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## Studies on Inbred Lines their Combining Ability and Development of High Productive Hybrid in Rice (*Oryza sativa* L.)

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**Abstract:** The technology of hybrid in rice (*Oryza sativa* L.) utilizing heterosis is an essential requirement for achieving food security. The current study was aimed at assessing the genetic parameters and the gene actions of 15 yield-component traits associated with heterosis, in 9 new parental lines of hybrid rice and their generated hybrids. Five cytoplasmic male sterile (CMS) lines were crossed with four restorer (R) lines using twenty generated line × tester designation hybrid combinations. The results revealed that all the traits were controlled by additive and non-additive gene actions. However, the additive variance was the main component of the total genotypic variance. Assessment of the general combining ability (GCA) detected the best combiners among the genotypes. The hybrid combinations that expressed the highest-positive specific combining ability (SCA) for grain-yield were detected. The correlation between the GCA and SCA was evaluated. The hybrid crosses with high-positive heterosis, due to having a better parent for grain yield, were detected. The principal component analysis (PCA) recorded the first four principal axis displayed Eigenvalues >1 and existing variation cumulative of 83.92% in the genotypes for yield component characteristics. Three-dimensional plots corresponding to the studied traits illustrated that the genotypes Guang8A × Giza181, Quan-9311A × Giza179, II-32A × Giza181, and II-32A × Giza179 are classified as possessing superior grain yield.

**Keywords:** inbred, high, productive, hybrid, inlines, rice, *Oryza sativa* L., grain, genotypes

### Introduction:

Heterosis is a phenomenon in which F1 hybrids derived from diverse parents show superiority over their parents in vigor, yield, panicle size, number of spikelets per panicle, number of productive tillers, etc. Heterosis is expressed in the first generation only. Heterosis varies according to the level of parental diversity and or presence of heterotic gene blocks in parental lines; indica × japonica crosses show maximum heterosis vis-à-vis any other combination between other subspecies. The crosses showing heterosis in descending order are indica × japonica > indica × javanica > japonica × javanica > indica × indica > japonica × japonica > javanica × javanica. Heterosis can be positive or negative. Both positive and negative heterosis can be useful depending on the trait, for example, positive heterosis for yield and negative heterosis for growth duration. Farmers tend to use a lower seed rate for hybrids than for

conventional varieties because of their better seed quality and higher seed cost. However, it is necessary to purchase fresh seeds every season to raise a commercial crop. Heterosis is expressed in three ways, depending on the reference used to compare the performance of a hybrid:

Mid-parent heterosis is the increase or decrease in the performance of a hybrid in comparison with the mid-parental value. Heterobeltiosis is the increase or decrease in the performance of a hybrid in comparison with the better parent of the cross combination. Standard heterosis is the increase or decrease in the performance of a hybrid in comparison with the standard check variety of the region. From the practical viewpoint, standard heterosis is the most important because we aim to develop hybrids that are better than the existing high-yielding varieties grown commercially by farmers.[1,2]

