Chapter 10

Chinese Experiences in Breeding Three-Line, Two-Line and Super Hybrid Rice

Liyong Cao and Xiaodeng Zhan

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1. Introduction

Because rice is a staple food for over half of the world's population, it is estimated that the world rice production must be annually increased by approximately 1% to meet the growing demand for food, resulting from population growth and economic development (Rosegrant et al., 1995). Rice is one of the main food crops in China with the second largest planting area, most total yield and highest per unit yield (Table 1), it feeds more than 60% of the population and contributing nearly 40% of total calorie intake in China (Cheng and Li, 2007). China is the largest producer and consumer of rice, and also a pioneer in the utilization of hybrid rice technology in the world. Hybrid rice has resulted in a substantial increase of food production in China over the past 40 years. China average rice yield has risen from 1.89 tons per ha (t/ha) in 1949 to 6.71 t/ha in 2012, which created the highest historical record (http://futures.xinhua08.com/a/20121018/1042507.shtml). Hybrid rice has played an important role for total grain production to consecutively increase for nine years in China (http://www.aqzyzx.com/system/2012/10/31/006110920.shtml).

Crop	Planting Area (10^4 ha)	Total output (m t)	Yield (kg/ha)
Corn	33541.67	19.28	5741.7
Rice	30057.04	20.10	6680.7
Wheat	24270.00	11.74	4832.4

Source: http://datacenter.cngrain.com/IndexProduce.aspx?Flag=1&IsHome=0&TId=74&Str=PP

Table 1. Planting area, total production and yield of three main food crops in China in 2011

Variety improvement plays a leading role in increasing of grain yield characterized as two quantum leaps in rice (Chen et al., 2002). The first one was brought about by the development of semi-dwarf varieties in the late 1950s in China and early 1960s at the International Rice Research Institute (IRRI). In 1956, a dwarf mutant was found in *indica* variety Nantehao in Guangdong Province, China. Since then, Huang et al. (2001) had initiated dwarf rice breeding in Guangdong and southern China, and released semi-dwarf *indica* rice varieties Guangluai 4, Guichao 2, Teqin, etc. subsequently. The semi-dwarf varieties displayed a yield potential up to 7.5 t/ha, which is 20%–30% higher than the traditional tall varieties, owing to the improved tolerance to lodging for standing higher rates of fertilizer. In 1966, IR8 as the first semi-dwarf variety from IRRI was released to tropical irrigated lowlands (Peng et al., 1994; 2008). The second leap arose from commercial use of hybrid rice in 1976 in China (Yuan et al., 1994). Compared with inbred rice, hybrid rice can increase grain yield by approximately 20%. These two major breakthroughs have brought China's average rice yield up to a new level in the mid-1970s and mid-1980s, respectively. Thereafter, with popularization of hybrid rice due to improved hybrid seed production methods, rice yield was further elevated to 6.0 t/ha in early 1990s in China, which was the world level, then. However, the yield ceiling witnessed in various crop species has also been encountered in the rice production in China since 1990. Considering that the annual per capita rice consumption is 150 kg and rice cropping area maintains at 31.57 million hectares in China, it is estimated that rice total production and yield per unit must be increased by 35% and 32%, respectively in 2030 (Cheng et al., 2005). This estimation implies a great challenge to rice community, and the 3rd leap of rice yield is definitely needed for the challenge.

In response to the challenge, Chinese Ministry of Agriculture (CMOA) organized China National Super Hybrid Rice Symposium at Shenyang in 1996, where rice scientists from all over China united to design a national proposal to breed super hybrid rice and develop cultivation methodologies to realize yield potential of the super hybrid. In 2000, because of the leadership of CMOA and the leading role of China National Rice Research Institute, China Super Rice Cooperative Research Group released super hybrid rice cultivars and reached the phase◎target of 10.5 t/ha. In 2005, the phase II target of 12 t/ha was accomplished, and cultivation of super hybrid rice cultivars developed in the phase I was dramatically extended to a large area nationwide. Till 2012, the grain production has consecutively increased for nine years, and nationally produced grain of more than 500 million tons has maintained for five consecutive years in China. The 500 million tons set a new record of grain production in China, which is the planning level of grain production in 2020. The abundant harvest will play an important role in maintaining economy to develop steadily in China.

Meanwhile, steady rice production in China has to keep dealing with decreasing growth area along with an increasing population, biotic and abiotic stresses, extensive use of chemical fertilizers, and water shortage. Therefore, it seems at present that the most effective and economic way is to develop and extend super inbred rice and hybrid rice cultivars with wide adaptation and super high yielding potential, which is also an alternative solution to China's future food security problem and an important way to maintain social stability (Chen et al., 2007).

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2. Genetic mechanism of rice heterosis

Heterosis, or hybrid vigor, refers to the phenomenon that progeny of diverse inbred varieties is superior over both parents on yield, panicle size, number of spikelets per panicle, number of productive tillers, stress tolerance etc. This phenomenon to be a powerful force in the evolution of plants has been exploited extensively in crop production. Successful development of hybrid maize in 1930 gave great impetus to breeders of other crops including rice to utilize the principle of hybrid production by exploiting heterosis. In fact, the exploitation of heterosis has been the greatest practical achievement of the science of genetics and plant breeding (Alam et al., 2004). The impact of this phenomenon can be judged by the fact that rice dramatically varies on the number of grains per square meter among 1) wild ancestors with only a few hundreds, 2) improved inbred varieties with about 40, 000, and 3) rice hybrids with about 52, 000 (Mir, 2002). Rice heterosis was first reported by Jones (1926) who observed that some F_1 hybrids had more culms and greater yield than their parents. Between 1962 and 1967, a number of suggestions came from different places of the world for commercial exploitation of heterosis to become a major component of rice improvement programs at national and international level. For example, rice breeders from Japan, China, United States, India, the former Soviet Union and Philippines started their projects to utilize rice heterosis. However, progress had not been sound because of the difficulty for rice to be a strictly self-pollinated crop unlike corn, which made out crossing absolutely essential for hybrid seed production extremely difficult.

