

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Hybrid Rice Research: Current Status and Prospects

*Diptibala Rout, Debarchana Jena, Vineeta Singh,  
Manish Kumar, Pandurang Arsode, Prakash Singh,  
Jawahar Lal Katara, Sanghamitra Samantaray  
and Ramlakhan Verma*

## Abstract

Heterosis is a solitary means of exploiting hybrid vigor in crop plants. Given its yield advantage and economic importance, several hybrids in rice have been commercialized in more than 40 countries, which has created a huge seed industry worldwide. India has made commendable progress and commercialized 117 three-line *indica* hybrids for different ecology and duration (115–150 days), which accounted for 6.8% of total rice area in the country. Besides, several indigenous CMS lines developed in diversified genetic and cytoplasmic backgrounds are being utilized in hybrid rice breeding. NRRI, which has been pioneering to start with the technology, has developed three popular rice hybrids, viz., Ajay, Rajalaxmi, and CR Dhan 701 for irrigated-shallow lowland ecosystem. Biotechnological intervention has supplemented immensely in excavating desirable genomic regions and their deployment for further genetic enhancement and sustainability in rice hybrids. Besides, hybrid seed production creates additional job opportunity (100–105 more-man days) and comparatively more net income (70% more than production cost) than HYVs. Hence, this technology has great scope for further enhancement in *per se* rice productivity and livelihood of the nation.

**Keywords:** hybrid rice, CMS, genetic gain, heterosis, restorer, breeding value

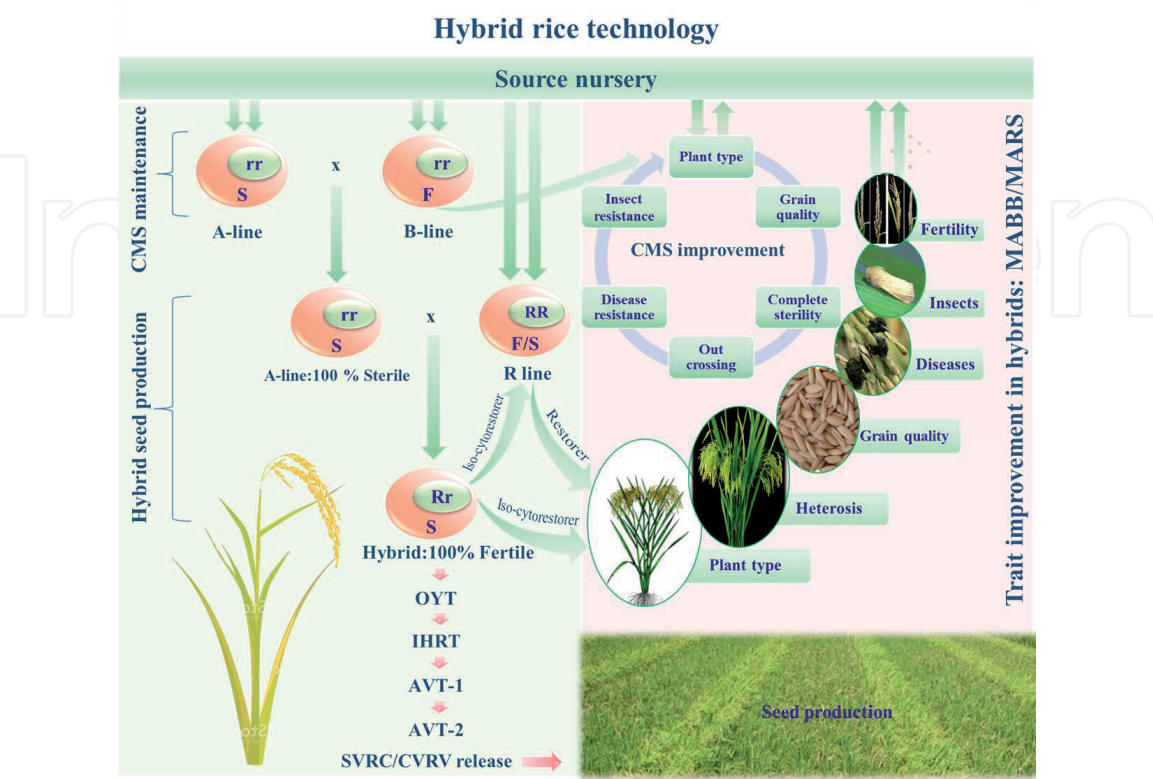
## 1. Introduction

Heterosis is the superiority of  $F_1$  offspring over either parent, a solitary means of harnessing complete hybrid vigor in crop plants. This phenomenon has aided agriculture and captivated geneticists for over centuries for the development of superior cultivar in many crops [1]. Suitable allelic combination and manipulation has made yield advantage in hybrid than HYVs. It covers large acreage for many crops, including rice, and has affected agrarian practices and the seed business across the world. Heterosis had been exploited in several practical ways for centuries before Darwin provided an early scientific explanation in maize. In rice, heterosis was first reported by Jonse [2]. However, owing to its self-pollinating nature (0.3–3.0% out-crossing), heterosis could be realized during middle of second half of the twentieth century after identification and development of the cytoplasmic male sterile (CMS) source. Subsequently, China, under the leadership of Yuan Long Ping, started work

on the development of hybrid rice (HR) with a vision to make it possible to be commercial. He identified a natural male sterile mutant plant in rice (*indica*) and pollen abortive genotypes in the wild rice (*Oryza rufipogon*; Li 1970), which later served as donor of male sterile source (male sterile cytoplasm) for CMS development. In 1973, through recurrent back-cross breeding, several promising *indica* wild abortive CMS, viz., Erjiunan1A, Zhenshan 97A, and V20A CMS-WA, and good restorers, viz., Taiyin1, IR4, and IR1, were developed. Later during 1974, first *indica* rice hybrid, Nanyou 2, was released for cultivation in China. Afterward, relatively more heterotic hybrid rice (HR) breeding approaches like two-line system (1987 AD) and super hybrids (1996 AD) were adopted which supplemented substantially toward Chinese food security and livelihood.

In India, systematic research on hybrid rice was initiated during 1989 when the Indian Council of Agricultural Research (ICAR) launched a special goal-oriented and time-bound project, “Promotion of Research and Development Efforts on Hybrids in Selected Crops,” for rice at 12 network centers. Around 4 years (1989–1993) of rigorous research efforts have rewarded substantially, and India became the second country after China to develop and commercialize hybrid rice. The first hybrid variety APRH-1 was released by APRRI, Maruteru, for Andhra Pradesh in 1993–1994. So far, 117 rice hybrids (36 from public organization and 81 from private sector) were developed, suitable for different ecology and duration ranging from 115 to 150 days, covering 3.0 mha, which accounted for ~7.0% of the total rice acreage in India (Varietal Improvement, Progress report) [3].

Hybrid rice technology has substantial yielding ability that is able to enhance farm productivity ~15–25% more than inbred varieties. Given its yield advantage and economic importance, several hybrids in rice have been commercialized in more than 40 countries, which has created a huge seed industry worldwide. Moreover, this venture also has great service opportunity and generates additional employment for the poorer [1]. However, it has some limitations in generation of



**Figure 1.** A schematic representation of hybrid rice technology (seed production, trait improvement, yield evaluation, etc.).

hybrids, seed production, and marginal heterosis. Success of the hybrid depends on their parental combination, adaptability, and allelic interactions, and hence, faces several problems like unstable male sterility (MS), non-abundance in cytoplasmic diversity, inherited CMS load, low seed producibility in seed parent, poor grain and eating quality, lack of responsive parents for biotic and abiotic stresses, hybrid sterility, marginal heterosis in *indica* hybrids, etc. This chapter deals with information on: (i) research status of HR, (ii) breeding system and methods involved in hybrid rice development and production, (iii) trait-specific parental line improvement, (iv) molecular dissection of genes and QTLs for parental line improvement, and (v) economic opportunity (**Figure 1**).

