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## Techniques hybrid seed production in rice

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### Abstract

Commercialization of hybrid rice is linked to the development of hybrid rice seed production. Rice is basically a self-pollinated crop and the requirement of seed per unit area is high; therefore, the development of hybrid rice exploits the phenomenon of hybrid vigor and involves raising a commercial crop from  $F_1$  Seeds. Research at IRRI indicates that development of hybrid rice technology offers opportunities for increasing rice varietal yields by 15-20% compared to results achievable with improved, semi dwarf and inbred varieties. Rice must involve use of an effective male sterility system to develop and produce hybrids on commercial scale; and as for now, there are only two ways to produce hybrid rice seed successfully and these include three-line and two-line system. The three line system of seed production is used for large scale hybrid rice seed production in the world; while as two-line approach involving environmental sensitive genetic male sterility for successful hybrid rice seed production

**Keywords:** Rice, hybrid, technology, genetic engineering, sterility, emasculation

### Introduction

On 22 February 2018, at Yogyakarta, Indonesia-The International Rice Research Institute (IRRI) and the Indonesian Agency for Agricultural Research and Development conducted a 3 day conference to address the newest solutions toward addressing the hybrid rice adoption, constraints, challenges and future in Southeast Asia (IAARD, 2018) [19]. This 7<sup>th</sup> International Hybrid Rice Symposium (IHRS 2018) is grounded on the theme *Food Security through Hybrid Rice under Changing Climatic Conditions*. The symposium addresses the Hybrid Rice Genetics and Breeding, Seed Production, New Technology Applications, Crop and Resource Management, Hybrid Rice Economics, and National Policies and Public-Private Engagement on international level.

Rice is an important cereal crop and is most widely consumed staple food for a large part of the world's human population. The first hybrid rice variety for commercial cultivation was released by China in 1976. In 1964, the father of hybrid rice, Professor Long-Ping-Yuan and his group started research on heterosis of rice with Indica type rice. Then in November, 1970 the discovery of male sterile line in Hainan Province, make a breakthrough in rice breeding. In India, the Hybrid Rice program was launched in 1989, through a systematic, goal oriented and time bound network project with the financial assistance from Indian Council of Agricultural Research (ICAR). The government of India has set a target of expanding the cultivation of hybrid rice to 25% of the area occupied by the crop by 2015 (Spielman *et al.* 2013) [24]. Indian Agriculture Research Institute (IARI) released the India's first basmati hybrid *viz.* Pusa RH-10. So far, 43 hybrids have been released for commercial cultivation. Among these, 28 have been released from the public sector while remaining 15 have been developed and released by the private sector. More than 80% of total hybrid rice area is in U.P, Jharkhand, Bihar, Chhattisgarh, Punjab and Haryana. Total 15 million ha (50%) of rice in China are hybrid varieties, producing 103.5 million MT of paddy annually (an average yield of 6.9 mt/ha). The remaining 50%, planted in inbred high-yielding varieties, produces 81 million mt (5.4 mt/ha). Thus, on average hybrid rice in China yield about 27% (1.5 mt/ha) more than the inbred high-yielding varieties.

### Pollination and Sterility in Rice

Since rice is a self-pollinated crop, hybrid seed production must be based on male sterility systems which include cytoplasmic genetic male sterility (CGMS) system, thermo-sensitive genetic sterility (TGMS) system or photo-sensitive genetic male sterility (PGMS) system, both PGMS and TGMS are combined called as environmental genetic male sterility (EGMS) system. The oldest and most popular technology used for development of commercial rice hybrids in China and elsewhere. The cytoplasmic genetic male sterility system popularly known as the three-line system, utilizes lines, cytoplasmic male sterile line (A line), a maintainer (B line), and a restorer (R line). For the commercial production of hybrid, it

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it involves two major steps viz., (i) multiplication of the A line (female sterile parent) by crossing it with maintainer or B line and then (ii) crossing of A line with restorer or R line to produce hybrid seed. TGMS and PGMS systems are also gaining popularity.

### Male sterility

It is essential to look at how male sterility manifests in plants before classifying them into various categories. One of the higher level manifestations is

- i. The absence or malformation of male organs (stamens) in bisexual plants or no male flowers in dioecious plants.
- ii. Failure to develop normal micro-sporogenous tissue-anther.
- iii. Abnormal microsporogenesis leading to deformed or inviable pollen.
- iv. Abnormal pollen maturation: inability to germinate on compatible stigma.
- v. No dehiscent anthers but viable pollen-saprophytic control.
- vi. Barriers other than incompatibility preventing pollen from reaching ovule.

