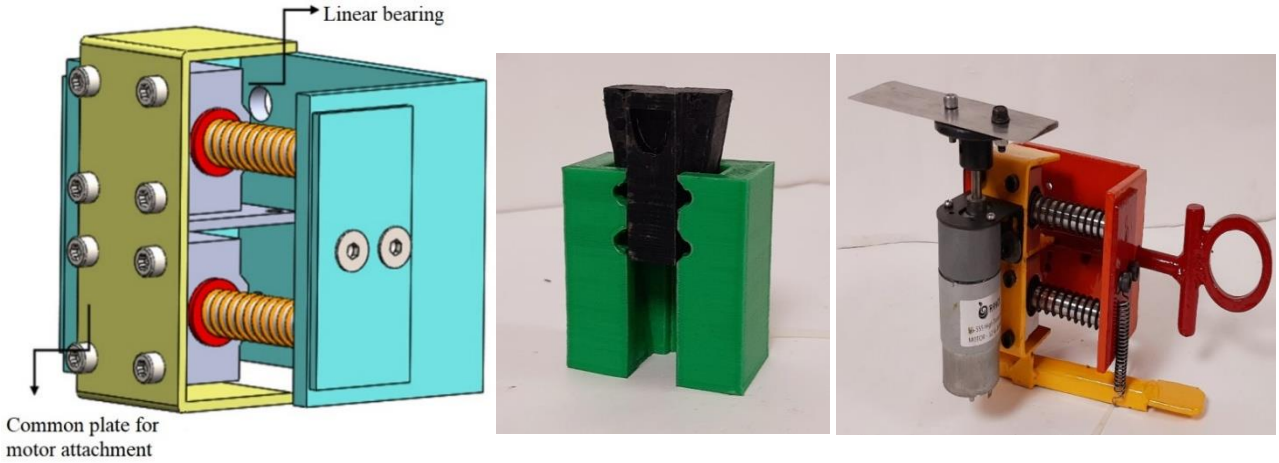
Based on the results obtained from crop characteristics and effect of operation parameters, a device was developed for real time measurement of crop cutting energy under actual field conditions. The device consisted of following main functional components.

Crop cutting unit

The crop cutting unit consisted of a cutting blade powered through 12V DC motor DC motor. Design values obtained from the experimental data were taken into consideration for fabri-cation of blade with optimum bevel angle (minimum required energy).

Compression unit

Cutting unit required inertia force to shear the crop stalk. Hence, spring compression system was developed to cause required momentum generation in cutting blade. A 12V DC motor was fitted on a compression system with a bracket attachment. Cutting was achieved when the DC motor fitted on the compression system was released from its initial position with a release knob (Figure 1).



(a) (b) (c)

Figure 1. Functional components of smart device for crop cutting energy measurement. (a) Compression unit; (b) 3D printed Crop holding unit; (c) Cutting blade with DC motor.

Crop holding unit

During on-field cutting operation, crop stalks required to be held in position to prevent de-flection due to high velocity of blade movement. Crop holder was designed to accommodate the required stalks with respect to their stem diameter. Crop holder was fabricated using 3D printer. It was consisted of two interlocking i.e. a tapered component sliding in the grooved component to facilitate working during experimentation. Stalk holder was fixed to the sus-pended part of load cell sensor.