## 2. Breeding component and system in hybrid rice development

Rice is a strict self-pollinated crop; commercial exploitation of heterosis requires some parental specificity which could exclude manual emasculation. The invention of naturally occurred male sterility (MS) in rice thus played substantial role in realization of heterosis in rice. Following are the genetic tools as mentioned in various heads are required for development and commercialization of hybrid in rice:

### 2.1 Male sterile system

The male sterility (MS) in plants is the condition where the male reproductive organ, anthers, loses its ability to dehisce and produce viable pollen and thus encourages the allogamous nature of reproduction. This is crucial breeding tools to harness heterosis that exclude additional efforts of emasculation which is cumbersome process. In plants, male sterility is conditioned either by mitochondrial or nucleus genome or in associations. The male sterility in plant was first observed by Joseph Gottlieb Kolreuter in 1763 and later it was reported in >610 plant species. In rice, it was reported by Sampath and Mohanty [4] at ICAR-NRRI (formerly CRRI), Cuttack by studying the differences in male fertility in *indica/japonica* reciprocal crosses. The male sterility in plant is found to be determined by several biological as well as environmental factors. In rice, it is conditioned either by cytoplasmic genes in association with nuclear genes (CMS) or nuclear genes alone (GMS) which cause abnormal development in sporogenous tissue (either sporophytic or gametophytic tissue). The sporophytic male sterility is governed by genetic constitutions of sporogenous tissues like tapetal and meiocytes which creates improper nourishing to developing microspores and cause pollen abortion, whereas in gametophytic male sterility, microspore and pollen development get affected. Sporophytic male sterility is quite useful in hybrid rice breeding as it gets fertile in heterozygous state and encourages complete fertility in resulting hybrids. To date, several types of male sterile system, viz., cytoplasmic male sterile (CMS), environment sensitive male sterile (GMS), viz., thermo-sensitive genetic male sterility (TGMS), photo-sensitive genetic male sterility (PGMS) and reverse photo-sensitive genetic male sterility (rPGMS), etc. have been identified and substantially being utilized in hybrid development (**Table 1**).

### 2.2 Diversity in male sterile system and their mechanism

The CMS is a maternally hereditary trait instigated by improper communication between cytoplasmic and nuclear genome [5]. Gene(s)/genic block(s)-conditioned cytoplasmic male sterility is chimeric construct, which evolved due to rearrangement of the mitochondrial genome (**Figure 2**). In rice, several types of CMS have

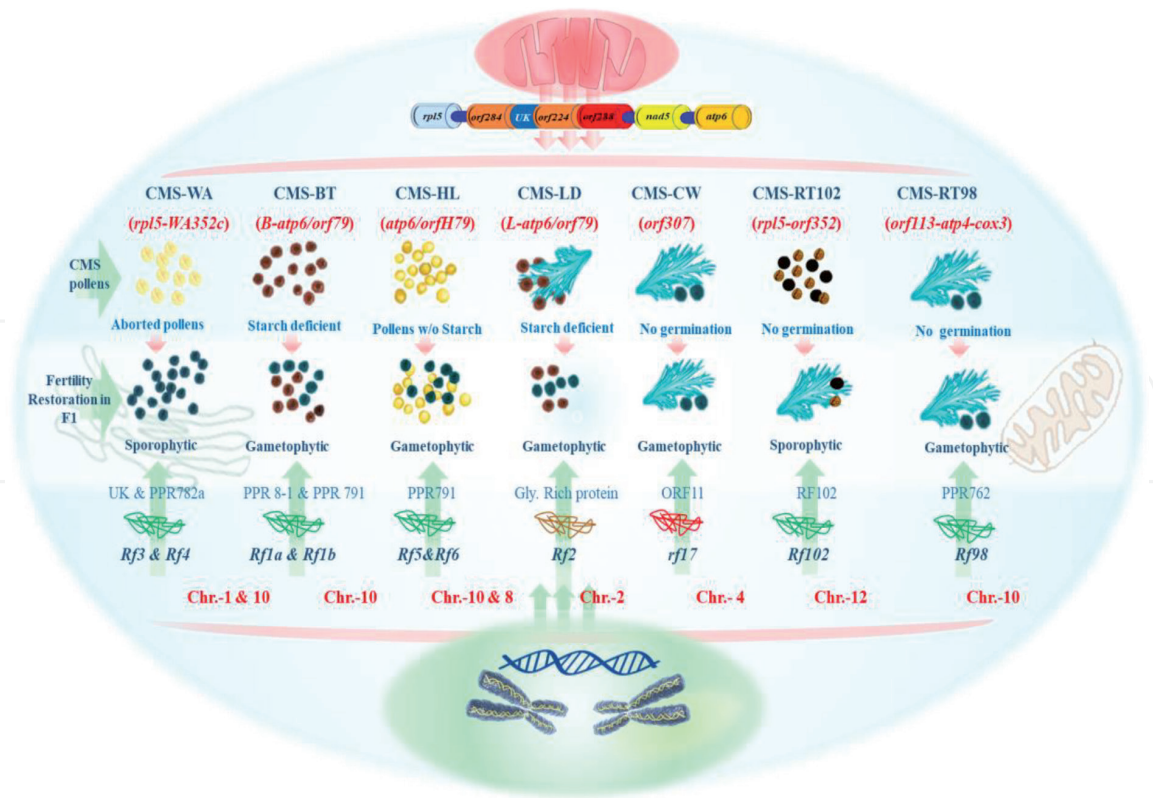
CMS group	Associated ORF	Protein	Cytoplasm source	Representative CMS-line
1. Cytoplasmic male sterile line				
a. BT-CMS and their lineage				
BT-CMS (G)	<i>B-atp6-orf79</i>	Membrane protein	Chinsurah Boro II/ Taichong 65	Liming A, Xu 9201A
LD-CMS (G)	UK	UK	Lead Rice (Burmese <i>indica</i> variety) × Fujisaka 5 ( <i>japonica</i> variety)	Fujisaka 5A
Dian1-CMS (G)	UK	UK	Yunnan high altitude landrace rice ( <i>indica</i> ) cytoplasm	Yongjing2A, Ning67A
HL-CMS (G)	<i>atp6-orfH79</i>	Membrane protein	Red-awned wild rice ( <i>Oryza rufipogon</i> ) cytoplasm	Yuetai A, Luohong 3A4
b. WA-CMS and their lineage				
WA-CMS (S)	<i>rpl5-WA352</i>	Membrane protein	Wild abortive rice ( <i>Oryza rufipogon</i> ) cytoplasm	Zhenshan97 A, V20A, IR58025A, CRMS31A, etc.
Kalinga-I-CMS (S)	UK	UK	Kalinga-I ( <i>indica</i> ) cytoplasm	CRMS 32A
D-CMS (S)	UK	UK	<i>Indica</i> rice Dissi D52/37	D-Shan A, D62A
DA-CMS (S)	UK	UK	Dwarf abortive rice ( <i>Oryza rufipogon</i> ) cytoplasm	Xieqingzao A
GA-CMS (S)	UK	UK	Gambiaca ( <i>indica</i> ) cytoplasm	Gang 46A
ID-CMS (S)	UK	UK	Indonesia paddy rice ( <i>indica</i> ) cytoplasm	II 32A, You1A
K-CMS (S)	UK	UK	K52( <i>japonica</i> ) cytoplasm	K-17A
CMS-RT102 (S)	<i>rpl5-orf352</i>	Membrane protein	<i>Oryza rufipogon</i> , W1125	RT102A
CMS-RT98A (G)	<i>orf113-atp4-cox3</i>	Membrane protein	<i>Oryza rufipogon</i> Griff, W1109	RT98A



CMS group	Associated ORF	Protein	Cytoplasm source	Representative CMS-line
LX-CMS	UK	UK	Luihui rice ( <i>indica</i> ) cytoplasm	Yue 4A
Maxie-CMS	UK	UK	MS mutant of Maweizhan ( <i>indica</i> ) with Xieqingzao ( <i>indica</i> )	Maxie A
NX-CMS	UK	UK	Selected from F2 male sterile plants in the progeny of Wanhui 88 ( <i>indica</i> ) × Neihui 92-4 ( <i>indica</i> ) nucleus	Neixiang 2A, Neixiang5A
Y-CMS	UK	UK	Yegong ( <i>indica</i> landrace) cytoplasm	Y Huanong A
CW-CMS (G)	<i>orf307</i>	Mitochondrial protein	<i>Oryza rufipogon</i> Griff.	IR24A, IR64A
2. Environment-sensitive genetic male sterility (EGMS)				
PGMS	<i>pms3</i>	Noncoding RNA	Nongken 58S, PGMS mutant of <i>japonica</i> cultivar Nongken 58	7001S, N5088S
P/TGMS	<i>p/tms12-1</i>	noncoding RNA	Photoperiod and temperature sensitive genic male sterile (P/TGMS) derived from Nongken 58S	Pei'ai 64S
TGMS	<i>tms5</i> , <i>RNase Z</i> <sup>S1</sup> (loss in function)	Nuclease enzyme	Spontaneous TGMS mutants of Annong S-1 and Zhu 1S	Guangzhan 63S5, Xinan S
rPGMS	<i>csa</i> OsMST8	MYB transcript regulator	Carbon starved anther ( <i>csa</i> ) mutant of <i>japonica</i> cultivar 9522	9522S
Note: “S” stands for sporophytic male sterility and “G” stands for gametophytic male sterility.				