2.1. Genetic hypotheses for crop heterosis

Classic quantitative genetic explanations for heterosis center on two concepts, dominance and over dominance (Crow, 1952). With advances on genetic study of quantitative traits and high density molecular linkage maps, many research groups prefer epistasis as a major genetic basis of heterosis (Wright, 1968; Hallauer and Miranda, 1988).

"Dominance" originally means that heterosis is resulted from action and interaction of favorable dominant genes brought together in an F_1 hybrid from two parents. This hypothesis assumes that genes that are favorable for vigor and growth are dominant, and the genes contributed by another parent result in more favorable combination of dominant genes in the F_1 than either parent. For instance, we have a combination of five dominant genes ABCDE favorable for yields, patent one (P1) has the genotype AAbbCCDDee (possessing three dominant genes ACD) and parent two (P2) has the genotype aaBBccddEE (possessing two dominant genes BE); the F_1 hybrid derived from the two parents will have five dominant genes as shown below (Fig. 1).

The F_1 hybrid therefore would exhibit higher yield than either of the parents because each parenthas only a part of five dominant genes. According to this hypothesis, inbreeding depression occurs when unfavorable recessive genes hidden in the heterozygous conditions in the F_1 generation become homozygous in subsequent generations with inbreeding. Crossing unrelated homozygous lines obscures the deleterious recessives and restores vigor.

P1 AAbbCCDDee × P2 aaBBccddEE

↓

F1 AaBbCcDdEe (performance of heterosis)

Figure 1. Illustration of dominance hypothesis

The second historical explanation for heterosis is "over dominance, " which refers to allelic interactions in the hybrid, such that the heterozygous class performs better than either homozygous class (Fig. 2). Thus, an individual such as the F_1 hybrid with the greatest number of heterozygous alleles will be mostly vigorous compared to two parents. Because these two explanations for heterosis were developed under the conditions with non-additive effects and supposed all the genes have the same influences to different traits, they have limitations and can't explain the heterosis in molecular level. Therefore, they are of diminished utility for describing the molecular parameters that accompany heterosis.

P a1a1b1b1c1c1d1d1e1e1× a2a2b2b2c2c2d2d2e2e2

↓

F1 a1a2b1b2c1c2d1d2e1e2 (performance of heterosis)

Figure 2. Illustration of over-dominance hypothesis

The hypothesis of over dominance advocating that the hybrids exhibit superiority to the better parent has been agreed by increasing number of studies. However, this hypothesis completely denies the function of dominant genes in heterosis. It is well known that heterosis doesn't perfectly comply with heterozygosity of alleles. For instance, some rice hybrids do not perform better than their homozygous parents at some specific traits.

The hypothesis of epistasis regards heterosis to be genetically controlled by many genes because a complex character such as yield includes many components. Heterozygosity with gene interaction is the primary genetic basis for explanation of heterosis because the hybrid is heterozygous across all genetic loci that different between the parents. Thus, the degree of heterosis depends on which loci are heterozygous and how within locus alleles and inter-locus alleles interact with each other. Interaction of within locus alleles results in dominance, partial dominance, or over dominance, with a theoretical range of dominance degree from zero (no dominance) to larger than 1 (over dominance). Interaction of inter-locus alleles results in epistasis. Genetic mapping results have indicated that most QTLs involve in heterosis and other quantitative traits have a dominance effect. Epistasis has been found more frequently and proven to be a common phenomenon in the genetic control of quantitative traits including heterosis (Yu et al., 1997; Luo et al., 2001; Hua et al., 2003). Study of Yu et al. (1997) provided strong evidence for two-loci and multi-loci interactions (epistasis) especially for traits such as grain yield, which are complex in nature. They found that heterosis is not controlled by a single locus, even the locus behaves in dominant or overdominant patten, linkage and epistasis has a major role. Thus, the effects of dominance, over dominance and epistasis of various forms are not mutually exclusive in the genetic basis of heterosis, as opposed to what was previously debated in favor of different hypothesis. All of these components have a role to play depending on the genetic architecture of the population (Hua et al., 2003), i. e. single-locus heterotic effects (caused by partial, full-and over-dominance), all three forms of digenic interactions (AA/AD/DA and DD) and probably multi-locus interactions.

Thus, these results may help reconcile the century long debate on the role of dominance, over-dominance and epistasis as genetic basis of heterosis. Two different types of allelic interaction, both within-locus and inter-locus, should play an important role in the genetic control of heterosis. A full understanding of heterosis has to wait for breakthrough achievements on cloning and functional analysis of all genes related to heterosis. This process would be very similar to the understanding of disease resistance genes with aid of standard check variety.

