Principles of Hybrid Rice Seed Production

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1. Introduction

The success of hybrid technology in any crop, largely depends on two essential prerequisites; 1) distinct yield advantage compared to open pollinated varieties and 2) ability to produce hybrid seed on commercial scale at economic level. Unlike other crops, where heterosis has been commercially exploited, rice is basically a self pollinated crop, the requirement of seed per unit area is high. Therefore, development of appropriate seed production technology is essential to exploit heterosis in rice. Hybrid rice seed production practices were standardized initially in China during 1976 which paved the way for commercialization of hybrid rice technology. Rice flowers are not amenable for hand emasculation and pollination to produce hybrid seed on commercial scale. Being a self pollinated crop, rice must involve use of an effective male sterility system to develop and produce hybrids on commercial scale. The three line system of seed production involving CMS, maintainer and restorer lines is being commonly used for large scale hybrid rice seed production in the world. Of late two-line approach involving environmental sensitive genic male sterility is also being practiced in many countries. The general principles of hybrid rice seed production through three and two-line approaches have been outlined in this chapter.

2. Classification of male sterility

It is essential to look at how male sterility manifests in plants before classifying them in to various categories. One of the higher level manifestations is 1) the absence or mal formation of male organs (stamens) in bisexual plants or no male flowers in dioecious plants 2) failure to develop normal microsporogenous tissue – anther 3) abnormal microsporogenesis leading to deformed or inviable pollen 4) abnormal pollen maturation: inability to germinate on compatible stigma 5) no dehiscent anthers but viable pollen – sporophytic control 6) barriers other than incompatibility preventing pollen from reaching ovule. There are basically, two types of classification of male sterility. One is the phenotypic classification which includes structural, sporogenous and functional male sterility. While, the genotypic classification includes genic male sterility, cytoplasmic male sterility and gene-cytoplasmic male sterility.
1) Structural male sterility: anomalies in male sex organs / missing altogether
2) Sporogenous male sterility: stamens form, but pollen absent or rare due to microsporogenous cell abortion before / during / after meiosis
3) Functional male sterility: viable pollen form, but barrier prevents fertilization (anther indehiscence, no exine formation, inability of pollen to migrate to stigma or other factors that affect fertilization.

Generally, male sterility classification and its exploitation is crop plants is on genotypic basis which includes

1) Cytoplasmic Male Sterility (CMS)
2) Cytoplasmic Genetic Male Sterility (CGMS)
3) Genetic Male Sterility
4) Genetically Engineered Male Sterility
5) Chemically Induced Male Sterility
6) Environmental sensitive genic male sterility

In rice cytoplasmic genetic male sterility system involving (three-line) CMS, maintainer and restorer lines is commonly used for commercial hybrid seed production in many hybrid rice growing countries of the world. Of late in addition to three line system, hybrid rice seed is being produced in China by using environmental sensitive genic male sterility system (two-line system).

3. Three line system of hybrid seed production

The cytoplasmic-genetic male sterility (genic-cytoplasmic male sterility) system is the result of interaction between specific sterility inducing cytoplasm and the nuclear genes. To get male sterility expression both sterile cytoplasm and recessive (rf) nuclear genes are required. In other words, a combination of sterile (S) cytoplasm and dominant (Rf) nuclear genes or normal (N) cytoplasm and recessive (rf) nuclear genes result in fertile plants. The CGMS system basically consists of three lines viz., a CMS line (A line), a maintainer line (B line) and a restorer (R) line. Hybrid Seed Production using the CGMS system involves the following two steps.

- Production of `A’ line (A x B)
- Production of Hybrid Seed (A x R)
The `B’ and `R’ lines are multiplied in the same way as inbred varieties.

<table>
<thead>
<tr>
<th>Seed Parent</th>
<th>Maintainer</th>
<th>Seed Parent</th>
<th>Pollen parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A line</td>
<td>B line</td>
<td>A line</td>
<td>R line</td>
</tr>
</tbody>
</table>

```
X
```

```
X
```

A line

Produces unviable pollen grains

Hybrid

Produces viable pollen and sets seeds which are used to plant commercial rice crop

A CMS line is always multiplied by crossing it with its maintainer (B) line. Restorer or `R' line possesses dominant fertility restorer genes, when crossed to a CMS line it restores fertility in the derived hybrid. Since this system involves three lines (A, B and R line) it is called as three line system and the hybrids developed by utilizing this system are called as three line hybrids. In this system of seed production, two major steps involved are (1) CMS multiplication (A x B) and (2) Commercial hybrid seed production (A x R).