**Table 1.**  
Cytoplasmic diversity in rice CMS.

been identified and characterized, having diversified mechanism in MS expression. Wild abortive (WA-CMS), a sporophytic MS system, is widely utilized in hybrid development. It is found to be caused by a constitutive mitochondrial gene WA352c



**Figure 2.** Schematic presentation of rice CMS types, where WA stands for wild abortive, BT is for boro type, HL for Honglian, LD for lead rice, CW is for Chinese wild rice, RT102A and RT98A, respectively.

located downstream of *rpl5* (comprised four mitochondrial genomic segments, *orf284*, *orf224*, *orf288*, and *cs4-cs6*) and encodes a putative protein (352-residue) with three transmembrane segments. The WA352c inhibits nuclear-encoded mitochondrial protein COX11 (essential for the assembly of cytochrome c oxidase, TCA) and triggered premature tapetal programmed cell death and pollen abortion [6]. In contrast, BT-CMS is a gametophytic MS reported in the Indian rice variety, Chinsurah Boro-II, in which pollen development get arrested at the tri-nucleate stage. The mitochondrial chimeric (dicistronic) gene *B-atp6-orf79* encodes a transmembrane protein, cytotoxic peptide ORF79 [7], which accumulates preferentially in the microspore, was found to be responsible for male sterility. The *orf79* reside downstream to the *atp6* and interact with P61 and mitochondrial complex III and impair the activity of this complex which lead to dysfunctional energy metabolism and elevate oxidative stress and thus causing sterility. However, in HL-CMS, which is also a gametophytic MS system, pollen development gets arrested at di-nucleate stage. A chimeric aberrant transcript of the mitochondrial gene *atp6-orfH79*, located downstream of *atp6* is confirmed as candidate gene of this MS. Transcript of *orfH79* gene preferentially accumulates in mitochondria which interacts with P61 (a subunit of ETC complex III) and impairs mitochondrial function [8] and leads to MS. The MS in CW-CMS is conditioned by mitochondrial *orf307*, which causes anther-specific mitochondrial retrograde regulation for nuclear gene expression. It is a gametophytic MS in which pollen grain appears normal but unable to germinate.

2.3 Genetic male sterility (GMS)

The GMS in rice is conditioned generally by recessive nuclear genes and exert showing normal Mendelian inheritance. Owing to difficulties in their maintenance (occurrence of only 50% sterility in F<sub>1</sub>), GMS could not be part of rice hybrid breeding program. Some GMS lines has shown threshold nature in MS expression

where male sterility occurs in specific environmental regime (high temperature and long day length); hence called environment sensitive genetic male sterile (EGMS). The GMS line shows male sterility at elevated temperature, that is,  $>30^{\circ}\text{C}$  is called temperature sensitive male sterility (TGMS) whereas male sterility in long day length, that is,  $>13.5$  h is called photoperiod-sensitive genetic male sterility (PGMS). The male sterility in EGMS line is found to be revert into male fertile in favorable temperature ( $<30^{\circ}\text{C}$ ) and day length ( $<12.5$  h) which provide its unique opportunity to be utilized in hybrid rice breeding program. The rice lines exert MS impression under long photoperiod and elevated temperature are referred as P/TGMS, for example, Pei'ai 64S. The EGMS lines, PGMS-Nongken 58S (NK58S) and TGMS-Annong S-1 and Zhu1S or derivatives are utilized extensively in majority ( $>95\%$ ) of the two-line hybrid program. Among, derivatives of NK58S are exerts either P/TGMS or even TGMS (e.g., Guangzhan 63S), the mechanism underlying to such dramatic changes yet to be revealed. Recently, a novel type of EGMS (*csa*-carban starved anther mutant) in rice called rPGMS (reverse PGMS). These lines expresses MS under short photoperiod ( $<12.5$  h) and revert to normal fertile when exposed to long days ( $>13.5$  h). This is found to be suitable for seed production of two-line hybrids in tropics and subtropics [9].

## 2.4 Transgenic cytoplasmic male sterility

The genetically engineered male sterile line M2BSin rice is developed by transformation of *indica* rice maintainer M2B with partial-lengthHcPDIL5-2a (Hibiscus cannabinus protein disulfide isomerase-like) genetic construct. Male fertility in this CMS is reported to be arrested due to tapetum degeneration which leads pollen abortion. The genetic analysis revealed this MS a maternally inherited inability as of CMS. Besides, by combining cysteine-protease gene (BnCysP1) of Brassica napus with rice anther-specific P12 promoter (promoter region of *Os12bglu38* gene), a transgenic MS system was successfully created which is restored by transgenic rice plants carrying BnCysP1Si silencing system [10]. Zhou and co-workers [11] could develop 11 “transgene clean” TGMS lines by editing most widely utilized TGMS gene *tms5* through CRISPR/Cas9.

## 2.5 Genetics of fertility restorer gene

The rice CMS is found to be restored by nuclear genome, that is, mono or oligo nuclear loci called restorer gene. In rice, a total of 10 *Rf* genes (*Rf1a*, *Rf1b*, *Rf2*, *Rf3*, *Rf4*, *Rf5*, *Rf6* and *Rf17*, *Rf98* and *Rf102*) have been identified, of those seven (*Rf1a*, *Rf1b*, *Rf2*, *Rf4*, *Rf5*, *Rf17*, and *Rf98*) are characterized. All *Rf* genes are found to be dominant in nature (except *Rf17*, restores fertility in CW-CMS), which can restore male fertility in heterozygous state. Restorer genes are very specific to male sterile genome in the mechanism of fertility restoration. Genes *Rf1a* and *Rf1b* (Chr.-10) encode pentatricopeptide-repeat (PPR)-containing proteins and have functional affinity of fertility restoration in BT-CMS; RF1A promotes endonucleolytic cleavage of the *atp6-orf79* mRNA and RF1B promotes degradation of *atp6-orf79* mRNA [7] and revert the male sterility into fertility. Whereas, HL-CMS is restored either by *Rf5* or *Rf6* gene, these genes can produce 50% normal pollen grains in F1 plants individually; however, both genes in complementation could restore more than 80% spikelets' fertility in hybrids. The *Rf5* encodes a PPR family protein PPR791 and which bind with GRP162 (glycine rich protein) and *atp6-orfH79* transcripts and makes a RFC (restoration of fertility complex). The RFC cleave the aberrant transcript of *atp6-orfH79* at 1169 nucleotides position [12]. The *Rf6* gene encodes a novel PPR family protein (duplicate PPR motif 3–5)



S.No.	Rf genes	Locality	Marker	CMS system	Restorer line	Causative gene	Encoded product	Reference
1	Rf1a, Rf1b	Chr-10	InDel-Rf1a	CMS-BT	BTR, IR24, MTC10R; C 9083	PPR8–1, PPR791, Rf1A, Rf1B	PPR	[13]
2	Rf2	Chr-2	CAPS42–1	CMS-LD	Kasalath, Minghui 63	LOC_ Os02g17380.1	Gly. Rich protein	[14]
3	Rf3	Chr-1	DRRM-Rf3–10	CMS-WA	Swarna, PUSA 33	—	PPR	[15]
4	Rf4	Chr-10	RM6100	CMS-WA	IR 24, Pusa 33, CRL 22R	PPR782a	PPR	[15]
5	Rf5(t)	Chr-10	RM3150	CMS-HL	Milyang 23	PPR791	PPR	[16]
6	Rf6	Chr-10 & 8	RM5373	CMS-HL	—	—	—	[16]
7	Rf17	Chr-4	AT10.5–1, SNP 7–16	CMS-CW	CWR	PPR2	RNA interference	[17]
8	Rf98	Chr-10	UK	CMS-RT98A	RT98C	PPR762	PPR	[18]
9	Rf102	Chr-12	UN	CMS-RT102A	RT102C, K102-Oryza rufipogon. T	UK	UK	[19]

**Table 2.**  
*Restorer genes in rice plants.*

which in association with hexokinase (*osHXX6*) targets mitochondria and process defective transcript of *atp6-orfH79* at 1238 nucleotide position. Thus, PPR protein family cause editing of aberrant transcript, inhibit their translation, and at the end, fertility restoration. Besides, male fertility in WA-CMS is found to be counteracted by *Rf3* and *Rf4* genes (chrom.-1 and 10, respectively). The genes *Rf3* and *Rf4* encode a pentatricopeptide protein (PPR) where RF4 cleave the abnormal WA352 transcript and RF3 suppress translation of WA352 into polypeptide and helps in restoring fertility in WA-CMS. Fertility in LD-CMS is reported to be restored by either *Rf1* or *Rf2*. The *Rf2* gene encodes a glycine-rich protein in mitochondrial; replacement of isoleucine by threonine at amino acid 78 of the *RF2* protein causes functional loss of the *rf2* allele. Moreover, CW-CMS is reported to be restored by a single recessive gene (*Rf17*) which is a retrograde-regulated male sterility (*rms*) gene (**Table 2**) [20].

2.6 Breeding system

Commercial hybrid seed production in rice where natural out-crossing (ranged only 0.3–3.0%) is very low, cumbersome, and an expansive task. To be practical and readily adoptable, it requires some specific parental requirements and agro-man-agement practices. Invention of male sterile lines thus provided unique opportunity to start with the technology in rice. Based on mechanism of male sterility, threshold nature in male sterility expression and number of parental lines used, three types of hybrid seed production system namely three-line system (involving three parents, A, B, and R), two-line system (two parents, A and R), and one-line system (apo-mictic-based) exist. Among them, CGMS-based three-line system is more suitable,

hence widely utilized (>90% of world's hybrids developed utilizing this) in hybrid rice varietal development and seed production.

