

# Hybrid Rice Breeding Manual

S. S. Virmani  
B. C. Viraktamath  
C. L. Casal  
R. S. Toledo  
M. T. Lopez  
J. O. Manalo

**IRRI**  
INTERNATIONAL RICE RESEARCH INSTITUTE

# Hybrid Rice Breeding Manual

HR2-01

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1997

International Rice Research Institute  
Los Baños, Laguna, Philippines



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# FOREWORD

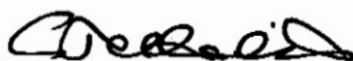
By 2030 the world must produce 60% more rice than it produced in 1995 to meet demands created by increasing populations and rising incomes. This production increase must be achieved on less land, with less labor, less water, and less pesticides, and must be sustainable. Experience in China, India, and Vietnam have established that hybrid rice offers an economically viable option to increase varietal yields beyond the level of semidwarf rice varieties. Several other countries such as Bangladesh, Brazil, Colombia, Egypt, Democratic People's Republic of Korea, Japan, Malaysia, Myanmar, Pakistan, Philippines, Republic of Korea, Sri Lanka, Thailand and USA are currently exploring the prospects of hybrid rice. Availability of adequately trained human resources is an essential prerequisite for developing an effective national hybrid rice breeding program.

Hybrid rice breeding uses several concepts, skills, and procedures which are strikingly different from those used for inbreds rice breeding. These must be learned by plant breeders before initiating a comprehensive hybrid rice breeding program.

The International Rice Research Institute has offered several short-term training courses in hybrid rice breeding. The experience in these courses indicated that there was a need for a training manual on the subject which describes concepts and illustrates the procedures stepwise. This manual has been prepared to serve this need. It is based on the experiences attained at IRRI and those reported from China and India. The authors have described and presented hybrid rice breeding procedures stepwise in a systematic manner.

From now on, IRRI will use this manual as a primary courseware for hybrid rice breeding training courses and recommend its use in national programs interested to develop human resources in hybrid rice breeding.

I compliment the authors and all others involved in developing this extremely useful training ware.



**G. H. Rothschild**

*Director General*

# ACKNOWLEDGMENT

*Adviser* : Robert T. Raab

*Editor* : Madeline B. Quiamco

*Supervisor* : Gina E. Zarsadias

*Layout  
and graphic design* : Marie A. Clabita

*Secretarial  
assistance* : Leni B. Nazarea



# 1. Heterosis Breeding and Hybrid Rice

*Heterosis breeding, which exploits the phenomenon of hybrid vigor, has proven to be a practical method of crop improvement, especially for increasing yield potential in many crops. This phenomenon has been exploited primarily in several cross and often cross-pollinated crops such as maize, pearl millet, onion, sorghum, cotton, etc. but its application is also being extended to several self-pollinated crops including rice. Rice is the staple food providing about 35-59% of the total calorie intake of people in South and Southeast Asia. The demand for rice would be 800 million t by 2020. This means that we have to produce about 350 million t more rice by 2020 than what we are producing today to feed the ever increasing population. Among the different approaches contemplated to meet this challenge, hybrid rice technology has already shown potential. In the past, adoption of hybrid technology in rice was considered impractical because of the strict self-pollinating nature of the crop and skepticism about the practical feasibility of producing hybrid seed on a commercial scale. Fortunately, rice breeders have overcome these hurdles by developing a usable system of cytoplasmic-genetic male sterility and packages for efficient and economic seed production. More than 50% of the total rice area in China is planted to hybrid rice, and many countries outside China are developing and exploiting hybrid rice technology.*

## Heterosis breeding

### *What is heterosis?*

The term heterosis, often used synonymously with hybrid vigor, refers to the superiority of the  $F_1$  hybrid over its parents.

- Expression of heterosis is confined to the first generation only. Thus, farmers have to buy fresh seeds every season to raise a commercial crop.
- Heterosis may be positive or negative. Both positive and negative heterosis are useful in crop improvement, depending on the breeding objectives. For example, positive heterosis is desired for yield, but we look for negative heterosis for traits like days to maturity and height.

### *Types of heterosis*

Heterosis is expressed in three ways, depending on the reference which is used to compare the performance of a hybrid (Fig. 1.1).

- **Mid-parent heterosis** -- The increase or decrease in the performance of the hybrid in comparison with the mid-parental value.
- **Heterobeltiosis** -- The increase or decrease in the performance level of the hybrid in comparison with the better parent of the cross combination.
- **Standard heterosis** -- The increase or decrease in the performance of a hybrid in comparison with the standard check variety of the region.

From the practical point of view, standard heterosis is most important because we are trying to develop hybrids which are better than the existing high yielding varieties grown commercially by farmers.

## Illustration of different types of heterosis

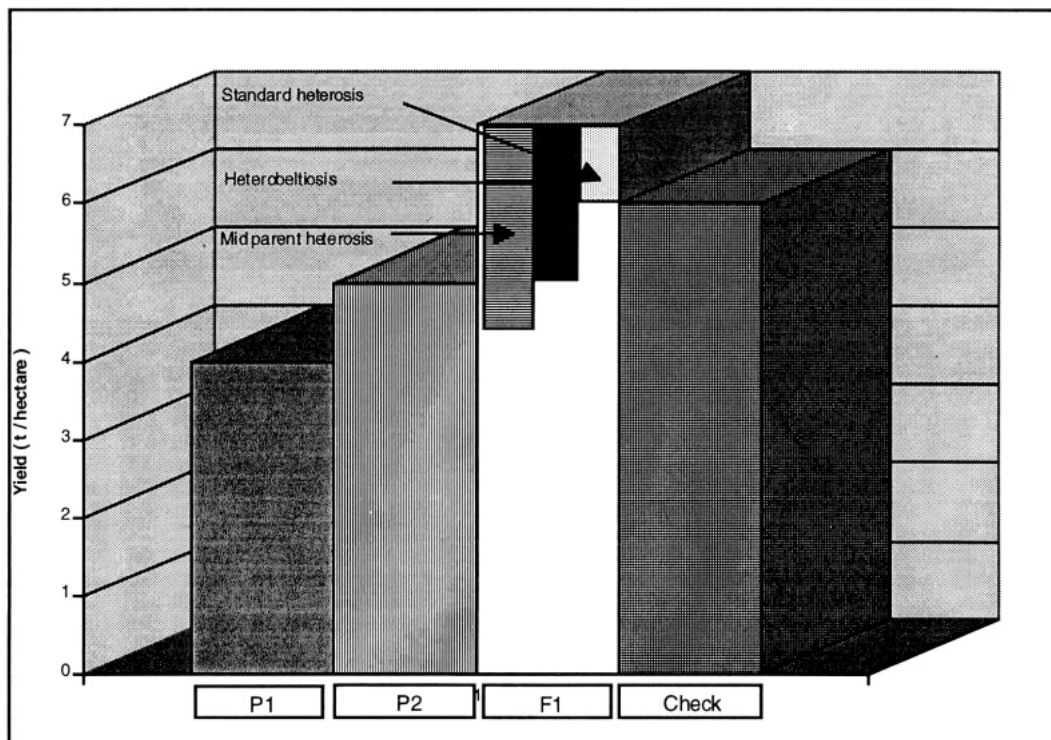


Fig. 1.1 Different types of heterosis.



## ***How is heterosis measured?***

Measurement of heterosis is quite simple. It is generally expressed as percent increase or decrease in the performance of a hybrid in comparison with the reference variety or a parameter.

$$\text{Mid-parent heterosis (\%)} = \frac{F_1 - \text{Mid parent}}{\text{Mid parent}} \times 100$$

$$\text{Heterobeltiosis (\%)} = \frac{F_1 - \text{Better parent}}{\text{Better parent}} \times 100$$

$$\text{Standard heterosis (\%)} = \frac{F_1 - \text{Check variety}}{\text{Check variety}} \times 100$$

## ***Genetic basis of heterosis***

Two major hypotheses have been proposed to explain the genetic basis of heterosis: dominance hypothesis (Davenport, 1908), and overdominance hypothesis (East, 1908 and 1936).

- Dominance hypothesis
  - states that heterosis is due to the accumulation of favorable dominant genes in a hybrid derived from the two parents (Fig. 1.2).

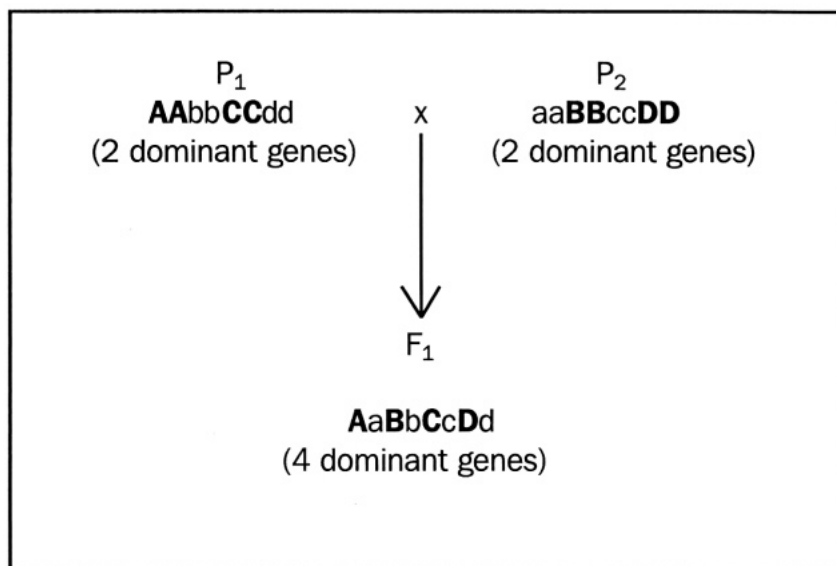


Fig. 1.4 Illustration of dominance hypothesis to explain genetic basis for heterosis.

This was demonstrated in a pea hybrid whose parents had different dominant genes for node number and internodal length. The hybrid was much taller than either parents. The increased height was due to the accumulation of both dominant genes in a hybrid.

- **Overdominance hypothesis**

- states that heterozygotes (Aa) are more vigorous and productive than either homozygotes (AA or aa). This has been proven in traits controlled by single or few genes. Heterozygotes perform a given function, over a range of environments, more efficiently than either homozygotes (East, 1936).

Studies on genetic basis of heterosis for polygenic traits in various crops have shown that heterosis is the result of partial to complete dominance, overdominance, and epistasis, and may be a combination of all these (Comstock and Robinson, 1952).

Evidence of real overdominance for quantitative traits is hard to find. However, apparent overdominance due to non-allelic interaction and linkage disequilibrium is a common contributor to heterosis (Jinks, 1983).

Heterosis may also be due to the specific positive effects of the cytoplasm of the maternal parent on the nuclear component of the paternal parent. Differential heterosis observed between the same pollen parent and CMS lines of different cytoplasmic sources is an example of this kind of heterosis.

It is indeed difficult to explain the genetic basis of heterosis for a complex trait like yield because of the complexity of its inheritance. Hybrid crop breeders believe that no single hypothesis can explain the basis of heterosis; perhaps all the above stated hypotheses may work jointly to explain this phenomenon. Lack of clear understanding of the genetic basis of heterosis has not prevented plant breeders from exploiting this phenomenon to raise crop yields.

# Hybrid rice

## *What is hybrid rice?*

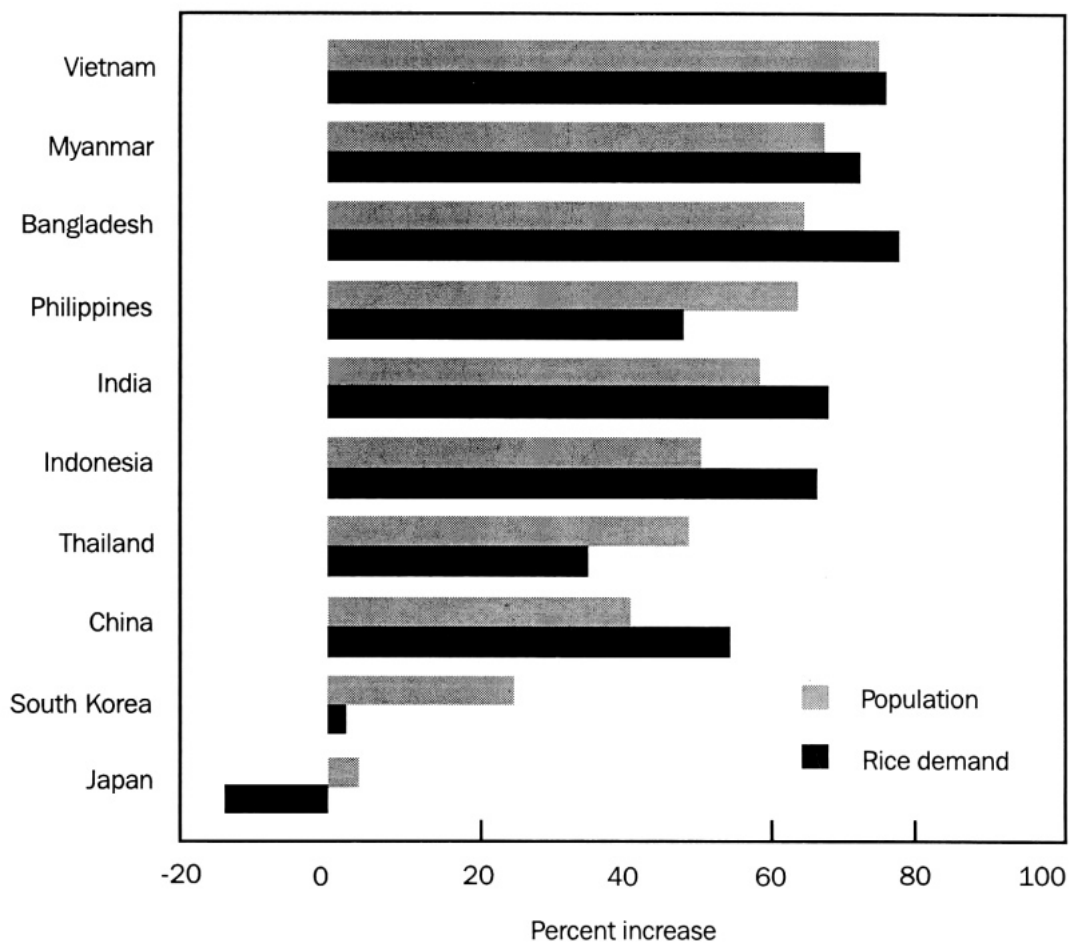
Hybrid rice is the commercial rice crop grown from  $F_1$  seeds of a cross between two genetically dissimilar parents.

- 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.

## *Why hybrid rice?*

We need to go for hybrid rice because

- yield levels of semi-dwarf varieties of the green revolution era have plateaued.
- more and more rice has to be produced on less land and with less inputs.
- demand for rice is rapidly increasing with the increase in population, especially in less developed countries (Fig. 1.3).
- hybrid rice varieties have shown 15-20% higher yield potential than inbred rice varieties under farmers' field conditions.
- hybrids have shown their ability to perform better under adverse conditions of drought and salinity.



**Fig 1.3** Projection of population growth and demand for rice, 1990-2025.

## ***How is hybrid rice developed?***

Rice is a strictly self-pollinated crop. Therefore, for developing commercial rice hybrids, use of a male sterility system is essential. Male sterility by genetic or non-genetic means makes the pollen unviable and such rice spikelets are incapable of setting seeds through selfing. Thus, a male sterile line can be used as female parent of a hybrid.

A male sterile line, when grown side by side with a pollen parent 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.



## 2. Male Sterility Systems in Rice

*The use of a male sterility system is a prerequisite for commercial exploitation of heterosis in rice. Though several male sterility systems are known to occur in rice, cytoplasmic-genetic male sterility has been widely used for developing rice hybrids. Recent discovery of a genetic male sterility mechanism influenced by environmental factors is getting serious attention from hybrid rice breeders. To a limited extent, chemical gametocides have also been used to induce male sterility in rice.*

### Male sterility systems

The following genetic and non-genetic male sterility systems are known for developing rice hybrids:

- Cytoplasmic-genetic male sterility
- Environment-sensitive genetic male sterility
- Chemically-induced male sterility

#### *Cytoplasmic-genetic male sterility*

It is caused by an interaction between genetic factor(s) present in cytoplasm and the nucleus. Absence of a sterility inducing factor either in the cytoplasm or in the nucleus makes a line male fertile (Fig. 2.1).

Presence of certain dominant restorer gene(s) in the nucleus makes a line capable of restoring fertility in the hybrid derived from it and a CMS line (Fig. 2.1).



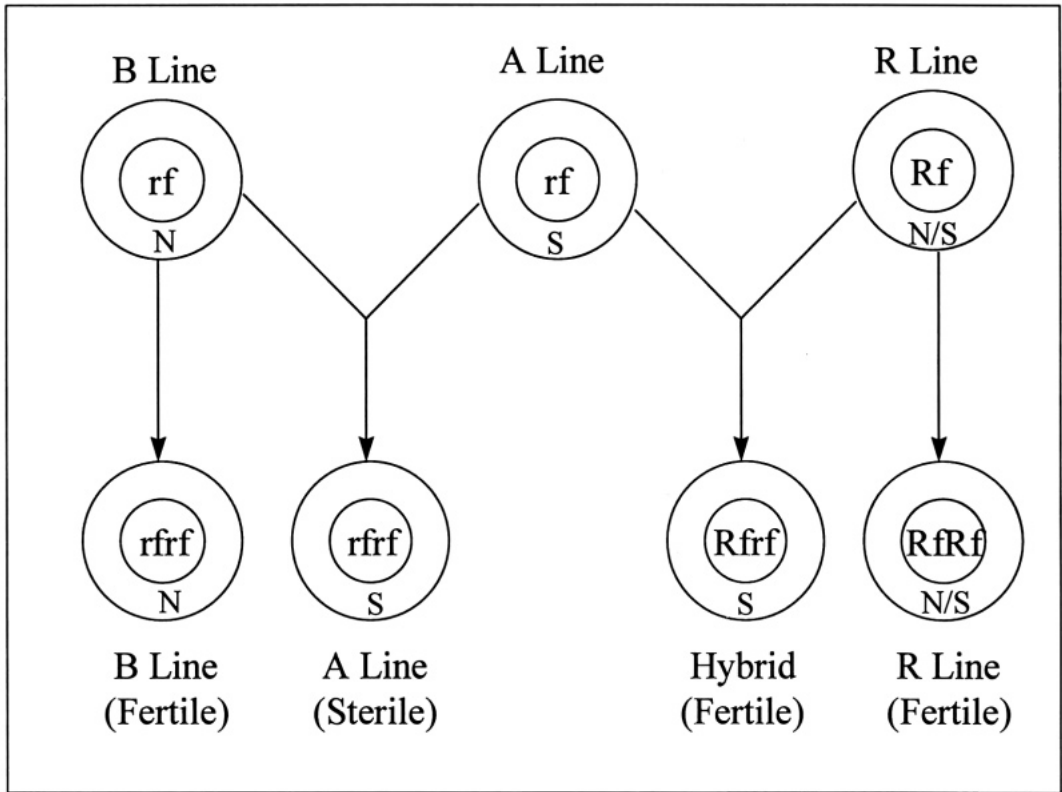


Fig. 2.1 Schematic description of cytoplasmic genetic male sterility system.

- **The cytoplasmic-genetic male sterility system involves**

1. a CMS (A) line
2. a maintainer (B) line
3. a restorer (R) line

A CMS line is multiplied always by crossing it with its maintainer line, either by hand-crossing (to produce small quantities of seed) or by out-crossing in an isolated plot (to produce bulk quantities of seed). Since the CMS line is always maintained by crossing it with its maintainer line, the two lines (A and B) are similar morphologically except that 'A' line is male sterile and 'B' line is male fertile. Occasionally, the two lines may show differences in some morphological and agronomical traits which are influenced by the cytoplasmic factors inducing male sterility. Restorer or R line possesses dominant fertility-restoring genes. When crossed with the CMS line, it restores fertility in the derived  $F_1$  hybrid.

Since this system involves the use of three lines (A, B, and R lines), the hybrids developed by using this male sterility system are known as three-line hybrids.

### **Procedures for identifying a CMS source**

CMS sources can be identified in

- inter-varietal reciprocal crosses
- inter-specific crosses

## Identifying CMS sources in inter-varietal crosses

Differences in reciprocal crosses between varieties with respect to male sterility is attributed to the cytoplasmic-genetic interaction (Fig. 2.2).

Example: Chinsurah Boro-II source

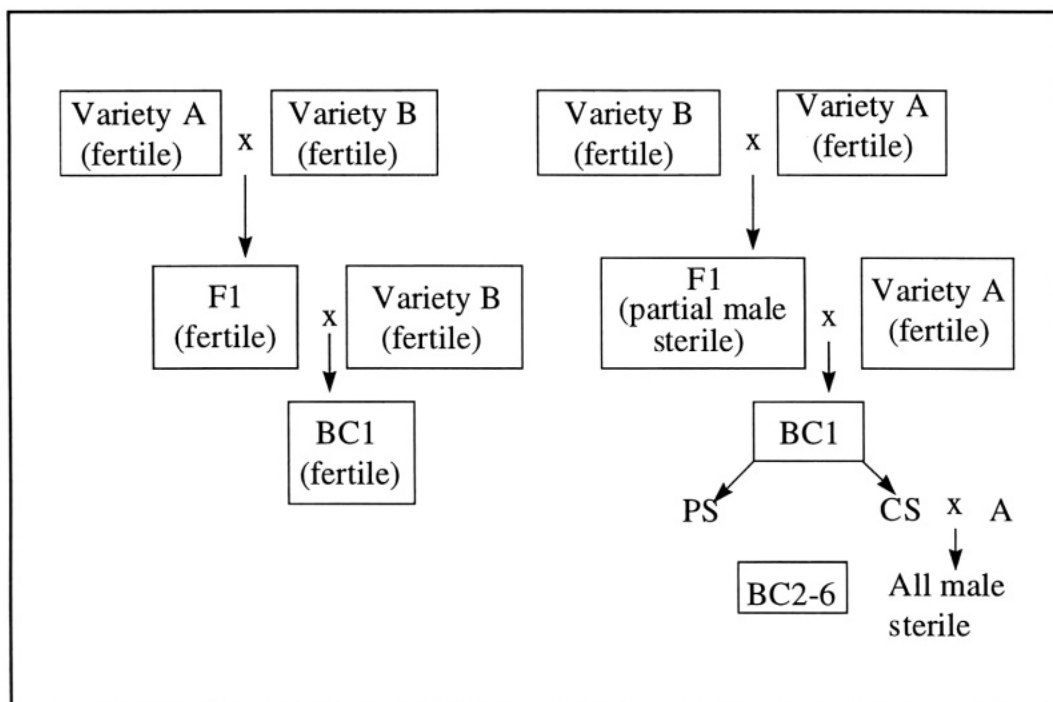


Fig. 2.4 Identification of new CMS sources in inter-varietal crosses.

The occurrence of high frequency of completely male sterile plants in  $BC_2$  generation indicates that variety B is a donor of cytoplasmic factor-inducing male sterility and variety A is a maintainer. Several rice varieties, viz., Chinsurah Boro II, Taichung Native 1, ARC 13829 etc., possess male sterility-inducing factors in their cytoplasm.

### Identifying CMS sources in inter-specific crosses

Crossing between wild species and cultivated varieties can also help to identify new CMS sources (Fig.2.3).

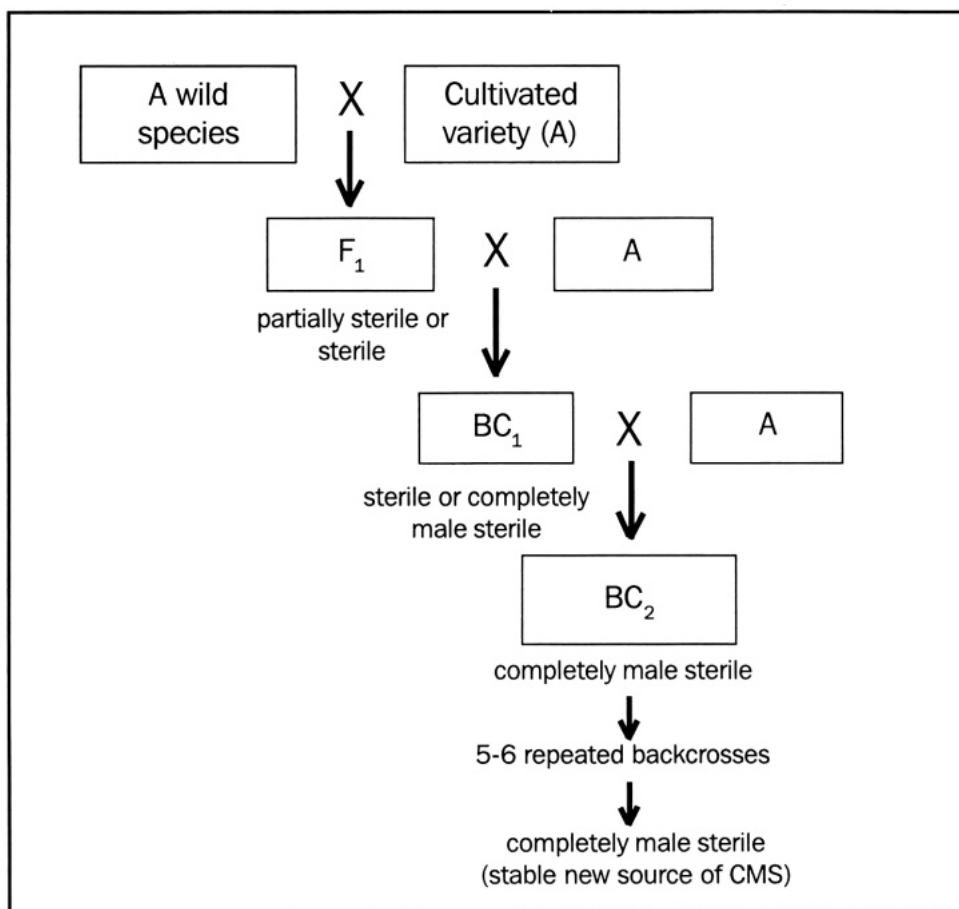


Fig. 2.3 Identification of new CMS sources in inter-specific crosses.