2.2. Molecular basis of heterosis

Since heterosis is a phenomenon of superior growth, development, differentiation, and maturation caused by the interaction of genes, metabolism and environment, a simple explanation of heterosis solely based on the nuclear genome heterozygosity appears untenable. Several distinct lines of evidence from biochemical, physiological, ultrastructural and restriction endonuclease DNA fragment analyses in a variety of organisms are available to show that all three genetic sources of nuclear genome, mitochondrial genome and chloroplast genome, in staed just one of them, are at work during the manifestation of heterosis.

Some molecular studies support the over dominance hypothesis (Stuber et al., 1992, Yu et al., 1997, Li et al., 2001), but another supports the dominance hypothesis (Xiao et al 1995). Yu et al. (1997) reported over dominance at several main-effect quantitative trait loci (QTLs) and a stronger additive epistasis affecting grain yield and its components in F_3 progenies from Shan You 63, the most widely grown hybrid in China. Furthermore, Li et al. (2001) concluded that most QTLs associated with inbreeding depression and heterosis in rice appeared to be involved in epistasis, and almost 90% of the QTLs contributing to heterosis appeared to be over dominant. Zhang et al. (2001) assessed the relationship between gene expression and heterosis by assaying the patterns of different genes expression in hybrids related their parents using a diallel cross. The analysis revealed that differentially expressing fragments occurred in only one parent of the cross were positively correlated with heterosis, but the fragments detected only in F_1 generation not in the respective parents were negatively correlated with heterosis. Using a total of 384 fragments recovered from gels which hybridized with the mRNAs from seedling and flag-leaf tissues, Zhang et al. (2000) detected an overall elevated level of gene expression in the hybrid compared with the parents, where several fragments showed a higher expression in the high-heterotic hybrid than in the low-heterotic hybrids. Studying the molecular mechanism of differential gene expression between Chinese super-hybrid rice cultivars and their parental lines concluded that many genes were up-regulated in the super-hybrid, whereas other genes were down-regulated (Zhang et al, 2006). These findings pointed out a role of enhanced photosynthesis in the heterosis of the super-hybrid combinations. Using different display techniques for a set of diallel cross involved eight elite hybrid rice parents, Xiong et al. (1998) studied the relationship between banding patterns of differentially displayed gene expressions and the level of heterosis, and showed that dominant type of differential gene expression in flag leaf tissue failed to be correlated with heterosis on yield traits, while differential inhibition of gene expression in the hybrids appeared to be significantly correlated with heterosis. Huang et al. (2006) analyzed gene expression profiles of an elite rice hybrid with the parents at three stages of young panicle development, a cDNA microarray consisting of 9198 expressed sequence tags (ESTs) was used for the objective to reveal gene expression patterns that may be associated with heterosis in yield. The results showed that the biochemical and physiological activities took place in the hybrid relatively rather than in the parents. Identification of genes showing expression polymorphisms among different genotypes and heterotic expression in the hybrid may provide new avenues for exploring the biological mechanisms underlying heterosis.

Nonetheless, a lack of a clear understanding of the genetic or molecular basis of heterosis has not prevented plant breeders from exploiting this phenomenon to raise crop yields.

3. Methods and strategies in hybrid rice breeding

3.1. The methods in hybrid breeding

Prof. Yuan proposed the breeding strategy to Chinese scientists for developing hybrid rice in the following phases. Three approaches are for breeding methodology, 1) three-line method or CMS (cytoplasmic male sterility) system, 2) two-line method or PTGMS (photo/temperature sensitive genic male sterility) system and 3) one-line method or apomixis system. Three ways are for increasing the degree of heterosis, 1) inter-varietal hybrids, 2) inter-sub-specific hybrids and 3) inter-specific or intergeneric hybrids (distant hybrids).

Indica and *japonica* (both tropical and temperate) are two main subspecies of *Oryza sativa* in Asia. *Javanica* is genetically between *indica* and *japonica*. Among them, *indica* and *temperate japonica* subspecies have the most apparent difference in morphological and agronomic traits. Many studies indicate that the degree of heterosis in different kinds of hybrid rice varieties has the following general trend: *indica/japonica*>*indica/javanica*>*japonica/javanica*>*indica/indica*>*japonica/japonica* (Yuan, 1996). The first three kinds are inter-subspecific hybrid, and the last two are inter-varietal. Currently, hybrid rice technology mainly uses inter-varietal heterosis, *indica* × *indica* and *japonica* × *japonica*. The high-yielding inter-sub-specific hybrids yield 15% to 20% more than the best inbred varieties when they are grown under similar conditions. But now, it has been quite difficult to make the genetic difference between parents great enough for the inter-subspecific hybrids, so that their yields have almost reached a ceiling. In *japonica/javanica* hybrids, there are a few fertility problems till present. Therefore, using heterosis between *japonica/japonica* and *indica/japonica* would be an effective approach for increasing rice yields. *Indica/japonica* hybrids posses the highest yield potential in both sink and source. Their theoretical yield can be 30% more

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than the best existing inter-varietal hybrid varieties. But inter-subspecific heterosis has not been commercially utilized because of high spikelet sterility and long growth duration.

The discovery of wide compatibility (WC) genes provides a possibility to resolve the problems of seed setting and growth duration in the inter-subspecific hybrids. IRRI, China, and India are making a great effort to develop inter-subspecific hybrids. Recently, Chinese scientists have developed super high-yielding rice hybrids from crosses involving *indica/japonica* derivative parents. Most of the inter-specific crosses in cultivated species are within either *O. sativa* or *O. glaberrima*, and their hybrids are heterotic but not so useful in terms of yield and plant stature. Most inter-specific hybrids from wide hybridization have elevation of genetic variability and bring in desirable genes for resistance to several biotic and abiotic stresses, such as the progeny of *O. sativa* × *O. longistaminata*, *O. sativa* × *O. rufipogon*, and *O. sativa* × *O. perennis*.

