



## Research Article

# TWO-LINE HYBRID PRODUCTION SYSTEM AND THEIR APPLICATIONS IN RICE

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**Abstract-** Rice is one of the most important world food crops, serving as the staple food for over one-third of the world's population. Three line method or CMS system, which is possible and has been found to be most effective genetic tool for developing hybrids. Another efficient method is identification of a pollen sterility system that is dependent on temperature known as the thermo-sensitive genic male sterility called two line system. The TGMS system have three main advantages over the three line methods; no need of restorer line, no need of maintainer line and does not have any negative effect of cytoplasm. TGMS lines become sterile when the environmental temperature is higher than 25–30°C, during the panicle initiation and anthesis stages, and it convert fertile condition, when the temperature goes down below the 25–30°C. Two line method of hybrid seed production is more economic and efficient than to three line method.

**Keywords-** TGMS, EGMS, CMS, Temperature, Hybrid and rice.

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## Introduction

Rice is one of the most important world food crops, serving as the staple food for over one-third of the world's population [1]. Reports of hybrid rice yields, which are at least 15%–20% higher than the best semi-dwarf inbred local varieties, have been made in several countries [2]. The breeding methodology involves the three approaches (a) Three line method or CMS system which is possible and has been found to be most effective genetic tool for developing hybrids, (b) Two line method or TGMS, PGMS and PTGMS system which is governed by environment and (c) One line system or apomictic system. The identification of a pollen sterility system dependent on temperature [3, 4], known as the thermo-sensitive genic male sterility (TGMS), has contributed to obtain male-sterile lines under specific ranges of temperature, opening new perspectives for hybrid rice exploitation. The TGMS lines remain sterile when the temperature goes higher than the 25–30°C, at the time panicle initiation and anthesis stages, and it convert to fertile stage, when temperature goes to lower than 25–30°C. The Two line method of hybrid seed production is more economic and efficient than to three line method[5]. As an alternative to some of the limitations of CMS system, the TGMS system does not require a maintainer line (B line) in hybrid seed production, does not require a restorer line (R line) and the TGMS system reduces the risk of genetic vulnerability, since the expression of male sterility is not dependent on the cytoplasm, so the hybrid can be developed with diverse genetic background[5].

## What is hybrid rice?

The hybrid generation ( $F_1$ ) of rice obtained by crossing the two genetically different parents or it may be varieties. In rice the cyto-plasmic male sterile (CMS) used as a female parent ('A' line) because it is a self pollinated crop. The fertility restoring line which is called 'pollinator' to the female parent is known as male parent. It is generally referred to as 'R' line, and is used for hybrid seed production. The hybrid combines the desirable characters from CMS line and R line. They exhibit vigour for several quantitative characters including yield. They exhibit buffering capacity to counteract several biotic and abiotic factors that limit the productivity.

## Hybrid rice Status

India was one of the first countries to start academic studies on hybrid rice. The Indian Council of Agricultural Research established a goal-oriented network project on hybrid rice in 1989 [6]. The situation for the development and commercialization of hybrid rice in India is very encouraging. Between 1990 and 1996, more than 700 hybrids were developed and evaluated, and the yields of over 100 combinations exceeded that of the best traditional variety by more than 1 tone/ha. Several hybrid varieties have been released for commercial cultivation. In the current situation hybrid rice is growing in 2 lakh hectares area. This area may be increased in future after identifying the good heterotic hybrids with high productivity and production in the main rice growing areas. An effective transfer of technology is taken up vigorously in the target regions [7].

In fact, the hybrid rice research was initiated in 1964 [8]. The genetic tools essential for breeding hybrid rice varieties are as the male sterile line (A-line), maintainer line (B-line) and restorer line (R-line) were developed during 1973 [9]. The breeding methodology involves the three approaches (a) Three line method or CMS system which is possible and has been found to be most effective genetic tool for developing hybrids, (b) Two line method or TGMS, PGMS and PTGMS system which is governed by environment and (c) One line system or apomictic system which would enable farmers to use their own seed for the successive crops without experiencing genetic segregation. Among these, three line approaches is being widely adopted in India and had fruitful results in the development of more than thirty five varieties of rice hybrids.