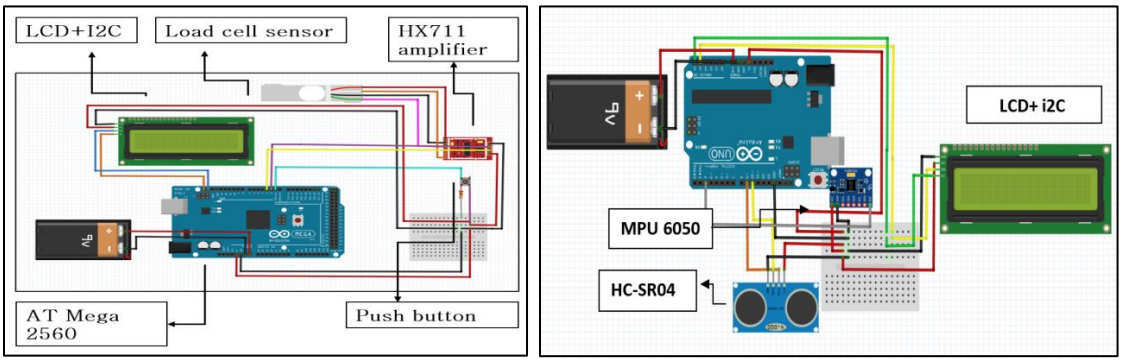
Force measurement unit

It consisted of a beam load cell (Power cell, Mettler-Toledo India Private Limited, Mumbai, India; capacity: 5 kg; precision: 0.05%; overload: 150% capacity) fitted between the s-shaped aluminum blocks arranged in a way that one side of S-shaped load cell was attached to cantilever plate and

another had a crop stalk holder without any fixture to allow free movement.

Sensor system

The system was developed to have a real time measurement of cutting speed, cutting angle, and cutting height at field level. For real time data acquisition related to cutting angle, cutting speed, and cutting height three sensors were integrated in the design. Ultrasonic sensor (HC SR04 ultrasonic sensor, Shenzhen Ef Technology Co limited Shenzhen, China; Voltage: 5V, Current: 15mA, Range: 2cm-4m, Frequency: 40Hz, Angle: 15º) was incorporated in the de-sign to measure the height at which the blade makes a cut with the stalk. Gyroscope sensor (InvenSense Inc. 1197 Borregas Ave, CA, USA; MPU 6050; 3-5V, i2c protocol) was used to measure the cutting angle. Sensor was attached to the device such that the angle made by blade with longitudinal axis of stalk was measured. Code for both the sensors i.e ultrasonic sensor and gyroscope sensor was loaded to Arduino board fitted with LCD display. The speed of cutting blade was controlled by regulating the voltage of DC motor using pulse width modulation (PWM) controller (Figure 2).



(a) (b)

Figure 2. Sensor circuit for force, distance, and angle measurement. (a) Circuit design for force measurement (b) Circuit design for distance and angle measurement

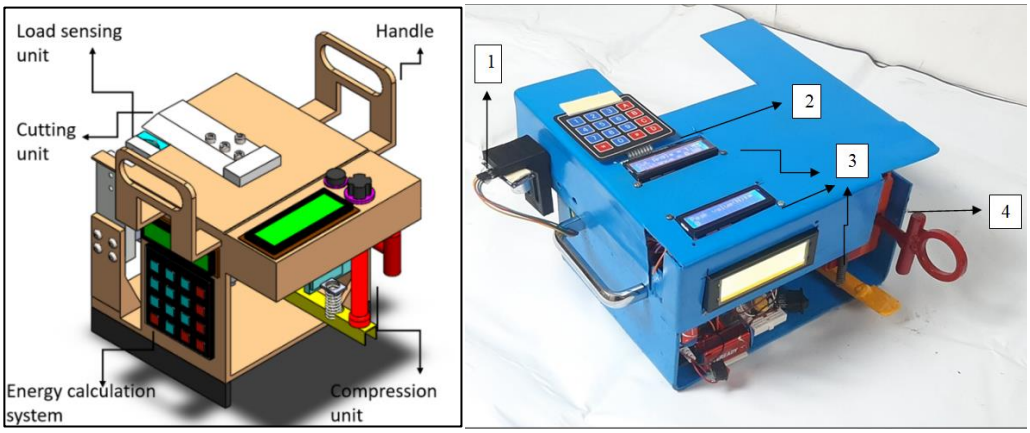
Arithmetic unit

Cutting energy was calculated as a product of peak cutting force and cutting distance (steam diameter). To have on field calculation of cutting energy, the system was incorporated with cutting energy calculation system. Therefore, the required C++ programming code in Ar-duino IDE was fed into the Arduino board fitted to the device for making the device work in real time basis. A 4*4 matrix keypad with 16*2 LCD display was used to build the calculator via the Arduino board.