3.2. The principles for parental selection

High-yield, good quality and multiple resistances are the eternal targets in rice breeding. Matching proper parents together is the key and basis to breed excellent hybrid combinations. Selection of excellent hybrid combinations must base on heterosis with the following specific considerations.

3.2.1. Selection of parents with big variation in genetic basis

Hereditary diversity is the basis of heterosis, and within a certain range, the genetic diversity decides heterosis. Because rice varieties are different in kinship, geographical origin or ecotype, the heterosis is produced by genetic diversity between both parents. Widely commercialized hybrid rice combinations usually have widely different parents in ecological types and geographic origins, such as the parents for Shanyou63, ShanYou10 and XieYou46. Furthermore, the parents may differ in *indica-japonica* affinity.

3.2.2. Screening the parents with good traits

Presently, hybrid rice varieties in commercial production have complementary good traits from their parents. Thus, hybrid rice combinations are comprehensively better than the parents, such as Shanyou63, Shanyou10, Liangyou Peijiu, Zhongzhe You 1 and so on. The hybrid combinations gather many good characteristics of parents such as disease-resistance, late mid-maturity, strong tillering ability and high grain weight. Studies on heredity law of essential traits in hybrid rice showed that some traits of hybrids have certain relationships with the average of parents, such as number of grains per panicle, filled grain number per panicle, grain weight, efficient panicles per unit area, growth period and plant height. These traits have highly significant correlations between a hybrid and the average of its parents. According to this relationship, we may choose the combinations with excellent parents to breed new restorer or maintainer lines.

3.2.3. Selection of parents with good combining ability

General combining ability (GCA) refers to the average performance of an offspring from one parent that is crossed with many other parents. GCA is determined by the number of favorable genes and the size of gene function in parents, and often influenced by additive effects. Specific combining ability (SCA) refers to the performance of a unique offspring deviation from a specific pair of parents. SCA is mainly controlled by non-additive effects. Therefore, not only the yield of parents is necessary for, but also the combining ability of parents is very important for hybrid rice breeding.

3.3. Strategies for hybrid rice breeding

In addition to the breeding principles described above, the following strategies are also very important in hybrid rice breeding to result in hybrid cultivars with high yield, good quality and disease resistance.

3.3.1. Amplification of genetic diversity in parents

Through pedigree analysis, most rice cultivars originate from few parents which genetic diversity is small. The main reason for hybrid rice yield to stand still since 1980s maybe relate to short in genetic diversity in China. Since the discovery of semi-dwarfism in *indica*, genetic diversity among cultivars has become increasingly narrow, so that strong hybrid vigour has hardly been achieved by combining *indicas* cultivars. *Japonica* materials are full developed and utilized in temperate zone, resulting in a narrow genetic diversity among cultivars. The narrow diversity makes it very hard to achieve hybrid preponderance in inter varietal crosses. Tropic *japonica* (*Javanica*) and *nuda* types of rice (*japonica*) are not widely utilized in breeding because they are limited in special regions with some particular traits. Recently breeders have paid much attention to wide compatibility germplasm derived from tropic *japonica* and *nuda* rice. After Yuan (1987) proposed the strategy to utilize heterosis between *indica-japonica*, breeders have made a great effort to amplify parents' genetic diversity and breed super high-yield *indica-japonica* hybrid through effective utilization of wide compatibility. *Indica-japonica* combinations from direct crosses hardly have super high-yield because of sterility. Introgressing *japonica* genes into *indica* restorer lines in the south and introgressing indica genes into *japonica* in the north are proven to be effective for super high-yield hybrid rice breeding in China. Taking this strategy, three new *indica* restorer lines, Zhong413, R9308 and T2070, are bred by China National Rice Research Institute. They all contain about 25% of *japonica* components, and their hybrid combinations have super high-yield.

3.3.2. Increasing the biological outputs

Ideotype is an ideal plant model which is expected to yield the most for a specific environment. Hybrid rice ideotype (HRI) is a best parental combination to yield the most grains with good quality in a certain ecological environment. HRI does not only contain desire morphological characteristics but also have resistances to biotic and abiotic stresses for the environment. Otherwise, the most grains with good quality are not achievable. For instance, Taoyuan in

Yongsheng County, Yunnan Province, China is a perfect ecological environment for rice, where the yield of indica hybrid rice Shanyou63 can be up to 15.27 t/ha with a harvest index up to 0.54 in small field trial. However, under normal conditions in most areas of China, it is not possible for Shanyou63 to yield 15.27 t/ha. This difference may imply the importance of plant biomass for grain yield. Increasing plant biomass with maintaining or improving harvest index may lead to raise grain yield directly. It is well known that high biological-outputs need abundance of sunshine and high levels of nitrogen nutrition. In addition to high plant biomass, high grain yield needs a series of good characteristics such as tough stems, erect leaves and rational operation of photo synthetic products. Otherwise, the population will lodge to flat and the leaves shade each other, favorable for pest and disease infestations. At the end, rice yields will decline instead of increasing.