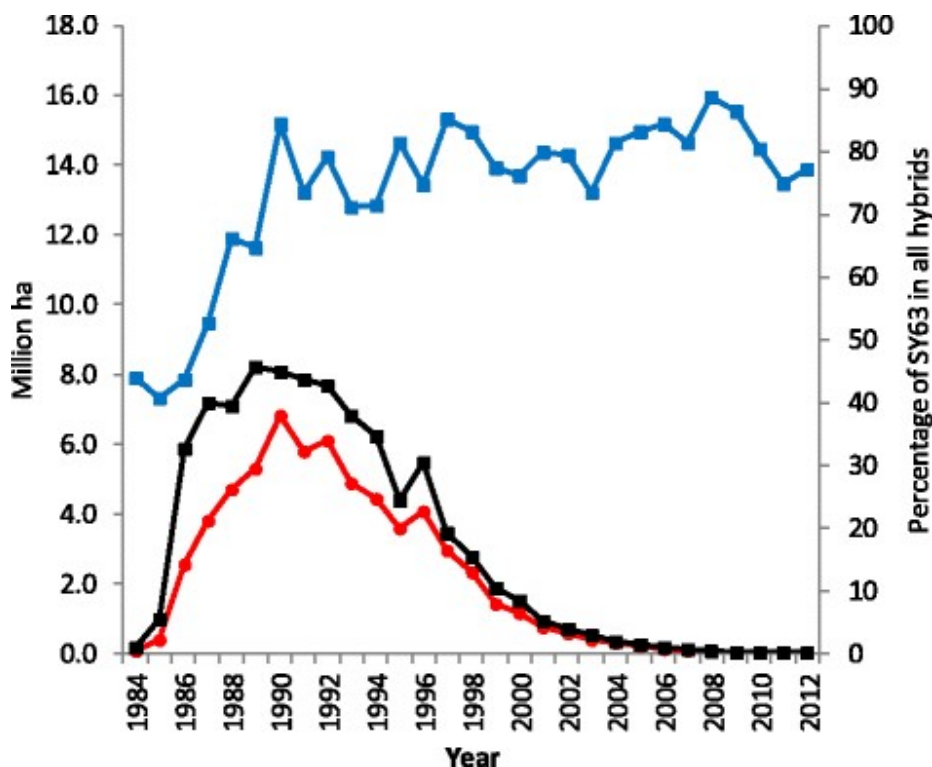


Hybrid rice is the commercial rice crop grown from F1 seeds of a cross between two genetically dissimilar parents.

- Good rice hybrids have the potential of yielding 15–20% more than the best inbred variety grown under similar conditions.
- To exploit the benefits of hybrid rice, farmers have to buy fresh seeds every cropping season
- The need for hybrid rice has been felt because:-
- Yield levels of semidwarf varieties of the Green Revolution era have plateaued.
- The demand for rice is increasing rapidly with the increase in population, especially in less developed countries.
- More and more rice has to be produced on less land and with less inputs. [3,4]
- Hybrid rice varieties have already shown a 15–20% higher yield potential than inbred rice varieties under farmers' field conditions in several countries.
- Hybrids have also shown an ability to perform better under adverse conditions of drought and salinity.

Hybrid rice is developed by exploiting the phenomenon of heterosis. Rice, being a strictly selfpollinated crop, requires the use of a male sterility system to develop commercial rice hybrids. Male sterility (genetic or nongenetic) makes the pollen unviable so that rice spikelets are incapable of setting seeds through

selfing. A male sterile line is used as a female parent and grown side by side with a pollen parent in an isolated plot to produce a bulk quantity of hybrid seed because of cross pollination with the adjoining fertile pollen parent. The seed set on male sterile plants is the hybrid seed that is used to grow the commercial hybrid crop.[5,6]