There are basically, two types of classification of male sterility viz., phenotypic and genotypic classification. Phenotypic classification of male sterility includes structural, sporogenous and functional male sterility. The genotypic classification of male sterility includes genetic male sterility, cytoplasmic male sterility and cytoplasmic genetic male sterility.

### Genetic Male Sterility (GMS)

Genetic male sterility is ordinarily governed by single recessive gene, *ms*, occurs widely in plants, and in a given plant species, several different *ms* genes act monogenically to produce male sterility. Genetic male sterile line is maintained by crossing recessive male sterile plants (*ms*) with heterozygous male fertile plants (*Ms*). Such cross will yield 50% sterile plants and 50% fertile plants. The male sterile plants are used as female parent in the development of hybrid. The fertile plants are rouged out. Crosses between recessive male sterile plants and heterozygous male fertile plants are carried out every year for maintaining the male sterility.

### Types of genetic male sterility

#### Environmental sensitive genetic male sterility (EGMS)

The male sterility where the expression of male sterile gene (*ms*) depends on a specific range of temperature and photoperiod regimes is called as environmental sensitive genetic male sterility (EGMS). This type of male sterility has been observed in rice and other crops such as tomato, cotton and some varieties of wheat. The environmental sensitive genetic male sterility is further divided into two groups viz.

#### (a) Temperature sensitive genetic male sterility (TGMS)

The sterility which is influenced by temperature is called as thermo sensitive genetic male sterility e.g. in rice, the sterile plants become fertile at temperature below 23 °C and at 23 °C or above, complete male sterility is produced by the *ms* gene.

#### (b) Photosensitive genetic male sterility (PGMS):

The sterility which is drastically influenced by the prevailing photoperiod or day length is called as photosensitive genetic male sterility (PGMS) e.g. in rice, long day condition leads to

male sterility and short day produces male fertility providing the temperature ranges between 23-29 °C.

### ii. Transgenic or genetically engineered male sterility

The male sterility which is induced by the technique of genetic engineering is called as transgenic or genetically engineered male sterility. Transgenic male sterility has been induced in tobacco and rapeseed by transferring a gene from *Bacillus amyloliquefaciens*.

### Cytoplasmic Male Sterility (CMS)

The pollen study which is controlled by cytoplasmic genes or plasmagenes is known as cytoplasmic male sterility. This system consists of *a* line and *B* line. *A* is male sterile and *B* is male fertile. The cytoplasmic male sterile is maintained by crossing of *a* line with *B* Cytoplasmic Genetic Male Sterility (CGMS)

This type of sterility is governed by both nuclear and cytoplasmic genes. Here, nuclear genes for fertility restoration (*Rf*) are available. The fertility restorer gene *R*, is dominant and is found in certain strains of the species, or may be transferred from a related species.

The parents and steps involved in CGMS or three line systems are briefly described below:

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

**b) B line:** It is isogenic to a line and is used as pollen parent to maintain male sterility in a line. This line is maintained by growing in isolation, at least 5 m away from any rice variety.

**c) R line:** This is also called as fertility restorer or pollinator line. This is used in hybrid seed production by growing along with a line in a standard row ratio. It is also maintained by growing in isolation, at least 5 m away from any rice variety.

### Chemically Induced Male Sterility

The chemical which induces male sterility artificially is called as male gametocides. It is rapid method but the sterility is non-heritable. In this system A, B and R lines are not maintained. Some of the male gametocides used are gibberellins (rice, maize), Sodium methyl arsenate and zinc methyl arsenate (rice) and Maleic hydrazide (wheat, onion).

### Three-line breeding

In the Source Nursery, each line is grown isolated from each other and any other outside plants as to avoid accidental cross pollination until the right time. The restorer and maintainer lines are selfed here along with CMS line maintaining. After the correct number of *a* line and *R* line seeds are made, the test-cross and re-test cross phases are next.

### Two-line breeding

The second method for producing hybrid rice uses a plant type that has an abnormality in the section of gene that dictates whether the plant is male fertile or sterile depending on day-and/or temperature-length. This type of plant is known as Photo or Thermal-sensitive Genetic Male Sterile variety (P [T] GMS).