Increasing the biological outputs is the key to further improve yield potential under a certain harvest index condition for high-yielding dwarf rice. It is no doubt to properly raise plant height beneficial for increasing biological products, but we must enhance the capacity of lodging resistance at the same time and ensure all set grains harvestable. In current high-yielding rice fields, when the number of spikelets per square meter is about 40, 000 or slightly less, 50, 000 to 60, 000 and 60, 000 to 70, 000, the corresponding grain yield could be 7.5 t/ha, 11.25 t/ha and 15 t/ha, respectively. To a specific rice variety, the panicle number per unit and grains per panicle varied upon to ecological conditions, thus, we must improve leaf position and leaf quality to increase the number of spikelets per unit and the total output. Meanwhile, strong root activity, no immature stems and leaves, long time of photosynthesis, rough vascular bundles in stems and spikes are all very important for grain production.

3.3.3. Improvement of leaf posture and quality

Plant growing, tillering and expanding ability of tillers at early stage, stand upright, and enough green leaves at later stage are all extremely important for rice to use solar energy highly efficiently. Thickness of leaves and maximum leaf nitrogen content should be exploited under intensive cultivation conditions because their advantages for improving photosynthetic capacity. Besides maintaining the photosynthetic ability of leaves, maintaining the flag leaves upright is also very important. Studies have indicated that the thicker the leaves are, the more mesophyll cells per unit area and the larger intercellular spaces there are (Zhu et al., 2001; Fu et al, 2012). Therefore, it is also very important to select thicker leaves in hybrid breeding because they are beneficial for diffusing carbon dioxide and maintaining high level of chlorophyll and nitrogen content inside the leaves.

Curling degree of leaf is another important index for leaf posture in super-rice breeding due to its benefit for photopermeability by increasing the under surface. Zhu et al. (2001) studied the relationships between curl degree and photopermeability, and classified the leaf curl degrees into high, intermediate and low with 44^{o}-47^{o}, 15^{o}-16^{o} and 10^{o}-11^{o}, respectively. Testing the photosynthesis rate between under and upper surface of leaves resulted in 1.19-1.32 in the high, 0.90-1.02 in the intermediate and 0.82-0.85 in the low curl degree hybrid combinations, respectively. Compared with the low curl degree combinations, the high and intermediate curl degree combinations had smaller leaf angle, higher leaf straightness and lower extinction efficient. So, we should select the lines with high or middle leaf curl degree in breeding.

3.3.4. Increasing the ability of root system

Long time ago, researchers were attracted by the influence of root system on rice associated to grain yield. Nagai proposed the concept of root type for the first time in 1957. Ling et al (1989) studied the close relationship between the direction of root stretching and leaf angle, and proposed that cultivating root type was favorable for ideo-type as well as rice high-yield cultivation. Root vigour, especial during the filling stage, guarantees a super high-yield of rice, undoubtedly. A radical reason for bad grain plumpness in hybrid rice is root senescence. However, improving root system properties has not been embodied in rice breeding plan till now, so we should do further and more research in root vigour at various aspects such as methodology study for root characteristic, root physiological characteristic, relationship with ground part, diversity among breeding materials and genetic utilization to construct ideal root type.

4. Three-line system hybrid rice

4.1. Identification and utilization of cytoplasm male sterility

The role of cytoplasm on causing male sterility of rice was first reported in 1954 (Sampath and Mohanty 1954). In 1965, Sasahara and Katsuo studied cytoplasmic differences among rice varieties and developed, for the first time, a male sterile line by transferring the nuclear genotype of rice cultivar Fujisaka 5. However, this cytoplasm male sterility (CMS) line could not be used for breeding rice hybrids because of its instability, poor plant type and photoperiod sensitivity. In 1964, Yuan Long Ping put forward the idea to utilize the heterosis in rice and initiated the research on hybrid rice in China for the first time. In November 1970, a pollen abortive wild rice plant (shortly called wild abortive, i.e., WA) was discovered among the plants of common wild rice (*Oryza rufipogon Griff*. L.) at Nanhong Farm of Ya County, Hainan Island which is the south most province of China. After the discovery of WA, a nationwide cooperative program was immediately established to extensively testcross with the WA and screen for its maintainers and restorers. Soon in 1972, the first group of CMS lines such as Erjiunan 1A, Zhenshan 97A and V20A were developed all using WA as the donor of male sterile genes and all using successive backcrossing method. In 1973, the first group of restorer lines such as Taiyin 1, IR24 and IR661 were screened out through direct test crossing method. In 1974, the hybrids with strong heterosis such as Nanyou 2 and Nanyou 3 were released. In another word, the discovery of WA resulted in the subsequent and successful breakthrough in hybrid rice development, so that the three-line hybrid rice system was established. Therefore, China became the first country to commercialize hybrid rice for food production in the world.

Three-line hybrid system includes the CMS line (A), maintainer line (B) and restorer line (R) for a commercial production of rice hybrids. The A line cannot produce viable pollen due to the interaction between cytoplasmic and nuclear genes, so called cytoplasmic male sterile, which anthers are pale or white and shriveled. The A line is used as a female parent for hybrid seed production, so it is commonly called the CMS line and the seed parent as well. Because the CMS line is male sterile, it cannot be self-reproduced and has to have a maintainer. The B line is the maintainer line, which morphology is highly similar to its corresponding CMS line except its reproductive function. The B line has viable pollen grains and normal seed setting, so can pollinate the A line and the F_1 plants from this pollination are male sterile, again. In this way, the male sterility of the A line is maintained, and the A line is reproduced for further use or commercial use in a large scale. Similarly, the R line has viable pollen grains and normal seed setting and can pollinate the A line. Differently from the pollination with B line, the F_1 plants from the pollination with R line are highly fertile, or the male sterility of the A line is restored into fertility by R line in their progeny. Therefore, the R line also called the pollen parent, male parent, and/or restoring line.