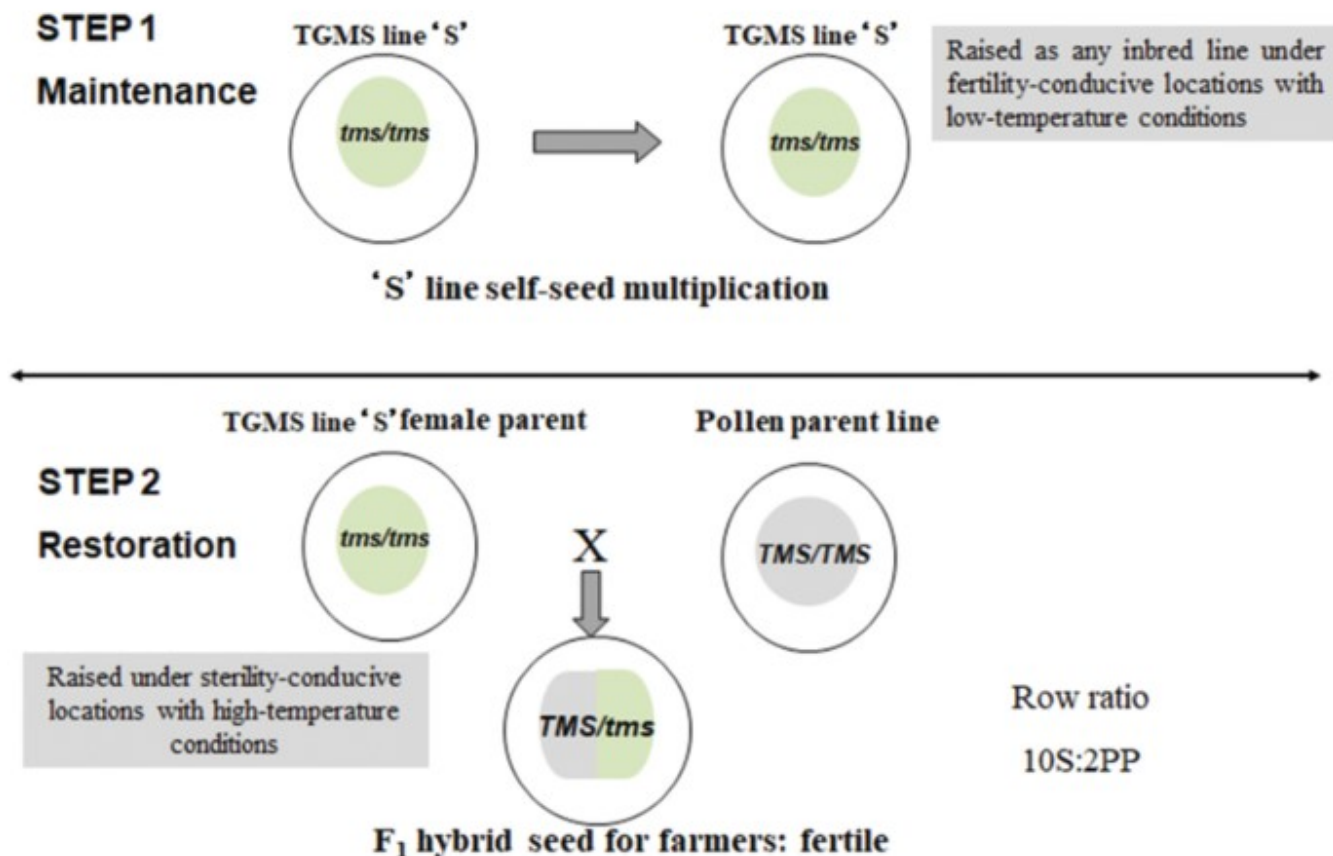
## Discussion

The exploitation of hybrid vigor in rice has shown the way to increasing rice production after yield stagnation with the use of semi dwarf inbred rice varieties in irrigated ecosystems. The deployment of three-line rice hybrids in China and elsewhere in Asia has substantially increased the hopes of sustaining Asian food security. Hybrid rice technology has caught the attention of rice farmers outside China and, during the next ten years, several countries should have a large area covered with rice hybrids. There is a continuous need to reduce the cost and increase the efficiency of hybrid rice seed production. The discovery of the EGMS system in China and later in Japan, at IRRI, and in India has improved the chances of substantially reducing the cost of seed production by using two-line rice hybrids. These hybrids also help to increase heterosis beyond the level of three-line rice hybrids. The two-line hybrids have already created an impact in China, with their area reaching 2.6 million ha. Among the several two line rice hybrids, Liangyou PeiJiu (Peiai64S/9311) gave the highest average yields of 11 t ha<sup>-1</sup> on 10-ha farms in two successive years. The highest yield recorded was about 12.1 t ha<sup>-1</sup>, a new record in these areas, clearly revealing the enhanced potential of two-line rice hybrids. Outside China, two-line rice hybrids are also being developed at IRRI and in Vietnam, India, Korea, the Philippines, Thailand, and Egypt. Future research priorities include the following:

Development of stable EGMS lines. Stable elite EGMS lines with a precise fertility alteration mechanism hold the key to success in developing two-line commercial hybrid rice. The underlying genetic mechanism of fertility alteration needs to be understood clearly to properly enhance the efficacy of EGMS seed multiplication and hybrid rice seed production. Breeding of TGMS lines with a low CSP is important for developing two-line commercial rice hybrids in the tropics. The genetic characterization of the loci of

the EGMS genes from different sources in relation to closely tagged molecular markers is useful for marker-assisted selection. 2. Use of anther culture to develop and/or purify elite EGMS lines.