Another important tool for hybrid seed production is Environment-sensitive genic male sterility system, controlled by nuclear gene expression, which is influenced by environmental factors such as temperature, day-length, or both. This male sterility system was first observed in pepper by Martin and Crawford in 1951 and subsequently in different crops. The advantages of the EGMS system over the three line method there is no need for a maintainer and restorer lines for the multiplication of seed. That is why the system becomes simpler and more cost-

effective for seed production. Any of the fertile line can be used as a pollen parent to restore the fertility of female parent; therefore, the frequency of heterotic hybrids is higher among two-line hybrids than among three-line hybrids, thereby increasing hybrid breeding efficiency. Negative effects of sterility-inducing cytoplasm are not encountered. The EGMS trait is governed by major genes, thus enabling their easy transfer to any genetic background and thus increasing diversity among the female (EGMS) parents, which helps in reducing potential genetic vulnerability among the hybrids. Since there is no need for restorer genes in the male parents of two-line hybrids, this system is ideal for developing indica/japonica hybrids because most japonica lines do not possess restorer genes.

The discovery in 1973 of Nongken 58 S, a PGMS/TGMS japonica rice line [10, 11], provided the first genetic source for the development of two-line system hybrid rice. The major feature of such PGMS/TGMS lines is that, under longer day length and higher temperatures they show complete pollen sterility, in which case they can be used for hybrid seed production, while under shorter day length and moderate temperatures they show almost normal fertility and thus can multiply themselves by selfing. In 1987, China initiated a collaborative research project involving the exploitation of PGMS/TGMS lines to develop two-line system rice hybrids. The degree of male sterility was 99-100% at heading under artificial light of more than 14 hours but plants were male fertile under artificial light of less than 13 hrs 45 min [12]. This mutant was designated as Hubei-photo sensitive genetic male sterile rice (PHGMS) and this male sterility was controlled by single recessive gene [12, 13].

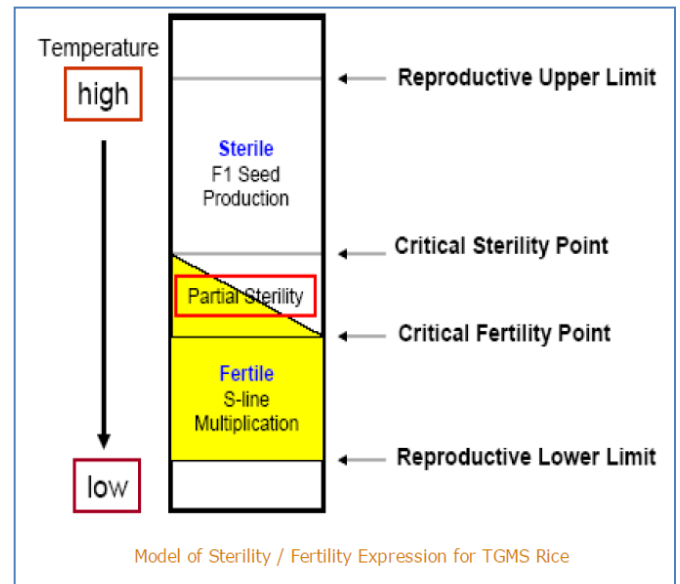
#### Two-line hybrid rice

Based on the environment behavior EGMS systems are categorized in to three partitions that affect the sterility/fertility: 1. photoperiod-sensitive genic male sterility (PGMS), 2. Thermo-sensitive male sterility (TGMS) and 3. Photo thermo-sensitive male sterility (PTGMS). The EGMS in rice first became possible when Nongken 58S was discovered in China by Shi (1985). This spontaneous mutant of the japonica variety Nongken 58 was male-sterile under long day and convert to fertile under short-day conditions. In china and other countries were developed so many PGMS and TGMS lines. In the tropics, where photoperiod differences are minimal but wide temperature differences exist among different altitudes or different seasons, it is more practical to use the TGMS system [14]. The temperature fluctuation is the main constraints for commercialization and adapting the two-line hybrid system for seed production and the mixing of selfed seeds with the TGMS seeds. One of the strategies can be solved this problem by breeding the TGMS lines with low critical sterility point (CSP) temperature lines. When used in hybrid seed production, TGMS lines possessing low CSP remain completely sterile despite sudden temperature changes during the sensitive growth stage. This eliminates selfing. The CSP is the critical temperature during the sensitive stage of a TGMS line that results in complete sterility. Hybrid rice breeders use either of the 2 parameters in determining the CSP of a TGMS line: maximum temperature or mean temperature, depending on geographical location. The maximum temperature was influences the fertility/sterility expression of the TGMS line in the tropics[15]. The ideal CSP for a TGMS line under tropical conditions is at maximum temperatures between 30 and 32°C. In China, which is a temperate country, the daily mean temperature is used to identify the CSP of TGMS lines, since it is a function of both minimum and maximum temperatures [16]. The mean temperature is computed from 4 temperature readings at 6-hour intervals. The ideal CSP for a TGMS line is a daily mean temperature of 23-25°C [17]. The objective of the study firstly screen out the TGMS lines which are having low CSP and determine the correct timing for TGMS evaluation in the tropics areas. There are mainly two types systems of two line hybrid seed production generally used:

1. Photoperiod Sensitive and
2. Thermo-sensitive Genetic Male Sterility

The male sterile lines in which sterility expression is controlled by temperature are called thermo-sensitive male sterile (TGMS) lines and those in which expression is controlled by day-length period are called photoperiod-sensitive male sterile (PGMS) lines. In 1973, Chinese scientist Shi Mingsun discovered a natural male sterile plant in the field of Nongken 58, a *japonica* late maturing variety, at Shahu

Farm of Mianyang County, Hubei Province, China. After eight years of in-house study for confirmation, he announced his discovery as a dual-purpose rice line Nongken 58S in 1981, and proposed a new strategy to utilize heterosis in rice, namely two-line system based on his research results [11]. When Nongken 58S heads during August it is male-sterile (99.5-100%). In the summer season sterility condition of pollen is stable, but in autumn season the fertility is unstable due to over the locations and years. The first stable line was Nongken 58S i.e., completely sterile under long day period and high temperature conditions, and partially fertile under short day period and low temperature conditions.



Source: Rice Knowledge bank IRRI

#### Breeding of two-line hybrid rice

In china, by backcrossing method in several varieties the PGMS trait has been transferred in *Indica* and *Japonica* rice. The hybrids those have been developed by this system are being further evaluated in multi-location trials. In two-line hybrid system higher the possibility to get heterotic combinations compare to the three-line hybrid rice. Unlike three-line hybrids, the male parent of two-line hybrid is not restricted by restorer genes, so we can use not only the good restorer lines with high combining ability as the male parent, but also the good conventional varieties without restorer genes as male parent. The non-restriction of restorer genes brings about greater opportunity to breed elite hybrids. The developed PTGMS lines have many advantages for hybrid combinations, such as larger freedom for crossing, higher yielding, better quality and resistance to diseases than CMS lines.

#### Breed new restorers from crossing

Although in case of different traits e.g., quality traits, resistance and yielding the two-line hybrid system in rice is superior over the three-line hybrid rice. But we have to create or expand the genetic variations in parents, by making the crosses and breeding for new restorers lines in two-line hybrid system, and reduce the shortage of parents using all the complementary effects.

#### Achievements in two-line hybrid rice breeding

Because the PTGMS lines can be used to produce hybrid seeds in the sterile period and to multiply themselves in the fertile period, a nationwide research was organized to study the mechanism of PTGMS and its application after the discovery of Nongken 58S. Soon after, many *japonica* and *indica* PTGMS lines have been released using male sterile genes in the original Nongken 58S. Furthermore, some other germplasms with fertility alteration such as AnnongS-1, 5460 S and Hengnong S-1 are also identified. Up to now, tens of practical PTGMS lines in rice for hybrid seed production have been identified. At present, the PTGMS lines e.g. Nongken 58S or TGMS Annong S. used in rice production. More attention should be paid to the following areas in order to improve screening and utilizing efficiency of photothermo-sensitive male sterility [18].

### Future research prospectus of two line system [19]

**Development of stable EGMS lines**-Stable elite EGMS lines are with a precise fertility alteration mechanism hold the key 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 (critical sterility point) 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.

**Use of anther culture to develop and purify elite EGMS lines**-Anther culture techniques involving dihaploidization can be used to expedite the development and/or purification of EGMS lines possessing major genes and QTLs in influencing the PGMS/TGMS trait.

**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.

**Incorporation of hybrids with resistance to biotic and abiotic stress**-Two-line rice hybrids possessing multiple resistances to diseases-insects and abiotic stresses 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.

**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.

### Conclusion

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. This system is more economic compare to the three line method but main problem is that unavailability of stable TGMS lines and fluctuation in the temperature/photoperiod. 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. In India no any hybrid developed so far developed through two-line based hybrid system in rice because of the flexibility in the environmental condition and lack of stable TGMS lines.

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**Abbreviations:** CMS- Cytoplasmic Male Sterility, EGMS- Environmental-sensitive Genetic Male Sterility, PGMS- Photoperiod-sensitive Genic Male Sterility, TGMS- Thermo-sensitive Genic Male Sterility, PTGMS- Photo-thermo-sensitive Genic Male Sterility, PHGMS- Hubei-photo sensitive Genetic Male Sterile, CSP- Critical Sterility Point, WA- Wild Abortive, QTL- Quantitative Trait Loci and IRRI- International Rice Research Institute.

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of Interest:** None declared

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