### 2.6.1 CGMS system

This system involves three parents such as male sterile line (A-line, cytoplasmic male sterile), B-line (maintainer), and R (restorer) lines and two steps in seed production, that is, CMS multiplication and hybrid seed production under strict isolation (spatial or temporal or physical barrier). Male sterile line (A-line), because of their eliminated manual emasculation needs, served as seed parent and facilitates large-scale seed production. A suitable CMS line to be utilized as seed parent should have complete and stable male sterility, substantial seed producibility, wide compatibility, and good combining ability with minimum CMS load. The wealthy panicle and narrow semi-erect leaf configuration in seed parent has additional impact, assures more seed production. In Indian perspective, hybrid seed production is a major dilemma, generally keen to *Rabi* season, hence, CMS lines should have substantial cold tolerance at seedling stage and heat at flowering stage.

The maintainer (B-line), on the other hand, is an isogenic to the CMS line (differs only for fertility/sterility) in their genetic constitution, able to produce functional pollen and maintain the sterility in male sterile line/seed parent. The maintainer line can maintain 100% male sterility in seed parent thus utilized to perpetuate CMS with their inherent male sterile ability.

In contrast, restorer line can restore male fertility in  $F_1$ s produced on male sterile parent, thus utilized as pollen parent in hybrid seed production. A good restorer should have substantial genetic distance with seed parent which is prerequisite and major determinant of the extent of heterosis in hybrids (more genetic distance more heterosis and *vice-versa*). Restorer is the major contributor of heterosis in three-line hybrids, hence, should have good combining, strong fertility restoration ability (dominant *Rf* gene(s) responsible for fertility restoration in CMS). Besides, restorer line with ideal plant type, acceptable grain quality parameters, substantial source-sink balance, heavy pollen load, and broad spectrum of resistance/tolerance against multiple biotic/abiotic stresses is imperative in maximization of genetic gain in hybrids.

### 2.6.2 EGMS system

This system is a simple and more efficient hybrid breeding system in rice, involves only two parents, that is, A and R line in seed production, thus, referred as two-line system. This is a threshold of genetic male sterility (EGMS)-based hybrid rice breeding system, where male sterility is conditioned in specific environmental regimes such as long photoperiod (>13.5 h day length) and at elevated temperature (>30°C). In this system, male sterile parents are to be maintained by selfing under favorable conditions (below critical sterility point, i.e., <30°C temperature and at below CSP of photoperiod length, <12.5 h.).

Two-line hybrid seed production system is an easy and effective alternative to CMS and has specific advantages as it requires only one step for seed production. In this system, any good combiner genotype irrespective of their fertility restoration ability can be utilized as a pollen parent. EGMS system is normal and does not exert any ill effect in the growth and development of carrier plant, and thus, exploits comparatively higher extent of heterosis (up to 5–10%) in  $F_1$  than the CGMS-based three-line system. The EGMS traits are governed by major genes, thus are easily

transferable to any genetic background; besides, no CMS load could be helpful in reducing the potential vulnerability among the hybrids. Because of its eliminating needs for restorer genes in the male parents, this is ideal for developing inter-subspecific (*indica/japonica*) hybrids.

### 2.6.3 One-line system (apomictic-based)

In this system, seeds of rice hybrid once generated need not to be further produced in the hybrid seed production plot. This system is solely based on apomixes phenomenon (embryo developed apart from mixing of sexual gametes/fertilization) where the embryo developed without fertilization. In this system, hybrid seeds once generated will be maintained through apomixes in their original heterozygous form. The apomictic embryo is formed in the ovule via two fundamentally different pathways, sporophytic or gametophytic, which define the origin of the apomictic embryo [21]. In sporophytic apomixes, the embryo arises directly from the nucellus or the integument of the ovule in a process generally called adventitious embryony. In gametophytic apomixis, two mechanisms are generally recognized, diplospory and apospory. In both of these, an embryo sac is formed and the two mechanisms are distinguished by the origin of the cells that give rise to the apomictic embryo sac. In diplospory, the embryo sac originates from megaspore mother cells either directly by mitosis and/or after interrupted meiosis. In apospory, the embryo sac originates from nucellar cells. In both gametophytic mechanisms, the resulting nuclei forming the embryo sac are of the same ploidy as those found in the female parent because the reduction division cycle of meiosis does not occur. The embryo arises autonomously from one of the cells in the embryo sac.

In a recent adventure, Delphine et al. reported three genes such as SPO11-1, REC8, and OSD1 in the sexual model plant *Arabidopsis thaliana*, which were combinationally mutated to turn meiosis into mitosis and its nourishing tissue from the female gametophyte without contribution of a male genome. This results in the production of clonal male and female gametes, but leads to doubling of ploidy at each generation when self-fertilized. Crossing a *MiMe* plant as male or female with a line whose genome is eliminated following fertilization (lines expressing modified CENH3) leads to the production of clonal offspring [22]. The *MiMe* technology was also implemented in rice to get diploid gametes. Furthermore, a study was conducted by Reda et al. to induce apomixis and fix heterosis in the sterile Egyptian Hybrid1 line using 0.2% colchicines [23]. It was observed that as colchicine is an alkaloid, which during cell division binds to tubulin protein of the spindle fiber and stops microtubules formation, and during meiosis, it prevents chromatids separation and inhibits cytokinesis. So ultimately, colchicines lead to meiosis aberrations, which produce aberrant microspores, pollen sterility, ovule sterility, as well as loss of fertility. Recently, a strategy based on the advanced technique, that is, CRISPR/Cas 9, has been utilized to introduce apomixis into rice (*Oryza sativa*) by mutating the three combined genes OsSPO11-1, OsREC8, OsOSD1, and OsMATL to get a *MiMe* phenotype [24].

## 3. Progress in hybrid rice research and development

### 3.1 International status

Hybrid technology is one of the greatest innovations in the modern era, contributed greatly in yield enhancement in several important crops. Over the decades of rigorous research, Chinese could develop parental lines, that is, cytoplasmic male-sterile line, maintainer line, and restorer line which assisted in the realization

of heterosis exploitation in rice. Subsequently, hybrid seed production system was refined and world's first hybrid rice was released for commercial cultivation during 1974 AD. The first generation wild abortive CMS line, that is, Zhenshan 97A was widely utilized and several elite hybrid rice varieties were commercialized. Besides, several CMS with altered genetic mechanism of male sterility expression were also identified and characterized.

At beginning, low seed producibility with WA-CMS was a concern for its commercialization. However, with the keen interest of agronomist, management practices for hybrid seed production were sustainably rationalized. The Chinese government has supported this venture in pilot mode and established large and effective hybrid rice seed businesses in the late 1970s at all levels. Besides, intensive mechanization of hybrid seed production helped in modification of planting ratio (2R: A as 6–8 rows to 40–80 rows) and reducing the cost of production. Therefore, China could achieve seed yield by 2.7–3.0 t/ha on a large scale in hybrid rice seed production, which is further enhanced to 3400 kg/ha and maximizes their acreage.

Over past three decades, hybrid rice varieties have been substantial for national food security in the China which accounted for approximately 57% of the total 30-million-hectare rice planting area. The Ministry of Agriculture, China, has launched project on super hybrid rice development during 1996 which resulted altogether 73 super hybrids (52 three-line hybrids and 21 two-line hybrids) for commercial cultivation. Super hybrid P64S/E32 released recently has recorded new height of yield potential of 17.1 t/ha with some striking characteristics [25].

Beside China, this technology has also been introduced and promoted by more than 40 countries around the world. At beginning, IRRI helped technically and supplied prerequisite parental materials. Later, most of the countries could establish their own hybrid rice breeding program and developed several heterotic hybrids. India was the second country after China that adopted this technology in 1989 and made substantial progress. At present, hybrid rice covers around 3.0 mha in India that has 6.8% of total rice area. Vietnam was the next to adopt this technology in 1992, harnessing yield of 6.3–6.8 t/ha from 0.7 mha, which covers around 10% of their rice area. In Philippines, it was introduced in 1993. Several popular hybrids like Magat, Mestizo, Mestizo 2, Mestizo 3, Bigante, Magilla, SL8H, Rizalina 28, etc. were developed and commercialized. Hybrid seed production in Philippines has been handled by “seed growers” cooperatives that are to produce around 60–70%. In Bangladesh, several rice hybrids were introduced and commercialized from China, India, and Philippines. They are almost self-sufficient in hybrid seed production, producing around 8000 tons to cover about 800,000 ha. In order, Indonesia also has substantial hybrid rice area, developed several good rice hybrids like Hipa7, Hipa 8, Hipa9, Hipa10, Hipa11, Hipa12 SBU, Hipa13, Hipa14 SBU, Hipa Jatim1, Hipa Jatim2 and Hipa Jatim3 were extensively commercialized, having yield superiority of 0.7–1.5 tons/ha over the lowland inbred varieties.