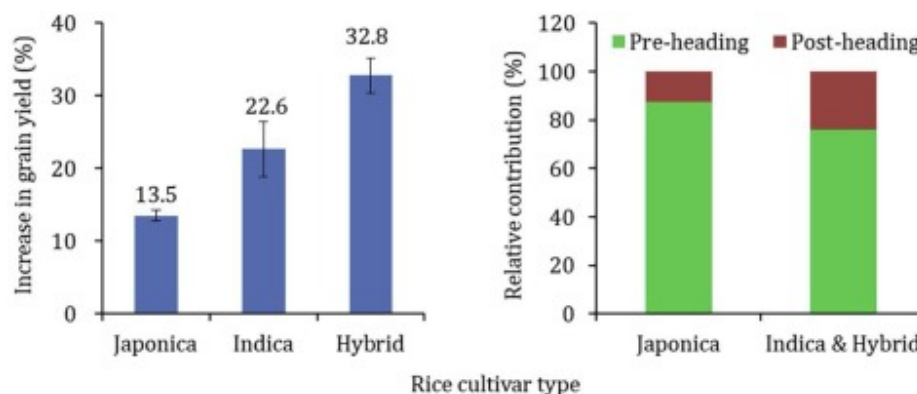
### TGMS system for two-line rice hybrids



Anther culture techniques involving di haploidization can be used to expedite the development and/or purification of EGMS lines possessing major genes and QTLs in influencing the PGMS/TGMS trait. 3. Breeding for super high-yielding two-line hybrids. Two-line hybrid rice technology involving EGMS lines allows the choice of a wider range of parental combinations and avoids the negative effects of male-sterility-inducing cytoplasm. Rice scientists at IRRI and in China can now use new plant type (NPT) lines developed in the tropical japonica and indica/tropical japonica background as male and/or female parents to develop hybrids with enhanced heterosis. Two-line breeding technology can overcome the major problems of wide incompatibility and the narrow range of restorers in exploiting indica-japonica heterosis. 4. Incorporation of hybrids with resistance to biotic stress. Two-line rice hybrids possessing multiple resistance to diseases and in- 70 sects can be developed more expeditiously than three-line hybrids since the desired resistance genes need to be incorporated in two rather than three parental lines. 5. Abiotic stress tolerance. Hybrid rice technology so far has been used under the irrigated rice ecosystem. Environmental degradation resulting from salinity and water shortages has posed a major threat to sustaining food production. Researchers at IRRI and in Egypt, India, and China have found that hybrid rice technology can be extended to saline-prone irrigated conditions because hybrids have performed exceedingly well under moderate to high saline soil conditions. Since the high cost of seed is a major constraint to the adoption of this technology by resource-poor farmers and two-line hybrid breeding and the seed production approach make it more cost-effective, this technology can be adopted by resource-poor farmers. 6. Quality. The negative influence of WA cytoplasm on certain quality parameters (such as



grain chalkiness) allows the alternative use of EGMS-based two-line hybrid rice technology to overcome such drawbacks. A multidisciplinary approach in developing superior EGMS lines and pollen parents can help to develop two-line rice hybrids suitable for the different ecological situations in which rice is grown. Despite the promise that two-line hybrid rice technology holds, it would be wise to have a harmonious balance in using two-line and three line hybrids and conventional rice varieties in an appropriate manner in national rice production programs.[7,8]