3.1 Production of nucleus and breeder seed

Parental lines get contaminated at different stages of handling and, it is necessary to regularly purify them through numbers seed production (at least once in three years). Parental
lines have to be purified under the direct supervision of the rice breeder. Purification process essentially involves four steps: i) Growing the source material (Source Nursery); ii) Test crossing (Test Cross Nursery); iii) Evaluating the test crosses (Identification Nursery); and iv) Multiplication of the lines (Multiplication Nursery).

Breeder seed production involves the further multiplication of A, B and R lines using nucleus seed. The seed material obtained from systematic paired crossing can be used to produce the breeder seed. Breeder seed production has to be taken up in a field where no rice crop is grown during previous crop season. Recommended isolation distance is 300-500 meters.

A row ratio of 2 : 4 and 2 : 6 can be adopted for nucleus and breeder seed production. Utmost care is needed for meticulous rouging as the seed has to be very pure. Other practices are similar to those recommended for hybrid seed production. Further multiplication as foundation seed of A-, B- and R- lines can be done in similar fashion. The seed chain of nucleus, breeder, foundation and certified seed production should be maintained regularly with highest standard of genetic and physical purity at each of the stages. Details on purification of parental lines are given in a separate chapter.

4. Two-line System of hybrid seed production

Besides CGMS system another new kind of genetic (genic) male sterility i.e. Environment Sensitive Genic Male Sterility (EGMS) has been deployed for developing commercial hybrids particularly in rice. In this system, male sterility condition is due to the interaction of nuclear genes with environmental factors such as photoperiod, temperature or both. A particular range, duration or concentration of environmental factor, at sensitive stage of the plant induces male sterility, whereas some other range, duration or concentration induces fertility in the same plant (Virmani and Ahmed, 2001).

1 Environment-sensitive Genic Male Sterility (EGMS) system
Two Commercial Systems for Hybrid Rice

Photoperiod-sensitive
Genic male sterility (PGMS)

Temperature-sensitive
Genic male sterility (TGMS)

Micronutrient-sensitive genic male sterility (MGMS)
The EGMS comprises of the following three types:

i. Photoperiod sensitive genic male sterility (PGMS): The line is sterile when the photoperiod (day light) exceeds 14 hrs and the same line becomes fertile when subjected to photoperiod of < 13 hrs.

PGMS system is useful and can be deployed in temperate countries where the day length differs considerably during different seasons.

ii. Temperature sensitive genic male sterility (TGMS): It is sterile when temperature exceeds 32°C/24°C (day/night) and becomes fertile when the temperature is below 24°C/18°C (day/night). However, in few cases, sterility is observed at lower temperatures and fertility is observed at higher temperatures. Such type of male sterility is referred to as ‘Reverse TGMS type’.

TGMS system can be utilized in tropical and sub-tropical countries, where there are large temperature differences across locations, regions, seasons and at different attitudes. For a vast country like India, with various regions and seasons and with attitude ranging from sea level to several thousand meters in hilly areas, TGMS system is an ideal one for deployment and development of two-line hybrids.

iii. Photo-thermo sensitive genic male sterility (PTGMS): This line is controlled by the interaction of photoperiod and temperature. Most of the PGMS lines earlier discovered such as the classical Nongken 58S were later reported to fall in this category. PTGMS is just similar to the TGMS system in all respects except for the temperature regime in between the CSP and CFP, where the photoperiod sensitivity is observed. At relatively low temperatures, short light hours ensure complete fertility, while at relatively higher temperature, still more short light hours are needed to make it completely fertile.
4.2 Advantages of Two-line v/s Three-line System of Heterosis Breeding

♦ **Wide choice of parental lines; hence increased chances of identifying heterotic hybrids.**

Any genotype can be used as a male parent, unlike in three line system where only those genotypes possessing restorer gene(s) can only be utilized as male parent. Similarly in three line system, only those genotype which show maintainer reaction, can only be converted into CMS lines. Normal frequency of restorers and maintainers in most of germplasm collections, does not exceed 30 percent. So in three line system at best only 30% of the germplasm is useful as parental lines and can be utilized in heterosis breeding. Remaining more than 70% of the germplasm which may posses may useful genes/trait cannot be utilized. There is no such restriction in two-line system. Any genotype can be converted into TGMS line and any other genotype can be utilized as male parent. Hence the chances of developing and identifying heterotic hybrids are greatly increased.