Assembly of device

All the functional units were assembled in the form of portable device. The individual sensor circuits were connected to external power supply with separate 9V batteries. Cutting unit consisting of 12V DC motor was connected to an independent lead acid battery (Voltage: 12V, Capacity: 7Ah, weight: 2.2 kg) through a DC motor controller (PWM Speed control, 12V, 8A, 13 kHz) to obtain speed ranges of cutting. Load cell sensor was used to obtain the force value during cutting the crop stalks. The code of the sensor was altered to obtain the force value from the load

value of the sensor. Obtained values were amplified using a HX711 amplifier and further connecting to the Arduino board. Peak force value during the cutting process was obtained by designing the code with a logic function, such that the highest value was directly displayed on the LCD when push button was pressed at the end of every cutting process (Figure 3).



(a) (b)

Figure 3. Smart device for on-field crop cutting energy measurement. a) Conceptual design of smart device for cutting energy measurement; (b) Orthographic projection of developed smart device for cutting energy measurement (1. Distance sensor; 2. Calculator keypad unit; 3. LCD display units; 4. Compression unit).

Working of smart device for on-field cropping cutting energy measurement

The sensor based smart device was designed based on the values obtained on physical dimensions and engineering properties of rice stems. Force measurement unit was designed based on cutting force range of selected cultivars and relative error of different load cells evaluated during the experiment. A load cell of 5 kg was considered suitable due to low error and drift in output values, in addition to higher input sensitivity. As the device was to be used for crop cutting energy measurement at varying crop stem heights, considering the height of prominent Indian rice and wheat cultivars, therefore, ultrasonic sensor (HC SR-04, Range=2-400 cm) was selected to measure the cutting height with respect to ground level. Similarly, gyroscope sensor (MPU 6050, Range = 0-180) was selected to measure the on-field cutting angle. The crop holder unit of device was designed considering the overall stem di-ameter of the Indian rice and wheat cultivars. Bevel angle of 15º was selected for fabrication of cutting blade in reference to minimum cutting force and cutting energy value for particular bevel in range of bevel angles (15º, 20º, 25º) obtained from the experimental setup during experimentation. Cutting unit of smart device consisted of blade mounted on DC motor. To achieve to and fro motion, the compression system present in the system for initial momentum required springs of adequate compression. For this, springs of 6.12 N.cm-1 spring constant were selected for obtaining a retracting motion of 20 mm. Sensor systems designed for experimental setup performed well with microcontroller board Arduino Mega 2560. Sensor

units on the developed device were interfaced with Arduino Uno R3 and Arduino Mega 2560 microcontroller board which were highly compatible for all different sensors mounted on device.

Performance of developed device at field level

Cutting force and cutting energy of wheat stem determined at field condition with the de-veloped device ranged from 1.85-1.45 N and 10.81-8.48 N.mm, respectively for three range of cutting speeds i.e. 50, 70 and 90 m.min-1. Similarly, cutting force and cutting energy was in the order of 1.79, 1.66 and 1.44 N and 11.92, 9.89 and 6.74 N.mm, respectively during measurement from ground level at 10, 20, and 30 cm stem height. Cutting operation carried out in the range of 10-30 cm stem height from ground level, revealed decreased cutting en-ergy consumption. Similarly, cutting energy consumption was found to increase as stalk size increased. Sensitivity analysis of the force sensor fitted on device revealed that trend observed in cutting force and cutting energy was similar to the laboratory values. Sensors fitted on the device were analyzed for their accuracy in field conditions, particularly the ultrasonic sensors for cutting at particular stem height were compared with manual measurements, and accuracy of 97.5% was observed. Similarly, MPU 6050 gyroscope sensor to measure the cutting angle was compared with a magnetic angle indicator, revealed accuracy of 94.29%.

Conclusions

A sensor based smart device was developed for real time crop cutting energy measurement. The developed device has wide scope for real time measurement and recording of data on cutting energy, heights, speed and angle of cut during on-field crop cutting.

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