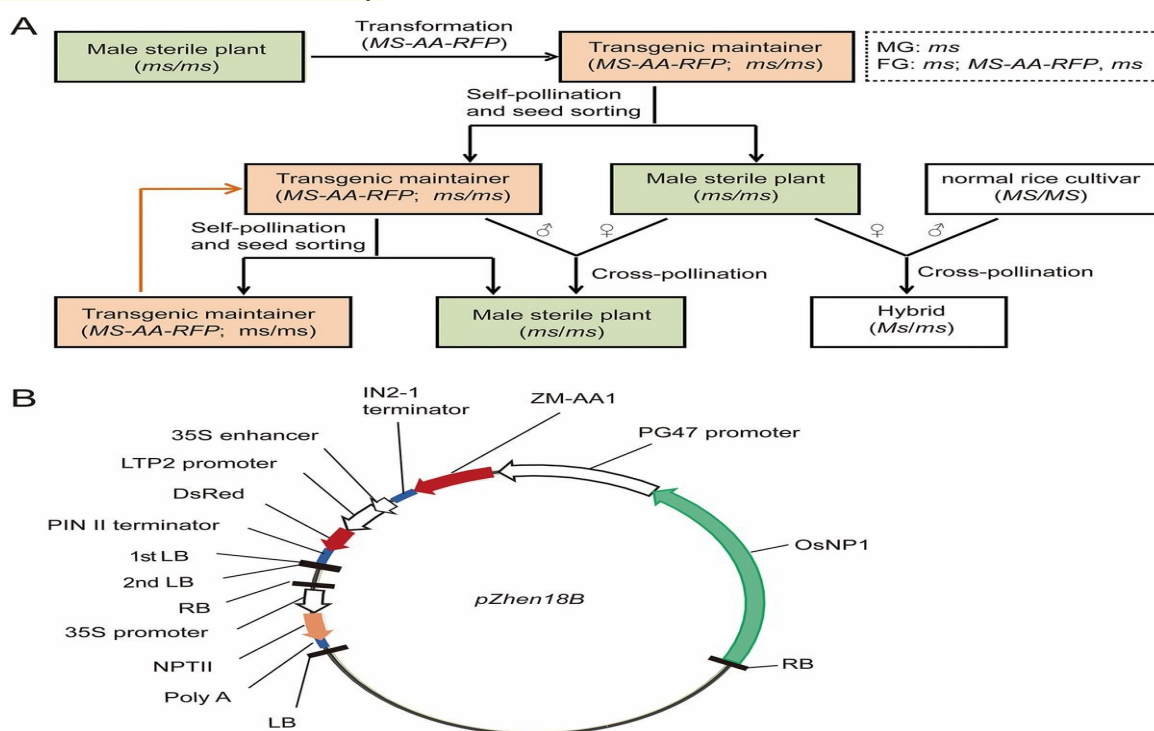


In another case, The 21 parental seed materials [six Lines and 15 Testers (Testers = eight R lines and seven aerobic rice varieties)] were sown in a raised nursery bed during the month of June, 2009. The source materials of A, B and R lines were sown adopting line sowing in raised beds of one meter width and convenient length in a fertile well leveled plot. Thin sowing in the nursery was followed by good water and nutrient management to obtain healthy seedlings with three to four tillers at the time of planting. Seedlings of A, B, R lines which attained the age of 29 days were transplanted in three meter length row with the spacing of 30 cm between rows and 15 cm between plants of each genotype in four rows. R lines were planted separately with an isolation of 300 meter. The row ratio obtained for planting the A and B lines was 8:2. Recommended package of practices and need based plant protection measures were adopted. Crosses were effected in a 'Line x Tester' mating design. The spikelets which were likely to open in the same day were selected during early hours between 6.30 and 8.30 A.M. in the female parents. Wet cloth method of emasculation as suggested by Chaisang et al. (1967) was followed to emasculate the selected spikelets. In this method, Panicles of the A lines on the 3rd or 4th day of its blooming were selected. The immature already opened top and lower spikelets were removed leaving only the middle spikelets. The panicle was covered with wet cloth and hot air was blown through the mouth. Due to increase in temperature and humidity inside the wet cloth, the spikelets were forced to open in the preanthesis time. All the six stamens that protruded out of the opened spikelets were removed one by one carefully by using a pointed forceps without damaging the style and stigma. The unopened spikelets were clipped off. At the time of anthesis, the matured anthers from the male parents were collected and dusted on the stigma of the emasculated spikelets of the female parents. The crossed panicles were labeled and covered with red colored butter paper covers. The butter paper covers were removed three days after pollination. Crossing was repeated till sufficient number of crossed seeds were obtained in each of the cross combinations. Selfing of parents was also done by putting white colored butter paper covers on the panicles before the opening of spikelets. Thus, hybrid seeds of 90 cross combinations and selfed seeds from all the 21 parents were collected after maturity. The seeds were dried at 12 %moisture and preserved at room temperature (28±1°C).[9,10]