### Hybrid Seed Production Mechanism in Rice

A sufficient number of pollen grains must be deposited on the stigma lobes of each spikelet of the seed (male sterile) parent for successful hybrid production. It helps if the pollen parent grows to a greater height than the seed parent. Other plant characteristics which influence this include small and horizontal flag leaves, the number of panicles per square meter, the number of spikelets per panicle, good panicle exertion, and synchronized flowering of seed and pollen parents. In China, conditions favorable for good out-crossing in rice have been identified as: a daily temperature of 24-28 °C, a relative humidity of 70-80%, a diurnal difference in temperature 8-10 °C and sunny days with a breeze. Suitable field conditions include: fertile soil, a dependable irrigation and drainage system and a low risk of disease and insect infestations.

### Guidelines for Hybrid Rice Seed Production

Extensive research has led to the identification of the following guidelines for successful hybrid rice seed production.

- Selection of seed and pollen parents with synchronized time of anthesis.
- Selection of seed parents with long, exerted stigma, longer duration, and wider angle of floret opening.
- Selection of a pollen parent with a high percentage of residual pollen per anther after anther exertion. High pollen shedding potential is attained by getting 2000-3000 spikelets/m<sup>2</sup> to bloom per hour during peak flowering period.
- Synchronization of flowering time of the two parents by seeding them at different dates depending on their growth duration or estimated accumulated temperature requirements for initiation of flowering.
- Use of optimum seed parent: pollen parent row ratio such that the ratio of spikelet number per unit area of seed parent and pollen parent is about 3.5:1.
- Use of seed and pollen parents with small and horizontal flag leaves, or cutting long and erect flag leaves.
- Use of gibberellic acid (GA<sub>3</sub>) to improve panicle exertion and prolong duration of floret opening and stigma receptivity.
- Planting of seed parent pollen parent rows across the prevailing wind direction and use of supplementary pollination with a rope or stick when wind velocity is below 2.5 m/sec.
- Selection of optimum time of flowering of parental lines in seed production plots.

### Vegetative Propagation of Hybrid Rice

Vegetative propagation of rice is well-known. Adult plants can be raised from seedlings, tillers, culm cuttings or by ratooning. Clonal propagation of rice was proposed in early sixties to exploit hybrid vigor in rice. However, no serious attempt was made to develop this as a technology. Since the commercialization of hybrid rice technology in China in 1976, agronomists and farmers have tried using vegetative propagation in various ways to reduce the seed rate, and hence the seed cost, of commercial rice hybrids. These approaches include double transplanting and ratooning.

Hybrid rice seed production technology is considered labor- and knowledge-intensive. It involves various risks, especially in the early stages when seed producers are still lacking in experience. Typical problems are poor synchronization of the parental lines, and unfavorable weather. At present,

commercial production of hybrid rice seed outside China is limited to India, Vietnam, the United States and the Philippines.

### India

Government of India has not fixed any target for increasing acreage of hybrid rice in the country unlike Philippines. However, efforts are being made to promote cultivation of hybrid rice through various crop development programmes such as National Food Security Mission, Bringing Green Revolution to Eastern India and Rashtriya Krishi Vikas Yojana (Vadlamani, 2016) [21]. Studies conducted in the Indian states of Andhra Pradesh, Karnataka and West Bengal showed that on average, labor accounted for about 48% of the total cost of hybrid rice seed. Hybrid seed production requires additional labor for extra farm operations such as thin and row planting, supplementary pollination, filling gaps, rouging, GA<sub>3</sub> application, and manual harvesting, threshing and cleaning. It is estimated that around 3 + Million Hectares is under Hybrid Rice cultivation in India in 2016 which is around 7% of the Total Rice cropped area in India (Vadlamani, 2016) [21].

**Table 1:** Biological yield (q ha<sup>-1</sup>) of the rice as influenced by varieties, N level and Time of application