Some sources of male sterility inducing cytoplasm in rice.

Designation	Cytoplasmic Source	First Nuclear Donor
CMS-WA	Wild rice with abortive pollen	Zhen shan 97 V20 V41
CMS-DA	Dwarf wild rice with abortive pollen	Xue Qin Zhao
CMS-IP	Indonesian Paddy	II-32
CMS-DI	Dissi type	297
CMS-HL	Hong Lian	Lian-Tana Zhao
CMS-KR	<i>Oryza rufipogon</i>	Taichung 65
CMS-BT	Chinsurah boro II	Taichung 65
CMS-TN	TN1	Pankhari 203
CMS-GAM	Gambiac	Chao Yang 1
CMS-ARC	Assam Rice Collection IRRI Acc. 13829	IR10179-3-2-1
CMS- <i>O. perennis</i>	<i>O. perennis</i> , Acc. 104823	IR64R

- **Need for diversified CMS sources.**

Most of the rice hybrids cultivated in China and elsewhere are based on the WA system of cytoplasmic male sterility. Such overdependence on a single cytoplasmic source may be disastrous in case there is a sudden outbreak of pests and diseases, and if susceptibility is associated with a CMS-inducing factor. Therefore, diversification of CMS sources should be an important component of a strong hybrid rice breeding program.

Although several cytoplasmic sterility sources have been identified, not all of them are usable. To be usable, the CMS source should

- have stable and complete pollen sterility across environments,
- be easily maintained so that diverse genotypes can be converted into new CMS lines,
- be easily restored so that diverse genotypes can be used as male parents, and
- not have adverse effects on agronomic traits.

The most commonly used cytoplasmic sterility sources are WA, BT, DI, DA and IP

- **Procedure used to transfer a CMS source into an elite line**

Elite lines are first testcrossed with the CMS line of a desired cytoplasmic 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 cytoplasmic sterility source (Fig. 2.4).

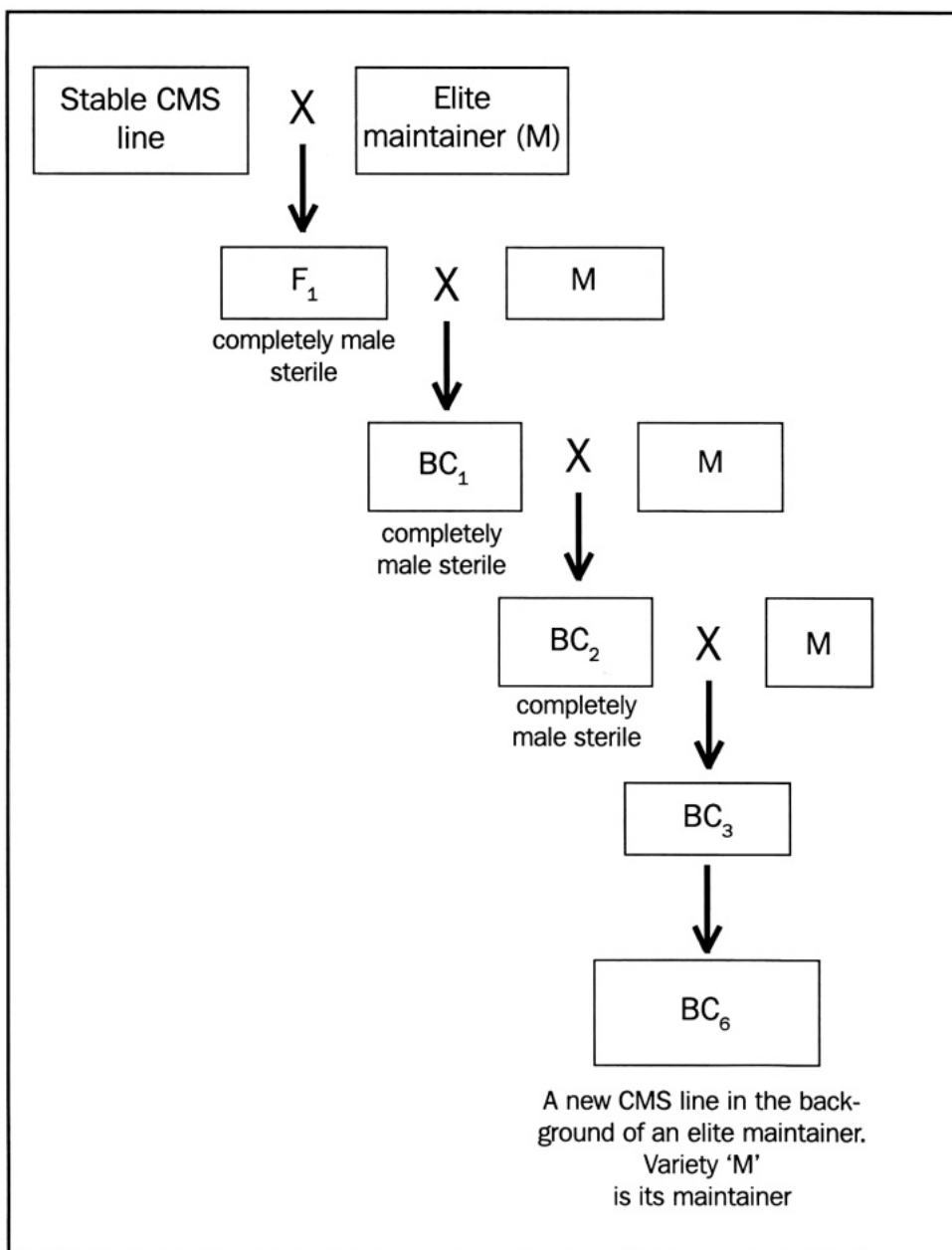


Fig. 2.4 Procedure of transferring a CMS system into an elite maintainer line.

- **Characteristics of a commercially usable CMS line**

An ideal CMS line should have

- stable male sterility over environments;
- adaptability to target environment for which rice hybrids have to be developed;
- easy restorability, so that many elite lines can be used as male parents;
- good outcrossing ability to result in higher seed yield;
- good combining ability; and
- good grain quality so that rice hybrids can be developed with acceptable grain quality.

- **Restoring ability**

Availability of a wide range of restorers is an essential prerequisite for exploitation of heterosis. The frequency of restorers vary between ecotypes and geographic regions. The following are the general observations regarding restoration ability.

- Indica rices have higher frequency of restorers than japonica types.
- Among indicas, *aman* and *boro* cultivars have higher frequency of restorers than *aus* types.
- *Bulu* rices are weaker restorers than *tejereh* cultivars of Java.
- Frequency of restorer lines is generally higher in rice varieties originating from lower altitudes than those from higher altitudes.



- Frequency of effective restorers is higher in south and southeast Asia and southern China while nonrestorers are concentrated in Northern China and Far Eastern Asia.

- **Inheritance of fertility restoration**

- Fertility restoration of CMS-Boro cytoplasm is controlled by a dominant gene  $Rf_1$  carried by a restorer line.
- In case of CMS-WA cytoplasm, fertility restoration is governed by two dominant genes with differential strengths of restoration. One of the two fertility restorer genes is stronger than the other.
- The effect of the restorer gene on CMS-boro cytoplasm is gametophytic, causing partial pollen fertility but normal spikelet fertility in  $F_1$  hybrids.
- The effect of restorer genes on CMS-WA cytoplasm is sporophytic which causes normal pollen and spikelet fertility in  $F_1$  hybrids.
- The restorer gene identified for CMS-boro cytoplasm is located on chromosome 10 (Shinjyo, 1975). For CMS-WA cytoplasm, the stronger restorer gene is located on chromosome 7, while the weaker one is located on chromosome 10 (Bharaj, Virmani, and Khush, 1995).

- **Advantages of the CMS system**

Among all the male sterility systems, the CMS system is the most effective and proven method of commercial hybrid rice production.

- **Disadvantages of the CMS system**

- Seed production is quite cumbersome as it is done in two steps, i.e., AxB multiplication and AxR F<sub>1</sub> production.
- The choice of male parents is limited to only those genotypes which are identified as restorers.
- Sometimes the sterility-inducing cytoplasm exerts adverse negative effects on the expression of agronomic traits.
- A CMS system may cause a genetic vulnerability of the derived hybrids if this system gets associated with susceptibility to a biotic stress.

### ***Environment Sensitive Genic Male Sterility (EGMS)***

This is a genetic male sterility system in which sterility expression is conditioned by environmental factors.

- **Types of EGMS**

There are two types of EGMS which are currently being used in rice:

- **PGMS** - Photoperiod sensitive genic male sterility includes genic male sterile lines which respond to the photoperiod or duration of day length for expression of pollen sterility and fertility behavior. For example, 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.

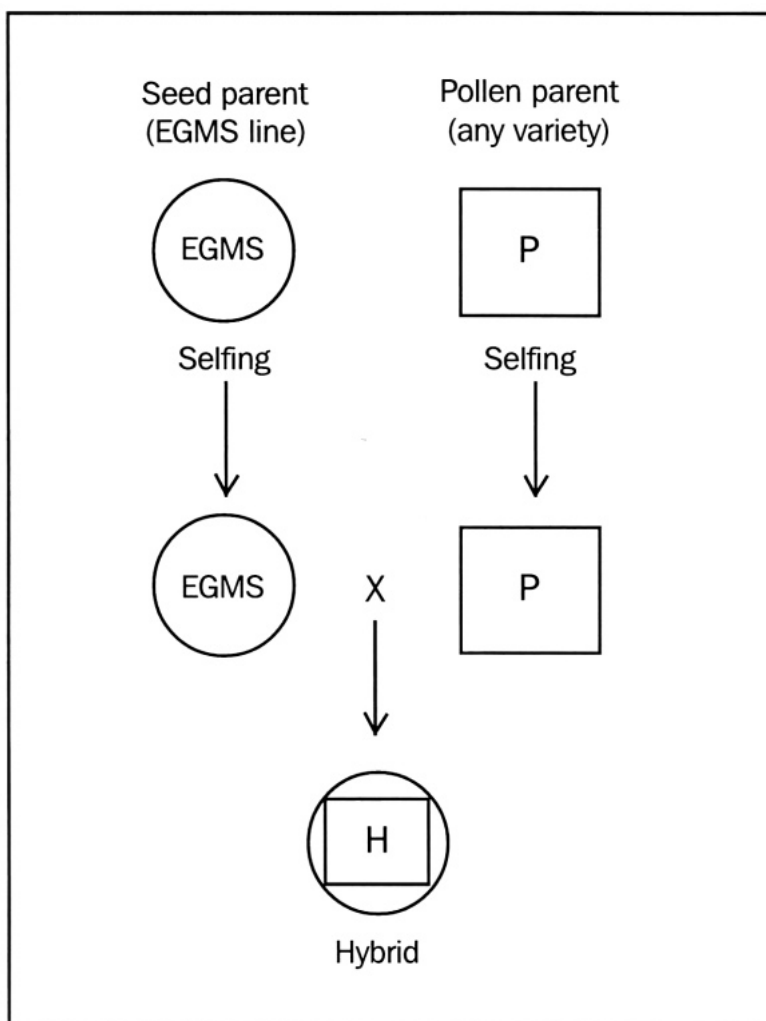
- **TGMS** - Thermosensitive Genic Male Sterile lines are genic male sterile lines whose male sterility/fertility alteration is conditioned by different temperature regimes. For example, most of the TGMS lines remain male sterile at a high temperature (maximum >30°C) and they revert back to partial fertility at a lower temperature (maximum <30°C). The critical sterility/fertility points vary from genotype to genotype.
- The critical thermosensitive stage for fertility alteration in the TGMS line varies from 15 to 25 days before heading or 5-15 days after panicle initiation.

Some EGMS lines identified

EGMS Lines	
PGMS	TGMS
Nongken 58 S (China)	Annong 810 S (China)
EGMS (USA)	Hennong S (China)
201 (USA)	5460 S (China)
CIS 28-10S (China)	R59TS (China)
Zhenong S (China)	Norin PL12 (Japan)
X 88 (Japan)	IR 32364 (IRRI)
Peiai S (China)	IR68945 (IRRI)
7001 S(China)	IR68949 (IRRI)

### Deployment of EGMS lines for developing two-line hybrids.

Unlike the CMS system, seed production in EGMS system is relatively simple as no maintainer is required for multiplying the EGMS line. EGMS lines are multiplied by selfing like any other varieties when they are grown under conditions favorable for inducing fertility. Only the EGMS line and the pollen parent are needed to produce a hybrid. Hence, the hybrids developed by using EGMS system are called 'two-line hybrids' (See fig. 2.5 for details).



**Fig. 2.5** Schematic description of the use of EGMS lines for developing two-line hybrids.

## *Chemically induced male sterility*

This non-genetic method of inducing male sterility involves the use of chemicals called Chemical Hybridizing Agents (CHA) or gametocides. This method is very useful for plants with bisexual flowers in which it is difficult to obtain genetic or cytoplasmic-genetic male sterility.

- In this method of developing hybrids, male sterility is induced by spraying a rice variety with chemical gametocide that can kill pollen grains of treated plants without affecting the pistil. In hybrid seed production, two parents are planted in alternate strips. One is sprayed with chemicals at appropriate growth stage, and the other is used as pollen source to produce the hybrid seed.
- The ideal gametocides should
  - selectively induce male sterility without adversely affecting the female fertility.
  - have systemic effects so as to sterilize both early and late panicles.
  - have a broad range of effectivity in order to withstand adverse environmental conditions
  - have minimum side effects on plant growth and panicle development

- Important gametocides found useful in rice are given below:

Chemical	Concentration	Growth stage for application
Ethrel	8000-10,000 ppm	pre-boot and boot stage
Monosodium Methyl Arsenate (MGI)	0.02% or 2000 ppm	uni-nucleate pollen stage
Sodium methyl Arsenate	0.02% or 2000 ppm	5 days before heading

- For developing hybrids by using gametocides:
  - the female line should have a synchronous flowering habit
  - the line should respond to chemical treatment
  - the parents should possess good outcrossing traits
- Important factors that decide the efficiency of chemical gametocides are
  - the correct dosage of the chemical.
  - appropriate stage of treatment.
  - even coverage while spraying.
  - synchronized flowering of the tillers in the female parent.

- Other practices for hybrid seed production by using CHA are similar to those followed for three-line hybrids.
- Hybrids produced by using chemically induced male sterility are also called two-line hybrids.

In rice, chemically induced male sterility is used sporadically because effective and safe chemicals inducing male sterility are not available. Besides, effective CMS and EGMS systems are available.

### **3. Organization of Hybrid Rice Breeding Program Using CMS System**

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*Procedures for developing rice hybrids are quite distinct from those employed for breeding conventional varieties. In hybrid breeding, productivity genes are assembled and exploited under a heterozygous condition for only one generation. On the contrary, conventional breeding involves the accumulation of productivity genes that perform well under a homozygous condition year after year. Hybrid rice breeding broadly covers: i) development of parental lines, ii) seed production of parental lines and experimental rice hybrids, and iii) evaluation of hybrids. For the efficient development of parental lines, breeding materials should be grouped into separate nurseries, i.e., source, testcross, retestcross, and backcross nurseries. The genetic base of the material used should be as wide as possible. The evaluation of heterosis is the most crucial part of hybrid rice breeding and experimental hybrids should pass through each and every stage, and be finally tested in the farmer's field before their release.*

## **Components of hybrid rice breeding**

### *Development of parental lines*

#### **Source Nursery (SN)**

This nursery includes elite lines which have the potential to become parents of commercial hybrids. The best available CMS and TGMS lines are also included in this nursery.



## **CMS Line Maintenance and Evaluation Nursery (CMSN)**

It is the breeding nursery in which the CMS lines, both developed locally and those which are introduced from outside, are maintained and evaluated.

## **Testcross Nursery (TN)**

It is the breeding nursery wherein  $F_1$ s of cytoplasmic male sterile lines and test varieties from the source nursery are screened for pollen sterility/fertility, spikelet fertility, and other agronomic traits to identify the potential maintainers and restorers and heterotic hybrids.

## **Restorer Purification Nursery (RPN)**

This breeding nursery comprises the progenies of the CMS line and individual plants of restorer lines which are selected for purification and seed multiplication purposes.

## **Backcross Nursery (BN)**

It is a breeding nursery wherein the CMS system from the available CMS lines is transferred into the genetic background of elite maintainer lines identified in the testcross nursery by consecutive backcrossing.

## **Combining Ability Nursery (CAN)**

A breeding nursery comprising a set of crosses derived from promising CMS and restorer lines which are evaluated along with their parents to assess their combining ability or their ability to produce superior progenies when crossed with another parent.

## *Seed production of parental lines and experimental hybrids*

### **Nucleus and breeder seed production of parental lines**

Nucleus and breeder seeds are the seeds of highest genetic purity to be produced under the strict supervision of the breeder/agency sponsoring a hybrid, which is further distributed to produce foundation seed.

### **Seed production for evaluation of experimental hybrids**

This involves the seed production plots for producing a small quantity of seeds of a large number of experimental hybrids which are to be tested in various yield trials.

## *Evaluation of experimental hybrids*

The experimental rice hybrids are evaluated in comparison to check varieties to identify those which are commercially usable. This is done by stepwise evaluation of experimental hybrids in a series of yield trials such as observational, preliminary, advanced, and multilocation yield trials (Fig. 3).

### **Trials for commercial release of rice hybrids**

The experimental hybrids found promising in advanced yield trials are further tested in the farmer's field on larger plots along with the check varieties of the region, before their eventual release for commercial cultivation.

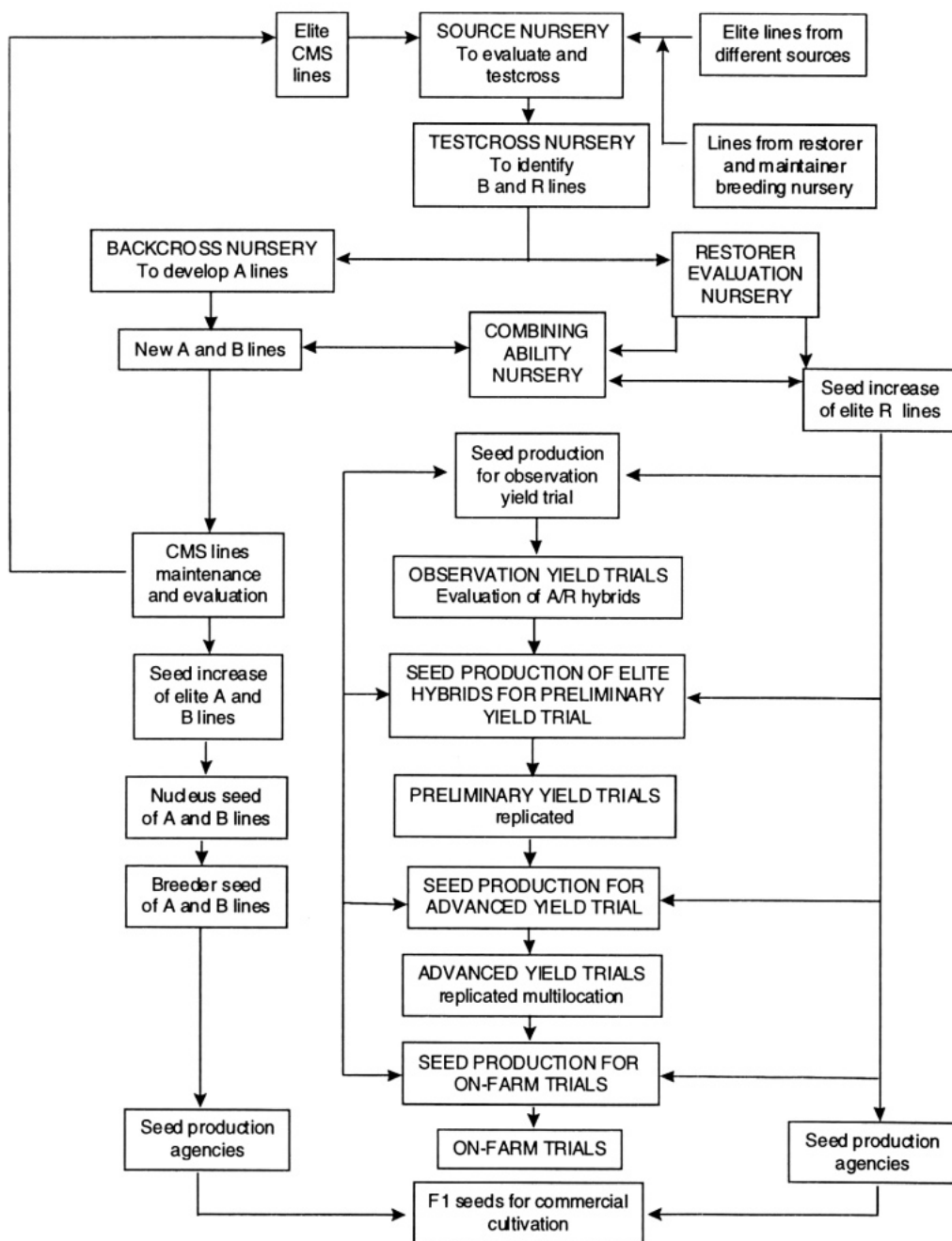


Fig. 3. Operational flowchart of hybrid rice breeding using CMS system-

## 4. Source Nursery

*Constituting a source nursery is one of the basic steps in organizing a hybrid rice breeding program. The success of hybrid rice breeding depends to a great extent on the quality and diversity of elite lines included in the source nursery. Hybrid rice breeders should keep close contact with the inbred varieties breeding program and should have access to promising materials developed within the province, country, and abroad.*

### Objectives

- To assemble at one place prospective maternal and paternal parents of diverse genetic origin, for making experimental rice hybrids
- To use the assembled genotypes for making testcrosses with the best available CMS and TGMS lines

### Composition

Following are the types of materials which are included in the source nursery:

- Stable CMS lines with different cytoplasmic sterility sources
- Released varieties of the target area for which hybrids are to be developed
- Elite breeding lines in on-farm trials, advanced yield trials, regional, and multilocation yield trials
- Locally adapted lines selected from national and international yield trials

## General considerations

- Total number of elite lines in a source nursery can vary from 50-300 depending on the availability of new materials and the capacity of a hybrid rice breeder to make testcrosses.
- Group the elite lines in the source nursery according to their major characteristics such as growth duration, grain quality, adaptability to specific ecosystem, etc.
- The site of source nursery should be close to facilities where crosses are made.

## Field layout

- Divide the source nursery field into small strips of 6 m width and of a convenient length to facilitate easy observation of lines and collection of their panicles for testcrossing purposes (Fig. 4).
- Plant only a single seedling per hill.
- In order to synchronize flowering of elite lines and CMS lines included in the source nursery, stagger seeding/planting of CMS lines 3-4 times at 10-15-day intervals. This will ensure the availability of CMS plants for crossing with elite lines of different growth durations.
- Plant each genotype in a single row with 12 plants/row at a spacing of 20 x 20 cm.
- CMS lines for crossing should be planted near the elite lines for easy monitoring of flowering.

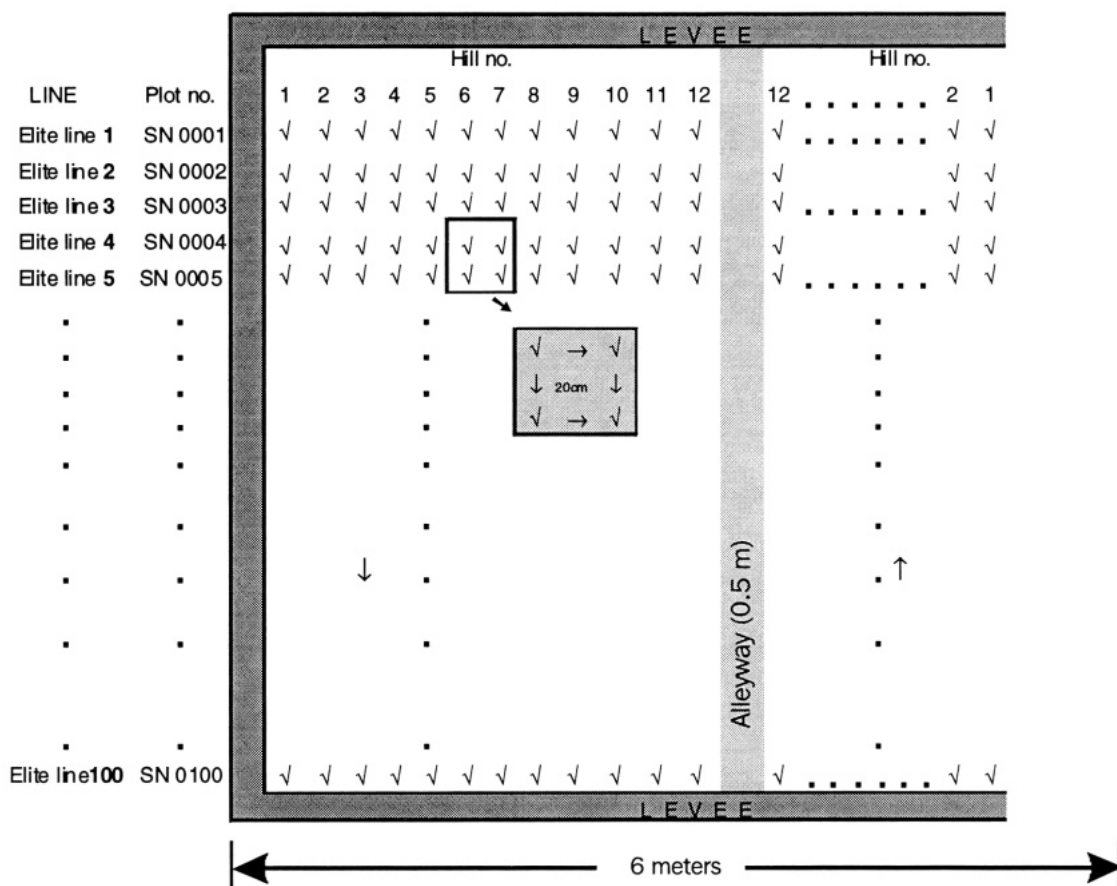


Fig. 4 Field layout for source nursery.