♦ **Seed Production system is simpler and more efficient.**

In two-line system multiplication of female line is very simple, since it is multiplied as any ordinary genotype through self pollination under fertile phase. There are no problems of alternative rows, synchronization, supplementary pollination etc. unlike in three line system where CMS lines are multiplied or maintained utilizing maintainer line through A x B seed production plots.

♦ **Risk of outbreak of epidemics associated with large scale use of unitary source of cytoplasm as well as the negative effects of sterility inducing cytoplasm are avoided altogether.**

One of the major risks in large scale use of unitary source of sterility inducing cytoplasm is the threat of epidemics as has happened in case of maize (susceptibility of T-cytoplasm to leaf blight) and Bajra (susceptibility of Tift 23A cytoplasm to downy mildew). Such risks are avoided altogether in case of two-line system.

♦ **In Rice, two-line system is specifically useful for developing hybrids in Basmati and Japonica type.**

Frequency of restorer gene(s) in japonica and basmati type of rices is very low. It is very difficult to find useable restorers in these types. Two-line system can be easily deployed for
development of hybrids in Japonica and basmati types, since in this system any genotype can be used as male parent.

- Magnitude of heterosis in two-line hybrids is 5 to 10% higher than in three line hybrids.

The major constraints to develop and using TMGS lines in the tropics are:

- Limited availability of stable TGMS germplasm.
- Insufficient training and experience of researchers in breeding and using TGMS lines.

The EGMS lines are multiplied by sowing these lines in such a way that the sensitive period coincides with photoperiod/temperature that is conducive for inducing fertility. The extent of reversion to fertility may vary from 30-80%. Hybrid seed production is taken up by sowing these lines in such a way that the sensitive stage coincides with the photoperiod or temperature conducive for inducing complete male sterility. Detailed studies have shown that the period 5 days after panicle initiation to 10 days before flowering is most sensitive to environmental factors.

Since the PGMS, TGMS and PTGMS are controlled by recessive gene(s), when these lines are crossed with a fertile line, the hybrids are fully fertile, irrespective of the day length and temperature conditions prevailing during the growth season.

Although attractive and potential as a tool for exploiting heterosis, the EGMS system has some problems. During the hybrid seed production, if there is sudden change in the environmental condition, there will be reversion to fertility which may lead to impurity of hybrid seed. Perhaps, this is one of the main drawbacks of EGMS system.

4.3 Seed production of two line hybrids

Seed production of two-line rice hybrids is not much different from that of three line hybrids. Most important consideration is to precisely determine the location or season which is ideal for inducing complete male sterility. Currently the seed yields obtained in two-line hybrid seed production in China range from 2.3 to 3.0 t/ha which is comparable with seed yields obtained
with three line hybrids (Virmani and Ahmed 2001). Hybrid seed production with EGMS lines involved two steps.

### 4.3.1 Multiplication of EGMS lines

EGMS lines are multiplied at appropriate locations and seasons where stable fertility inducing environmental (photoperiod/temperature) conditions prevail for a continuous period of 30 days. Let us take the example of TGMS lines which turn to fertility at lower temperature and the most ideal temperature regime to induce higher fertility is 27/21°C. In this case, the TGMS line has to be planted in such a way that the sensitive stage (5-20 days after planting) occurs in the middle of the fertility inducing phase. The seed yields in EGMS multiplication plots may vary depending upon the critical fertility inducing factors and their duration. If the conditions are highly favourable, seed yields of 4.0 – 4.5 t/ha could be obtained as experienced in China with the PGMS line N 5088 S and 7001 S (Lu et al. 1998).

### 4.3.2 Maintaining the purity of TGMS lines

Maintaining the purity of EGMS lines is extremely important for developing and using two line hybrids. When the EGMS lines are reproduced generation after generation, without any selection, plants in a population segregate with respect to their critical sterility/fertility points. The method of maintaining the genetic purity of EGMs lines is described below by Deng and Fu (1998).