USA has adopted this technology during 2000 and has developed and commercialized several two-line and three-line hybrids. Most of the hybrid rice cultivars in USA employed Clearfield (CL) technology offering selective control of weedy red rice. Rice hybrids, viz., Clearfield XL729, Clearfield XL745, Clearfield XP756 (a late maturing) and Clearfield XP4534 (new plant type) has shown yield advantage ranging from 16 to 39% over inbred cultivars are being commercialized by RiceTec.

### **3.2 National status**

In India, systematic hybrid rice research was initiated in 1989. The first hybrid rice was released in Andhra Pradesh during 1993–1994 and India became the second



country after China to commercialize hybrid rice. India has made substantial progress and developed total 117 (*indica/indica*) rice hybrids having 15–20% yield superiority with 115–150 days duration for various rice ecosystems. Recently, Savannah Private Limited from India has made another landmark by developing two two-line rice hybrids, viz., SAVA-124 and SAVA-134, for commercial cultivation. In addition, more than 100 CMS in diversified genetic and cytoplasmic backgrounds have been developed and utilized. Among, the promising CMS lines CRMS 31A, CRMS 32A, CRMS 8A, PMS10A, PMS 17A, APMS 6A, DR8A, PUSA 5A, PUSA6A, RTN 12A, etc. are substantially being utilized in development of rice hybrids in India and abroad. Notably, medium-duration seedling stage cold-tolerant CMS, CRMS 32A, developed at NRRI under Kalinga-I cytoplasm is more suitable for development of hybrids for *boro* ecosystem. Two popular hybrid rice varieties, namely, Rajalaxmi and KRH 4 were developed using CRMS 32A as one among the parent.

Hybrids released in India having unambiguous specificity like specific to ecosystem, tolerant to several abiotic/biotic stresses and consumer preferences (**Table 3**). These hybrid varieties can be utilized to up scale the hybrid rice cultivation and productivity enhancement *per se* in the respective area.

Hybrids like CRHR 105, CRHR 106, 25P25, 27P31 are suitable for high-temperature regime which has a more deleterious effect on seed development in hybrids. The hybrid varieties, US 382, Indam 200–17, US 312, DRRH3, and JKRH 401 having high N use efficiency are thus found suitable for cultivation in N-deficient soil. Besides, hybrids PNPH 24, RH 1531, and Arize Tej are under mid-early maturity group which can sustain substantially under drought situations. The problems of coastal and shallow lowland ecosystem sharing around 32% of total rice area can be addressed by adopting long-duration hybrids like CRHR 32, Arize Dhani, CRHR 34, CRHR 102, and Sahyadri 5 (**Table 4**).

3.3 ICAR-National Rice Research Institute’s contribution

The ICAR-National Rice Research Institute, Cuttack has been pioneer to start with the technology in late of seventh decade of last century, quite before the beginning of their project mode program in 1989 by ICAR. In the beginning, ICAR-NRRI has acquired all the prerequisite materials (CMS lines, viz., V 20A, Yar Ai Zhao A, Wu10A, MS 577A, *Pankhari* 203A, V 41A, Er-Jiu nanA, respective maintainers, nine other maintainers, and 13 restorers) from the IRRI (NRRI annual report 1981–1982). Systematic hybrid rice breeding was initiated in an interdisciplinary mode with objectives to develop desirable parental lines, viz., cytoplasmic genetic male sterile (CGMS) lines, maintainers, and restorers for the development of rice hybrids for irrigated and shallow submergence. The farmers

S. No.	Stress	Promising hybrids
1	Rain-fed upland	DRRH-2, Pant Sankar Dhan-1, Pant Sankar Dhan-3, and KJTRH-4
2	Salinity	DRRH-28, Pant Sankar Dhan-3, KRH-2, HRI-148, JRH-8, PHB-71, and Rajalaxmi
3	Alkalinity	Suruchi, PHB-71, JKRH-2000, CRHR-5, DRRH-2, DRRH-44, and Rajalaxmi
4	<i>Boro</i> /Summer season	Rajalaxmi, CRHR-4, CRHR-32, NPH 924–1, PA 6444, Sahyadri, and KRH 2
5	BB resistant	BS 6444G, Arize Prima, Rajalaxmi, Ajay, CR Dhan 701, PRH 10, etc.

**Table 3.**  
*Rice hybrids tolerant to various stresses.*

Aerobic condition	PSD 3, PSD 1, Rajalaxmi, Ajay, ADTRH 1, PRH 122, DRRH 44, HRI 126, JKRH 3333, and KRH 2
Early duration	CRHR 105, CRHR 106, 25P25, 27P31 (heat-tolerant), US 382, Indam 200–17, US 312`, DRRH3, JKRH 401high N use efficient; PNPH 24 and RH 1531, Arize Tej-mid-early drought-tolerant; DRRH2, and KJTRH-4 (upland)
Long duration	CRHR 32, CRHR 34, CRHR 100, and Sahyadri 5
SRI	TNRH CO-4, KRH 4
Idly making	VNR 2355+
MS grains	CRHR 32, DRRH 3, 27P63, 25P25, and Suruchi
Aromatic	PRH 122 (slight aroma), PRH 10

**Table 4.**  
*Hybrids suitable for specific condition.*

in the rain fed shallow lowland ecosystem would be extremely benefited if the hybrid rice technology can be extended to this ecosystem, which need hybrids of Swarna duration. Keeping in views, ICAR-NRRI has developed three rice hybrids, viz., Ajay, Rajalaxmi, and CR Dhan 701 for this fragile ecosystem. Among them CR Dhan 701 is the country’s first long-duration hybrid, substitute for popular variety Swarna. Besides, NRRI has developed several promising CMS lines which have stable male sterility (WA, Kalinga-I and O. perennis, etc. cytoplasmic back-ground), maintainers, and effective restorers. More than 45 CMS lines in diverse genetic and cytoplasmic backgrounds have been developed among Sarasa A, Pusa 33A (WA), Annada A (WA), Kiran A (WA), Deepa A (WA), Manipuri A (WA), Moti A (WA), Krishna A (O. perennis), Krishna A (Kalinga I), Mirai (Kalinga I), Padmini A, PS 92A and Sahabthagidhan A, etc., which are more prominent to be utilized in hybrid development. The medium-duration CMS, CRMS 31A (WA) and CRMS 32A (Kalinga-I) are significantly utilized for hybrid development at NRRI and elsewhere in the country. The CRMS 24A and CRMS 40A, developed under the nucleus background of Moti and Padmini are found suitable for late-duration hybrid breeding. Moreover, short-duration CMS, CRMS 8A, CRMS 51A and CRMS 52A and CRMS 53A having drought tolerance are also being used for development of hybrids for drought prone ecosystem.

The latest release CR Dhan 701 (CRHR32) found suitable for irrigated-shallow lowland of Bihar, Gujarat and Odisha having MS grain type with an average yield capacity of 7.5 t/ha. This hybrid shows substantial tolerance to low light intensity, thus having great scope in eastern Indian states where low light limits the potential expression of hybrids/varieties during wet season. Moreover, hybrid Rajalaxmi (125–130 days) was developed utilizing native CMS line CRMS 32A, released by SVRC 2006/CVRC 2010 for irrigated-shallow lowland of Odisha and *boro* ecosystem of Odisha and Assam as it has seedling stage cold tolerance. Ajay is a medium-duration, long slender grain-type hybrid, released for cultivation in irrigated-shallow lowland of Odisha. As these hybrids are adaptable for eastern Indian climatic condition with assured remuneration, 12 private seed agencies over five states have commercialized them.

To make this technology more sustainable and amenable to farmers, trait development strategy among the parental lines becomes mandatory. The parents of ICAR-NRRI bred hybrids Ajay, Rajalaxmi and CR Dhan 701 has been improved for bacterial blight, the most devastating disease of rice [26]. The submergence and salinity are the major abiotic stresses occur frequently in rain-fed shallow lowland area and causes substantial yield loss in rice. Hence, to cope up with the problems, and make hybrid rice more sustainable during these adversity, ICAR-NRRI has successfully stacked submergence and salinity-tolerant QTLs in

the seed parents CRMS 31A and CRMS 32A. To enhance the seed producibility in seed parents, introgression of stigma exertion trait from *O. longistaminata* into CRMS 31A and CRMS 32A, are under progress. To excavate the genetic region responding heterosis in rice, transcriptomic analysis of hybrids Rajalaxmi and Ajay are completed and interpreted. Availability of restorers for WA-CMS lines is very stumpy in nature, only 15% of total rice genotypes having the ability to restore complete fertility in WA-CMS-based hybrid rice [15]. Hence, good combiner genotypes having partial fertility restorers Mahalaxmi and Gayatri were improved by introgressing fertility restorer gene(s) *Rf3* and *Rf4* through MABB approach. Further, to make clear cut identity and ensure pure seed of parents/hybrids to the stack-holder, 12 signature markers that unambiguously distinguish 32 rice hybrids were developed, which can be utilized for DNA fingerprinting and genetic purity testing of hybrids.