## Results

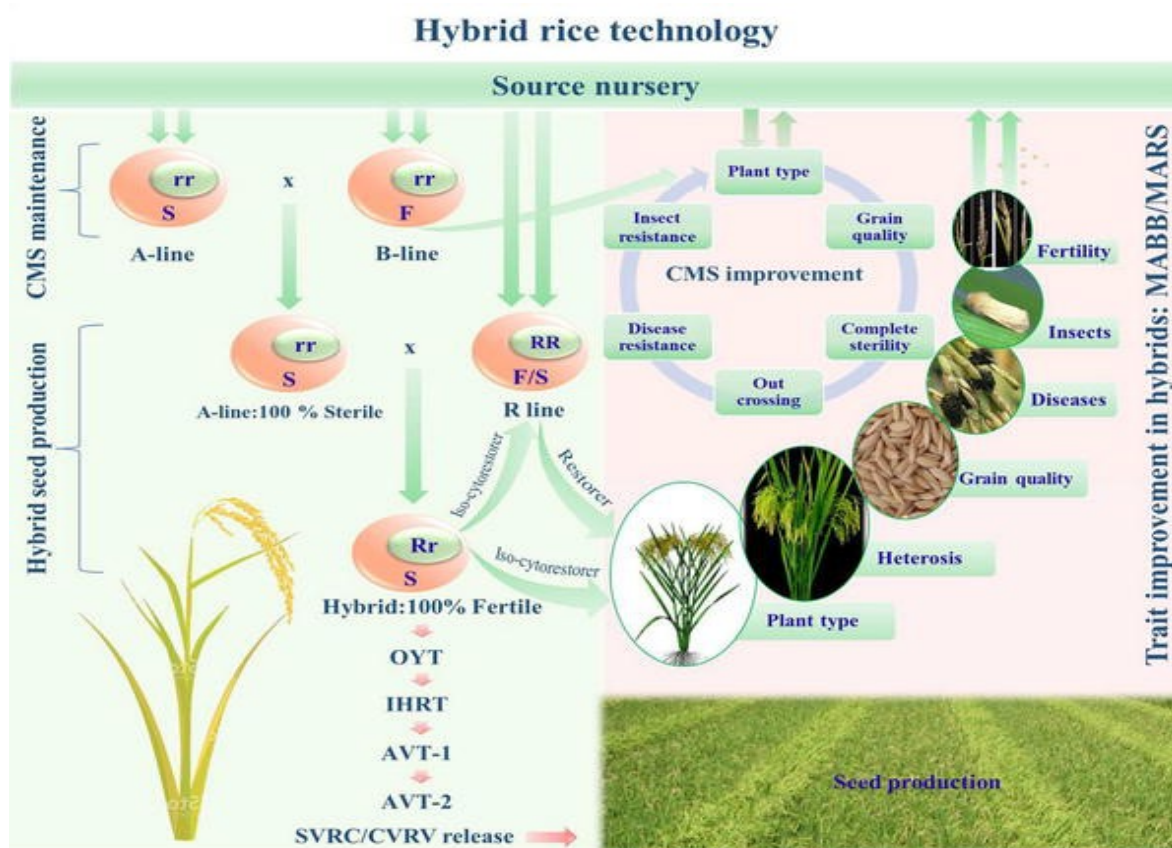
Heterosis is a solitary means of exploiting hybrid vigor in crop plants. Given its yield advantage and economic importance, several hybrids in rice have been commercialized in more than 40 countries, which

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.[11,12]