- Selecting about 100 plants with the typical characteristics of the original EGMS lines and planting them separately in pots.
- Transferring the pots at the sensitive stage into a glasshouse with a controlled temperature or phytotron where appropriate temperature and photoperiod are set.
- Monitoring pollen sterility critically at the time of heading and selecting plants with 100 percent sterility.
- Ratooning selected plants in suitable short-photoperiod/low temperature conditions and collecting their selfed seed (nucleus seed).
• Bulking nucleus seed from each selected plant in a row and comparing the agronomic characters and fertility or sterility traits of the selected rows or lines with those of the original line.

• Selected those lines that are identical/similar to the original ones and harvesting. The harvested seed is also called nucleus seed.

• Multiplying nucleus seed to produced breeder seed and multiplying breeder seed to produce foundation seed of EGMS lines.

Virmani et al. (1997) have also described a procedure based on field testing for maintaining the purity and production of nucleus seed of TGMS lines in the tropics. Thus by following these procedures purity of EGMS lines can be maintained.

Foundation seed of EGMS lines is directly used for producing the hybrid seed. All the procedures are similar to those employed for producing three line hybrids. Important requirement is that sowing of the EGMS line for hybrid seed production has to be taken in location/season in such a way that the sensitive stage of the EGMS lines (5-20 days after planting) occurs during the sterility inducing range of photoperiod (>13.75 hrs) and temperature (> 32/24 0C). The EGMS lines are planted with specified row ratios along with the male parent. All other procedures are similar to the production of three line hybrids.

5. Three line breeding-Steps involved in seed production

Hybrid rice seed production requires specialized techniques, which need to be thoroughly understood before embarking upon this venture. The success of hybrid seed production depends on various factors such as choice of field, isolation, seeding time, planting pattern and weather conditions during the period of flowering, roguing synchronization in flowering of parental lines, supplementary pollination techniques, proper harvesting, processing, packing and effective seed distribution etc.

5.1. Choice of location:
Choosing a desirable location for hybrid seed production is very important. In the well isolated area, the paddy field with fertile soil, a desired irrigation and drainage system, sufficient sunshine, and no serious disease and insect problems are essentially needed.
5.2 Isolation:

Rice pollen grains are very small and light, and can travel very far with the wind. In order to ensure the purity of hybrid seed and avoid pollination by unwanted rice varieties, the hybrid seed production plots should be strictly isolated by the following methods.

**Space isolation:** A space isolation of 50 – 100 m would be satisfactory for hybrid seed production, which implies that within this range no other rice varieties should be grown except the pollen parent.

**Time isolation:** Wherever, it is difficult to have space isolation, a time isolation of over 21 days would also be effective. It means that the heading stage of the parental lines in hybrid seed production plot should be 21 days earlier or later than that of other varieties grown within the vicinity.
**Time Isolation**

Sowing/Planting has to be adjusted in such a way that difference between flowering in seed production plot and the neighboring rice crop is at least 21 days.

**Barrier isolation**: In some places, the natural topographic features such as mountains, rivers, forests can serve as the most effective barrier. A crop barrier with maize, sugarcane, sesbania covering a distance of 30 m would also serve the purpose of isolation. Artificial barrier with polythene sheets of about 2 m height can also be used for small scale seed production. However, the most ideal locations are the areas covered with hillocks and mountains, which act as natural barriers.

**Barrier Isolation**

Barriers like plastic sheets, tall crops such as maize, dhahincha, sugarcane etc. can be used.
5.3 Favorable climatic conditions:

Climatic conditions have profound influence on the seed yields. Detailed information on the weather data of a given locality is necessary for fixing the seeding dates. Seeding of the parental lines should be planned in such a way that the flowering coincides with the most favorable climatic conditions, which are as follows:

- Daily mean temperature of 24 – 30°C
- Relative humidity ranging from 70 – 80%
- The differences between day and night temperatures should not be more than 8–10°C, preferably 5 – 7°C
- Sufficient sun shine with moderate wind velocity.
- There should not be rains continuously for three days during the period of flowering.
- Seed yields will be adversely affected if the temperature is below 20°C and above 35°C.
- The Seed Production areas near forest, rivulets and valleys are better for getting higher seed production.