#### **4. Potential application of OMICS approaches in hybrid rice breeding**

Recent advancement in molecular biology has offered tremendous opportunities to the breeder and breeding *per se* in enhancement in their efficacy and speed up the varietal development process. It has diverse applications like mapping, tagging, amplification-based cloning, gene pyramiding, marker-assisted selection (MAS/MARS), fingerprinting applications, including varietal identification, ensuring seed purity, phylogeny and evolution studies, diversity analysis, and elimination of germplasm duplication. The progress in research related to application of DNA marker technology in hybrid rice improvement may be valuable in following way.

##### **4.1 DNA fingerprinting and genetic purity testing**

Varietal identity of hybrids and parents is imperative to assure the ownership (IPR issue) and pure seeds to the stakeholders. The genetic purity testing of hybrid seed is done by conducting Grow-Out-Test (GOT) which is time taking (needs one full growing season), tedious and very expensive. Molecular markers in this context found to be a suitable alternative, provide an unbiased means of identifying crop varieties. Among available DNA-based markers, sequence-tagged microsatellites (STMSs), which are co-dominant in nature, are widely used for speedy genetic purity assessment of the hybrids and parental lines [27, 28]. Besides, ICAR-NRRI has developed another set of nine signature markers which can distinguish parents CRMS 31A, CRMS 32A; and hybrids Ajay, Rajalaxmi and CR Dhan 701, unambiguously.

##### **4.2 Trait improvement in parental lines and hybrids**

Hybrid rice has been one of the innovations that led the quantum jump in rice productivity last century. However, the challenge of meeting the increasing demand for rice and making hybrid more sustainable under impending climatic changes, trait development in parental lines for ideal plant type with substantial yield, grain quality, and resistance/tolerance to multiple biotic and abiotic stresses is necessary. In this context, conventional breeding is more cumbersome, time taking and less précised. The advancement in molecular breeding techniques makes it convenient to improve the parents and hybrids for desirable traits with great precision. Marker-assisted selection/MABB has provided strong utensils for indirect selection/trace the trait of interest at any plant growth stage. The bacterial blight and blast are the



two-major destructive diseases affecting rice plant at different growth stages and caused substantial yield loss. Resistant genes for BB diseases have been deployed successfully in popular hybrids like Rajalaxmi, Ajay [26], BS 6444G, PRH 10 [29], Shanyou 63, Guangzhan63-4S; seed parent of CR Dhan 701; restorers Minghui 63 and Mianhui 725 [5, 26], Zhonghui 8006 and Zhonghui 218, etc. The popular CMS line Rongfeng A, Pusa 6A female parent of popular basmati hybrids PRH 10, RGD-7S, and RGD-8S [30] were successfully stacked with blast and BB resistant gene(s). Besides, CRMS 31A and CRMS 32A were deployed with submergence and salinity tolerance QTLs (NRRI newsletter 2015). Grain and eating quality in hybrids are concerns which are addressed by stacking QTLs/genes for quality traits in parents. Zhenshan 97A seed parent of several hybrids in China has been stacked with QTLs of AC, GC and GT [31]. Efforts were made toward quality improvement of both the parental lines of popular indica hybrids, viz., Xieyou57, using marker-assisted selection for *Wx* locus [32]. Yield-enhancing QTLs, *yld1.1* and *yld2.1*, from *O. rufipogon* to restorer “Ce64” [33] are successfully stacked. Hybrid sterility in inter-subspecific (*indica/japonica*) hybrids is reported to be effectively addressed by utilizing genome editing tool “CRISPR/Cas9” [34].

### 4.3 Screening of Rf genes in parents

Limited availability of fertility restorer system in rice makes three-line system very selective and less heterotic. Rice genotypes have fertility restorer ability can only be utilized as pollen parent in three-line hybrid breeding. Identification of genetically compatible, well combining restorers is tedious process, involve laborious test cross generation and evaluation steps. However, prior information on fertility restorer genes in the pollen parent excludes test cross steps thus make it convenient for saving time of hybrid development. Plenty of co-segregating molecular markers (tightly linked or functional markers) for fertility restorer gene(s) having functional specificity to diverse CMS systems are available (Table 2). The genic/functional markers, RM6100 and DRRM Rf3-10 of restorer gene(s) *Rf4* and *Rf3*, respectively, are widely utilized for screening the fertility restoration efficacy of unknown pollen parents for WA and lineage CMS well in advance [15].

### 4.4 Screening of parental lines for wide compatibility genes

Hybrid sterility is common nuisance menacing breeder to exploiting heterosis in inter-subspecific (5–10% more heterosis) hybrids. Generally, *indica* × *japonica* hybrids are sterile due to lack of wide compatibility (WC) between parents. It is reported that hybrid sterility in inter-subspecific crosses is mainly affected by the genes at *Sb*, *Sc*, *Sd*, and *Se* [35] loci causes male sterility in F<sub>1</sub> and the gene at *S5* locus cause female sterility in F<sub>1</sub>. Presence of these genic regions in at least one parent ensures complete fertility in resulting hybrids. These gene(s) can be assessed in advance by utilizing co-segregating markers (*S5*-InDel, functional marker to *S5<sup>n</sup>*) [36] and G02-14827 (genic marker) PSM8, PSM12, and PSM180 (linked SSR); IND19 and ID5 (indel markers) to *Sb*, *Sc*, *Sd*, and *Se*, loci). Thus, it helps breeder in selection of WC-positive parent in more predictable way which circumvents laborious test-cross and their evaluations steps.

### 4.5 Prediction of heterosis

Genetic distance and level of genetic gain/breeding value in parents are major determinants of extent of heterosis in the resulting hybrid. Molecular markers help in assessing the genetic diversity among parents and breeding values in progenies



(through genomic selection, high-density SNP genotyping) with great convenient. There are abundant STMS and SNP markers available which can be utilized for assessment of genetic diversity/genetic distance between parents and genomic selection in progenies easily [37]. Hence, this is helpful in the selection of diverse parents with maximum breeding values in turn higher heterosis or genetic gain in hybrids.

#### 4.6 Determination of heterotic group and heterosis pattern

The extent of genetic variation and selection strategies are keys to the success of heterosis breeding. Accurate assessment and assignment of parental lines into heterotic groups “group of genotypes (related or unrelated) having similar combining ability and heterosis response when crossed with the genotypes of other diverse group” are fundamental prerequisites. Usually it is evaluated by combining ability analysis of parents and hybrids in multi-environment trials. However, advances in molecular marker technology have made it possible to combine information on parental pedigree and field trials with molecular marker data to detect and establish heterotic groups. Several heterotic groups have been developed and utilized for three-line and two-line hybrid development in rice [38].

#### 4.7 Excavating QTLs/gene(s) responses heterosis

Omics techniques reported to have great potential in excavation of QTLs/gene(s) responses heterosis in rice. By utilizing genomics tools, many QTLs/genes for several important traits has been mapped, validated, and deployed in trait development in rice. The transcriptomics, an emerging technique helps in genome-scale comparisons of the transcripts of different individuals within the same species/population. It helps in understanding the level of variation for gene expression, as measured by transcript abundance that exists within plant species and between hybrids and their parents. This is useful for identification of transcript and gene *per se* involves in heterotic expression. Moreover, epigenetics, a posttranslational biochemical regulation of gene is found to be playing substantial role in trait expression. Individuals of the same species can have epigenetic variation in addition to genome and transcriptome content variation. A potential role for epigenetic regulation in heterosis has been proposed. It is possible for epigenetic variation to affect heterosis by creating stable epialleles that would behave similarly to the genomic or transcriptomic differences. Alternatively, hybrids may exhibit unique epigenomic states that lead to heterosis.

### 5. Major challenges and potential research opportunities

#### 5.1 Major challenges

Despite of being remunerative and varietal abundancy, HR technology could not make substantial dent in the rice farming system outside China. The following are the inherited void led poor acceptability and acreage expansion of hybrids:

##### 5.1.1 Lack of cytoplasmic diversity in countries outside China

Outside China, WA-CMS or their lineages are commonly utilized as seed parent in more than 90%rice hybrids. Several alternative MS cytoplasmic sources such as BT-CMS, HL-CMS, and CW-CMS are identified in China, but the hybrid breeding program of other countries relied only on WA-CMS which has several

inherited abnormalities. These narrowed genetics of sterile cytoplasm limits the extent heterosis exploitation and make hybrids vulnerable to many biotic and abiotic stresses.