## Observations

The following observations should be recorded in the source nursery:

- Days to 50% flowering
- Phenotypic acceptability (1-9 scale)

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

- Grain type (as per 1-9 scale)

Scale	Shape	L/B ratio
1	Slender	> 3.0
3	Medium	2.1 to 3.0
5	Bold	1.1 to 2.0
9	Round	< 1.1

- Remarks (note down the striking features, if any).

## Utilization

- Cross a single plant of each selected line of the source nursery with the CMS lines included in the nursery, and mark the specific plant of male parents used for crossing to collect their seeds for inclusion in the test-cross nursery.
- It would suffice to include one or two of the most stable CMS lines representing each cyto sterility source for testcrossing.





## 5. CMS Maintenance and Evaluation Nursery

*Maintenance and evaluation of cytoplasmic male sterile lines and their maintainers is essential in implementing the hybrid rice breeding program based on CMS system. The desirable characteristics of a commercially usable CMS lines are: its stability for pollen sterility, adaptability to target environments, good outcrossing rate, good combining ability, and easy restorability. An active hybrid rice breeding program has to maintain an array of CMS lines in different cytoplasmic and nuclear backgrounds. These must be maintained and evaluated systematically to select those which can be used for developing commercial hybrids. The others are retained as germplasm to maintain cytoplasmic diversity in the collection for future use. In this nursery, we maintain and evaluate the available CMS lines on a continuous basis.*

### Objectives

- To collect and maintain genetically diverse CMS lines introduced from outside and bred locally
- To evaluate available CMS lines for their stability of pollen sterility, outcrossing rate, useful agronomic characteristics, and phenotypic acceptability

### Composition

- All CMS lines (along with their respective maintainers) which have been introduced from outside and/or developed locally.

- Group newly developed and designated CMS lines and those introduced along with their maintainers, based on CMS source (WA, ARC, Gam, etc.) and respective ecology (irrigated, boro, rainfed, lowland, etc.).

## **Field layout**

- CMS lines and their maintainers are planted as single plant progenies, side by side (Fig. 5).
- Three to five pairs are grown for each CMS line; three for those which are to be maintained as germplasm and five for those which are commercially usable.
- Planting the CMS nursery in small strips 6 m in width and of convenient length is preferred. This facilitates close observation and collection of pollen samples at frequent intervals for microscopic examination.
- Single rows of 12 plants each are planted with a single seedling per hill and at a spacing of 20 x 20 cm.
- The pairs are planted side by side.

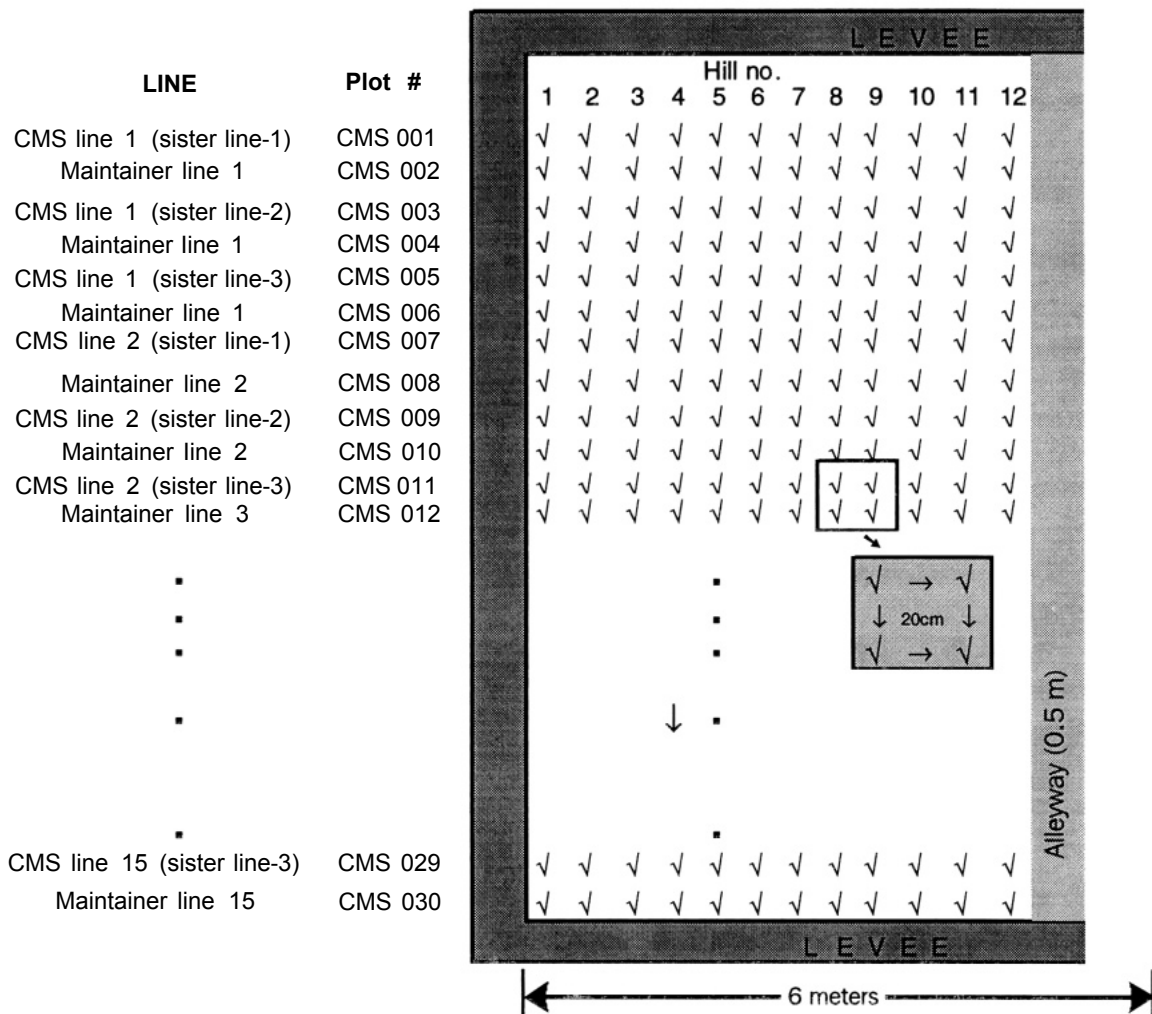


Fig. 5 Field layout for CMS maintenance and evaluation nursery.



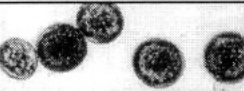
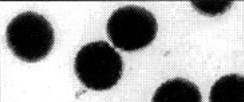
## Observations

- **Days to initial flowering (heading)** - Number of days required for protrusion of panicles from the boot leaf in 5% of the plants.
- **Days to 50% flowering** - Number of days required for 50% of panicle emergence in most of the plants of a line.
- **Pollen sterility (%)** - Ratio of sterile pollen to the total pollen in 3 microscopic fields expressed in percent.

Each plant must be tested for pollen sterility as described below:

- Collect 15-20 spikelets from the just emerged panicles of all the 12 plants in a vial containing 70% ethanol.
- Take a glass slide, put a drop of 1% iodine potassium iodide (IKI) stain (this stain is prepared by dissolving 1 g of iodine and 2 g of potassium iodide in 100 ml of water). All the anthers from at least 6 spikelets are taken out with the help of a forcep and placed in the stain. These are gently crushed by using a needle to release the pollen grains. After removing the debris, a cover slip is placed and the slide is ready for observation.
- Scan the entire slide and take the pollen sterility count in 3 random fields. The pollen grains are classified based on their shape, size, and extent of staining.

Classification of pollen based on sterility/fertility:

Category	Appearance	Classification
Unstained withered sterile (UWS)		Sterile
Unstained spherical sterile (USS)		Sterile
Stained round (light) sterile (SRS)		Sterile
Stained round fertile (SRF)		Fertile

- The CMS lines are classified as follows, based on the extent of pollen sterility:

Pollen sterility (%)	Category
100	Completely sterile (CS)
91-99	Sterile (S)
71-90	Partially sterile (PS)
31-70	Partially fertile (PF)
21-30	Fertile (F)
0-20	Fully fertile (FF)

- **Panicle exertion rate (PER)** - refers to the proportion of the panicle that is exerted from the flagleaf to the total panicle length after the full blooming, which is expressed in percentage.

$$\text{PER (\%)} = \frac{\text{Length (cm) of exerted panicle}}{\text{Total length (cm) of panicle}} \times 100$$

This character can also be scored with a 1-9 scale.

Scale	Description	% Coverage of panicle by flagleaf sheath
1	Well exerted	0
3	Moderately exerted	1-10
5	Just exerted	11-25
7	Partially exerted	26-40
9	Enclosed	Above 40

- **Stigma exertion rate (SER %)** - It is the ratio of spikelets with exerted stigma (one or both side) to the total number of spikelets expressed in percentage.

$$\text{SER (\%)} = \frac{\text{No. of spikelets with exerted stigma}}{\text{Total no. of spikelets}} \times 100$$

This character can be scored with a 1-9 scale.

Scale	SER %
1	Above 70
3	41- 70
5	21- 40
7	11- 20
9	0- 10

- **Outcrossing rate (OCR%)** - refers to the extent of seed set on open pollinated panicles which is expressed in percentage.

$$\text{OCR (\%)} = \frac{\text{No. of filled spikelets}}{\text{Total no. of spikelets}} \times 100$$

- Outcrossing rate can also be scored on a 1-9 scale.

Scale	No. of seeds set on open pollinated panicle
1	> 35
3	25-35
5	15-25
7	5-15
9	0-5



- Phenotypic acceptability - scored on a 1-9 scale.

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

## Utilization

- Data on pollen sterility are useful to identify the stable CMS lines. CMS lines showing all completely sterile plants are classified as stable. If any pair is segregating, it should be discarded and not used to maintain the CMS line. Only stable CMS lines should be selected for maintenance. Maintenance is done by crossing completely sterile plants of a stable CMS line with 3-5 single plants of its respective maintainer line. Produce 300-500 seeds for maintenance and utilization for nucleus seed production. For other CMS lines, produce 100-200 seeds for their maintenance only.
- Mark those CMS lines as promising which show stable pollen sterility, good phenotypic acceptability, an outcrossing score of 1-5 and a panicle exertion of 1-5.

## 6. Testcross Nursery

*A testcross nursery helps to identify maintainers, restorers, and partial restorers among the lines included in the previous season's source nursery. It is also helpful in the selection of apparently heterotic rice hybrids which are further evaluated in preliminary yield trial. Maintainer lines are used for conversion into new CMS lines and restorer lines are used subsequently as male parents of the experimental rice hybrids.*

### Objectives

- To identify maintainers and restorers from among the entries of the source nursery, which were used as pollen parents of testcrosses
- To select apparently heterotic rice hybrids
- To initiate backcrossing for conversion of maintainers into new CMS lines

### Composition

- Testcross  $F_1$ s made between CMS lines and elite lines included in the previous season's source nursery.
- Respective pollen parents of each of the above test crosses.
- Standard check variety of the region.

## Field layout

- It is preferable to grow TN in small strips 6 m in width and of convenient length and with walking alleys to observe the nursery frequently and clearly (Fig. 6).
- Plant 21-25-day-old seedlings in single rows of 12 plants each, with a single seedling per hill and a spacing of 20 x 20 cm.
- Plant the pollen parent beside the testcross  $F_1$  in single rows.
- Plant a standard check variety/released  $F_1$  hybrid after every 10 test-cross  $F_1$ s and their respective male parents. If there are four check varieties of different growth durations (i.e., very early, early, medium, and late), these will be repeated after a set of 40 testcrosses of  $F_1$ s and their male parents.

## Observations

- **Days to 50% flowering (DFF)**
- **Anther color (AC)** - scored as yellow and plumpy (suspected to contain fertile pollen) and white and shrivelled (sterile pollen)
- **Pollen fertility** - Collect 15-20 spikelets from 3 randomly selected test-cross  $F_1$  plants, and observe these under the microscope. Mark as F, PF, PS, or S as described earlier in chapter 5
- Evaluate pollen sterility of all the plants in those lines marked as 'S' on a single plant basis.
- **Spikelet fertility (SF)** - Observe the open pollinated panicles of test-cross  $F_1$ s for seed setting in comparison to the corresponding male parent; mark as fertile (F) if the seed set is comparable, and partial fertile (PF) if seed setting is lower. Male parents of testcross  $F_1$ s categorized as 'F' are marked as **restorers**.

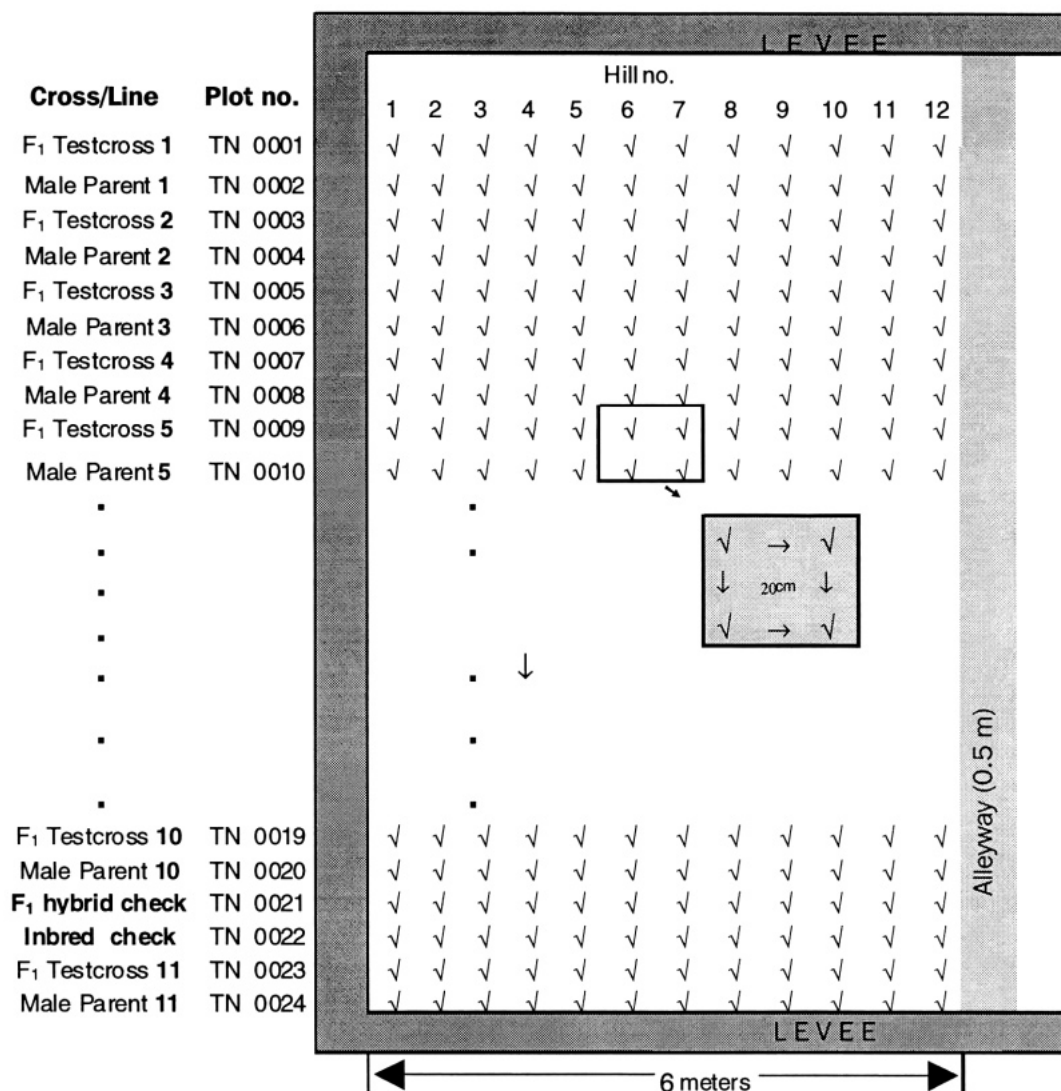


Fig. 6 Field layout for testcross nursery.

## Utilization

Results of this nursery are utilized as below:

- Male parents of testcross  $F_1$ s which show completely pollen sterile plants are labeled as maintainers. These are backcrossed with single plants of the maintainer line to initiate the backcrossing program for converting maintainers into new CMS lines.
- Male parents of the testcross  $F_1$ s which show normal spikelet fertility are designated as restorers. Seeds of these restorers are harvested and kept for producing experimental hybrids.
- Testcross  $F_1$ s which show normal spikelet fertility and appear to be more productive than the male parent and the check varieties included in the testcross nursery are marked as prospective hybrids for evaluation in observational yield trials.

## 7. Restorer Purification Nursery

*Restorers identified among released varieties and elite breeding lines have not been bred for fertility restoration. Therefore, these may show differential restoration between and within testcross progenies. With differential restoration, the  $F_1$  progenies segregate for fertility restoration which would adversely affect the yield heterosis of the hybrid. Therefore, purification of promising restorers becomes extremely important before their use in producing rice hybrids.*

*Restorer purification involves re-testcrossing of a large number of single plants of a restorer line with the CMS line, and evaluation of the re-testcross  $F_1$  progenies for uniformity or segregation for spikelet fertility. The progenies of the single plants of the restorer used in the re-testcrosses are also grown for observation. Seeds of uniform-looking restorer progenies, i.e., those that give normally fertile and uniform re-testcross  $F_1$  progenies, are bulked to obtain a nucleus seed of the restorer line.*

### Objectives

- To purify promising restorer lines
- To produce nucleus seeds of the restorer lines

### Composition

- Restorer purification nursery consists of 50-100 re-testcross  $F_1$  progenies and their corresponding pollen parent progenies from each promising restorer line.

## Procedures

- About 200 plants of the restorer line intended to be purified and the corresponding CMS line tester are planted in adjacent plots.
- The first step of purification involves making of crosses between 50-100 single plants of the selected restorer line and its CMS line tester.
- Single plants being used either as male or female parents in these crosses are carefully selected on the basis of known agronomic and morphological traits of the parental lines. In addition, plants of the CMS line being used as female parent of each testcross should have white shrivelled anthers indicative of their complete pollen sterility.
- Specific restorer plants used as pollen parents are properly tagged so that their seeds can be collected at maturity for the succeeding progeny testing.

## Field layout and evaluation

- In the succeeding season, 24 plants of each re-testcross are planted in 2 rows with 12 hills each. Likewise, 12 plants of the progenies of the corresponding pollen parents are planted in single rows (Refer to Fig.7).

## Observations

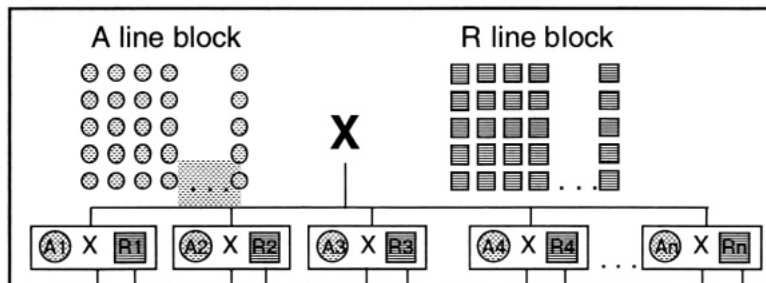
The following data are obtained from the re-testcross plots:

- Days to 50% flowering
- Qualitative assessment of spikelet fertility of each plot (80% and above - F or fertile, <80% - PF or partially fertile)
- Within-plot uniformity of agronomic traits
- Visual assessment of productivity of re-testcross plot

**Season 1**

**Step 1**

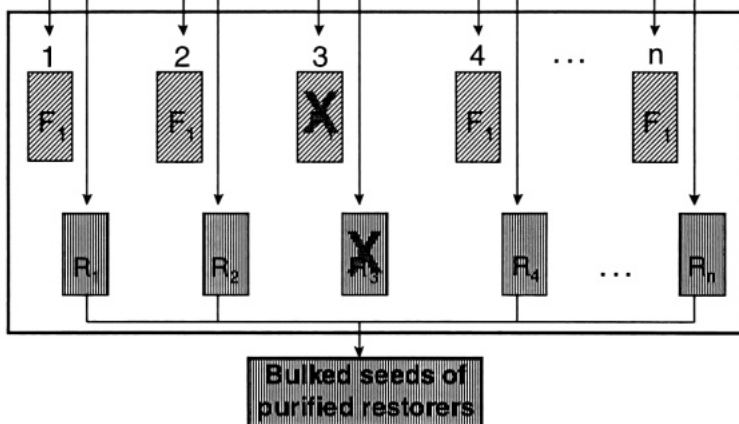
Production of testcrosses



**Season 2**

**Step 2**

Evaluation of testcrosses



**Step 3**

Evaluation and multiplication of purified restorer

**Fig. 7.1 Procedure for restorer line (R line) purification.**



- Relative appearance of plot compared with the rest
- Reaction to pests and diseases

All the above data are also collected from progenies of the restorer line.

## **General considerations**

- The selection of restorer line progenies to be included in the bulked nucleus seed is based on all observations made on the progeny rows and their retestcrosses.
- If flowering of a restorer progeny plot deviates from the rest of the progenies, it is discarded. Likewise, if a re-testcross deviates from the other testcrosses, its corresponding pollen parent progeny is also discarded.
- Progeny rows of pollen parents with corresponding re-testcrosses showing inferior spikelet fertility should also be discarded. The minimum acceptable spikelet fertility for the re-testcrosses is 80%.
- Plants within the restorer progeny and re-testcross plots should have uniformity in terms of agronomic traits. Otherwise, these should be discarded.
- Restorer progenies of retestcrosses showing better-than-average productivity should be selected.
- The disease reactions of both restorer progeny rows and re-testcrosses are also considered in selection.
- Before final selection of restorer progenies is made, all likely candidates should be examined thoroughly to ensure that there is uniformity between progeny plots before bulking.

## Utilization

- All selected restorer progeny rows are bulk-harvested to form the purified nucleus seed of the restorer line which is used for breeder seed production.



## 8. Backcross Nursery

*A backcross nursery is the backbone of an effective and efficient hybrid rice breeding program. Breeders need to have a wide range of CMS lines to develop desired hybrids. CMS lines introduced from elsewhere may not be adaptable and/or suitable for a given target area. For example, Chinese CMS lines introduced to the tropics were found to be highly susceptible to major diseases and insect pests besides being poor in grain quality. These were, therefore, unsuitable for developing commercial rice hybrids for the tropics. Thus, it was necessary to transfer the available CMS systems into elite lines well adapted to the tropics. Such a transfer is feasible only when the elite lines are effective maintainers. These are identified and backcrossing is initiated in the testcross nursery. About 5-6 backcrosses are generally required to transfer a CMS system into the genetic background of elite maintainer lines. Backcross progenies from  $BC_1$  to  $BC_6$  are handled in the backcross nursery.*

### Objectives

- To transfer a cytoplasmic male sterility system into the nuclear background of elite maintainer lines.

### Composition

- Successive backcross progenies ranging from  $BC_1$ - $BC_6$  along with their corresponding maintainer lines.

## Field layout

- Narrow strips 6 m in width and of convenient length are desirable for evaluation of backcross nursery.
- Each pair of backcross progeny can be grown in 3-5 rows of 10-12 plants each, using a single seedling/hill (Fig. 8).
- In BC<sub>1</sub>-BC<sub>3</sub> generations, the number of rows should be more than in BC<sub>4</sub>-BC<sub>6</sub> generations.
- The backcross F<sub>1</sub>s should be grown side by side with single plant progenies of corresponding maintainer lines. Such a layout facilitates the comparison of BC progenies with corresponding maintainer lines to determine how closely they resemble each other in each BC generation.
- The backcross progenies are arranged by generation (BC<sub>6</sub>, BC<sub>5</sub>, BC<sub>4</sub>, ..... BC<sub>1</sub>) for the sake of convenience for monitoring the material.

## Evaluation procedure

- Critically evaluate each plant in a BC progeny for pollen sterility.
- Backcross completely male sterile plants from only stable BC progenies (showing all completely male sterile plants) successively to three single plants of the corresponding maintainer lines.
- Evaluate stable BC progenies used for further backcrossing for their outcrossing ability using 1-9 scale.
- During the process of evaluation, you should discard those pairs which segregate for pollen sterility because these progenies can not be converted into stable CMS lines.