5.4 Seeding of parental lines in the seedbed

- Puddle the seedbed field properly. Puddle the field twice at an interval of 6-7 days to destroy weeds, weed seeds and germinated rice seeds.
- Prepare raised seedbeds (5-10 cm height) of 1m width of any convenient length.
- Provide drainage channels in between seedbeds to drain excess water.
- Apply recommended fertilizer to the nursery beds
- Sow pregerminated seed uniformly on the seedbed (1-2 kg seed/20m²)
- Use 15 kg of ‘A’ line seed and 5 kg of ‘R’ line seed to produce sufficient seedlings to grow one hectare.
- Manage the seedbed properly for getting healthy and vigorous seedlings for transplanting.
5.5 Transplanting

Commence transplanting seedlings of A and R lines as and when they attain the age of 21-25 days, which ensures timely heading, and flowering of parental lines. Transplanting of older seedlings delays flowering and transplanting of younger seedlings advances flowering. If the transplanting of seedlings of `A' line is delayed, then delay transplanting the `R' line seedlings by the same number of days to synchronize flowering. Transplant one or two seedlings per hill of the `A' line and two seedlings per hill of `R' lines.

5.5.1 Transplanting in a specific Row Ratio & Row direction: In hybrid rice seed production the seed parent and pollen parent are planted in a certain row ratio at certain spacing. The row ratio and spacing of pollen parent and seed parent have a distinct effect on the hybrid seed yields.

The row ratio or row proportion refers to the number of rows of the male parent (R line) to that of the female parent (A line) in a seed production plot. Suppose if we plant 2 rows of `R' line followed by 8 rows of `A', the row ratio can be taken as 2:8. In hybrid rice seed production plot the recommended male (R) to female (A) row ratio is 2:8. However, the row ratio may vary from region to region, depending on weather, management and parental lines. R and A lines can be planted in several row ratios of 2:8; 2:12; 3:10 etc.
5.5.2 Factors Influencing Row Ratio: The ratio of pollen parent (R line) to seed parent (A line) is determined by the characteristics of the parental lines.

- Plant height of pollinator
- Growth and vigour of the pollinator
- Size of the panicles and amount of residual pollen
- Duration and angle of floret opening in CMS lines
- Stigma exsertion of CMS lines

To facilitate out crossing, the rows of male and female in the seed production plot should be perpendicular to the prevailing wind direction expected at flowering time of the parents.

5.5.3 Transplanting of the R line

- Transplant the seedlings of R line in paired rows
- Leave a space of 145 cms inside block between paired rows of `R’ line seedlings for transplanting 8 row blocks of `A’ line seedlings.
- Transplant 2-3 seedlings per hill with a row-to-row distance of 30 cms and plant-to-plant spacing of 15 cms.

5.5.4 Transplanting of CMS line (A line)

- Transplant `A’ line seedlings in blocks of 8 rows in between the paired rows of `R’ lines
- Transplant with 1-2 seedlings per hill at a spacing of 15 x 15 cms
- Leave a 20 cms wide alleyway between A line rows and nearest R line row.

Field Layout

```
    R line   A line   R line
X X O O O O O O O X X
X X O O O O O O O X X
X X O O O O O O O X X
X X O O O O O O O X X
X X O O O O O O O X X
```

Wind direction
5.5.5. Transplanting Sequence

The transplanting sequence of seed parent and pollen parent in the hybrid rice seed production plot depends on the growth duration of seed parent (A line) and pollen parent (R line). 5.5.6. Seed parent (A line) has 10 day longer growth duration than pollen parent (R line):

Transplant 25 day old seedlings of the ‘A’ line, 10 days earlier than the second ‘R’ line seedlings. The seedlings of the R line are transplanted when the seedlings from the second R line seeding are 25 days old. At this time the age of seedlings from the first R line seeding will be 21 days old and the age of seedlings from third R line seeding will be 29 days old.