#### 5.1.2 Marginal heterosis in intra-subspecific hybrids

Two-lines and inter-subspecific (*indica/japonica*) hybrids are comparatively more heterotic (5–10%) than three-line *indica* hybrids. But owing to several inevitable difficulties in seed production of two-line hybrids and poor grain and eating quality in inter-subspecific hybrids, both could not be exploited in the countries like India who has vast climatic and food affection diversity. We are utilizing three-line *indica* hybrids which are comparatively less heterotic hybrid breeding systems giving low yields. Hence, focused and intensive research is proposed to make above said hitches be addressed in future.

#### 5.1.3 Poor grain and eating quality

In hybrids, consumable parts are  $F_2$  grains, segregating for various quality traits hence very poor in quality limits its acceptability among stakeholders. Therefore, make hybrids more sustainable and popular, quality traits in hybrids needs to be addressed urgently in the country like India where people have vast category of food fondness. Hence, a strong breeding strategy for quality concern in hybrids is needs to be devised and implemented.

#### 5.1.4 Subtle information on QTLs/gene(s) responding heterosis

Although heterosis, or hybrid vigor, is widely exploited in agriculture, but despite extensive investigation, complete description of its molecular underpinnings has remained elusive. It appears that there is not a single, simple explanation for heterosis. Instead, it is likely that heterosis arises in crosses between genetically distinct individuals because of a diversity of mechanisms. Hence, mining factors responding heterosis in rice will have a substantial role in development and exploiting heterosis in most precise way.

#### 5.1.5 Inter-subspecific hybrid sterility

Hybrid sterility is key nuisance in inter-subspecific hybrids, limiting development and commercialization of more heterotic *indica/japonica* hybrid in rice. The sterility in hybrids (*inter-subspecific*) generally occurs due to non-functional pollens as well as sterility in female reproductive organs. It is reported that mutant of *S-i* alleles at *Sb*, *Sc*, *Sd*, and *Se* loci produce sterile pollens; and mutants of *S5* locus causes sterility in female gamete. Hence, trait development for wide compatibility in either parent has great opportunity in addressing the hybrid sterility in rice.

### 5.2 Potential research opportunity

#### 5.2.1 Exploitation of inter-specific heterosis

Inter-subspecific (*indica/japonica*) hybrids as discussed in earlier section are more heterotic than intra-subspecific hybrids. However, owing to hybrid sterility

and poor grain quality, this genetic pool remains untapped. Grain quality of inter-subspecific hybrids proposed to be improved by utilizing parental combinations having good combining ability but similar in quality parameters, might reduce the concern of segregation for quality traits. Hybrid sterility problem in inter-subspecific hybrids can be addressed by stacking *indica* allele (*S-i*) at *Sb*, *Sc*, *Sd*, and *Se* loci and the neutral allele (*S-n*) at *S5* locus in to *japonica* genetic background [35] or by silencing the *S-i* and *S5* mutant loci through genome editing tools [34].

#### 5.2.2 Utilization of Iso-cytoplasmic restorers

In three-line hybrid system, cytoplasm of CMS exerts various unwanted effect (called CMS penalty) and reduces the complete heterosis expression (up to 5–10%) in CGMS hybrids. Iso-cytoplasmic restorer is fertile transgressive segregant of CGMS hybrid, having same cytoplasm as of CMS. In combination with iso-cytoplasmic CMS, it can normalize the fatal cyto-nuclear conflicts, hence enhances the heterosis to substantial extent. In rice, several iso-cytoplasmic restorers have been developed and utilized in hybrid rice research [39].

#### 5.2.3 Out-crossing enhancement in seed parent

Low seed producibility (1.5–2.5 t/ha) in the CMS remains a concern, restricts seed abundance, and area expansion in India. Trait development in seed parent for out-crossing traits like stigma exertion, complete panicle exertion is important and needs to be addressed strategically. Recently, a CMS line, IR-79156A possessing more than 50% out-crossing, developed by IRRI showed seed producibility of 3.5 t/ha.

#### 5.2.4 Ideotype hybrid breeding

To maximize genetic gain in rice, breeding of ideal plant type was started long back in Japan and subsequently adopted by China. Through morphological improvement and adopting inter-subspecific (*indica/japonica*) hybrid strategies, substantial progress in ideotype hybrid breeding “super hybrid” have been achieved. China, indeed has made considerable progress and released more than 100 high yielding super hybrids [25]. Hence, inclusion of inter-subspecific quality type inbreds “super rice” in hybrid development will have substantial impact in attaining quantum genetic gains in hybrids.

## 6. Economic importance

In spite of being more cumbersome and high input intense practice, hybrid rice seed production is a profitable venture. It creates additional job opportunity (requires 100–105 more-man days) and provides more net income (around 1050 USD/ha net income, 70% more than the unit production cost) as compared to seed production of HYV (192.0 USD/ha, only 18% more than production cost) (Table 5). The market price of hybrid seed is 3.5–4.25 USD per kg. The farmers producing the hybrid seed get only 1.15–1.30 USD per kg. In case of low production (<5 quintal/acre) farmers get minimum 635.0 USD as compensation from seed production agencies.

Item		Quantity/number (per hectare)	Cost/income (USD)	
			Hybrid seed	HYV
Seed cost	Male	5 kg @ 0.71 USD/kg	4	28
	Female	15 kg @ 5.65 USD/kg	42	Nil
Labor cost		250/145 @ 2.83 USD/labor/day	707	410
FYM and fertilizer cost		N:P:K (100:50:50) (based on market price)	76	76
Irrigation		18–20 Irrigation (weekly) @ 21.20USD/ha/irrigation	425	425
Gibberellic acid			28	Nil
Others			212	141
Total cost			1494	1080
Average production			2.0 t/ha	4.5
Gross income		Price @ 1.27USD/kg and 0.28USD/kg <sup>a</sup>	2544	1272
Net income			1050	192

<sup>a</sup>Price of seed is the price given to the farmer.  
Source: Verma et al., [40].

**Table 5.**  
*Cost analysis of hybrid rice seed.*

## 7. Way forward

Hybrid technology has been substantial in enhancement of rice productivity *per se* production in temperate countries, however, owing to low photo-intensity during growing period in tropics, its impact remains meager. Under changing climatic and agriculture scenario, rice hybrid is likely to face stiff competition to sustain in future. Despite having great potential to enhance production and productivity, it has not been adopted on large scale as was expected. This is due to several constraints like lack of acceptability of hybrids in some regions such as Southern India due to region-specific grain quality requirement. Moderate (15–20%) yield advantage in hybrids is not economically very attractive and there is a need to increase the magnitude of heterosis further. Lower market price offered for the hybrid rice produce by millers/trader sis acting as a deterrent for many farmers to take up hybrid rice cultivation. Higher seed cost is another restrain for large-scale adoption and hence there is a need to enhance the seed yield in hybrid rice seed production plots. Efforts for creating awareness and for technology transfer were inadequate in initial stages. Involvement of public sector seed corporations in large-scale seed production has been less than expected. Hybrid rice for aerobic/upland, *boro* season and long-duration hybrids for shallow lowland conditions are to be developed. Most of the constraints mentioned above are being addressed with right earnestness through the ongoing research projects and transfer of technology efforts.

## 8. Conclusion

Since inception, this technology has a substantial impact in enhancing the productivity and production in crop plant and livelihood of the farming community.



In rice, it is adopted worldwide over 40 countries; however, it could not make a substantial dent in outside of China. This chapter has represented the holistic status of hybrid technology in rice along with future research and developmental road map to make this venture more substantial and sustainable for benefiting all stakeholder involves. This chapter identifies the ambiguities held responsible for slow adoption of this technology and probable strategies to get rid of those. Therefore, this chapter will be helpful for researchers and students in planning of future hybrid rice breeding strategies.