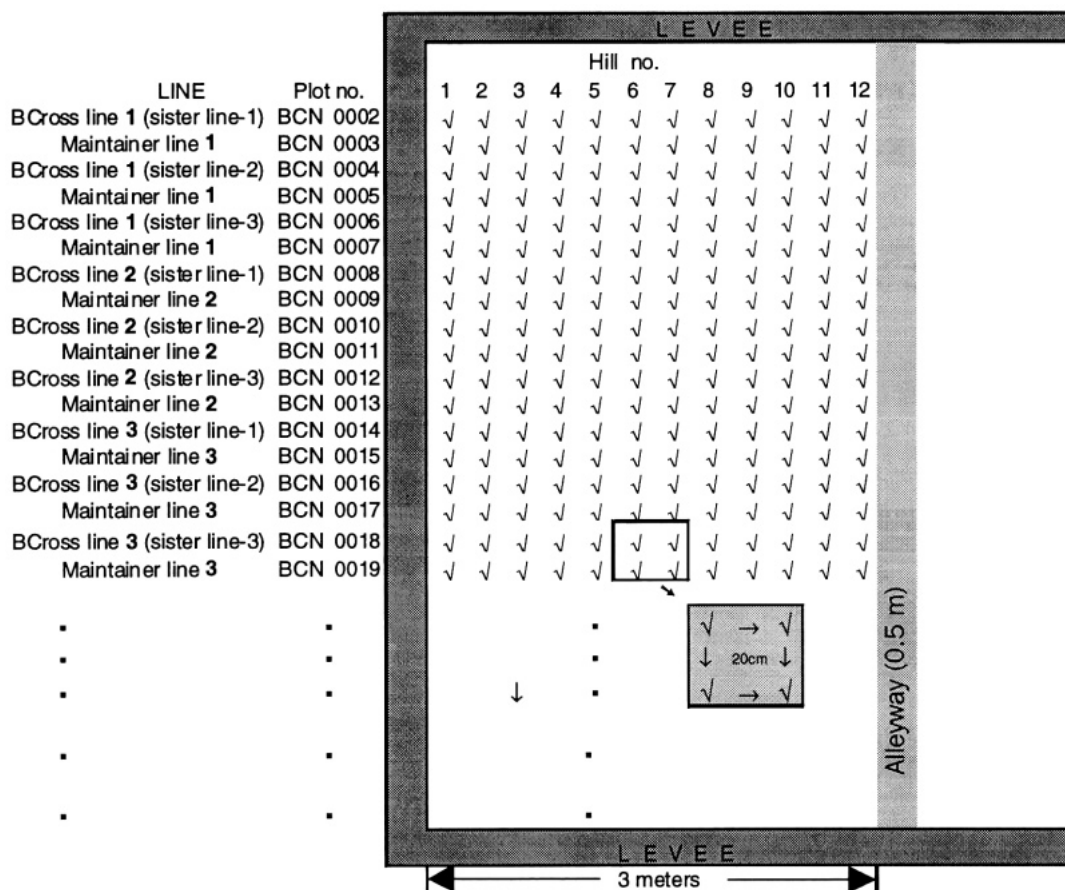


Fig. 8.1 Field layout for backcross nursery

- In the BC<sub>5</sub>-BC<sub>6</sub> generations, designate as a new CMS line only one pair that is most stable for pollen sterility and showing an outcrossing score of 1-5, and include this in the CMS line maintenance and evaluation nursery.
- In each BC generation, take care to mark the single plants used as pollinators for backcrosses.

## Observations

- Days to 50% flowering
- Pollen sterility
- Outcrossing rate (1-9 scale)
- Phenotypic acceptability (1-9 scale)
- Remarks

## Utilization

- The final products of a backcross nursery are the new CMS lines in the nuclear background of the elite maintainer lines. Such lines should be given a new designation and entered in a CMS line maintenance and evaluation nursery.

## 9. Combining Ability Nursery

*Assessing the combining ability of parental lines is extremely useful in a hybrid breeding program, especially when a large number of prospective parental lines are available and most promising ones are to be identified on the basis of their ability to give superior hybrids. Line x tester method (Kempthorne, 1957) is commonly used for the purpose.*

### Definitions

- **Combining ability** - refers to the ability of a genotype to transfer its desirable traits to its progenies.
- **General combining ability (GCA)** - is the average performance of a parent in a series of crosses.
- **Specific Combining ability (SCA)** - is the deviation in the performance of a hybrid from the performance predicted based on the general combining ability of its parents.

### Types of lines to be evaluated for combining ability

- The most stable CMS and TGMS lines possessing high phenotypic acceptability and fair to excellent outcrossing rate
- Effective restorers/pollen parents adapted to the target area



## **The line x tester procedure**

- Let us suppose we have 'l' lines (elite restorers) and 't' testers (elite CMS/TGMS lines).
- All the 'l' lines should be crossed to each of the 't' testers so as to produce 'l x t' experimental hybrids.

## **Composition of combining ability nursery**

- All the 'l x t' hybrids along with parents (lines + testers).
- Suitable check varieties may also be included for working out standard heterosis.

## **Field layout**

- Choose a fairly homogeneous plot for growing combining ability nursery in a replicated trial using randomized complete block design (RCBD).
- The number of replications should ensure a minimum of 12 degrees of freedom for error so as to have statistically valid comparisons.
- Plant one seedling per hill at a spacing of 20 x 15 or 20 x 20 cm.
- Plot size may depend on the amount of  $F_1$  seed available. However, a minimum of 50 plants per plot is essential. The larger the plot size, the better it is for evaluation.
- Avoid collecting data from border plants. Each three-row plot of hybrids can be flanked by a border row of check variety.

Layout of a combining ability trial:

R-1					R-2					R-3				
5	25	15	18	13	24	C	4	21	6	25	17	27	14	7
5	25	15	18	13	24	C	4	21	6	25	17	27	14	7
16	9	11	2	28	19	14	23	13	26	19	10	1	20	12
12	21	1	17	8	5	28	16	9	17	21	29	3	16	2
3	7	23	4	27	29	7	3	11	10	26	6	23	8	C
10	20	C	26	29	12	1	18	22	20	29	13	5	24	22
14	22	6	24	19	27	25	15	8	2	9	18	15	11	4

1-20 Hybrids; 21-25 Lines; 26-29 Testers; C Check variety

## Statistical analysis

- Assume that we have 5 lines (R lines) and 4 testers (A lines).
- The total number of crosses will be  $l \times t = 5 \times 4 = 20$ .
- Test these 20 crosses, along with 5 lines and 4 testers (29 entries), in a randomized complete block design (RCBD) with 3 replications.

## *Analysis of variance*

$$\text{Correction Factor (CF)} = \frac{(\text{Grand Total})^2}{\text{Total No. of Observations}}$$

$$\text{Total S.S. (TSS)} = \sum Y_{ij}^2 - \text{CF}$$

$$\text{Replication S.S. (RSS)} = \frac{\sum Y_{.j}^2}{t} - \text{CF}$$

$$\text{Treatment S.S. (Tr.SS)} = \frac{\sum Y_i^2}{r} - \text{CF}$$

$$\text{Error SS (Er SS)} = \text{TSS} - \text{Tr.S.S.} - \text{RSS}$$

## *Analysis of variance table*

Source	DF	SS	MSS	F
Replications	(r-1) [2]		RSS/2	
Treatments	(t-1) [28]		Tr.SS/28	
Error	(r-1)(t-1) [56]		Er.SS/56	
Total	(rt-1) [86]			

To test the significance of genotypic difference, compare the calculated F (Tr.M.S.S./Er.S.S.) with the table value of F for 28 and 56 degrees of freedom at 5% or 1% level of significance.

Treatment SS can be further partitioned into SS due to parents, SS due to crosses, and SS due to the interaction of parents vs. crosses.

$$\text{Treatment SS} = \frac{\sum C^2_{ij} + \sum P^2_{ii}}{r} - CF$$

$C_{ij}$  = Observation for  $ij$ th cross

$P_{ii}$  = Observation for  $i$ th parent

$r$  = Number of replications

$$\text{SS due to crosses} = \frac{\sum C^2_{ij}}{r} - CF \text{ (crosses with 19 DF)}$$

$$\text{SS due to parents} = \frac{\sum P^2_{ii}}{r} - CF \text{ (parents with 8 DF)}$$

$$\begin{aligned} \text{SS parents vs crosses} &= \text{Tr.SS} - \text{SS (crosses)} \\ &\quad - \text{SS (parents) (with 1 DF)} \end{aligned}$$

### *ANOVA with parents and crosses*

Source	DF	SS	MSS	F
Replications	2			
Treatments	28			
Crosses	19			
Parents	8			
Parents vs crosses	1			
Error	56			

Test all sources of variation against error variance.

### *Line x tester analysis*

Construct a two-way table.

	Testers				
Lines	1	2	3	4	Total
1	$Y_{ij}$				$Y_{i..}$
2					
3					
4					
5					
Total	$Y_{.j.}$				$Y_{..}$

$$\text{SS due to lines} = \frac{\sum Y_{i..}^2}{r \times t} - \text{CF (crosses)}$$

$r$  = replications

$t$  = testers

$$\text{SS due to testers} = \frac{\sum Y_{.j.}^2}{l \times r} - \text{CF (crosses)}$$

$$\text{SS due to lines x testers} = \text{SS (crosses)} - \text{SS (lines)} - \text{SS (testers)}$$

*ANOVA for line x tester analysis*

Source	DF	SS	MSS	F
Lines	4			
Testers	3			
Lines x Testers	12			
Error	56			

*ANOVA for line x tester analysis including parents.*

Source	DF	SS	MSS	F
Replications	2			
Treatments	28			
Parents	8			
Parents vs crosses	1			
Crosses	19			
Lines	4			
Testers	3			
Lines x testers	12			
Error	56			
Total	86			

**Note:** MS due to lines and MS due to testers are to be tested against MS due to lines x testers. MS due to lines x testers is to be tested against MS due to error.

Sometimes line x tester analysis is done by using cross means (means of crosses over replications). In that case, MS due to error which is used for testing the significance of MS (lines x tester), should be divided by the number of replications before testing.

Estimation of general combining ability (GCA) effects

i) GCA effects of lines

$$g_i = \frac{Y_{i..}}{tr} - \frac{Y_{..}}{ltr}$$

Where:  $Y_{i..}$  - Total of  $i$ th line over testers

$Y_{..}$  - Grand total

$l, t, r$  - Number of lines, testers and replications, respectively

Work out GCA effects for  $g_1$  to  $g_5$ .

Check  $\sum g_i = 0$

ii) GCA effects of testers

$$g_t = \frac{\sum Y_{.j.}}{lr} - \frac{Y_{..}}{ltr}$$

Where  $Y_{.j.}$  = Total of  $j$ th tester over lines

$Y_{..}$  = Grand total

$l, t, r$  = Number of lines, testers, and replications, respectively

Work out GCA effects for  $g_5$  to  $g_9$

Check  $\sum g_t = 0$

iii) Estimation of SCA effects

$$S_{ij} = \frac{Y_{ij.}}{r} - \frac{Y_{i..}}{rt} - \frac{Y_{.j.}}{rl} - \frac{Y_{...}}{ltr}$$

Where  $Y_{ij.}$  - Value of  $j$ th line with  $i$ th tester

$Y_{i..}$  - Total of  $i$ th line over all testers

$Y_{.j.}$  - Total of  $j$ th tester over all lines

$Y_{...}$  - Grand total

$l, t, r$  - Number of lines, testers, and replications, respectively

Work out SCA effects for all hybrids

$$\text{Check } \sum_i \sum_j S_{ij} = 0$$

Testing the significance of combining ability effects

$$\text{S.E. (gca for lines)} = \left[ \frac{Me}{rt} \right]^{1/2}$$

$$\text{S.E. (gca for testers)rl} = \left[ \frac{Me}{rl} \right]^{1/2}$$



$$\text{S.E. (sca effects)} = \left[ \frac{M}{r} \right]^{1/2}$$

$$\text{S.E. (gi - gj) line} = \left[ \frac{2Me}{rt} \right]^{1/2}$$

$$\text{S.E. (gi - gj) testers} = \left[ \frac{2Me}{rl} \right]^{1/2}$$

$$\text{S.E. (Sij - Skl)} = \left[ \frac{2Me}{r} \right]^{1/2}$$

Me is the error mean sum of squares.

## Interpretation of results

- The statistical significance of treatments indicates that the entries have genotypic differences between them. If the treatment differences are significant, we can go for further partitioning.
- Partitioning of treatments SS into SS due to crosses and parents helps to test the significance of these two components individually.
- The parents with higher positive significant GCA effects are considered as good general combiners, while those with negative GCA effects are poor general combiners.

- The hybrids with significant SCA effects in a positive direction are considered as the most promising ones.

## **Utilization of results**

- The CMS lines with a good general combining ability are chosen for developing experimental hybrids for testing in observation yield trials.
- The restorers with a good general combining ability are used for crossing with other CMS lines to produce experimental hybrids for testing in observation yield trials.
- Hybrids with higher positive significant SCA effects are chosen for evaluation in the preliminary yield trials.



## 10. Breeding Rice Hybrids With TGMS System

*A genetic male sterility system in which alteration to fertility is conditioned due to the effect of temperature is known as Thermosensitive Genetic Male Sterility (TGMS). In the tropics, consistent temperature differences are found at different altitudes and during different seasons in the same location. Thus, we can use TGMS system for hybrid rice development. Unlike the CMS system, the TGMS system does not require a maintainer line for seed multiplication of a male sterile line; only a TGMS line and a pollen parent are needed to produce a hybrid. Hence, this system of developing rice hybrids is also known as two-line system. This chapter describes procedures to develop, characterize, evaluate, and use TGMS lines in a hybrid rice breeding program.*

### Advantages of the TGMS system

- There is no need for a maintainer line for seed multiplication. Hence, the seed production procedure is simplified.
- Any fertile line can be used as a male parent. Therefore, the frequency of heterotic hybrids is higher among two-line hybrids than three-line hybrids.
- The negative effects of a sterility-inducing cytoplasm are not encountered. Hence, the extent of heterosis in two-line hybrids can be higher than those of three-line hybrids.
- The TGMS system can be incorporated into any genetic background, so that the use of this system provides more genetic and cytoplasmic diversity among male sterile lines. Thus, the two-line system reduces the risk of genetic vulnerability among the hybrids.

- Since there is no need for restorer genes in the male parents of two-line hybrids, this system is ideal for developing indica/japonica hybrids, as most japonica lines do not possess restorer genes in them.

## **Characteristic features of ideal TGMS lines**

- The proportion of male sterile plants in a population of more than 1000 plants during the critical sterility period should be 100%.
- Pollen sterility of each male sterile plant should be more than 99.5%.
- The TGMS lines should have clearly defined sterility-fertility alteration regimes.
- The male sterile phase should last for more than 30 consecutive days.
- Seed setting during the fertile phase should be more than 30%.
- The critical temperature for inducing sterility should be as low as possible.

## **Development of TGMS lines**

TGMS lines can be developed by any of the following methods:

- Screening of existing varieties
- Induced mutagenesis
- Hybridization method

- **Screening of existing varieties**

1. Conduct a survey of rice fields just before the maturity of the crop during a high temperature period (during summer when the temperature goes beyond 32°C).
2. Select plants in which earlier panicles are partly fertile and recent ones are almost sterile. These are easily identified by the combination of partly-filled hanging panicles and erect panicles with sterile spikelets in the same plant.
3. Study the pollen sterility of younger panicles and confirm that sterility is higher than 99%.
4. Multiply the suspected plants by separating the tillers and by ratooning.
5. Evaluate plants for their fertility behavior under different temperature regimes either by using growth chambers, phytotron, or under field conditions (Methods to be described later).

- **Induced mutagenesis**

1. Select a popular high-yielding variety for inducing thermosensitive genetic male sterility.
2. Select any of the physical (gamma rays, neutrons) or chemical mutagens (EMS, MMS, NEU, DES, etc.). No specificity of mutagen in inducing TGMS has been observed yet.
3. Treat the seed material with an appropriate dose of mutagen and grow it as  $M_1$  generation.
4. Grow the  $M_2$  during the period of higher temperature. Select plants showing differential fertility of panicles within the same plant or complete sterility.

5. Multiply these plants by separating their stubbles and evaluate them under fertility induction conditions. Those which revert to fertility are suspected TGMS plants.

- **Hybridization method**

1. This method is most dependable and it is deployed when we wish to transfer a TGMS trait from a donor to a locally adapted variety.
2. Select a stable and suitable TGMS donor with well-defined critical sterility/fertility points. Cross it with the variety to which the TGMS trait has to be transferred.
3. Grow  $F_1$  generation to produce  $F_2$  seeds of the cross.
4. Grow  $F_2$  population during sterility-inducing temperature regime. Select desired fertile plants in segregating population.
5. Grow  $F_3$  to  $F_5$  generations during sterility-inducing temperature and select 8-10 desirable fertile plants from the progeny rows segregating for sterility. The reason for selecting so many fertile plants in the segregating population is to ensure the probability of selecting at least one heterozygous fertile plant which would segregate for sterility in the next generations.
6. Grow  $F_5$  and  $F_6$  population during sterility-inducing temperature. Select the most desirable male sterile plants and ratoon them.
7. Transfer the ratooned male sterile plants to a phytotron or glass-house with a day/night temperature of 27/21°C to induce fertility.

8. Select those plants which revert to fertility at low temperature conditions and collect their seeds. These are suspected TGMS plants.
9. Grow progenies of the suspected TGMS plants during sterility-inducing temperature conditions in the field and select those plants as TGMS which give completely male sterile progeny.

The TGMS lines identified by any of the above methods can be characterized for critical sterility (CSP) and fertility points (CFP) under

- field conditions
- controlled conditions

- **Characterizing TGMS lines under field conditions**

1. Have detailed meteorological data on minimum and maximum temperature, daylength, humidity, etc. of the location where the lines are to be characterized. It is better if the data of 10-15 years are available.
2. Identify 3-4 distinct periods of high and low temperatures during the year.
3. Arrange seeding/planting in such a way that the period 15-25 days before heading (5-15 after PI) coincides with the high temperature. Select such plants which remain sterile at high temperature.
4. Note the temperature data pertaining to 15-25 days before heading, this is the critical sterility point of a given line.
5. Multiply the plants (selected in #3) by ratooning and subject them to lower temperature regimes at the same growth stage. Identify those which exhibit partial fertility or become partially fertile.



6. Note the temperatures which prevailed during the period 15-25 days prior to heading. This is the critical fertility point of a TGMS line.

- **Characterization of TGMS lines under controlled conditions**

1. For determining more accurate CSP and CFP of TGMS lines, these can be grown in a phytotron, a walk in a growth chamber or a glasshouse. Temperature, humidity, light hours and intensity are controlled in a phytotron while in a glasshouse, the plants are grown under natural sunlight with controlled temperature and humidity.
2. Results obtained at IRRI suggest that maximum temperature determines the sterility-fertility behavior of indica TGMS lines.
3. The progeny of suspected TGMS plants is kept in separate growth chambers, with varying day and night temperature regimes.
4. The range of temperatures should include both lower and higher temperature so that both CFP and CSP are determined. For example, the temperature regimes can be as follows:

Day temp (°C)	24	25	26	27	28	30	32
Night temp (°C)	18	19	20	21	22	22	24

5. The critical stage for thermosensitivity is during 5-15 days after PI. The suspected TGMS plants grown in pots are observed for panicle initiation. When 2-3 panicles attain the PI stage, the plants are transferred to the growth chamber. Other panicles which reach the critical stage after PI in the growth chamber are tagged. Treated plants are removed from the chamber after a period of 15-20 days.
6. When the treated panicles flower, they are examined for pollen sterility. The temperature at which the panicles become completely male sterile is its critical sterility point. On the other hand, the temperature at which the plants revert to highest fertility is considered as its critical fertility point.
7. CSP and CFP vary from genotype to genotype. It is, therefore, essential to know the CSP and CFP of every TGMS line before using it for seed production.
8. Sterile plants which remain completely sterile in different temperature regimes are considered as non-TGMS types and are discarded.

## Evaluation of TGMS lines

- The TGMS lines are evaluated for their outcrossing rate, phenotypic acceptability, and combining ability as per methods described for evaluating the CMS lines. The operational flow chart describing the procedure for using the TGMS system is presented in Fig. 10.

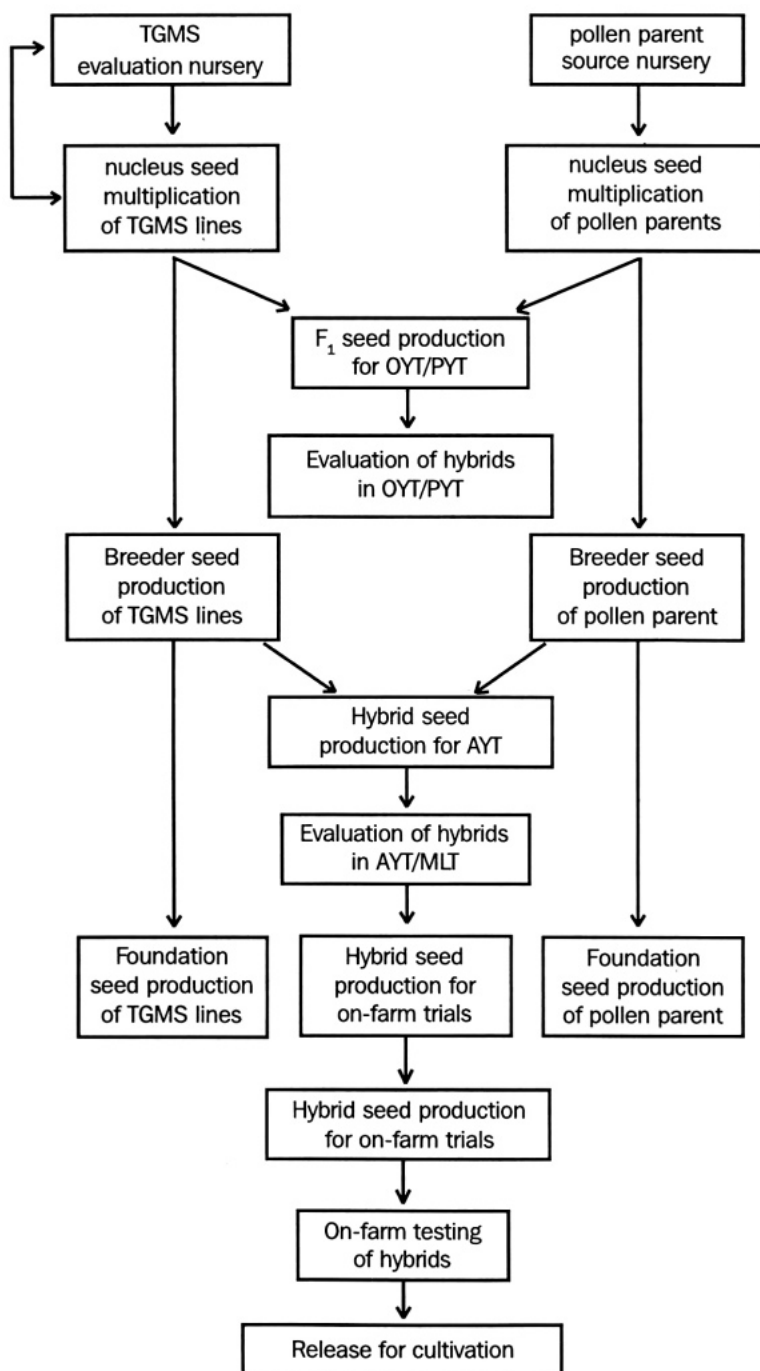


Fig. 10 Flow chart of hybrid rice breeding using TGMS system.

## 11. Nucleus and Breeder Seed Production of A, B, R, and TGMS Lines

*The availability of a genetically pure and good quality seed is a primary prerequisite for exploiting the full potential of hybrids. Lack of purity in parental lines and improper isolation conditions in seed production are the major causes of poor hybrid seed quality. Chinese scientists have reported that with every 1% decrease in purity of the hybrid seed, the eventual yield loss in the  $F_1$  hybrids would be about 100 kg/ha. The parental lines get contaminated or deteriorate during the process of handling by foundation seed growers. Therefore, it is most necessary to produce pure nucleus and breeder seed of parental lines under the strict supervision of plant breeders.*

### Definitions

- **Nucleus seed** - a class of a seed of parental lines produced in small quantities under the direct supervision of a qualified plant breeder.
- **Breeder seed** - a class of seed produced from a nucleus seed under the supervision of a concerned breeder or an institution.

### Choice of lines

- Parental lines (A, B, R, and TGMS) of most promising and/or released hybrids

## Procedure

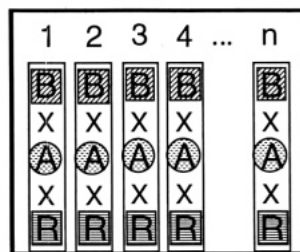
### *For A, B, and R lines*

Nucleus and breeder seed of A, B, and R lines can be produced simultaneously by following the method described below:

- Select about 50-100 typical and completely male sterile single plants of the selected CMS line (Fig. 11.1).
- Make 50-100 crosses of the selected CMS plants with corresponding single plants of the maintainer and restorer lines of the promising hybrids.
- Sow and plant few seeds of A x B crosses and all the seeds of A x R crosses in an identification nursery to select the best pairs that produce typical, uniform, and stable progeny.
- Plant balance seeds of A x B crosses and their corresponding B line progeny in an isolated multiplication block 21 days after the planting of A x B crosses in an identification nursery.
- Based on the observations made in A x B crosses grown in the first planting, mark those which lack uniformity in growth and flowering and show lack of stable male sterility. Such A x B crosses and their corresponding B lines are removed from the A x B multiplication block before flowering. The remaining A x B pairs are allowed to get cross-pollinated to produce the nucleus seed of the A line.
- Among the pairs of A x R crosses, identify those which exhibit poor restoration and lack of uniformity.
- Plant all the maintainer and restorer progenies of respective A x B crosses and A x R crosses in isolated plots for multiplication. Such lines whose F<sub>1</sub> progenies failed to meet the set standards based on the observations made in the identification nursery are discarded and the remaining lines are bulked to form the nucleus seed of 'B' and 'R' lines.