Table – 1: Seeding Sequence and seedlings age for transplanting
### 5.5.7. Seed parent (A line) has 10 day shorter growth duration than pollen parent (R line):

The seedlings of the R line are transplanted when the seedlings from the second R line seeding are 25 days old. At this time the age of seedlings from the first R line seeding will be 21 days old and the age of the seedlings from the third R line seeding will be 29 days old. Later transplant 25 days old seedlings of the A line 10 days later than the second R line seedlings.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Seed/pollen parent</th>
<th>Seeding sequence</th>
<th>Seedling age for transplanting (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A line</td>
<td>0 day</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>First R line</td>
<td>6th day</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>Second R line</td>
<td>10th day</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>Third R line</td>
<td>14th day</td>
<td>29</td>
</tr>
</tbody>
</table>

### 5.5.8. Seed parent (A line) has same growth duration as pollen parent (R line):

The planting of both R and A lines can be done simultaneously. First complete the A line plantings with 25
day old seedlings followed by R line plantings with the seedlings ages of 21 day old first R line, 25 days old second R line and 29 days old third R line.

Table – 3: Seeding Sequence and seedlings age for transplanting

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Seed/pollen parent</th>
<th>Seeding sequence</th>
<th>Seedling age for transplanting (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>First R line</td>
<td>0 day</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>Second R line and A line</td>
<td>4th day</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Third R line</td>
<td>8th day</td>
<td>29</td>
</tr>
</tbody>
</table>

5.6. Roguing

The purity of hybrid rice seeds used in commercial production must be more than 98%. To meet this requirement, the purity of the restorer and CMS lines must be more than 99%. Therefore, in addition to ensuring strict isolation, it is necessary to remove all rogues from the seed production plots. Roguing is the removal of undesirable rice plants from the hybrid seed production plots. Undesirable rice plants are those plants either in A or R line rows that differ from plants that are true to type. Roguing helps to prevent the off-types from cross pollinating the true to type A line plants and thus enhancing the purity of hybrid seed.

The undesirable plants come from many sources. They may be voluntary plants from the previous crop. Contamination due to improper isolation also result in the occurrence of off-types. Admixing during the process of harvesting, threshing, packing and handling are also other sources from which the off-types occurred. Therefore, due care is to be taken to remove the off-types during the cropping season.
Roguing can be done at any time during the crop stage. Off-type rogues can be removed whenever they are identified – earlier the better. The most important stages for roguing are at maximum tillering, flowering and just before harvesting.

5.6.1 Roguing at maximum tillering: We can identify the off-types by their morphological differences from the true to type plants. Therefore, it is essential to know the characteristic features of parental lines, which help in easy identification of rogues and efficient roguing. As a basic step, any plant found out side the rows has to be removed as they may be volunteer plants. Remove all those plants which are either too tall or too short than the seed or pollen parent. We can also identify the off-type plants by difference in their leaf blade size, shape and leaf sheath colour.

5.6.2 Roguing at flowering: Roguing at flowering is extremely important as it is the stage when we can identify many off-types which look similar to the parental lines during the early stages of growth. All the off-type plants that flower very early or very late are to be removed. The plants which differ from parental line plants in respect of leaf size, shape, angle, panicle shape, size and pigmentation are to be carefully removed. Remove all the plants from A line that have plumpy yellow anthers. Plants in the A line should not have fertile pollen. The off-types in A lines can also be distinguished from their fully exserted panicles. Care should be taken to remove the plants which are highly infested from pests and diseases.

5.6.3 Roguing just before harvest: This is the last opportunity to keep away the off-types in order to maintain high purity. Before harvesting, the plants in A line rows are to be thoroughly checked and those plants which show normal seed set are to be removed. It is necessary to remove all the off-types that have different grain characters as compared to that of A line plants. The grain size, shape, colour and pigmentation of A line plants have to be critically examined for effective roguing.

5.7 Promotion of exertion of panicle: Most of the male sterile lines based on WA cytoplasm have imperfect exertion of panicle, with the result as much as 15% spikelets remain enclosed in the flag leaf and are not exposed for
out crossing. Through following methods, the exertion of the panicles can be promoted to a great extent.

5.7.1. Application of gibberellic acid (GA$_3$): It is an efficient and effective growth hormone, which stimulates the cell elongation, thus can be used to enhance panicle exertion in CMS line. Besides, GA$_3$ has the following favorable effects:

i. Increases the duration of floret opening

ii. Increases stigma exertion and receptivity

iii. Promotes plant height

iv. Influences flowering and hence flowering in parental lines can be adjusted

v. Widens the flag leaf angle

vi. Promotes exertion and growth rate of secondary and tertiary tillers.