## Author details

Diptibala Rout<sup>1</sup>, Debarchana Jena<sup>1</sup>, Vineeta Singh<sup>2</sup>, Manish Kumar<sup>3</sup>, Pandurang Arsode<sup>3</sup>, Prakash Singh<sup>4</sup>, Jawahar Lal Katara<sup>1</sup>, Sanghamitra Samantaray<sup>1</sup> and Ramlakhan Verma<sup>5\*</sup>

<sup>1</sup> Crop Improvement Division, National Rice Research Institute, Odisha, India

<sup>2</sup> Acharya Narendra Dev University of Agriculture and Technology, U.P., India


<sup>3</sup> Department of Genetics and Plant Breeding, I.Ag.Sc., Banaras Hindu University, U.P., India

<sup>4</sup> Department of Plant Breeding and Genetics, Veer Kunwar Singh College of Agriculture (Bihar Agricultural University-BAU, Sabour), Bihar, India

<sup>5</sup> Hybrid Rice Breeding, Crop Improvement Division, National Rice Research Institute, Odisha, India

\*Address all correspondence to: ram.pantvarsity@gmail.com

## IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] Verma RL, Katara JL, Sah RP, Sarkar S, Azaharudin TP, Patra BC, et al. CRMS 54A and CRMS 55A: Cytoplasmic male sterile lines with enhanced seed producibility. 2018, NRRI Newsletter. January-March 2018;**39**:1-14
- [2] Jonse JW. Hybrid vigour in rice. Journal of American Society of Agronomy. 1926;**18**:423-428
- [3] IIRR, Indian Institute of Rice Research, Technical Bulletin, 2019. IIRR: Hyderabad
- [4] Sampath S, Mohanty HK. Cytology of semi-sterile rice hybrid. Current Science. 1954;**23**:182-183
- [5] Chen S, Lin XH, Xu CG, Zhang Q. Improvement of bacterial blight resistance of “Minghui 63,” an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Science. 2014;**40**:239-244
- [6] Tang H, Zheng X, Li C, Xie X, Chen Y, Chen L, et al. Multi-step formation, evolution, and functionalization of new cytoplasmic male sterility genes in the plant mitochondrial genomes. Cell Research. 2017;**27**:130-146
- [7] Wang ZH, Zou YJ, Li XY, Zhang QY, Chen LT, Wu H. Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. Plant Cell. 2006;**18**:676-687
- [8] Wang K, Gao F, Ji Y, Liu Y, Dan Z. ORFH79 impairs mitochondrial function via interaction with a subunit of electron transport chain complex III in Honglian cytoplasmic male sterile rice. New Phytologist. 2013;**198**:408-418
- [9] Zhang H, Xu C, He Y, Zong J, Yang X. Mutation in CSA creates a new photoperiod-sensitive genic male sterile line applicable for hybrid rice seed production. Proceeding National Academy of Science, USA. 2013;**110**:76-81
- [10] Rao GS, Deveshwar P, Sharma M, Kapoor S, Rao KV. Evolvment of transgenic male-sterility and fertility-restoration system in rice for production of hybrid varieties. Plant Molecular Biology. 2018;**96**:35-51
- [11] Zhou H, He M, Li Z, Chen L, Huang Z, Zheng S, et al. Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated TMS5 editing system. Scientific Reports. 2016;**6**:373-395
- [12] Hu J, Wang K, Huang W, Liu G, Gao Y. The rice pentatricopeptide repeat protein *RF5* restores fertility in Honglian cytoplasmic male-sterile lines via a complex with the glycine rich protein GRP162. Plant Cell. 2012;**24**:109-122
- [13] Tao C, Ming L, Zya Z, Zhen Z, Ling Z, Qing Y, et al. Development and application of a functional marker associated with fertility restoring gene for BT-type cytoplasmic male sterility (CMS) in *japonica* rice. Chinese Journal of Rice Science. 2013;**27**(3):259-264
- [14] Itabashi E, Iwata N, Fujii S, Kazama T, Toriyama K. The fertility restorer gene, *Rf2* for lead rice-type cytoplasmic male sterility of rice encodes a mitochondrial glycine-rich protein. The Plant Journal. 2011;**65**:359-367
- [15] Katara JL, Verma RL, Nayak D, Umakanta N, Ray S, Subudhi H, et al. Frequency and fertility restoration efficiency of *Rf3* and *Rf4* genes in Indian rice. Plant Breeding. 2017;**136**:74-82
- [16] Liu XQ, Xu EX, Li SQ, Hu J, Huang JY, Yang DC, et al. Inheritance

- and molecular mapping of two fertility-restoring loci for Honglian gametophytic cytoplasmic male sterility in rice (*Oryza sativa* L.). *Molecular Genetics and Genomics*. 2004;**271**:586-594
- [17] Fujii S, Toriyama K. Molecular mapping of the fertility restorer gene for ms-CW-type cytoplasmic male sterility of rice. *Theoretical and Applied Genetics*. 2005;**111**:696-701
- [18] Igarashi K, Kazama T, Toriyama K. A gene encoding pentatricopeptide repeat protein partially restores fertility in RT98-type cytoplasmic male-sterile rice. *Plant Cell Physiology*. 2016;**57**(10):2187-2193
- [19] Okazaki M, Kazama T, Murata H, Motomura K, Toriyama K. Whole mitochondrial genome sequencing and transcriptional analysis to uncover an RT102-type cytoplasmic male sterility-associated candidate gene derived from *Oryza rufipogon*. *Plant Cell Physiology*. 2013;**54**(9):1560-1568
- [20] Toriyama K, Kazama T. Development of cytoplasmic male sterile IR24 and IR64 using CW-CMS/*Rf17* system. *Rice*. 2016;**9**:22-26
- [21] Koltunow AM, Bicknell RA, Chaudhury AM. Apomixis: Molecular strategies for the generation of genetically identical seeds without fertilization. *Plant Physiology*. 1995;**108**(4):1345
- [22] Mieulet D, Jolivet S, Rivard M, Cromer L, Vernet A, Mayonove P, et al. Turning rice meiosis into mitosis. *Cell Research*. 2016;**26**(11):1242-1254
- [23] Gaafar RM, El Shanshoury AR, El Hisseiw AA, AbdAlhak MA, Omar AF, Abd El Wahab MM, et al. Induction of apomixis and fixation of heterosis in Egyptian rice Hybrid1 line using colchicine mutagenesis. *Annals of Agricultural Science*. 2017;**62**(1):51-60
- [24] Xie E, Li Y, Tang D, Lv Y, Shen Y, Cheng Z. A strategy for generating rice apomixis by gene editing. *Journal of Integrative Plant Biology*. 2019;**61**(8):911-916
- [25] Yuan LP. Progress in super-hybrid rice breeding. *The Crop Journal*. 2017;**5**:100-102
- [26] Dash AK, Rao RN, Rao GJN, Verma RL, Katara JL, Mukherjee AK, et al. Phenotypic and marker-assisted genetic enhancement of parental lines of rajalaxmi, an elite rice hybrid. *Frontiers in Plant Science*. 2016;**7**:1005
- [27] Behera L, Patra BC, Sahoo RK, Nanda A, Patnaik A, Singh ON. Assessment of genetic diversity in medicinal rice using microsatellite markers. *Australian Journal of Crop Science*. 2012;**6**(9):1369-1376
- [28] Verma RL, Singh S, Singh P, Kumar V, Singh SP, Singh S, et al. Genetic purity assessment of *indica* rice hybrids through DNA fingerprinting and grow out test. *Journal of Environmental Biology*. 2017;**38**(6):1321-1331
- [29] Basavaraj SH, Singh VK, Singh A, Singh AK. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. *Molecular Breeding*. 2010;**26**(2):293-305
- [30] Liu W, Wang FM, Jin S. Improvement of rice blast resistance in TGMS line by pyramiding of Pi1 and Pi2 through molecular marker-assisted selection. *Acta Agronomica Sinica*. 2008;**34**:1128-1136
- [31] Zhou PH, Tan YF, He YQ, Zhang Q. Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. *Theoretical and Applied Genetics*. 2003;**106**:326-331
- [32] Ni D, Zhang S, Chen S, Xu Y, Li L, Li H, et al. Improving cooking and

eating quality of Xieyou57, an elite *indica* hybrid rice, by marker-assisted selection of the Wx locus. Euphytica. 2011;**179**(2):355-362

and Molecular Biology of Plants. 2017;**23**(4):891-909

[40] Verma et al. NRRI Technology Bulletin-114. 2015. p. 16

[33] Duan MJ, Sun ZZ, Shu LP. Genetic analysis of an elite super-hybrid rice parent using high-density SNP markers. Rice. 2013;**6**(1):21-36

[34] Shen R, Wang L, Liu X, Wu J, Jin W, Zhao X, et al. Genomic structural variation-mediated allelic suppression causes hybrid male sterility in rice. Nature Communications. 2017;**8**:1310-1320

[35] Guo J, Xu X, Li W, Zhu W, Zhu H, Liu Z, et al. Overcoming inter-subspecific hybrid sterility in rice by developing *indica*-compatible *japonica* lines. Scientific Reports. 2016;**6**:268-278

[36] Priyadarshi R, Hari Prasad SA, Singh AK, Khandekar D, Ulaganathan K, Shenoy V. Molecular stacking of wide compatibility gene, S5n and elongated uppermost internode (*eui*) gene into IR 58025B, an elite maintainer line of rice. Journal of Plant Biochemistry and Biotechnology. 2017;**26**(4):425-435

[37] Soni SK, Yadav VK, Bhadana VP, Yadav MC, Sundaram RM. Prediction of heterosis using hypervariable SSR markers in tropical *japonica* x *indica* rice hybrids (*Oryza sativa* L). International Journal of Current Microbiology and Applied Sciences. 2017;**6**(10):1419-1427

[38] Lu ZM, Xu B. On significance of heterotic group theory in hybrid rice breeding. Rice Science. 2010;**17**:94-98

[39] Kumar A, Bhowmick PK, Singh VJ, Malik M, Gupta AK, Seth R, et al. Marker-assisted identification of restorer gene(s) in iso-cytoplasmic restorer lines of WA cytoplasm in rice and assessment of their fertility restoration potential across environments. Physiology