4.2. Diversification of CMS sources

Chinese rice breeders designated various CMS sources arbitrarily without following any systematic nomenclature. Principally, the CMS sources are designated according to the cultivar name from which the male sterile cytoplasme is derived. In some cases, different symbols are assigned by different researchers for the same material. For example, Shinjyo designated the male sterile cytoplasm of Chinsurah Boro II as [CMS-boro] or [CMS-bo], but the Chinese workers designated it as BT (B for Chinsurah Boro ◎ and T for Taichung 65 which is the nuclear donor cultivar). The first series of released WA-type CMS lines include Zhenshan 97A, V20A, Erjiu Ai 4A, Erjiu Nan 1A and V41A (Mao, 1993). In order to diversify the genetic background of hybrid rice, other CMS types besides WA-type are developed for three-line hybrid rice varieties in China, including Dwarf Abortive (DA) type, Gambiaka and Dissi (G and D) type, Indonesla 6 (ID) type, K type and Hong Lian (HL) type. DA-type CMS lines are derived from the male sterile dwarf wild rice, including Xieqingzao A. G and D type CMS lines are developed from geographically distant crosses, where West Africa *indica* cultivars Gambiaka Kokoum and Dissi D52/37 are crossed with Chinese *indica* cultivar Aijonante, respectively to yield G46A and D62A, major representative of G and D CMS lines (Li 1997). ID type CMS lines are derived from an abortive plant in an Indonesia rice cultivar Indonesia 6. Zhong 9 A and II-32A are two representative CMS lines of ID type. K type CMS lines are derived from the cross of K52 and Luhongzao 1 with representative K qing A and K 17A. HL Type CMS lines are derived from the cross between red awn wild rice (*O.sativa spontanea* L.) and an indica rice cultivar Lian-tangzao with representative Yuetai A (Zeng et al, 2000). Virmani and Wan (1988) listed some of the CMS sources identified in and outside China, where the CMS sources are designated in principle according to the cultivar name from which the male sterile cytoplasme is derived, as well.

Outside China, IRRI used CMS sources from V20A, Kaliya 1, ARC and Gambiaka to develop CMS lines of IR58025A, IR68275A, 68281A, IR68273A, IR68888A, IR68891A and IR68893A. Also, IRRI developed CMS lines with male sterile cytoplasme sources of *Oryza perennis* (e.g.

IR66707A) and *O. rufipogon* (e.g. OMS1) (Virmani, 1996). Therefore, the genetic backgrund among three-line hybrid rice varieties are greatly broadened or diversified.

4.3. Genetic model of CMS line

As a self-pollinating crop, rice must use an effective male sterility system to develop and produce F_1 hybrid cultivars. The male sterility in CMS system is controlled by an interaction of cytoplasmic and nuclear genes. The presence of homozygous recessive nuclear genes for fertility restoration combining with cytoplasmic genes for sterility makes a plant male sterile. The cytoplasmic genes for sterility exist in mitochondrial DNA. The nucleo-cytoplasmic inter-reaction hypothesis explains genetics of three-line hybrids. In this hypothesis, a CMS or A line has sterility cytoplasm but no dominant restorer genes in nucleus, so sterile. A maintainer or B line also has no dominant restorer genes in nucleus, but has fertile cytoplasm, so fertile.

When B line pollinates A line, the progeny is male sterile because it has a complete sterility cytoplasm of A line with half nucleus from A line and another half nucleus from B line, and both A and B nuclei have no dominant restorer genes. A restoring or R line has dominant restorer genes in the nucleus. Regardless of sterile or fertile cytoplasm in R line, the progeny from crossing A with R line becomes fertile solely because of dominant restorer genes in the nucleus of R line. Accordingly, genetic constitutions can be expressed as *S* (sterile cytoplasm) with *rfrf* (sterile nucleus) in CMS line, *N (*fertile cytoplasm) wit *rfrf* in maintainer line, S/N with *RfRf* (fertile nucleus) in restorer line and S with *Rfrf* in hybrid rice. When the CMS line is crossed with corresponding maintainer, the sterility is maintained and seeds of the CMS line are multiplied. When the CMS line is crossed with the restorer line, the fertility is restored in F_1 generation, namely commercial hybrid seed production.

Zhang (1981) made the following conclusions on male sterility and cytoplasmic regulation of gene reaction in rice:

1. The occurrence of male sterility depends on “affinity” between the cytoplasm and nucleus. The greater the genetic distance between cytoplasm donor and nucleus donor cultivars is, the easier for their offsping to be male sterile and to breed male sterile line. If we assume that the evolutionary order of cultivated rice is wild, *indica* and *japonica,* the genetic distance between wild and *japonica* should be greater than it between wild and *indica*. Then, the cytoplasm of wild rice has less “affinity” with *japonica* than it with *indica*. However, the definition of “affinity” in genetic terms remains unexplained.

2. The cytoplasm and nucleus jointly decide pollen abortion. Pollen abortion is observed from uninucleate stage before first pollen mitosis, until binucleate stage just before anthesis. The earlier the abortion stage is, the more morphologically discernible pollen sterility there is.

3. A genotype can function as either a maintainer to one MS cytoplasm or a restorer to another MS cytoplasm, depending upon the ability of either complete sterility for maintaining or normal fertility for restoring. Therefore, the cytoplasmic differences between two male-sterile lines derived from two CMS sources can be ascertained through the reaction of maintainer and restorer by crossing with a set of cultivars.