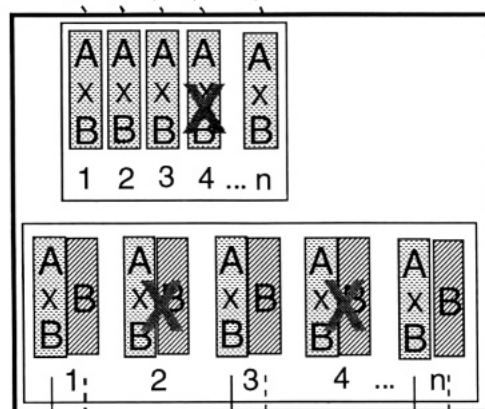
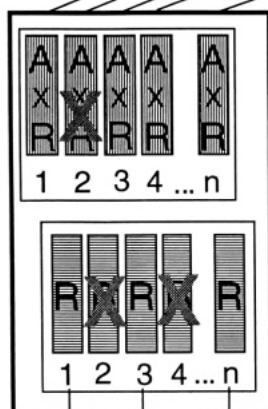
## Season 1

Testcross  
nursery

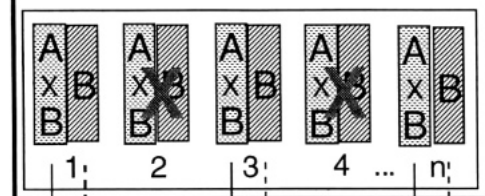
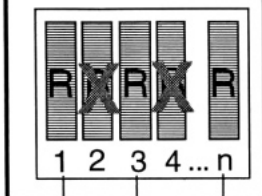


## Season 2

Identification  
nursery



Multiplication  
nursery



Purified R line

Purified A line

Purified B line

Fig. 11.1 Procedure for nucleus seed production of A and B lines.

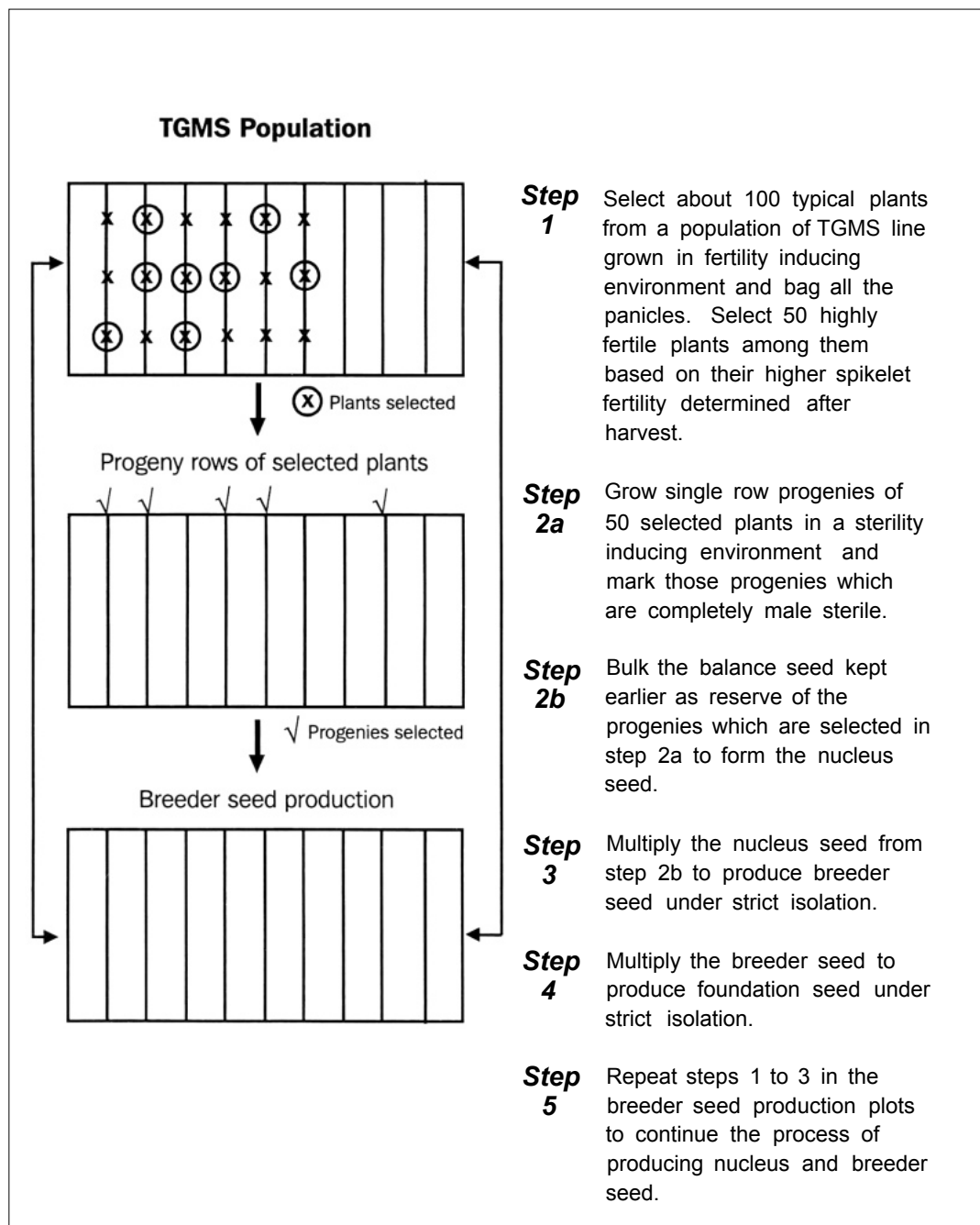
- The nucleus seed of 'A' and 'B' lines are used for producing breeder seed of the A line. Plant the A and B lines in strictly isolated plots (preferably 100 m away from other rice varieties) to produce the breeder seed of A line.
- Plant the nucleus seed of B and R lines in isolated plots as per certification standards for producing the breeder seed of respective B and R lines.

## Procedure for TGMS Lines

TGMS lines multiplied continuously for several generations without any selection may segregate for critical sterility point, causing major problems in maintaining purity. Therefore, nucleus and breeder seed production has to be taken up on a continual basis.

Nucleus seed production of a TGMS line is initiated in the fertility-inducing environment. Seeding of TGMS lines is arranged in such a way that the sensitive stage occurs when the temperature is favorable for higher seed set. At the time of flowering, about 100 typical plants are selected from the population of a TGMS line and their panicles are bagged. Selection process should be completed within a week. After the harvest, the selected plants are scored for spikelet fertility (based on the main panicle) and 50 plants with higher spikelet fertility are selected.

Progenies of the selected plants are grown in the sterility inducing environment. About 30 seeds are taken from each of the selected plants to grow single row progenies and the remaining seed is stored carefully. Progenies which are uniform and completely male sterile are marked. The balance seeds of such selected progenies (stored earlier) are bulked to form the nucleus seed (Fig. 11.2).



**Fig. 11.2 Procedure for nucleus and breeder seed production of TGMS lines.**



Nucleus seed is used to produce breeder seed under strict isolation. Breeder seed is produced in the fertility-inducing environment. The whole process of producing nucleus seed is again repeated on the breeder seed plot. Fresh breeder seed should be used by seed production agencies to produce foundation seed of TGMS lines. The latter should be used to produce hybrid seeds.

## **Utilization**

The nucleus seed produced under the direct supervision of the plant breeder has high genetic purity and is used for producing breeder seed on a large scale. The breeder seed will be distributed for producing foundation seed of parental lines, which in turn will be used for producing the hybrid seed.



- Twenty five-day-old seedlings of A and R lines are planted in alternate rows of five plants each at a spacing of 20 x 20 cm (Fig. 12.1).
- Frames of 1 x 1 x 1 m are prepared either with iron or aluminum angles.
- Cubicles of 1 x 1 x 1 m are stitched with muslin cloth with a flap at the top.
- The metal frame is placed around a 1 m<sup>2</sup> area where A and R plants are planted just before flowering.
- The frame is covered with a muslin cloth bag to prevent cross pollination.
- During the flowering period, the pollen plants are shaken to increase seed setting on A line. This can be facilitated by opening the flap.
- Restorer plants are harvested first and threshed separately. 'A' line plants are harvested and threshed later, to avoid possible mixing.

- ***Modified chimney isolation procedure***

The chimney isolation method of seed production has been modified to overcome the problem of synchronization and to simplify the supplementary pollination. The basic layout is the same as that of the chimney method except the following differences:

- All the 'R' lines are sown on the same day, while the 'A' lines are staggered 5-6 times with an interval of 6-7 days.
- Twenty five-day-old seedlings of 'R' lines are planted in a 1 m<sup>2</sup> area at a spacing of 15 x 15 cm in alternate rows, leaving a space for an 'A' line in between (Fig. 12.2).

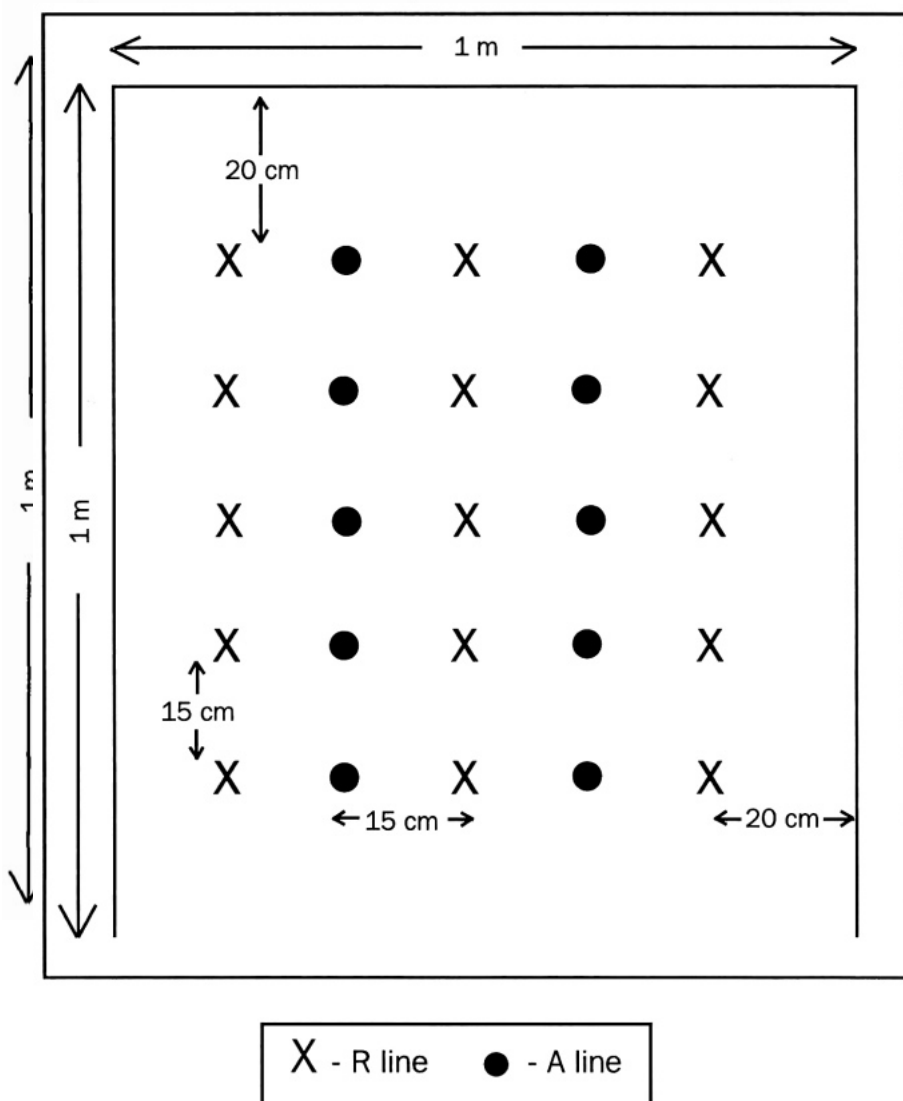
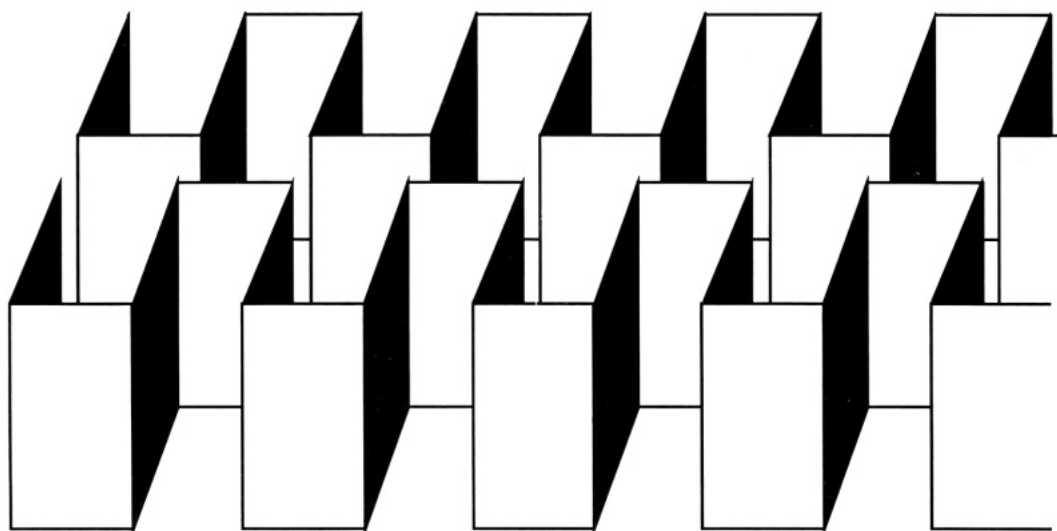


Fig. 12.1 Position of A (●) and R (X) line plants in a chimney.



**Fig. 12.2** Layout of a modified chimney method.

- At the boot leaf stage of 'R' lines, 2 m high barriers are erected to cover the three sides of a 1 m<sup>2</sup> plot, leaving a gap of 20 cm from the ground. The open side is covered by the barrier of the opposite plot. The space between the opposite plots is convenient for cultural operations, including supplementary pollination.
- Just before the panicle emergence of R lines, the plants of CMS lines which are in similar stage as that of the R line are removed in the morning hours and planted in the vacant spaces between the 'R' lines.
- Supplementary pollination is done by using sticks 3-4 times/day at peak anthesis during the flowering period of 7-10 days.
- About 30-35 g of hybrid seed can be obtained from each plot measuring 1 m<sup>2</sup>.

- ***Isolation free method***

An isolation free method developed at the International Rice Research Institute has been found to be more practical and popular in tropical countries. This method is most ideal for producing small quantities of hybrid seed required for OYT and PYT.

- Selected 'R' lines are grown side by side in 5 x 3 m plots. In each R line plot, four rows of 'R' line plants are planted as border rows at 20 x 20 cm spacing to provide isolation from adjoining plots. Four vacant spaces 40 cm in width are left in the middle of the plot which are interspersed by single rows of R line plants. About 68 CMS plants can be planted in these vacant spaces at the time of flowering (See layout in Fig. 12.3).
- Male sterile lines of experimental hybrids are staggered five times at 8-10-day intervals to ensure a continuous supply of CMS plants in the flowering stage to synchronize the flowering of R lines in different seed production plots.

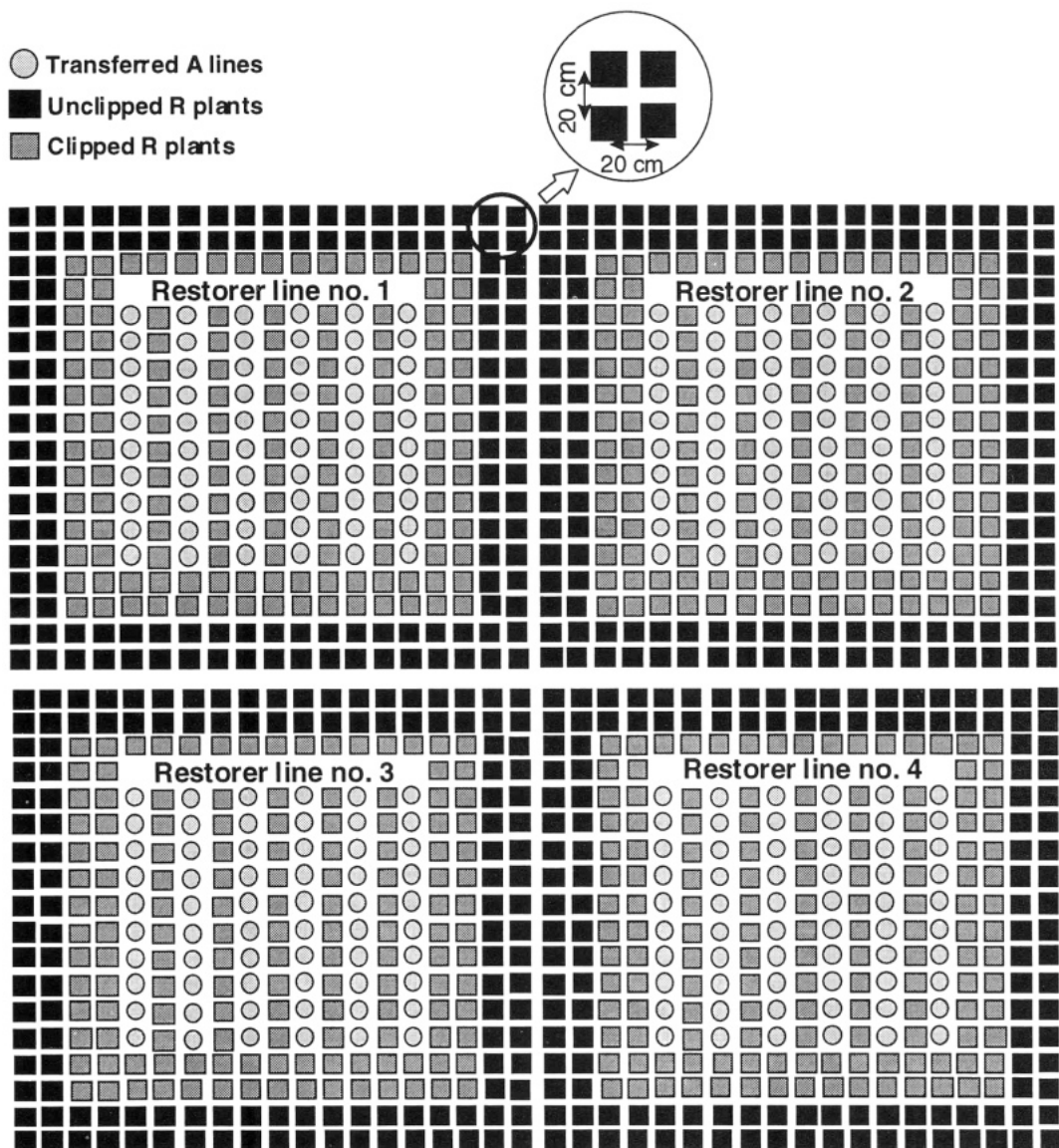


Fig. 12.3 Layout for isolation free system for producing seeds of experimental hybrids.

- When primary tillers of A and R lines are in the boot leaf stage, their flag leaves are clipped except for the two outermost border rows of R lines which act as a barrier for pollen from adjoining plots.
- Three to five days after leaf clipping, the A lines are uprooted (preferably in the morning, i.e., 6-8 a.m.), and are planted in the vacant spaces of plots.
- To enhance outcrossing, supplementary pollination is advocated at the peak anthesis period. Care should be taken to shake only those R lines which are flanking the A lines.
- R line plants are harvested first and threshed separately followed by A line plants bearing the hybrid seeds.
- By adopting this method, 3-5 g of hybrid seed can be obtained from each CMS plant. A plot with 15-40 CMS plants can yield 50-200 g hybrid seed which will be enough to conduct OYT for two seasons (20 g per season) and replicated preliminary yield trials also for two seasons (100 g per season).

- ***Seed production for advanced yield trials and multilocation trials***

#### **Strict isolation method**

The hybrid seed required for conducting advanced yield trials should be highly pure. About 1-2 kg of seed is required for this purpose. Therefore, the seed has to be produced in a larger area (100-200 m<sup>2</sup> plots) under strict isolation to ensure purity. The method is described below.



## **Isolation**

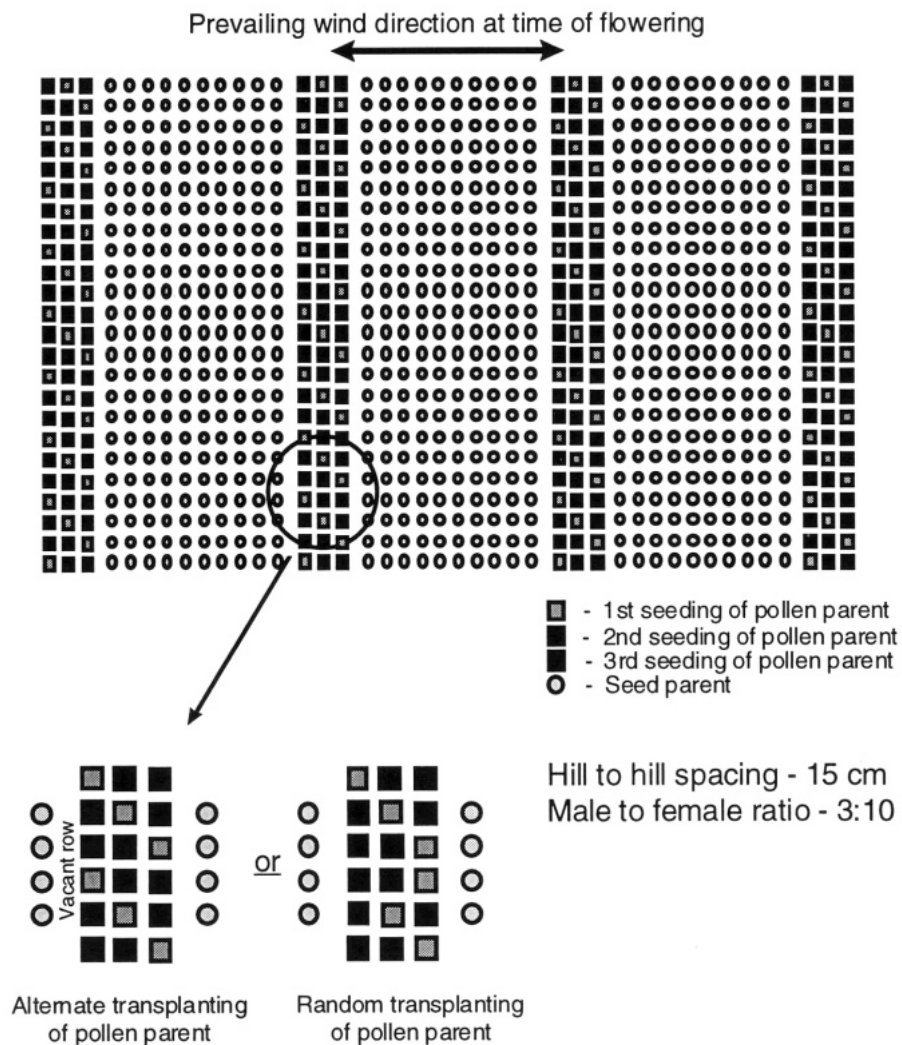
A space isolation of 50 m is ideal for hybrid seed production, which means that within this range no other rice varieties should be flowering except the pollen parent. If it is difficult to get space isolation, a time isolation of over 21 days would serve the purpose. Distance isolation can be reduced to 30-40 m if the hybrid seed production plot is surrounded by an additional 15-20 rows of pollen parents.

## **Seeding sequence**

Parental lines of hybrid combinations differ in their growth duration. Therefore, they have to be seeded on different dates so that their flowering would be synchronous. A late parent is sown first and the early parent is sown later, the difference being equal to the difference in their growth duration. The CMS line is seeded only once while the pollen parent is seeded three times, with three-day intervals, such that the difference between the second sowing of the pollen parent and that of the CMS line is equal to the seeding interval between the parental lines.

## **Row ratio and layout**

The optimum row ratio for hybrid seed production is 2-3 male: 8-10 female. Restorer seedlings from all the three seedings are evenly mixed and planted in three rows, at a spacing of 15 x 15 cm, leaving space for an A line in between. 'A' line seedlings are planted with a spacing of 30 x 15 cm. The spacing between the A line and adjacent R line should be 20 cm. Row direction should be perpendicular to the wind direction (Fig. 12.4).



**Fig. 12.4** Layout of breeder seed and hybrid seed production plots.

## **Roguing**

Roguing is an important operation in a hybrid seed production plot to ensure purity of hybrid seeds. The off-types observed during different growth stages up to flowering are to be removed. Roguing at flowering is extremely important as pollen from off-type plants can cause irreparable damage through cross-pollination with male sterile plants.

## **Flagleaf clipping**

At booting stage, the upper leaves of the CMS plants are held firmly, and they are cut with the help of a sharp sickle in such a way that 1/2 or 2/3 of the flagleaf is removed from just above the flagleaf joint with the tiller. Flagleaf clipping helps in easy dispersal of pollen and higher cross-pollination of panicles of a male sterile parent in a hybrid rice production plot.

## **GA<sub>3</sub> spray**

In most of the 'WA' based CMS lines, part of the panicle is enclosed in a flagleaf. Therefore, spraying of GA<sub>3</sub> is recommended to obtain good panicle exertion. A dose of 45-60 g/ha by a knapsack sprayer or 15-20 g/ha by a ULV sprayer is recommended for desired results. The spray liquid required is 500 l and 20 l for the knapsack and ULV sprayer, respectively. GA<sub>3</sub> should be sprayed two times, the first when 15-20% of the tillers have started heading, and the second should be done 2 days after the first spraying or when 35-40% of the panicles of the seed parent have emerged.

## **Supplementary pollination**

At the time of flowering, supplementary pollination is done by shaking the pollen parents either with a rope or bamboo sticks. This operation has to be done 3-4 times daily at peak anthesis for a period of 6-10 days.