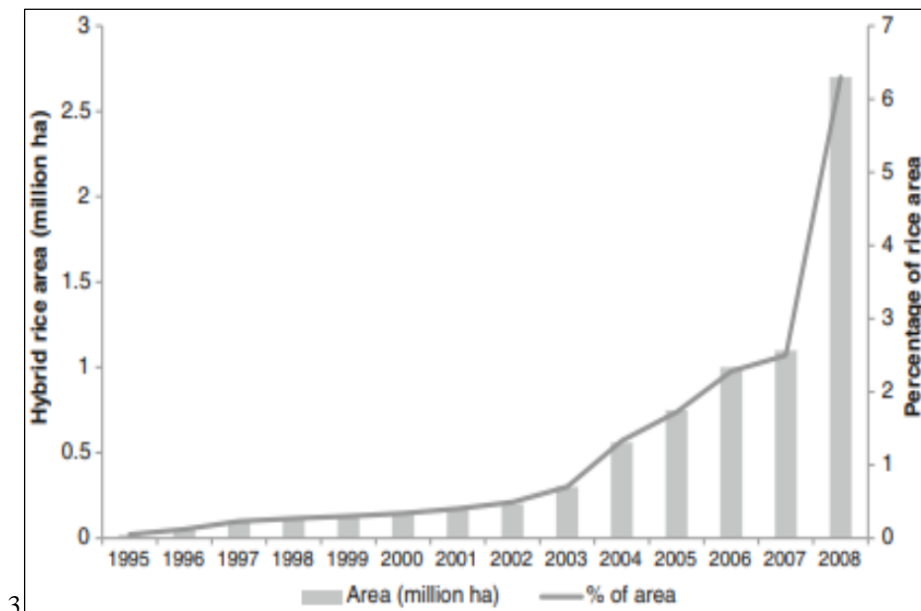
Treatment	Biological Yield (q/hac.)		
	Variety	2003	2004
PAC (832)	132.5	128.77	130.60
PAC (801)	131.20	127.07	129.15
F-test	NS	NS	-
SEM	-	-	-
CD. (P=0.05%)	-	-	-
<b>Nitrogen Levels</b>			
100	123.57	120.20	121.85
150	132.58	128.41	130.45
200	139.74	135.93	137.80
F-test	S	S	-
S.E.M	3.03	3.57	-
CD. (P=0.05%)	7.67	7.88	-
<b>Time of N-application</b>			
T1=1/2B+1/4MT+3/4P <sup>1</sup>	138.84	134.93	136.85
T2=1/2B+1/3MT+1/2P <sup>1</sup>	131.65	127.07	129.30
T3=2/3B+1/3MT	125.30	122.70	124.00
F-test	S	S	-
S.E.M	3.03	3.57	-
CD. (P=0.05%)	7.67	7.88	-

(Source Tanweer-Ul-Hussain, 2006)

**Table 2:** Harvest Index (%) of Rice as influenced by varieties, N-levels, and their time of application

Treatment	Harvest Index (%)		
	Variety	2003	2004
PAC (832)	70.73	71.37	71.05
PAC (801)	70.94	71.39	71.17
F-test	NS	NS	-
SEM	-	-	-
CD. (P=0.05%)	-	-	-
<b>Nitrogen Levels</b>			
100	70.33	71.00	70.87
150	71.13	71.21	71.17
200	71.05	71.09	71.07
F-test	S	S	-
S.E.M	0.38	0.43	-
CD. (P=0.05%)	0.76	0.76	-
<b>Time of N-application</b>			
T1=1/2B+1/4MT+3/4P <sup>1</sup>	69.44	69.80	69.62
T2=1/2B+1/3MT+1/2P <sup>1</sup>	70.62	71.94	71.28
T3=2/3B+1/3MT	72.44	72.40	72.24
F-test	S	S	-
S.E.M	0.38	0.43	-
CD. (P=0.05%)	0.76	0.87	-

(Source Tanweer-Ul-Hussain, 2006)



**Fig 1:** Area under hybrid rice cultivation in India, 1995-2008. Source: Authors' calculations based on Baig (2009) and Francis Kanoi Marketing Research (2009)

### China

In China, the production of hybrid rice seed takes about 375 mandays/ha, 75 mandays/ha more than ordinary rice.

The cost of female parental line seed (A seed) and gibberellic acid ( $GA_3$ ) (additional inputs for hybrid seed production) accounted for 9% of the total cost. The average cost of producing hybrid seed was US\$0.52/kg. The private sector was able to buy hybrid seed from seed growers at US\$0.85/kg, while government agencies paid US\$1.0-1.1/kg. At an average price of US\$1.0/kg, with an average seed yield of 1250 kg/ha, hybrid rice seed production was 65% more profitable than ordinary rice cultivation.

### Vietnam

Vietnam, because of its socio-economic and political structure, has been able to develop a strong research-extension-farmer linkage in seed production and distribution, similar to that of China. The top priority for Vietnam's hybrid rice program is to develop an efficient system for the production and distribution of hybrid seed at affordable prices. At present, about 20% of hybrid rice seed requirements are met by seed produced in Vietnam, while the remaining 80% is imported from China. About 600 ha in Vietnam were under hybrid rice seed production in the year 2000, compared to only 173 ha in 1992. Average seed yields increased from about 300 kg/ha to 2500 kg/ha during this period. According to Vietnam's Ministry of Agriculture and Rural Development, hybrid rice seed requirements are about 24,000 mt, if hybrid rice cultivation is to be increased to 800,000 ha by 2005.