4.4. Achievements on three-line hybrid rice

In order to reduce the potential threats from diseases due to MS cytoplasme uniformity, a variety of cytoplasmic male sterile sources have been utilized by Chinese rice breeders and a number of three-line hybrid CMS lines have been bred. WA CMS source used to be overwhelming for a long time. Other cytoplasm sources named Wild and D type, ID type etc. are gradually increasing lately in commercial extension of hybrid rice. For example, the monopoly of WA CMS source hybrids was broken by D-type CMS source hybrids with heavy panicles that are successully bred in Sichuan on commercial scale.

In recent years, great progresses have been made by Chinese rice breeders to improve grain quality and out-crossing rate of male sterile lines. ID type CMS line Zhong 9A (http://www.ricedata.cn/variety/varis/601141.htm), developed by China National Rice Research Institute, combines high quality and high outcrossing rate up to 80%. The grain quality of Yixiang 1A cultivated by Yibin Institute of Agricultural Sciences in Sichuan province (Jiang et al, 2008), and Yuefeng A cultivated by Guangdong Academy of Agricultural Sciences has reached international standards of first level high quality rice (Li, 2001). The breeding success of these CMS lines improved the quality of hybrid rice, especially for the significant improvement on milled rice rate, chalkiness and amylose content.

Rice statistics shows that three-line hybrids are still dominant in rice production in china (Table 2). From 2009 to 2011, the planting areas of top ten three-line hybrids ranged from 110, 700 ha for Jin you 207 in 2011 to 260, 000 ha for Yue you 9113 in 2009. Many three-line hybrids are elite, such as Yue you 9113, Gang you 188, Q you 6, Gang you 725, Tian you 998 and Zhongzhe you 1. Among them, Yue you 9113 is outstanding with the most total planting areas of 724, 7000 ha in the three years because it has good characteristics of high yield and premium quality, resistance to diseases and suitable maturity. Because three-line hybrid combinations have very significant yield increasing ability, it has proven that hybrid rice has a yield advantage of more than 20% over conventional rice. In recent years, hybrid rice covers about 15.5 million ha annually, accounts for 50% of the total rice area, and produces 60% of the total rice produced in China. From 1976 to 2011, the accumulated planting area of hybrid rice is 500 million ha, from which 500 million tons of paddy rice has increased over conventional rice. Up to now, three-line hybrids include Indica, Japonica and Indica/Japonica types with different maturities. Thus, hybrid rice production distributes to the entire China, from Hainan in the south to Liaoning in the north, and from Shanghai in the east to Yunnan in the west. Hybrid rice shows not only a high-yielding ability but also a wide adaptability. Chinese demonstration has also encouraged IRRI and National rice improvement programs of countries like India, Vietnam, Philippines, USA, Bangladesh and Indonesia to start hybrid rice breeding programs for utilization of heterosis.

Rank	2009		2010		2011	
	Variety name	Area	Variety name	Area	Variety name	Area
1	Yueyou 9113	26.00	Zhongzhe you 1	24.53	Yue you 9113	22.80
2	Tian you 998	25.53	Gangyou 188	23.87	Gang you 188	22.60
3	Gang you 188	24.73	Yueyou 9113	23.67	Q you 6	17.00
4	Jin you 207	21.93	Q you 6	22.80	Gang you 725	14.40
5	Jin you 402	20.33	Gangyou 725	17.93	Tian you 998	13.47
6	Q you 6	19.27	Jin you 207	14.73	Zhongzhe you 1	12.80
7	Zhongzhe you 1	17.33	Tian you 998	14.33	Wu you 308	12.67
8	Gangyou 725	16.60	Ganxin 688	12.80	Tianyou huazhan	12.60
9	II you 838	15.53	Jin you 402	12.47	Fengyuan you 299	11.73
10	Jin you 463	11.53	II you 838	12.13	Jin you 207	11.07

Table 2. Planting areas of top 10 three-line hybrid varieties during 2009~2011 (10^4 ha)

5. Breeding and application of two-line hybrid rice

5.1. Photoperiod Sensitive and Thermo-sensitive Genetic Male Sterility

In 1973, Chinese scientist Shi Mingsun discovered a natural male sterile plant in the field of Nongken 58, a *japonican* 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 (Shi, 1981). Further studies indicate that the critical stage for fertility transformation must be before the 1st or 2nd of September in Wuhan 30-31 N. When Nongken 58S heads during August 5th ~ 1st or 2nd of September, it is male-sterile (99.5-100%). However, its pollen sterility is reduced to 20% and seed setting rate varies between 10-40% when Nongken 58S heads after 1st or 2nd of September. This sterility-fertility change regulated by heading is true in many other regions. Pollen sterility during the sterile stage in summer is stable, but the fertility in autumn is unstable and varies over locations and years. The original sterile plant Nongken 58S becomes the first dual-purpose line in rice and possesses the characteristics of fertility alteration, i.e., completely sterile under long day period and high temperature conditions, and partially fertile under short day period and low temperature conditions.