## **Harvesting and threshing**

Extreme care should be taken while harvesting and threshing the hybrid rice plots. Harvest and thresh the pollen parent first. Thoroughly check and remove any panicles of the R line falling on the female rows. Harvest and thresh the seed parent separately. The seed should be dried, processed, bagged and properly labeled.





## *Experimental design*

- Since the number of experimental rice hybrids is large (100-200) and the amount of hybrid seed is limited, it is convenient to conduct OYT by using the augmented design.
  - In this design, the whole experimental area is divided into a number of blocks.
  - The check varieties are replicated in each block while the test entries are not replicated but assigned to the remaining plots randomly.
  - The yields of test entries are adjusted for block differences, based on the yield of check varieties in each block.
  - The block size is determined as follows:

If       $c$  = number of check varieties  
          $v$  = number of test hybrids  
          $b$  = number of blocks

Number of test entries in a block ( $n$ ) =  $v/b$

Number of plots/block ( $P$ ) =  $c + n$

Total number of plots ( $N$ ) =  $b(c + n)$

- The total number of blocks should ensure at least 12 df for error in ANOVA.

$$b > [12/c - 1] + 1$$

-- Let us take 40 hybrids and 4 check varieties.

$$\begin{aligned}\text{The number of blocks} &= [12/4-1] + 1 \\ &= 12/3 + 1 \\ &= 5\end{aligned}$$

$$\text{Number of check varieties} = 4$$

$$\text{Number of test hybrids} = 40$$

$$\text{Number of blocks} = 5$$

$$\text{Number of hybrids/block} = 40/5 = 8$$

$$\text{Number of plots/block} = 8 + 4 = 12$$

$$\text{Total number of plots} = 60$$

### ***Layout for augmented design***

- The plot size should be at least 5 m<sup>2</sup> for each entry.
- Plant a single seedling/hill with a spacing of 20 x 15 cm.
- First assign the check varieties randomly in each block.
- Assign the test hybrids randomly to the remaining plots.
- The field should be properly leveled.
- Fill the gaps 7-10 days after transplanting to obtain uniform plant population.
- Care should be taken for uniform distribution of fertilizers and plant protection chemicals.
- Uniform water control is a must for valid comparisons.



A worked example of OYT is described below.

Numbers 1-40 indicate hybrids and A, B, C and D are check varieties. Figures in parenthesis refer to yield in tons per hectare.

BLOCKS				
1	2	3	4	5
17 (4.6)	23 (7.1)	B (4.0)	12 (7.9)	2 (5.0)
C (3.8)	A (4.0)	28 (5.0)	37 (5.3)	25 (3.2)
9 (5.6)	3 (5.6)	14 (3.6)	A (3.9)	B (3.2)
13 (5.3)	36 (7.0)	D (4.3)	8 (3.4)	27 (5.4)
D (5.7)	B (5.1)	24 (7.5)	33 (5.2)	16 (6.0)
29 (5.2)	7 (6.3)	30 (6.2)	C (4.2)	35 (2.6)
6 (4.9)	38 (4.6)	A (4.5)	5 (2.9)	D (4.6)
A (4.5)	15 (3.9)	C (3.9)	40 (6.8)	22 (3.9)
31 (3.2)	C (4.1)	39 (4.3)	26 (7.8)	A (3.8)
18 (2.6)	20 (5.3)	1 (3.6)	D (3.9)	4 (4.7)
B (4.6)	11 (5.0)	32 (5.9)	34 (3.8)	C (3.3)
21 (6.1)	D (4.9)	19 (5.4)	B (4.9)	10 (5.2)

Layout and yield figures for OYT (augmented design).

- **Data recording**

Collect data on the following parameters:

- Vegetative vigor (1-9 scale)
- Days to 50% flowering
- Visual score for seed setting (1-9 scale)
- Yield/plot for conversion to yield/ha.
- Phenotypic acceptability score (1-9 scale).

- **Statistical analysis**

Construct a two-way table of check yields and means.

Check variety	BLOCKS						
	1	2	3	4	5	Total	Mean
A	4.5	4.0	4.5	3.9	3.8	20.7	4.14
B	4.6	5.1	4.0	4.9	3.2	21.8	4.36
C	3.8	4.1	3.9	4.2	3.3	19.3	3.86
D	5.7	4.9	4.3	3.9	4.6	23.4	4.68
Total	18.6	18.1	16.7	16.9	14.9	85.2	
Mean	4.65	4.52	4.17	4.22	3.72		4.26

- Compute the block effect.

$$r_j = B_j - M$$

where  $r_j$  = Block effect of  $j$ th block

$B_j$  = Mean of all checks in  $j$ th block

$M$  = Grand mean of the checks

Block effects of different blocks are:

Block	$r_j$
1	0.39
2	0.26
3	-0.09
4	-0.04
5	-0.52

Check  $\sum r_j = 0$

Construct a table of unadjusted and adjusted yields. Adjusted yields for each test entry is obtained by deducting the block effect to which it was allotted from the observed yield.

For example, the adjusted yield of hybrid no. 1 is calculated as follows:

Unadjusted yield of hybrid 1: 3.6  
 Block number to which  
 hybrid 1 is allotted: 3  
 Block effect of block number 3: - 0.09

Adjusted yield of hybrid 1 = 3.6 - (-0.09)  
 = 3.69

Adjusted (AD) and unadjusted (UA) yields of test hybrids in OYT.

Hybrid	Block	Yield		Hybrid	Block	Yield	
		UA	AD			UA	AD
1	3	3.6	3.69	21	1	6.1	5.71
2	5	5.0	5.52	22	5	3.9	4.42
3	2	5.6	4.74	23	2	7.1	6.84
4	5	4.7	5.22	24	3	7.5	7.59
5	4	2.9	2.94	25	5	3.2	3.72
6	1	4.9	4.51	26	4	7.8	7.84
7	2	6.3	6.04	27	5	5.4	5.92
8	4	3.4	3.44	28	3	5.0	5.09
9	1	5.6	5.21	29	1	5.2	4.81
10	5	5.20	5.72	30	3	6.2	6.29
11	2	5.0	4.74	31	1	3.2	2.81
12	4	7.9	7.94	32	3	5.9	5.99
13	1	5.3	4.91	33	4	5.2	5.24
14	3	3.6	3.69	34	4	3.8	3.84
15	2	3.9	3.64	35	5	2.6	3.12
16	5	6.0	6.52	36	2	7.0	6.74
17	1	4.6	4.21	37	4	5.3	5.34
18	1	2.6	2.21	38	2	4.6	4.34
19	3	5.4	5.49	39	3	4.3	4.39
20	2	5.3	5.04	40	4	6.8	6.84

For working out the standard errors for comparing the means, an ANOVA Table is prepared by using the replicated data of check varieties.

### ANOVA for check varieties

Source	df	SS	MSS	F
Block	4	2.068	0.517	
Checks	3	1.804	0.601	2.32 ns
Error	12	3.096	0.258	
Total	19	6.968		

- The standard errors are worked out as follows for different comparisons:

- Difference between two check means

$$\sqrt{2 \text{ MSE}/b} = \sqrt{2 \times 0.258/5} = 0.32$$

Differences between adjusted yields of two hybrids in the same block.

$$\sqrt{2 \text{ MSE}} = \sqrt{2 \times 0.258} = 0.72$$

Difference between adjusted yields of two hybrids in different blocks.

$$\sqrt{2 \text{ MSE} (1 + 1/c)} = 0.80$$

- Difference between an adjusted yield of a hybrid and a check mean.

$$\sqrt{\text{MSE} (b + 1) (C + 1)/bc} = 0.62$$

## *Utilization of results*

- The test entries are classified based on different maturity groups and their performance is compared with the check variety of corresponding duration by using the standard errors calculated for the purpose.
- The hybrids which give a significantly higher yield than check varieties are identified and promoted for the preliminary yield trial.

## **Preliminary yield trials**

### *Composition*

- Promising hybrids identified in observation yield trials
- Hybrids showing apparent heterosis in testcross nursery
- Check varieties of different growth durations (very early, early, medium, and late)

### *Experimental design and field layout*

- Randomized Complete Block (RCB) Design is most suitable for conducting the preliminary yield trials. The steps involved are as follows:
  - Number of blocks or replications should be such that the error degrees of freedom should be at least 12.
  - The ideal plot size would be about 10 m<sup>2</sup>.
  - If the fertility gradient is unidirectional, the blocks should be perpendicular to the fertility gradient.

- Hybrids should be grouped according to their growth duration. Each group or sub-group should have 15-20 hybrids and suitable checks.

### *An example*

- Let us assume there are 16 hybrids and 4 check varieties to be tested in 4 replications.
- Divide the field into four equal blocks (see the layout on the next page).
- Sub-divide each block into 20 experimental plots.
- Assign the treatments to each plot randomly. Each treatment should appear in every block.

Rep 1	Rep 2	Rep 3	Rep. 4
3 (5.3)	4 (8.3)	9 (4.7)	C (6.0)
D (4.3)	11 (5.6)	5 (7.3)	2 (7.1)
8 (6.5)	A (4.2)	7 (5.0)	13 (6.0)
10 (7.5)	2 (5.8)	C (6.9)	6 (5.9)
2 (6.0)	D (3.9)	10 (6.8)	B (6.0)
A (3.8)	6 (5.6)	1 (4.7)	11 (6.1)
7 (5.6)	3 (6.2)	15 (6.9)	4 (7.6)
4 (7.9)	13 (5.9)	D (5.2)	3 (6.0)
12 (6.0)	B (6.5)	8 (5.9)	12 (5.6)
6 (7.1)	9 (5.9)	14 (8.2)	7 (4.9)
14 (8.2)	15 (8.2)	A (4.6)	15 (7.8)
1 (3.8)	16 (4.9)	4 (8.0)	D (4.6)
B (5.9)	5 (7.6)	11 (5.9)	9 (5.0)
15 (7.9)	14 (7.6)	3 (5.8)	8 (6.7)
16 (5.8)	7 (4.6)	6 (5.3)	16 (6.1)
C (6.5)	10 (7.6)	2 (7.3)	A (5.0)
13 (7.2)	12 (5.9)	16 (5.9)	1 (5.1)
5 (6.9)	1 (4.6)	13 (6.3)	14 (7.2)
11 (4.9)	C (7.8)	12 (4.9)	10 (7.3)
9 (5.3)	8 (7.2)	B (5.2)	5 (8.1)

Layout and yield figures of PYT (RCB Design)

(1-16 are hybrids; A, B, C, and D are check varieties; values in parenthesis refer to yield in t/ha)



- **Data recording**

Observations are recorded on the following parameters:

- Days to 50% flowering
- Plant height
- Spikelet fertility (%)
- Grain yield (kg/ha)
- 1000 grain weight

- **Statistical Analysis**

- Group the data by treatments (entries) and replications and calculate the treatment total (T), replication total (R) and grand total (GT).

**Table 1. Yield (t/ha) arranged according to treatments and replications from the  
PYT using RCBD.**

<b>Treatments</b>	<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R4</b>	<b>Total</b>	<b>Mean</b>
1	3.8	4.6	4.7	5.1	18.2	4.55
2	6.0	5.8	7.3	7.1	26.2	6.55
3	5.3	6.2	5.8	6.0	23.3	5.82
4	7.9	8.3	8.0	7.6	31.8	7.95
5	6.9	7.6	7.3	8.1	29.9	7.47
6	7.1	5.6	5.3	5.9	23.9	5.97
7	5.6	4.6	5.0	4.9	20.1	5.02
8	6.5	7.2	5.9	6.7	26.3	6.57
9	5.3	5.9	4.7	5.0	20.9	5.22
10	7.5	7.6	6.8	7.3	29.2	7.30
11	4.9	5.6	5.9	6.1	22.5	5.62
12	6.0	5.9	4.9	5.6	22.4	5.60
13	7.2	5.9	6.3	6.0	25.4	6.35
14	8.2	7.6	8.2	7.2	31.2	7.80
15	7.9	8.2	6.9	7.8	30.8	7.70
16	5.8	4.9	5.9	6.1	22.7	5.67
A	3.8	4.2	4.6	5.0	17.6	4.40
B	5.9	6.5	5.2	6.0	23.6	5.90
C	6.5	7.8	6.9	6.0	27.2	6.80
D	4.3	3.9	5.2	4.6	18.0	4.50
<b>Total</b>	<b>122.4</b>	<b>123.9</b>	<b>120.8</b>	<b>124.1</b>	<b>491.2</b>	

- Compute the correction factor and various sums of squares as given below:

$$CF = \frac{(GT)^2}{N} = \frac{241277.4}{80} = 3015.96$$

$$\begin{aligned} \text{Total SS} &= (3.8)^2 + (6.0)^2 + \dots + (4.6)^2 - CF \\ &= 3128.76 - 3015.96 \\ &= 112.80 \end{aligned}$$

$$\begin{aligned} \text{Replication SS} &= \frac{(122.4)^2 + \dots + (124.1)^2}{t} - CF \\ &= 3016.32 - 3015.96 \\ &= 0.36 \end{aligned}$$

$$\begin{aligned} \text{Treatment SS} &= \frac{(18.2)^2 + (26.2)^2 + \dots + (18.0)^2}{r} - CF \\ &= 3110.82 - CF \\ &= 94.86 \end{aligned}$$

$$\begin{aligned} \text{Error SS} &= \text{Total SS} - \text{RSS} - \text{Tr.SS} \\ &= 112.80 - 0.38 - 94.86 \\ &= 17.58 \end{aligned}$$

- Compute the mean sum of squares by dividing each sum of squares by its corresponding degrees of freedom.

$$\text{Replication MSS} = \frac{\text{RSS}}{r-1} = \frac{0.36}{3} = 0.12$$

$$\text{Treatment MS} = \frac{\text{Tr.SS}}{t-1} = \frac{94.86}{19} = 4.99$$

$$\text{Error MS} = \frac{\text{Er.SS}}{(r-1)(t-1)} = \frac{17.58}{57} = 0.30$$

- Compute the 'F' value for testing the treatment differences.

$$\begin{aligned} \text{F value} &= \frac{\text{Treatment MS}}{\text{Error MS}} = \frac{4.99}{0.30} \\ &= 16.6 \end{aligned}$$

- Compare the calculated F value with table F value.
- Prepare the Analysis of Variance table by including all the computed values.

## Analysis of variance (ANOVA) table

Source	DF	SS	MSS	Computed F	Table F	
					5%	1%
Replication	3	0.36	0.12			
Treatment	19	94.86	4.99	16.6**		
Error	57	17.58	0.30			
Total	79	112.80				

A highly significant F value indicates that the test entries differ significantly among themselves.

- Compute the coefficient of variation (CV).

$$\begin{aligned}
 CV &= \frac{\sqrt{\text{Error MSS}}}{\text{GM}} \times 100 \\
 &= \frac{0.30}{6.14} \times 100 \\
 &= 8.92
 \end{aligned}$$

- Compute the critical difference

$$\begin{aligned}
 CD &= t_{0.05} \times \frac{\sqrt{2 \times \text{Error MSS}}}{r} \\
 &= 3.44 \times 0.387 \\
 &= 1.33
 \end{aligned}$$

- The hybrids whose difference from the check variety is more than the CD value are considered significantly superior to the check variety.

### **Utilization of results**

- The performance of the hybrids is compared with the check variety of corresponding duration or the highest yielding check variety.
- The hybrids which give significantly higher yield than check variety are identified and promoted to an Advanced Yield Trial. A significant yield advantage of more than one t/ha would also be the ideal criteria for selecting the best hybrids whenever the CD value is less than 1.
- Hybrids showing significant yield advantage should be critically evaluated for grain quality and disease/insect resistance.

## **Advanced yield trials (AYT)**

### ***Composition***

- The promising hybrids identified in the preliminary yield trials
- Three-four check varieties of different durations (very early, early, medium and late)

### ***Experimental design and field layout***

- A randomized complete block design is ideal for conducting AYT.
- The number of entries in AYT are much less than those in PYT. It is helpful to increase the plot size to 15 m<sup>2</sup>.

- Entries should be divided into at least two maturity groups (very early and early) and (medium and late).
- The field layout and agronomic management are similar to that for PYT.

### **Data recording**

The following observations are recorded for AYT:

- Plant height
- Days to 50% flowering
- Panicles/m<sup>2</sup>
- Number of filled grains/panicle
- Spikelet fertility (%)
- Yield/ha
- 1000 grain weight.
- Reactions to major pests and diseases.
- Remarks on special features.

- **Statistical analysis**

The method of statistical analysis is the same as the one explained for preliminary yield trials.

### **Utilization of results**

- The performance of hybrids is compared with check variety of corresponding duration or the highest yielding check variety.
- The hybrids which give significantly higher yield (> 1 t/ha) than the check variety are promoted for multilocation trials.

- Mere statistical significance is not a sufficient reason to consider a hybrid as promising. Therefore, an advantage of about 1 t/ha over the check variety is specified which would result in real benefit to the farmers.
- Hybrids selected for multilocation trials should be critically evaluated for grain quality and disease/insect resistance.

## **Multilocation yield trials (MLT)**

The major objective of multilocation yield trials is to identify the hybrids that have wider adaptability or those which are specifically adapted to a particular location. This exercise is essential as hybrids perform differently in different environments. This also provides an opportunity for the breeder to see the performance of hybrids bred by him in other locations. The concept of multilocation yield trials has really improved the efficiency of rice breeders and it is more so in hybrid rice breeding.

### ***Composition***

- The promising hybrids identified in AYT from different centers including those introduced from abroad.
- Three-to four check varieties of different durations (very early, early, medium and late). If the trials are constituted based on duration, it would suffice if a check variety of corresponding duration is included in the trial.

### ***Experimental design and field layout***

- The locations for MLT should be selected carefully so that each location serves as a distinct environment. The location selected for trials should be in the target areas for cultivation of hybrid rice.



- A randomized complete block design is commonly used for conducting MLT.
- It is necessary to have common guidelines for agronomic management and collection of data from different centers.

### ***General guidelines for conducting MLT***

- Experimental design to be adapted - RCBD with three to four replications.
- Entries and the check varieties should be specified. Besides the common check, each center can choose a local check for comparison.
- The trial should be conducted during the same season at all locations.
- Seedling age at transplanting should be similar -21-25 days old.
- Spacing adopted should be uniform --- 20 x 20 or 20 x 15 cm.
- The fertilizer dose may depend on the native fertility and recommendations in the local area; a top dressing of 20% N should be given at booting stage.
- Plant protection should be based on the needs; using the IPM strategy is preferred.
- The plot size should be uniform in all the locations to the extent possible (at least 15 m<sup>2</sup>).

## ***Agronomic management***

Agronomic management should be uniform in all locations so as to have valid comparisons, except for some specific recommendations made for a particular location.

## ***Data recording***

Data sheets are circulated to all the cooperators for collecting data on important parameters:

- Plant height
- Days to 50% flowering
- Panicles/m<sup>2</sup>
- Number of filled grains/panicle
- Spikelet fertility (%)
- Yield/plot
- Yield/ha
- 1000 grain weight.
- Reactions to pests and diseases.
- Weather data of each location.

## ***Utilization of results***

- The hybrids with higher yield potential and wider adaptability are identified based on stability analysis. These are promoted for on-farm testing in different areas, prior to their release for commercial cultivation.
- Those hybrids which are found suitable for particular location, are promoted for on-farm testing in that particular region only.

## ***Genotype-Environment interaction (GXE interaction)***

Experimental hybrids have to be evaluated in multilocation trials to identify those which have wider adaptability and others which are specifically adapted only to certain locations. In a multilocation trial, a hybrid giving highest yield in one location may perform poorly in another site. This differential behavior of genotypes in different environments is called genotype-environment interaction. Knowledge on stability of hybrids and their specific adaptability is necessary prior to their release for commercial cultivation.

Several models have been proposed by Finlay and Wilkinson(1963), Eberhart and Russell (1966), Perkins and Jinks (1968), Freeman and Perkins (1970) and Shukla (1972) to measure G x E interaction and to work out the stability of genotypes. These models were based on regression of varied performance on an environmental index rather than on their adaptability. Recent models extend the classical additive main effect model for genotypes and environment by including multiplicative terms for the interaction component. They are thus referred to as Additive Main Effects and Multiplicative Interaction (AMMI) models. These models provide insight on the environmental factors accounting for G x E interaction, and stability of genotypes, besides throwing light on the adaptability issues.

For a statistical analysis of the G x E interaction, adaptability of genotypes and interpretation of results, refer to some references cited at the end of this manual.



## ***The need for parental line breeding***

We need to improve parental lines:

- To increase the frequency of maintainers and restorers among elite lines, and
- To broaden the genetic base of parental lines.

## **Improvement of restorers**

Restorers can be improved by employing the following strategies:

- Exercising pedigree selection in A x R crosses (iso-cytoplasmic restorer improvement program)
- Exercising pedigree selection in R x R crosses (allo-cytoplasmic restorer improvement program)
- Exercising pedigree selection in a randomly mating composite population of restorers developed by using male sterility facilitated recurrent selection

### ***Development of restorers by using A x R crosses***

- Select promising A x R hybrids showing normal spikelet fertility.
- Grow an F<sub>2</sub> and select 100-200 individual plants which are more productive with normal spikelet fertility.
- Grow F<sub>3</sub> progenies and observe it for uniformity and higher spikelet fertility.
- Select the best F<sub>3</sub> progenies and the best plants showing normal spikelet fertility.

- Grow  $F_4$ - $F_6$  generations to select the normally fertile uniform progenies and make testcrosses with single plants of selected progenies (looking better than the R line used in A x R crosses) using the best available 'A' line.
- Since all the restorer lines derived from an A x R cross inherit the same cytoplasm, these are called iso-cytoplasmic restorers.
- These improved 'R' lines may or may not be restorers for CMS lines of different sources.

### *Development of restorers by R x R crosses*

- The main objective of this method is to increase the frequency of restorer genes and combine the desirable traits from two or more restorers.
- Select good restorers which have different desirable characteristics to be combined. The desirable traits most often sought after are higher yield potential, high grain number, good restoring ability, large anther, higher pollen load, disease and pest resistance, and good grain quality.
- Make crosses between the selected restorer parents and grow  $F_1$ s.
- Grow  $F_2$  progeny and select about 100-200 good recombinants with desirable characteristics of both parents.
- Grow  $F_3$ - $F_6$  generations, selecting the best plants in best progenies.
- From each R x R cross, select 15-20 elite lines.

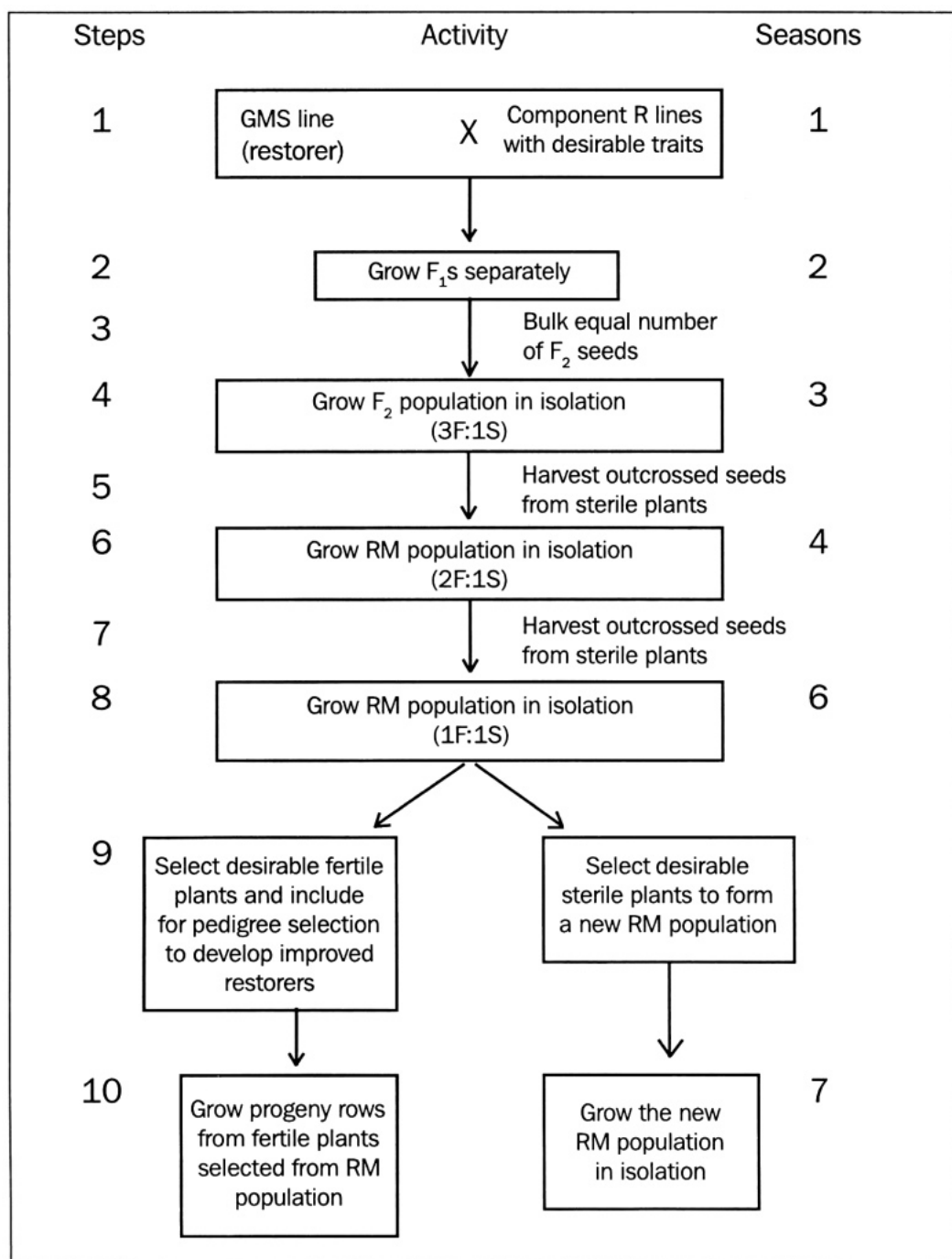
- Testcross these elite lines with a stable CMS line in a testcross nursery and assess their restoring ability and extent of apparent heterosis.
- Since the parents used for crossing are good restorers, the frequency of restorers developed by this method is quite high.