In hybrid seed production plots of rice, 5-10% panicle emergence stage is most appropriate for first spraying (40%) and the remaining 60% of GA$_3$ should be sprayed on the following day. The ideal time for spraying is from 8 to 10 AM and from 4 to 6 PM. Spraying should be avoided during cloudy weather and when the wind velocity is high. A dose of 45-60 g/ha of GA$_3$ is optimum. The hormone does not dissolve in water and it should be dissolved in 70% alcohol (1 g of GA$_3$ in 25-40 ml of alcohol).

5.7.2 Flag leaf clipping: Normally the flag leaves are erect and longer than the panicles and they come in the way of easy pollen dispersal thus effecting the out crossing rate. The clipping of flag leaf helps in free movement and wide dispersal of pollen grains to give higher seed production. The flag leaves should be clipped when the main culms are in booting stage. Only half or two-third portion of flag leaf should be removed. However, flag leaf cutting is not advisable in the plots infested with diseases as this operation may spread the disease further.
Flag Leaf Clipping

Crop Stage for Flag Leaf Clipping: When primary tillers are at booting stage

Do not clip the flag leaves in plots infected with BLB, BLS or Sheath blight

5.8. Supplementary pollination:

Rice is basically a self-pollinated crop and hence there is a need to go for supplementary pollination in order to enhance the extent of out crossing. Supplementary pollination is a technique of shaking the pollen parent so that the pollen is shed and effectively dispersed over the A line plants. Supplementary pollination can be done either by rope pulling or by shaking the pollen parent with the help of two bamboo sticks. Timing and frequency of supplementary pollination is very important. The first supplementary pollination should be done at peak anthesis time i.e. when 30-40% of the spikelets are opened. This process is repeated 3 – 4 times during the day at an interval of 30 minutes. Supplementary pollination has to be done for 7-10 days during the flowering period.

Supplementary pollination

To be done at peak anthesis time (30-40% of the spikelets are opened)
Repeat 3-4 times at 30 minutes interval
Continue for 7 to 10 days
5.9. Harvesting, threshing and processing

From the point of view of maintaining high purity, extreme care is needed while harvesting, threshing and processing of the hybrid rice plots.

5.9.1 Harvesting: Harvest all R lines rows first. Remove the R line harvest and keep it in a safe place separately. Carefully remove the left over R line panicles in the field.

5.9.2 Threshing: During threshing, the ‘A’ line parent and ‘R’ line parent harvests must be kept separate from each other. The A and R lines should be threshed separately. Before starting threshing, all the threshing equipment, threshing floor and tarpaulin to be thoroughly cleaned.

Use new gunny bags for storing the seeds. Prepare two labels for each bag – one to place inside the bag and one to attach to the bag outside. Each label should contain the following information.

1. Name and Address
2. Name of the parent
3. Name of the location
4. Season and year
5. Date of harvest

5.9.3 Seed drying:

- Seed drying helps seeds maintain their ability to germinate and their vigour for a longer period.
- Drying controls mold growth and the activity of the other organisms, that reduce the quality of stored grain
- Drying reduces seed discoloration
- Seeds can be safely stored when they have been dried to a moisture content of less than 13%.
**Seed drying methods:** Seeds can be dried by two methods viz., sun-drying and forced air-drying.

**Sun drying:** The seeds can be dried by placing them on jute bags or on a tarpaulin. Do not dry the seeds directly on the concrete threshing floor. While drying, stir the seeds occasionally to ensure uniform drying.

** Forced air-drying:** Seeds can be dried in a batch – type dryer by forced air heated to 40-45°C. The seed layer in a batch type drier should not be more than 45 cm deep. Dry the seeds slowly and do not dry abruptly to 13% moisture content.

**Seed Processing:** Seed Processing has to be done to remove impurities like trash, leaves, broken seeds sand etc., weed seeds and to remove immature, shriveled, unfilled and empty spikelets.

Seed processing usually done by public and private seed agencies by using Air screen machines. Air screen machines in addition to cleaning the seeds, grading also will be done by separating the seeds of uniform size from over size and under size seeds.

For **seed certification standards for paddy hybrids**, the manual on “Indian Minimum Seed Certification Standards” published by The Central seed Certification Board (Department of Agriculture & Cooperation, Ministry of Agriculture, Government of India, New Delhi, July 1988, pp 20-22) may kindly be referred.