### Future Demand

Successful development and large-scale dissemination of hybrid rice technology will have a major impact on the seed industry, in view of the fact that rice is a staple food crop in Asian countries. Usually, the private sector does not play much of a role in the early stage of technology development. At this stage, the public sector has to play a leading role.

There is a growing linkage between the public and private sectors, not only in sharing genetic resources, but also in the form of private sector support for public research. Some small seed companies have also started joint ventures with big

multinational companies for the production and/or import and marketing of hybrid rice seed in Bangladesh, Vietnam, the Philippines etc.

Some farmers' organizations have also begun hybrid rice seed production, either independently or as a joint venture with a private seed company. These developments may reduce the cost of hybrid rice seeds to as little as US\$20-35/ha. This would promote the further adoption of hybrid rice production by small-scale farmers.

### Constraints to Hybrid Seed Production in Asia

There are many constraints to the adoption of hybrid rice seed production technology. These are discussed below:

- Hybrid rice technology in countries outside China was adopted only a few years ago. Seed growers need time to become familiar with the technology, and need more experience of hybrid seed production at specific locations under local climatic conditions.
- There is a shortage of skilled manpower at the initial stages of seed production technology. Seed production can only be successful if it is supervised by well-trained technicians and carried out by experienced growers.
- Hybrid rice seed production is highly dependent on use of gibberellic acid ( $GA_3$ ). Outside China this is quite expensive (more than US\$1.00 per gram), because it is imported. In China it is quite cheap (US\$0.30 per gram) because it is produced locally, so it does not cost much to apply a high dosage of this growth regulator.
- The purification of parental lines is vital to the success of any hybrid seed production program. However, it has not received enough attention. Consequently, parental lines used for hybrid seed production are not pure, which results in poor-quality hybrid seeds.
- Hybrid rice seed production requires a well-organized seed industry. Apart from China, most countries in Asia have not yet achieved this for hybrid rice seed.
- Since hybrid rice technology is new in most countries, government policies do not yet motivate and encourage hybrid rice seed production on a large scale.
- The linkage between public sector research institutes and seed production agencies working on hybrid rice is weak.

### Future Outlook

Without hybrid rice technology, the world would require 6 million ha more land to produce the same quantity. Thus, the technology has contributed not only to food security, but it has also helped indirectly to protect the environment.

- Further genetic improvement of flowering behavior and floral traits of seed (e.g. exerted stigma) and pollen parents (e.g. abundant pollen);
- Modification of seed production practices; and,
- Selection of the most favorable seasons and locations for seed production.

GA<sub>3</sub> application is an important component of hybrid rice seed production technology. Its cost is very high in many countries. High seed yields in China have been achieved with a very high dosage of 150-300 grams per hectare. Outside China, it is essential to reduce the cost of GA<sub>3</sub>, either by reducing the dosage or by producing cheap GA<sub>3</sub> locally. Alternatively, a cheaper substitute for GA<sub>3</sub> needs to be found. The discoloration of hybrid rice seeds caused by tropical fungi is an important problem which needs to be tackled. In China and northwestern India, CMS lines in hybrid rice seed production plots have been found to have a higher incidence of seed-borne diseases (such as paddy bunt, caused by *Neovossia horinda*, and Tak and False Smut caused by *Ustilogonoids virens*) compared to the pollen parents. This can cause a serious outbreak of these diseases in commercial crops of hybrid rice, and therefore needs more attention.

Apomixis is the ultimate genetic tool to develop true breeding hybrids and facilitate commercial exploitation of heterosis by even resource poor farmers. (Apomixis is reproduction involving specialized tissue but not dependent upon fertilization: an example is parthenogenesis). So far, there is no confirmed report of apomixis in rice. However, research is in progress in Mainland China, at IRRI, and elsewhere, to discover, induce, and/or genetically engineer such rices.

### Conclusion

The Significant cross-pollination occurs in male sterile lines. This has made possible the development of economically viable hybrid rice seed production systems in and outside China, yielding 1-3 mt/ha of hybrid rice seeds. More than 30 public and private seed companies outside China are currently involved in developing and/or commercializing hybrid rice technology. These companies are creating additional rural employment opportunities. In the United States, the seed production system has already been mechanized. For resource-poor rice farmers, the prospect of vegetative propagation of rice hybrids is being explored. Already about 0.61 million ha of paddy land outside China is planted in hybrid rice. This area is likely to increase to about 2 million ha over the next five years. It has also created additional rural employment opportunities. Outside China, the same phenomena are now being seen, and their impact will be felt during the next five to ten years.

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