### ***Breeding of restorers using male sterility facilitated recurrent selection***

Although effective, the methods mentioned in preceding sections are cumbersome and require lots of land and labor resources. Besides, the resulting restorer lines may not have wide adaptability.

At IRRI, we have developed an alternative strategy to extract a high frequency of R lines regularly from a random mating composite population of restorers. This is done using the male sterility facilitated recurrent selection procedure illustrated in Fig. 14.1. The procedure involves:

- the development of base population derived from crosses of a genetic male sterile (IR36 ms) line in a restorer background with a series of selected restorer lines of 'WA' cytoplasm (steps 1-4).
- a random mating of male sterile plants with fertile plants occurring in base and subsequently derived populations until both male sterile and fertile plants were in equilibrium (steps 5-8).
- growing the random mating population and selection of desirable fertile plants to extract improved restorer lines for evaluation in a pedigree nursery and compositing the seed set on desirable male sterile plants occurring in the population to form a new random mating composite (step 9).
- growing the desirable fertile plants in a pedigree nursery to extract improved restorer lines.



**Fig. 14.1** Scheme used at IRRI to develop random mating composite populations for improvement of restorer lines.



The random mating populations can be shared with collaborating national and international hybrid rice breeding programs to enable them to extract locally adapted and genetically diverse restorer lines for use in local hybrid rice breeding programs. Elite restorer lines, selected from these populations, can again be composited by repeating the above cycle to develop new random mating composite populations for further improvement of restorer lines.

### ***Incorporation of restorer genes into japonica lines/basmati lines***

The frequency of restorers in japonica and basmati elite lines is very low. Hence, there is a need to transfer restorer genes into such lines from indica restorers to develop high yielding japonica/basmati hybrids. This can be done in the following ways:

- Select good japonica/basmati lines and a good restorer line.
- Make crosses between the japonica/basmati lines and a restorer source, and grow  $F_1$ s.
- Grow  $F_2$  populations and select 100-200 plants possessing japonica/basmati type grains and good agronomic characteristics.

Grow  $F_3$  progenies and select plants among best families using the criteria employed in  $F_2$  population.

- In the  $F_4$ - $F_5$  generations, select elite progenies and testcross the best single plants from each progeny to identify the ones having fertility restoring ability.
- Select few best lines having good restoring ability and japonica/basmati grains.

- Backcross the derived restorer lines with the original japonica/basmati lines and follow the above steps to concentrate genes for japonica/basmati germplasm.
- Repeat the above cycle one more time.

## Improvement of maintainers

Maintainers can be improved by the crossbreeding method and also by the genetic male sterility facilitated recurrent selection method.

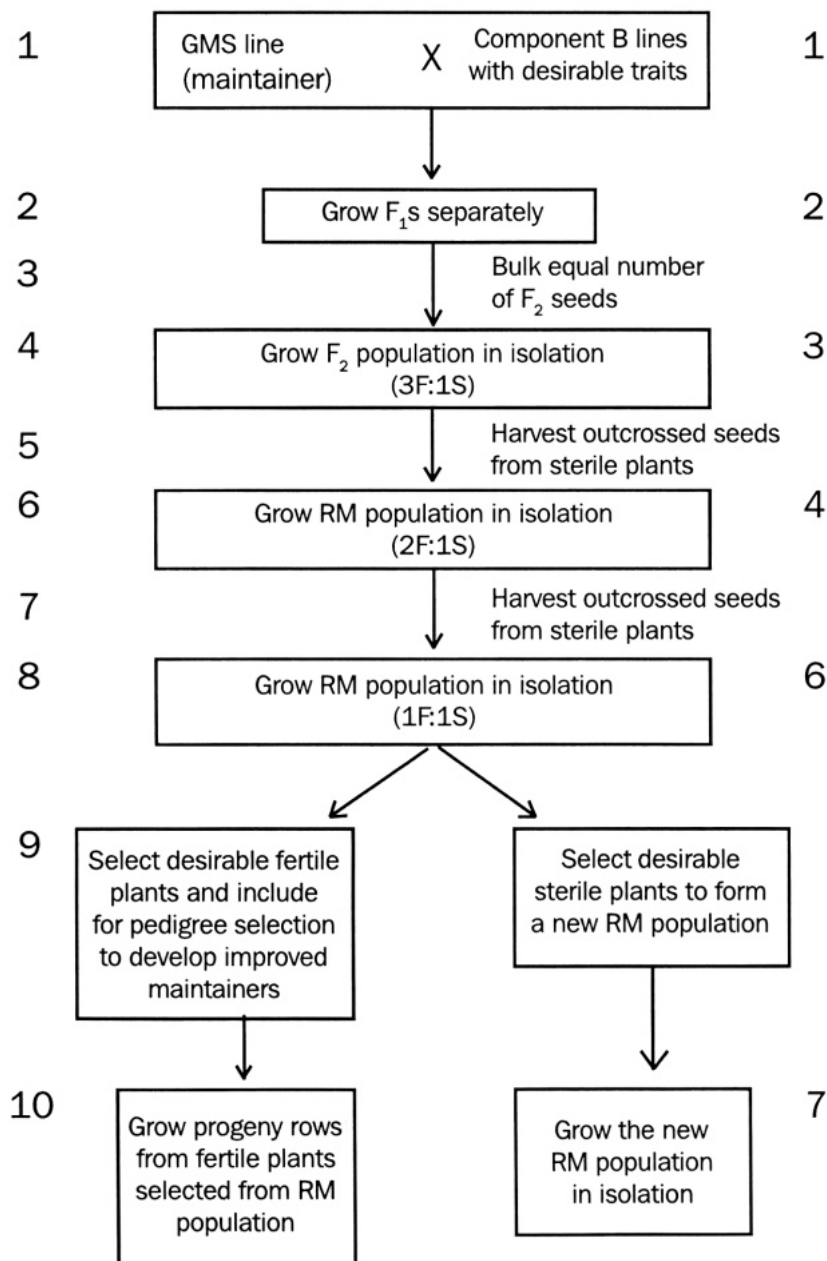
### ***Improvement of maintainers by B x B crosses***

- Select good maintainers having desirable traits which are to be recombined.
- Make crosses between them and grow an  $F_1$ .
- Grow an  $F_2$  population and select 100-200 best recombinants.
- Grow  $F_2$ - $F_5$  generations and continuously select the most productive progenies/plants.
- In  $F_4$ - $F_5$  generations, select elite progenies and testcross the best single plants from each progeny to confirm their maintaining ability.
- Backcross effective maintainers to convert them into new CMS lines.

### ***Extraction of maintainers from a random mating composite population***

Maintainers are improved by extracting them from a random mating composite population developed by using male sterility facilitated recurrent selection.

This method of improving maintainers is similar to the one described for the improvement of restorers. The main difference is that the genetic male sterile line should be in the background of a good maintainer and the component lines should be diverse maintainers with desirable traits. This method ensures the continuous supply of new maintainer lines from the random mating composite population. The details of the method are outlined in Fig. 14.2.



**Fig. 14.2** Scheme used at IRRI to develop random mating composite populations for improvement of maintaner lines.



## 15. Methods of Enhancing the Levels of Heterosis

*The extent of standard heterosis obtained in commercial rice hybrids is one of the critical factors that decide the economic viability of hybrid rice technology. Intervarietal indica hybrids grown in China and other countries have shown a yield advantage of 15-20% over the best check varieties. Although yield advantage of this magnitude is enough to ensure economic viability, a further increase in the levels of yield heterosis is needed for popularization and a wider adoption of this technology.*

*It has been possible to enhance the heterosis level by adopting the two-line approach to an extent of 5-6%. Exploitable heterosis has been maximum in indica/japonica crosses, followed by indica/Bulu (tropical japonica) crosses in temperate countries. From the practical point of view, indica/tropical japonica hybrids would be an ideal choice for the tropics on account of their better adaptability. The semi-sterility problem, generally encountered in indica/japonica crosses, can be overcome by deploying the wide compatibility genes which are found in specific indica and japonica varieties. The two-line approach appears to be rather simple for developing intersubspecific hybrids compared with the three-line approach.*

### Enhancing the levels of heterosis

Heterosis needs to be enhanced in order to

- provide more income to hybrid rice growers, and
- encourage a wider adoption of hybrid rice technology.

## ***Methods of enhancing yield heterosis***

Developing indica/japonica hybrids has been a widely accepted method. However, for the tropics, tropical japonicas are more suitable than typical temperate japonicas. These intersubspecific hybrids can be developed either by deploying the two-line approach or three-line approach, the former being more practical and easy to adopt. In either case, the use of a 'wide compatibility' mechanism is a prerequisite to overcome the semi-sterility problem.

### ***What is wide compatibility?***

Hybrids between indica and japonica rices show a varying degree of semi-sterility. This semi-sterility is the result of an allelic interaction at a locus designated as S-5 in indica/japonica hybrids ( $S-5^i/S-5^j$ ). On the contrary, some indica/japonica crosses show normal fertility because one of the parents has a neutral ( $S-5^n$ ) allele. Thus, the crosses having a genetic constitution of  $S-5^n/S-5^i$  or  $S-5^n/S-5^j$  are normally fertile. Such varieties which possess a  $S-5^n$  allele are called wide compatible varieties (WCV). We can define the wide compatibility of a variety as its ability to produce normally fertile progeny when crossed with both indica and japonica testers. This mechanism is a key factor in developing indica/japonica hybrids.

The following are some of the wide compatible varieties in different varietal groups.

Indica	Japonica	Tropical Japonica
BPI 76	NK 4	Banten
	Norin PL 9	Calotoc
	O2428	CP-SLO
Dular (Aus)		Ketan Nangka
N 22 (Aus)		Moroberekan
		Palawan
		Padi Bujang
		Pendec
		IR64446-7-3-2-2
		IR65598-112-2

### Developing two-line indica/japonica hybrids

The basic components for developing indica/tropical japonica hybrids are the TGMS lines in the background of either indicas or japonicas and a wide compatible gene in any one of the parents. In the initial stages, it is necessary to develop requisite parental lines for developing inter-subspecific hybrids.

- ***Incorporation of the 'WC' gene into TGMS lines***
  - Select a good TGMS line with distinct critical sterility/fertility points.
  - Identify a 'WC' donor variety.
  - Make a cross between a TGMS line and a 'WC' donor during its sterility phase.
  - Grow an F<sub>1</sub> progeny.



- Grow  $F_2$  in an environment conducive for expression of sterility and select sterile recombinants, with purple apiculus pigmentation (WC gene is tightly linked with purple apiculus and waxy endosperm). Alternatively, the selection for an Amp  $3^2$  allele of isozyme (found highly linked with WC gene) can also be practiced in segregating generations.
  - Ratoon and subject the selected TGMS plants to a lower temperature inducing fertility.
  - Grow  $F_3$ - $F_4$  generations, selecting the best plants in best progenies.
  - In the  $F_5$  generation, testcross with the counterpart genotype (cross with tropical japonica if TGMS is an indica and vice versa).
  - Evaluate the testcross  $F_1$ s for spikelet fertility.
  - Select those lines which show normal fertility. The resultant TGMS line has a 'WC' gene transferred from a donor parent.
- *Evaluation of indica/tropical japonica hybrids*
    - Make several crosses between TGMS lines (WC) and tropical japonica varieties. If some tropical japonicas have a 'WC' gene, they can be crossed to any indica TGMS line.
    - Evaluate the hybrids in OYT and a series of trials to identify the most promising hybrids with enhanced heterosis. The best hybrids which are grown in the locality should be used as standard checks. Special emphasis should be given to monitor the spikelet fertility during the evaluation process.

- ***Developing three-line indica/tropical japonica hybrids***

Developing indica/tropical japonica hybrids by deploying the CMS system is more complicated. In addition to the restorer gene which is a prerequisite for developing a three-line hybrid, one of the parents should also possess a 'WC' gene to make the indica/japonica hybrid really productive. The following are the options open to breeders to develop indica/tropical japonica hybrids using the CMS system:

- Indica CMS ( $WC^+$ ) x Tropical japonica ( $WC^- R^+$ )
- Indica CMS ( $WC^-$ ) x T. japonica ( $WC^+ R^+$ )
- T. japonica CMS ( $WC^+$ ) x indica ( $R^+$ )
- T. japonica CMS ( $WC^-$ ) x indica ( $R^+ WC^+$ )

The above options indicate the necessity of developing requisite parental lines as most of them are not readily available in large numbers.

- ***Developing CMS lines with the 'WC' gene***

- Testcross a series of wide compatible varieties both in indica and tropical japonica backgrounds and identify good maintainers.
- Develop these maintainers with the WC gene into CMS lines by repeated backcrossing as followed for the conversion program in a CMS system.
- If maintainers are not available with the 'WC' gene, this gene can be first incorporated into good maintainers and be converted into a new CMS line.

- Testcross the new CMS line with a restorer of other subspecies to confirm the transfer of WC gene.

- ***Developing tropical japonicas with restorer genes***

This has been described in the chapter on improvement of parental lines.

- ***Developing tropical japonicas with 'WC' and 'R' genes***

The same method described for transferring the 'R' gene into T. japonicas can be followed. However, the recipient tropical japonica should possess a 'WC' gene.

- ***Developing indica restorers with wide compatibility***

The frequency of restorers in indicas is quite high. Hence, they make good male parents to develop indica/tropical japonica hybrids, if only 'WC' genes are transferred to them. The procedure for this is as follows:

- Select indica varieties with a very good restoring ability and 'WC' donors with a marker gene (a purple apiculus or Amp<sup>32</sup>).
- Make crosses between indica restorers and WC donors.
- Grow F<sub>1</sub> progenies and evaluate all crosses for spikelet fertility. Choose the highly fertile cross for further selection.
- Grow F<sub>2</sub> and select good recombinants looking like the restorer, but with an apiculus pigmentation or Amp<sup>32</sup> allele.
- Grow F<sub>3</sub> and F<sub>4</sub> progenies and select the plants in best families. Keep track of the original plant type of the restorer and the apiculus pigmentation or Amp<sup>32</sup>.

- In  $F_5$  generation, testcross the selected lines on a single plant basis with a tropical japonica CMS line having no WC gene.
- Evaluate the testcross progeny for fertility. Select such lines which show high fertility. They have both restorer and wide compatibility genes.

Once the requisite parental lines are developed, they can be crossed with each other depending on their flowering behavior. Making testcrosses and evaluating experimental hybrids can be carried out as discussed in the chapters on evaluation of experimental hybrids.



## 16. Quality Assurance Procedures in Hybrid Rice Breeding

*Quality assurance procedures are a set of general guidelines which help to refine the existing techniques in order to enhance efficiency of any research project, including breeding program. Hybrid rice breeding involves several intricate steps and procedures which need intensive efforts. Careful planning and systematic implementation of these activities are crucial for obtaining useful results. In view of the generally declining trend in resource availability for research, it becomes imperative to achieve maximum efficiency with the limited inputs available. In the hybrid rice breeding program, the materials developed such as parental lines and hybrids must be very good in terms of their genetic potential and purity. The data generated should be meaningful and readily usable by a larger fraternity. Therefore, quality assurance procedures assume added significance.*

### What are the quality assurance procedures?

Quality assurance procedures are a set of general guidelines formed either by consensus or framed by experts with the aim of providing practical hints to the researchers to generate quality material and data.

- These procedures will refine the existing techniques and highlight some significant points which have profound influence on the outcome of experiments.

## Why quality assurance procedures?

Quality assurance procedures are important because there is a need for:

- careful utilization of limited sources,
- developing genetic materials of high quality,
- generating data of high standards,
- enhancing the efficiency of breeding procedures, and
- overcoming the common errors committed inadvertently during experimentation.

## How and when do we adopt quality assurance procedures?

Quality assurance procedures have to be followed at each and every step starting from planning the experiments till the delivery of output. The important points to be considered at different stages in a hybrid rice breeding program are described below.

### Source nursery

- Keeping in view the breeding objective, assemble the genetically diverse elite lines since genetic diversity is the key to higher heterosis.
- Be sure to plant a single seedling per hill in this nursery.
- Material planted in the source nursery may not always be pure. Remove off-types before using the elite lines for testcrossing.
- Include only one or two most stable and commercially usable CMS lines representing each CMS source in the source nursery. Testcrossing elite lines with several CMS lines of the same CMS source is a sheer waste of resources.

- Use only a single plant from each of the elite genotypes in the source nursery for testcrossing to CMS lines.
- Tag the pollen parent plant and collect seeds for future use.

### **Testcross nursery**

- Plant a single seedling per hill.
- Plant a single row of 10-12 plants each.
- Plant check varieties after every 10 testcross entries for comparison.
- Plant pollen parents by the side of testcross  $F_1$ s. This helps in valid comparisons and also expedites the backcrossing program.
- Record both pollen fertility and spikelet fertility of testcross  $F_1$ s to identify the maintainers and restorers.
- Follow the uniform criteria (given below) for classifying the elite genotypes as maintainers and restorers so that data will have wider use.

**Table 2. Classification of elite lines into maintainers and restorers.**

<b>Pollen Fertility (%)</b>	<b>Category</b>	<b>Spikelet Fertility (%)</b>
0-1	Maintainers	0
1.1-50	Partial maintainers	0.1-50
50.1-80	Partial restorers	50.1-75
>80	Restorers	>75



- **CMS nursery**

- Grow introduced CMS lines received from outside at least for two seasons to determine their stability.
- Maintain locally-bred and introduced CMS lines by paired crossing of selected single plants of A and B lines.
- Discard any pair that segregates for fertility.
- Collect data on 50% flowering, plant height, phenotypic acceptability, and outcrossing rate; there is no need to collect data on number of spikelets, panicle length, number of tillers, etc. unless the CMS line has been found commercially usable.

- **Backcross nursery**

- Before making a backcross, be sure that all plants in a particular testcross  $F_1$  progeny are completely male sterile.
- Make backcrosses on a single plant basis and 4-5 paired crosses which are necessary in the beginning.
- Examine all plants in each pair for pollen fertility.
- If any pair is found to segregate for pollen fertility, you must discard that cross.

- **Evaluation of hybrids**

Evaluation of hybrids forms an important part in hybrid rice breeding. The entries must be evaluated without any bias. Adopting suitable statistical designs helps to eliminate experimental errors, and the comparisons will become more valid.

- Choice of design and plot size is very important. The recommended guidelines for different trials are as follows:

<b>Trial</b>	<b>No. of entries</b>	<b>Design</b>	<b>Plot size (m<sup>2</sup>)</b>
OYT	20-100	Augmented	5
CAT	50-100	RBD	3-5
PYT	20-50	RBD	10
AYT	10-30	RBD	15
MLT	10-20	RBD	15-20
On-farm	4-5	-	1000-2000

- Grouping of test entries based on duration or ecosystems is desirable for better comparisons.
- Check varieties of corresponding duration should be included in each trial and the hybrids should be compared with checks of the corresponding duration only.
- Avoid taking yield data from border rows.
- Adjust yield values based on moisture content recorded at harvest. Otherwise, the results will be erroneous.
- It is necessary to follow common agronomic practices while conducting multilocation trials.
- Be strict in discarding poor entries. Rigorous selection can help save time and labor. Statistical significance alone is not enough for promoting entries. Yield advantage of at least 1 t/ha over the best check variety should be the guiding principle.

- **Seed production**

- Seed production activity should run concurrently for efficient functioning of a hybrid rice breeding program.
- Choose an appropriate method for seed production, depending upon the type of trials to be conducted. Go for the isolation free system for producing  $F_1$  seed for OYT, CAT and PYT. For advanced and multilocation trials, seeds should be produced under strict isolation.
- Seeds for advanced trials should be highly pure. Therefore, space isolation of 50-100 m or time isolation of 21 days is mandatory.
- Knowledge on growth duration of parental lines in different seasons and years is helpful to adjust seeding dates so as to obtain good synchronization.
- Adopt an optimum row ratio (2:8 or 2:10) for obtaining higher seed yields.
- Spraying the right quantity of  $GA_3$  at the right stage is crucial.
- Supplementary pollination at the peak anthesis enhances seed set.
- Harvest parental lines separately to avoid admixtures.

- **Handling of TGMS material**

- Handling TGMS lines is more complicated because their fertility/sterility expression is temperature-dependent.
- Weather data for a period of 10-15 years on maximum and minimum temperature, humidity, and daylength of the location or test site should be collected.

- It is essential to determine the critical sterility and fertility points of TGMS lines for a given location.
- For TGMS multiplication, adjust the seeding in such a way that the variety enters the critical stage when the temperature is favorable for inducing fertility.
- Absolute male sterility is a must for hybrid seed production using TGMS lines. The seeding date should be adjusted such that the TGMS plants enter the critical stage when the temperature is ideal for inducing sterility.
- Bag 100-200 panicles of TGMS plants in hybrid seed production plots. Any seed set due to temperature fluctuations can be detected and such plots should be discarded.

- **Use of innovative techniques**

- Transfer of desirable genes from donors is time consuming if we adopt the conventional pedigree system of breeding. Under such circumstances, techniques like anther culture can be employed to expedite the transfer of desired genes.
- Many useful genes (wide compatibility, TGMS, restorer) have been tagged with molecular markers. Use of marker-aided selection will not only enhance the accuracy of results, but also hasten the process of selection.

- **General field operations**

Field operations are very important to obtain good quality material and reliable data.

- Take care to break dormancy before seeding. The seeds used for seeding should be free from admixtures, weeds, and infection.

- Prepare nursery beds carefully. Perfect leveling is a must for healthy seedlings. Thin seeding is desirable to obtain a healthy multi-tillered seedling in 25 days.
- Do gap-filling 7 days after transplanting to ensure a uniform plant population.
- Do timely topdressing with fertilizers. A uniform distribution of fertilizer must be ensured. It is not desirable to apply fertilizers when it is raining and when the leaves are wet.
- Utmost care should be taken while harvesting. All fallen tillers should be removed before harvesting the next line.
- The presence of technical assistants in all field operations is a must to avoid mistakes in handling the material.

- **Data recording and presentation**

Voluminous data have often been collected by many researchers but only a fraction of it comes to the forefront due to several reasons. Systematic collection and presentation of data is as important as conducting the experiment itself.

- Collect data which are most relevant. A question may be asked as to how the data that are being collected can be used. It is a waste of time and resources to collect data which have no relevance (Refer to table 3 for details).
- Oftentimes, simple scoring based on an appropriate scale would be enough to judge the genetic worth of a material. Unless required, there is no need to record the quantitative measurement of such traits.
- Try to follow the common procedures for recording and analyzing data so that many people can benefit from these results. Use the generally accepted units of measurement.

- Regular calibration of equipment is a must to overcome systematic errors.
- Quick compilation of results is most desired because many decisions are to be taken based on the current season's findings.
- Carry out statistical analysis whenever necessary, and publish the findings immediately so that new information reaches the end users as early as possible.

- **Personnel management**

A well-organized workforce is a key to the success of any mission. Deployment of essential manpower is a must for effective implementation of a hybrid rice breeding program. Hybrid rice breeding involves diversified activities, and hence, it is essential to assign specific duties to different persons. Number of people required may vary with the size of the organization and the quantity of work to be carried out. To be reasonable and effective, a group of 4-5 scientists would be ideal. Each scientist will be assigned a major activity and he/she should be assisted by one or two technical assistants and two regular helpers. Casual workers can be hired as and when necessary.

At IRRI, the responsibilities for hybrid rice breeding and seed production are shared among five junior researchers as given below:

Scientist —1

**Breeding Nurseries**

Source nursery

CMS nursery

Testcross nursery

Backcross nursery

OYT seed production

Scientist-2

**Evaluation of Hybrids**

Observational yield trial

Preliminary yield trial

Combining ability trial

Advanced yield trial

Multilocation yield trial

Scientist-3

**Seed Production-I**

Nucleus seed production

Breeder seed production

Restorer purification

Seed management

Scientist-4

**Seed Production-II**

Hybrid seed production

Experiments to improve  
seed yields

Scientist-5

**Two Line Breeding**

TGMS development

TGMS evaluation

Indica/Japonica hybrids

**Table 3. Data to be recorded for hybrid rice experiments.**

Characters	Breeding nurseries				Evaluation trials				
	Source nurseries	CMS nursery	Testcross nurseries	Backcross nurseries	OYT	PYT	AYT	MLT	On-farm
Days to flowering (days)	√	√	√	√	√	√	√	√	√
Vegetative vigor (score)					√	√	√	0	
Plant height (cm)					√	√	√	√	
Panicles/m <sup>2</sup> (No.)					√	√	√	√	√
Anther color (yplws)		√							
Pollen fertility (%)		√	√	√					
Spikelet fertility (%)		√	√	√	√	√	√	√	
Panicle exertion rate (%)	√	0							
Stigma exertion rate (%)	√	0							
Outcrossing rate (%)	√	√							
No. of filled grains/panicle	√	√	√	√	√				
Apparent heterosis (scale)	√								
Grain yield (kg/ha)	√	√	√	√	√				
Grain type (scale)	√	√			√	√	√	√	√
Phenotypic acceptability (scale)	√	√			√	√	√	√	√
Reaction to pests/diseases		√			0	0	√	√	√
Weather data							√	√	0

= Essential; 0 = Optional  
 √ = Essential; 0 = Optional





## Selected References

- Bharaj T S, Virmani S S and Khush G S (1995) Chromosomal location of fertility restoring genes for 'wild abortive' cytoplasmic male sterility using primary trisomics in rice. *Euphytica* 83:169-173.
- Comstock R E and Robinson H F (1952) Estimation of average dominance of genes. In *Heterosis*, Iowa State College, Press, Ames, 494-516.
- Crossa J, Gauch, H G Jr., and Zobel R W (1989). Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. *Crop Sci.* 30(3):493-500.
- Davenport C B (1908) Degeneration, albinism and inbreeding. *Science* 28:454-455.
- East E M (1908) Inbreeding in corn. Rept. Connecticut Agric. Expt. Station for 1907. pp. 419-428.
- East E M (1936) Heterosis. *Genetics* 21:375-397.
- Eberhart S A and Russell W L (1966) Stability parameters for comparing varieties. *Crop Sci.* 6:36-40.
- Finlay K W and Wilkinson G M (1963) The analysis of adaptation in plant breeding programme. *Aust. J. Agric. Res.* 14:742-757.
- Freeman G H and Perkins J M (1971) Environment and genotype-environmental components of variability. VIII. Relations between genotypes grown in different environments and measure of these environments. *Heredity* 26:15-23.
- Gowen G W (ed.) (1952) "Heterosis" Iowa State College Press, Ames, Iowa. pp. 552.