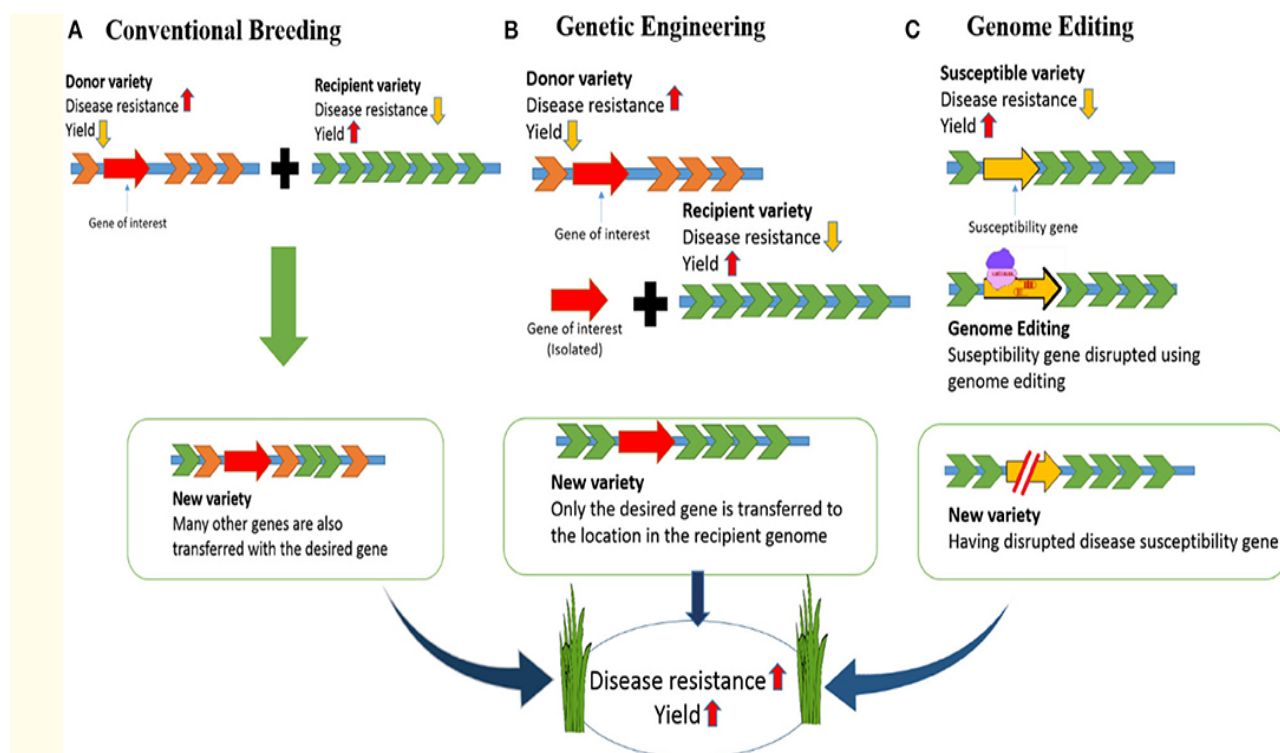
Hybrid technology is one of the greatest innovations in the modern era, contributed greatly in yield enhancement in several important crops. Over the decades of rigorous research, Chinese could develop parental lines, that is, cytoplasmic male-sterile line, maintainer line, and restorer line which assisted 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.[13]



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

### Conclusions

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[14,15]

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,[16,17] 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.[18,19] Hybrids released in India having unambiguous specificity like specific to ecosystem, tolerant to several abiotic/biotic stresses and consumer preferences. These hybrid varieties can be utilized to up scale the hybrid rice cultivation and productivity enhancement per se in the respective area.[20]



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

<b>S.No.</b>	<b>Stress</b>	<b>Promising hybrids</b>
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, KRRH-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	Boro/Summer season	Rajalaxmi, CRHR-4, CRHR-32, NPH 924-1, PA 6444, Sahyadri, and KRRH 2
5	BB resistant	BS 6444G, Arize Prima, Rajalaxmi, Ajay, CR Dhan 701, PRH 10, etc.

## 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