- Ikehashi H and Araki H (1984) Varietal screening for compatibility types revealed in F1 sterility of crosses in rice. *Jap. J. Breed.* 34(3):304-312.
- IRRI (1988) "Hybrid Rice." *Proc. of the International Symposium on Hybrid rice*, 6-10 October, 1996, Changsha, Hunan, China. International Rice Research Institute, Manila, Philippines.
- IRRI (1994) "Hybrid Rice Technology - New Development and Future Prospects." (S. S. Virmani, Ed.). International Rice Research Institute, Manila, Philippines.
- Jinks J L (1983) Biometrical genetics of heterosis. In: Frankel, R. (Ed.). *Heterosis-reappraisal of theory and practice*. Springer Berlin, Heidelberg, New York.
- Kempthorne O (1957) *An introduction to genetic statistics*. John Wiley and Sons, Inc., London.
- Kempthorne R A (1984) The use of biplots in interpreting variety by environment interactions. *J. Agric. Sci. Camb.* 104:123-135.
- Maruyama K, Araki H and Kato H (1991) Thermosensitive genic male sterility induced by irradiation. In "Rice Genetics II" pp. 227-232. International Rice Research Institute, Manila, Philippines.
- Perkins J M and Jinks J L (1968) Environmental and genotype-environmental components of variability, III. Multiple lines and crosses. *Heredity* 23:339-356.
- Shi M S (1985) The discovery and study of the photosensitive recessive male sterile rice (*Oryza sativa* L. subsp. *japonica*). *Sci. Agric. Sin.* 2:44-48.
- Shinjo C (1975) Genetical studies of cytoplasmic male sterility and fertility restoration in rice, *Oryza sativa* L. *Bull. Coll. Agric. Univ. Ryukyus* 22:1-57.

- Singh R K and Chaudaty B D (1977) Biometrical methods in quantitative genetic analysis. Kalyani Publishers, Ludhiana, India, pp. 300.
- Shull G H (1908) The composition of field of maize. Rept. Amer. Breeders Assoc. 4:296-301.
- Shukla G K (1972) Some statistical aspects of partitioning genotype-environmental components of variability. Heredity 29:237-245.
- Virmani S S (1994) "Heterosis and Hybrid Rice Breeding." Springer-Verlag, Berlin, pp. 189.
- Virmani S S (1996) Hybrid Rice. Adv. Agron. 57:328-462.
- Virmani S S and Edwards I B (1983) Current status and future prospects for breeding hybrid rice and wheat. Adv. Agron. 36:145-214.
- Virmani S S and Casal C L (1993) Isolation-free system for producing experimental hybrid seed for preliminary evaluation. International Rice Research Institute Newsletter 18(3):6-7.
- Virmani S S and Sharma H L (1993) "Manual for Hybrid Rice Seed Production." International Rice Research Institute, Manila, Philippines.
- Wescott B (1985) Some methods of analyzing genotype-environment interaction. Heredity 56:243-253.













## APPENDIX 5: FORMAT FOR BACKCROSS NURSERY FIELD BOOK

[illegible]

\* Score on 1-9 scale







## Appendix 9: DATA SHEETS FOR CMS AND HYBRID RICE SEED PRODUCTION

[illegible][illegible]

\* Score on 1-9 scale



# GLOSSARY

## A

**'A' line** - the male sterile parent involving cytoplasmic or cytoplasmic-genetic male sterility. It is also known as CMS line.

**adaptability** - an ability of a genotype to adjust to a given environment and give a reasonably good yield.

**allo-plasmic lines** - the CMS or restorer lines which have different cytoplasms.

**anther** - a terminal part of the stamen that contains the pollen grains (male gametes).

**anthesis** - the action of opening of a flower or spikelet of rice.

**apiculus** - a small acute point or tip of a rice spikelet; extension of the lemma or palea.

**apomixis** - a kind of asexual reproduction through seed in which the embryo develops from maternal cell without fertilization. The resulting seed has the same genetic constitution as that of the seed parent.

**apparent heterosis** - subjective superiority of a hybrid over its parents or a check variety based on visual observations.

**augmented design** - a statistical design used for evaluation of genotypes in which the check varieties are replicated and the test entries are not replicated but are allotted randomly to the blocks.



**auricles** - the small paired ear-like appendages on either side of the base of the rice leaf blade that may not be present in older leaves.

**awn** - a bristle-like extension of varying length originating from the lemma of the spikelet.

## B

**'B' line** - the fertile counterpart parent of the male sterile 'A' line of a cytoplasmic or cytoplasmic genetic male sterility system which is used as male parent to maintain the latter. It is also known as maintainer line.

**backcross** - the cross of a hybrid with one of its parents.

**backcross method** - a breeding method in which  $F_1$  hybrid is again crossed with either of its parents. The resulting progeny is called a backcross progeny.

**backcross nursery** - breeding nursery where male sterile plants identified among the testcrosses (CMS x elite lines) are crossed with the respective male parents in order to transfer cytoplasmic male sterility into the nuclear genotype of the elite line.

**boot** - a rapidly growing panicle enveloped by the flag leaf sheath. In tissue culture, this refers to the panicle collected when the distance between the collar of the flag leaf and subtending leaf is about 7 to 8 cm.

**booting** - bulging of the flag leaf sheath due to the growing panicle inside.

**border rows** - the recommended number of rows of the male parental line grown on all sides of the hybrid seed production plot to minimize the contamination by outcrossing with stray pollen.

**bract** - a leaf from the axis from which a flower arises.

**breeder seed** - breeder seed is the seed of the highest genetic purity and is produced by the agency sponsoring a variety, and is used to produce foundation seed

## C

**caryopsis** - a small one-seeded dry indehiscent fruit with a thin membranous pericarp adhering so closely to the seed that fruit and seed are incorporated in one body forming a single grain, as in wheat and barley. In rice, brown rice is the caryopsis.

**certified seed** - seeds used for commercial crop production produced from foundation, registered or certified seeds under the regulation of a legally constituted agency. In hybrid rice, it is F<sub>1</sub> seed produced directly from CMS x restorer lines grown as per certification standards.

**CHA (chemical hybridizing agent)** - any chemical which is used to induce male sterility in plants.

**check variety** - Any popular or high-yielding variety widely grown in a region.

**chemical mutagen** - any chemical used to induce mutations artificially.

**CMS (cytoplasmic male sterile) line** - a male sterile line whose anthers produce no pollen or abortive pollen. Genetic factors responsible to induce sterility are present in its cytoplasm. No seed is set on such line by selfing. But its pistil is normal and it can produce seeds when pollinated by any normal plant.

**combining ability** - the ability of a genotype (inbred, pure line, or synthetic) to transfer its desirable traits to its progeny: **general**, average performance of a strain in a series of crosses; **specific**, deviation from performance predicted on the basis of general combining ability of parental lines,

**correlation coefficient** - a measure of the degree of association between two variables which is computed as the ratio of the covariance of the two variables to the products of their standard errors. Its values vary between. -1 to + 1.

**covariance** - the mean of the product of the deviation of two variates from their individual means.

**critical difference** - a statistical parameter computed to test whether the observed differences between the means of entries are significant or not.

**critical fertility point** - the temperature or photoperiod at which the spikelet fertility of an EGMS line is maximum.

**critical sterility point** - the temperature or photoperiod at which the EGMS lines are completely male sterile.

**cross fertilization** - the fertilization of an egg nuclei (ovule) of one parent by the pollen of another parent.

**cross pollination** - the application of pollen from the flowers of one plant to the stigma of another plant. It may or may not lead to fertilization.

**cytoplasm** - all the protoplasm of the cell except the nucleus.

**cytoplasmic heredity/inheritance** - the transmission of characters from parent to offspring through the cytoplasm of the germ cell.

## D

**day length** - number of light hours in a day.

**diallel mating** - a mating design in plant breeding in which a set of parents are crossed in all possible combinations.

**dihybrid** - a hybrid for two different genes. Heterozygous for two pairs of alleles.

**diploid (2n)** - an organism having two chromosomes of each kind.

**disomic** - a plant having one or more chromosomes duplicated, but not the entire genome.

**diverse** - having or capable of having various forms or qualities.

**dominance** - intra-allelic/intragenic interaction with complete suppression of the effects of one allele by another.

## E

**effective accumulated temperature (EAT)** - the total effective temperature in centigrade received by the plant from seeding to flowering. It is useful for predicting flowering.

$$\text{EAT} = \text{Mean daily temperature (}^{\circ}\text{C)} - \text{temperature higher than } 30^{\circ}\text{C} \\ - \text{temperature of lower limit (} 18^{\circ}\text{C)}$$

**emasculatation** - the process of removal of anthers from the florets so as to make the plant male sterile.

**elite line** - an improved breeding line or a variety.

**endosperm** - the nutritive tissue of the ripened ovary. It consists of the aleurone layer and the starchy tissue, and serves as the source of food for the germinating embryo.

**environmental genic male sterility (EGMS)** - male sterility- fertility transformation controlled by environmental factors such as temperature and photoperiod.

**epistasis** - the interaction of different genes in the expression of a trait.

## F

**F<sub>1</sub>** - abbreviation for the first filial generation, usually the hybrid between two homozygous parents.

**fertility restoration** - an ability of a genotype to restore fertility to its progeny when crossed to a CMS line.

**fertilization** - fusion of the nuclei of male and female gametes.

**flag leaf** - the uppermost leaf (of rice plant) originating just below the panicle base.

**flag leaf clipping** - a method of cutting 1/2 to 2/3 of the flag leaf from its tip in CMS and restorer lines to facilitate easy pollen dispersal.

**floret** - a unit of the spikelet which includes the lemma, palea, and the flower.

**flower, rice** - the reproductive organ consisting of lemma, palea, two lodicules, six stamens, and the pistil.

**foundation seed** - Seed stock produced from breeder seed by or under the direct control of a breeder or a research station. Foundation seed is the source of certified seed, either directly or through registered seed.

## G

**GA<sub>3</sub>** - a form of gibberellic acid which is sprayed on CMS lines to obtain good panicle exertion.

**gamete** - a mature reproductive male or female germ cell, sperm, or egg specialized for fertilization.

**gametic (tissue or generation)** - having 'n' number of chromosomes (haploid), in contrast to zygotic tissue with '2n' (diploid).

**gametocide** - organic or inorganic chemicals used for killing the functional sexual parts (pollen, ovule) of the plant. These may be selective for male or female parts.

**gametophytic** - in this system, the sterility/ fertility reaction is imparted to the pollen by the genetic constitution of the pollen itself and is controlled by a single gene which may have a large number of allelic forms.

**genetic purity** - trueness to type; seeds/plants confirming to the characteristics of the line/variety/hybrid as described by the breeder.

**genetic shift** - change in the genetic makeup of the line/ variety/ hybrid if grown over a long period particularly in areas outside their adaptation.

**genic male sterility** - the type of male sterility governed entirely by the nuclear genes. It may be transmitted by either the male or the female parent.

**germination** - the resumption of growth by the embryo and development of young plant from the seed. Germination, precisely, is the emergence and development from the seed embryo of those essential structures which, for the kind of seed being tested, indicate the ability to develop into a normal plant under favorable conditions in the soil.

**grain** - the ripened ovary and its associated structures.

## H

**heading (flowering), rice** - growth stage of the rice plant marked by the emergence of the panicle from the boot followed by anthesis.

**heritability** - broadly, the proportion of observed variance which is inherited, the remainder being due to environmental effects. Strictly, the proportion of variance due to additive effect of genes.

**heterobeltiosis** - refers to the phenomenon in which an  $F_1$  hybrid obtained by the crossing of two genetically dissimilar parents shows superiority over the better parent in one or a combination of characters.

**heterosis** - refers to the phenomenon in which an  $F_1$  hybrid obtained by the crossing of two genetically dissimilar parents shows superiority over mid-parental values in one or a combination of characters.

**heterosis (standard)** - refers to the phenomena in which the  $F_1$  hybrid obtained by the crossing of two genetically dissimilar parents shows superiority over the best standard check prevailing at that time in one or a combination of characters.

**heterosis breeding** - a method of breeding to develop  $F_1$  hybrid obtained by the crossing of two genetically dissimilar parents.

**heterozygote** - an individual having different alleles for any gene pair and producing two kinds of gametes.

**heterozygous** - hybrid for any gene pair, with different alleles for the gene being considered.

**hill** - a group of rice plants directly adjacent to each other because the seeds or seedlings were planted together. A hill may also consist of only one plant.

**hybrid** - the product of a cross between genetically dissimilar parents.

**hybrid rice** - the  $F_1$  seed of rice bred for commercial use.

**hybrid vigor** - an increased vigor of hybrid over its parents in one or more characteristics.

**hybridization** - a breeding method in which two varieties are crossed to generate new variability and to produce desired recombinants. The hybrids are allowed to self-pollinate and the segregating populations are handled by an appropriate method.

## I

**inbred** - an individual resulting from the mating of closely related parents or by selfing.

**inbred line** - a nearly homozygous line produced by continued self fertilization.

**inbreeding** - the interbreeding of closely related individuals occurring naturally (as in a closed population), or as a deliberately chosen system of breeding and serving especially to preserve and fix desirable characters or to eliminate unfavorable characters from a suitably selected stock but tending to effect an unwanted decline (as in size, vigor, or fertility), through the fixation of undesirable and often recessive characters when the initial stock is in any way defective.



**indoor growth cabinets** - small indoor chambers wherein temperature, humidity, and light are artificially controlled.

**inter sub-specific hybrid** - a cross between different subspecies of a crop. For example in rice, hybrids between indica and japonica lines are considered as inter sub-specific hybrids.

**isolation** - the separation of one group from another so that mating between or among groups is prevented.

**isolation (barrier)** - the separation between two groups can be provided by topography surface features or artificial/natural obstacles to the height of at least 2.5 m, in case of rice.

**isolation-free method** - a method of producing hybrid seed for experimental purpose without isolation but by providing crop barriers of 2-4 lines of the restorer lines.

**isolation (space)** - the separation is provided by keeping a certain distance between two groups. A space isolation of 50-100 meters is ideal for hybrid rice seed production.

**isolation (time)** - the separation is provided by growing two groups at different times of the crop season so that one group is already mature (stopped providing pollen) when the other group comes to flowering. Generally a period of 21 days difference in flowering is sufficient in case of rice.

**isoplasmic** - these are the CMS or restorer lines differing in nuclear genetic constitution but have the common cytoplasm.

# L

**leaf number** - total number of leaves developed on the main culm of a plant which is a characteristic feature of each variety.

**lodicules** - the two scale-like structures adjoining to the base of the palea which control the opening of the lemma and palea during anthesis.

# M

**maintainer line** - a pollinator variety used to pollinate a cytoplasmic male sterile line and produce progenies which still remain male sterile. If there is no maintainer line, the male sterile line can not be maintained and multiplied generation after generation.

**male sterility** - absence or nonfunction of pollen in plants.

**mature grain stage (Rice)** - growth stage in which the individual grain is mature, fully developed in size and is hard, clear, and free from green tint.

**milk stage (Rice)** - stage occurring during the ripening phase when the inside of the grain is at first watery but later turns milky in consistency.

**milling yield** - the estimate of the quantity of head rice and of total milled rice that can be produced from a unit of a rough rice. It is generally expressed in percent.

**multilocation trial** - yield trials conducted in different locations to study the adaptability of varieties/hybrids over environments.

# N

**nuclear genes** - genes located on the chromosomes.

**nucleus seed** - a small quantity of genetically pure seed produced under the strict supervision of the plant breeder.

# O

**off type** - the plants/seeds of the same crop deviating significantly from the characteristics of the variety/hybrid as described by the breeder.

**outcrossing rate** - the extent of cross pollination measured on the basis of seed set to the total number of spikelets.

**outdoor growth cabinets** - the small cabinets located outside where temperature and humidity are artificially controlled while light provided is natural.

**ovary** - the bulbous, basal portion of the pistil containing one ovule.

**overdominance** - superiority of the heterozygote Aa over either of homozygote AA or aa.

# P

**panicle** - the terminal component of a rice plant which bears the rice spikelets.

**panicle development** - the growth stage of the rice plant in which the spikelets become distinguishable and the panicle extends upwards inside the flag leaf sheath.

**panicle exertion** - growth stage of the rice plant marked by the emergence of the panicle from the boot.

**panicle exertion rate** - the extent to which the panicle is exerted out of the flag leaf.

**panicle initiation (Rice)** - growth stage which starts when the primordium of the panicle has differentiated and becomes visible.

**partial restorer** - a pollinator variety used to pollinate male sterile line to produce  $F_1$  male fertile progenies which produce partial seed set upon selfing.

**pedigree** - the record of the ancestry of an individual or a cultivar.

**pedigree nursery** - a nursery consisting of segregating families in different generations derived from different crosses.

**PGMS - photoperiod sensitive genic male sterile line.** The genic male sterile plants which respond to the photoperiod or duration of day length in terms of pollen fertility and sterility behavior.

**phenotypic acceptability** - breeder's shorthand to record his observations on overall acceptability of breeding lines or populations. This can be done using an acceptability score from 1-9. For example: 1 - excellent plant type and absence of diseases. Promote to the next level of testing, and spread to other breeding programs. 3 - very good appearance. Promote to next level of testing. 5 - fair appearance, but has a few essential shortcomings (too early maturity, etc.). Use as parent in hybridization block. 7 - poor appearance, but has a few important traits that make it suitable as a donor. Make a few crosses. 9 - poor. Discard.

**photoperiod** - duration of day length.

**pistil** - the female reproductive organ consisting of the ovary, style, and stigma.

**plant growth substances** - natural and synthetic compounds that elicit growth, developmental or metabolic responses. These substances are usually not metabolites in the sense that they are not intermediates or products of the pathways they control, and they are active at very low concentrations.

**planting ratio** - the ratio in which the male and female parental lines are planted to make a crossing block in hybrid seed production or maintenance of the CMS line.

**plumule** - the leaves of the young plant in any embryo. It is enclosed by the coleoptile.

**pollen** - a mature reproductive male germ cell, microsporocyte, specialized for fertilization.

**pollen fertility/sterility** - the ratio of fertile/sterile pollen grains to the total pollen grains counted in 3-4 fields under a microscope, and expressed in percentage. Fertility/sterility of pollen grains is decided by their stainability with 1% IKI stain.

***Pollen fertility/sterility gradation***

<b>% sterile pollen</b>	<b>Category</b>	<b>% fertile pollen</b>
0 - 20	Fully fertile	81 - 100
21 - 40	Fertile	61 - 80
41 - 70	Partially fertile	31 - 60
71 - 90	Partially sterile	11 - 30
91 - 99	Sterile	1 - 20
100	Completely sterile	0

**pollen load** - the amount of air-borne pollen/liter per hour at peak anthesis on a specified day.

**pollen parent** - male parent of a cross combination.

**pollination** - transfer of pollen from the anther to the stigma of a flower.

**progeny** - offspring; individuals resulting from a mating.

**pure line** - a line that has been rendered almost homozygous by repeated self-pollination over generations.

**purity** - the composition by weight of the sample being tested, and by inference, the composition of seed lot; the identity of various kinds of seeds and inert matter constituting the sample.

## R

**random mating** - a system in which every individual plant in a population has an equal chance of getting pollinated by any other individual.

**randomization** - allotting the treatments to different plots without any bias.

**recurrent selection** - a method of breeding designed to concentrate favorable genes scattered among a number of individuals by selecting in each generation among the progeny produced by mating *interse* of the selected individuals (or their selfed progeny) of the previous generation.

**replication** - repeating the experiment under identical conditions with the objective of reducing the experimental error.

**restorer line** - a pollinator variety used to pollinate the male sterile line to produce  $F_1$  progenies which are male fertile, and thus produce seeds on selfing.

**retestcross** - a cross made between a cytoplasmic male sterile line and a test variety (identified to be a restorer in the testcross) to recheck the potentialities of the  $F_1$  to give normal seed set upon selfing.

**retestcross nursery** - breeding nursery to evaluate the retestcross  $F_1$ s and corresponding male parents.

**ripening phase. (Syn. maturity phase, grain-filling phase)** - the period from pollination to harvest.

**rogue** - a variation from the standard type of a variety or strain. Roguing is the removal of undesirable individuals to purify the stock.

**row ratio** - the proportion of seed parent and pollen parent planted to maintain cytoplasmic male sterile line or to produce  $F_1$  hybrid seed in a seed production plot.

## S

**secondary tillers** - tillers arising from primary tillers.

**second leaf** - the first differentiated leaf with blade and sheath.

**seed** - the fertilized and ripened ovule of a seed plant comprising an embryonic plant accompanied by a store of food (as endosperm or perisperm), enclosed in a protective seed coat, and capable under suitable conditions of independent development into a plant.

**seed dormancy** - the ability of mature seeds to delay their germination after reaching physiological maturity.

**seed parent** - a female parent of a cross combination.

**seed viability** - in general, the state of being alive; ability of the seed to germinate and produce normal seedlings.

**seedbed** - the bed on which rice seeds are sown, consisting of soil (wetbed method) or banana leaves, plastic sheets or concrete floor ("dapog method").

**seeding sequence** - the order of seeding the parental lines based on their growth duration so that they come to flowering at the same time.

**seedling (rice)** - from seed germination to early tillering; a juvenile plant.

**self-fertilization** - fusion of male and female gametes from the same individual.

**source nursery** - breeding nursery where all the genetic material, including sources imparting cytoplasmic male sterility, genotypes with specific traits useful for hybrid breeding program, and elite rice lines showing high general and specific combining ability, are maintained for use in hybrid breeding program.

**spikelet** - the basic unit of the rice inflorescence consisting of the two sterile lemmas, the rachilla, and the floret.

**spikelet fertility** - the number of filled spikelets to the total number of spikelets on a panicle.

**sporophytic** - this system, the sterility/fertility is imparted to the pollen by the plant upon which the pollen is borne and the genotype of the pollen has no bearing *per se*. It may be controlled by more than one gene with multiple alleles.



**staggered planting** - planting the restorer line on different dates to maintain a uniform and regular supply of the pollen to the spikelets of cytoplasmic male sterile line which continues to bloom for a longer period.

**stamen** - the male reproductive organ consisting of the anther and the filament.

**sterile** - failing to produce or incapable of producing offspring.

**stigma** - the apex of the pistil of a flower, upon which pollen is deposited at pollination.

**stigma exertion rate** - the proportion of spikelets with exerted stigma (either one or on both side) to the total number of spikelets in a panicle.

**supplementary pollination** - a method of shaking the male parent at the time of peak anthesis so as to disperse pollen grains to increase the seed set on CMS line. This is particularly necessary when the wind velocity is less than optimum (2-3 m/sec).

**synchronization (anthesis)** - refers to the simultaneous opening of the spikelets of the seed and pollen parents.

**synchronization (flowering)** - refers to the simultaneous flowering of seed and pollen parents despite having different growth durations.

## T

**TGMS (thermo sensitive genic male sterile line)** - the genic male sterile plants which respond to the temperature in terms of their fertility/sterility behavior.

**testcross** - a cross made between the cytoplasmic male sterile line and a test variety to identify maintainers and restorers.

**testcross nursery** - breeding nursery where  $F_1$  progenies of cytoplasmic male sterile lines and test varieties are screened for pollen sterility/fertility and spikelet fertility to identify maintainers and restorers.

**thermosensitivity** - sensitivity of a genotype to varying temperature regimes in terms of pollen or spikelet sterility/fertility.

**three line breeding** - breeding methodology/ where three lines -- viz., cytoplasmic male sterile, maintainer, and restorer -- are used for the production of  $F_1$  hybrids.

**tiller** - a vegetative branch of the rice plant composed of roots, culm, and leaves which may or may not develop a panicle.

**tillering** - growth stage of the rice plant that extends from the appearance of the first tiller until the maximum number is reached.

**topcross** - a cross between a selection, line, clone, etc., and a common pollen parent which may be a variety, inbred line, single cross, etc. The common pollen parent is called the top cross or tester parent.

**two line breeding** - breeding methodology where only two lines, a male sterile (either photosensitive, thermosensitive or chemically induced) and a pollen parent, are used for the production of  $F_1$  hybrid.

## U

**uniformity** - the extent of similarity between the individuals of a population.

# V

**variance** - the mean squared deviation of variates from their mean.

**vegetative phase** - the period from germination to panicle initiation.

**viability** - the ability to grow and develop.

**vigor** - the capacity for natural growth and survival, as of seeds, plants, or animals.

**volunteer plants** - unwanted plants growing from the seed (may or may not be of the same crop) that remains in the field from a previous crop.

# W

**wide compatibility** - the ability of a genotype to produce normally fertile progeny when crossed with both indica and japonica testers.

**wide hybridization** - a process of crossing between distantly related species.