Japanese rice scientists (Maruyama et a1., 1991a) reported thermo-sensitive genetic male sterility (TGMS) as a mutant from Japanese rice cultivar Remei treated by 20 kr gamma rays for the first time. This male-sterile mutant, designated as H89-1, sets no seeds under 31/24℃, some seeds under 28/21℃ and full seeds under 25/15℃. Pollen sterility in this mutant does not change along with change of day length period (viz.15, 13.5, 12 h). Behavior of this TGMS mutant is confirmed at IRRI. Like PGMS, TGMS can also be employed to develop rice hybrids rather than three lines. While PGMS can be used in the countries having large territory with striking differences in latitude, TGMS can be used in the area close to the equator where low temperature areas are on top of the hills. Thus, TGMS can be utilized in tropical and subtropical areas.

5.2. Breeding methods for two-line hybrid rice

Two-line hybrid rice research originates in China and successfully reached to production scale in 1995. 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. The PGMS trait has been transferred to several *Indica* and *Japonica* rice cultivars in China by backcrossing. Rice hybrids developed by this male sterility system are being evaluated in multi-location trials in China. Two-line hybrid rice has similar level of heterosis with three-line hybrid rice, but different in technique process. 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 such as PA64S, GZ63S, Zhun S, etc. have many advantages for hybrid combinations, such as larger freedom for crossing, higher yielding, better quality and resistance to diseases than CMS lines. Commonly, the yield of improved two-line hybrid rice combinations is higher than it of three-line hybrids as checks. Meanwhile, the techniques of seed production and cultivation for two-line hybrids have been sophisticated enough for production application. Breeding of elite restorer lines is the key for matching heterotic combinations. The following three ways are usually used in breeding restorers and new combinations for two-line hybrids.

5.2.1. Testing and screening strong combinations using conventional rice cultivars

China has a very long history for rice cultivation. Thousands of cultivars have been used for production and all these cultivars can be used as a restorer for testing new two-line combinations. Especially for some conventional cultivars bred in recent years, they have many advantages such as high-yield, good resistance and excellent quality, thus are easy to be used in two-line hybrid breeding. At present, two-line hybrid rice combinations applied in large production areas are mostly bred using conventional cultivars. For example, Teqing, Shagnqing11, Yuhong, and 9311 (Yangdao6) are the male parent for elite two-line hybrids Liangyouteqing, Peizashanqing, Peiliangyouyuhong and Liangyoupeijiu, respectively.

5.2.2. Testing and screening strong combinations using cytoplasmic male sterile restorers

Three-line hybrid rice breeding technologies in China are regarded the first class in the world. For the pasted four decades, a large number of restorer lines with strong general combining ability, good resistance to diseases and high quality have been bred. All these restorer lines can be used as male parent for testing two-line hybrid rice combinations. For example, many two-line hybrid rice combinations which are widely used in production such as Peiliangyou 288, 70 You 9 (Wandao20), Liangyou 2163 and Fuliangyou 63 are configured by three-line restorer R288, Wanhui9, Minghui63, respectively.

5.2.3. Breed new restorers from crossing

Although two-line hybrid rice is superior to three-line hybrid rice in quality traits, resistance and yielding, we also need to expand genetic differences of parents, make crosses and breed new two-line restorers, and overcome the shortcomings of parents using complementary effects. For example, researchers in Yahua Seed Industry in Hunan Academy of Agricultural Sciences have bred a restorer line ZR02 which combines good quality, stable growth period, strong resistance and good out-crossing ability together. We can use this restorer line to cross with other lines to improve the traits such as poor quality, late maturity and poor out-crossing ability. Using this restorer line, a new combination Zhuliangyou 02 is bred as a double-crop early maturing hybrid rice with stable and high yield and good quality. As a result, Zhuliangyou 02 has good prospects in Yangtze River.

5.3. Advantages of two-line hybrids

In the two-line system, only two lines are involved in hybrid rice seed production. One is the male sterile line in which male sterility is genetically controlled by recessive genes, which expression is influenced by environment (temperature, photoperiod, or both). Another is male parent or pollinator line that can be any inbred variety with dominant gene (s) for male sterile locus. There are no constraints for the restoration-maintenance relationship because the male sterility of PGMS and TGMS is controlled by only one or two pairs of recessive genes. There is no need for special R genes to restore fertility, so the choice of parents in developing heterotic hybrids is greatly broadened. Developing hybrid rice varieties with these systems has the following advantages over the classical three-line or CMS system:

1. **Maintainer lines are not needed.** The PGMS lines (under long day-length) and the TGMS lines (under high temperature) show complete pollen sterility and can thus be used for hybrid seed production. Under short day-length or low temperate conditions, they show almost normal fertility and can multiply themselves by selfing. Therefore, in the PGMS/TGMS system, no maintainer line is needed in seed multiplication of male sterile lines, thus the cost to produce hybrid seed is cut down because of the simplified production procedure.

2. **The parental choice for developing heterotic hybrids is greatly broadened.** Studies showed that more than 97% of tested varieties (within subspecies) could restore fertility of MS lines, indicating no need to identify restoring abilities. Thus, our choice of parents in developing heterotic hybrids is broadened in comparison with the CMS system. In addition, PGMS and TGMS genes can be easily transferred to almost any rice lines with desirable characteristics.

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3. **Negative effects from the sterile cytoplasm are avoided.** Therefore, the vulnerability to destructive diseases or insects due to uniform resource of male sterile cytoplasme may be eliminated.

Apparently, it is easier to develop rice hybrids that possess higher yield, earlier maturity, better grain quality and improved pest resistance by two-line system than by three-line system. The research findings and production experiences have also proven that two-line hybrid rice out-yields three-line hybrid rice by 5%-10%. Furthermore, it is promising to develop elite hybrids for the early cropping rice with both high yield and early maturity, and to develop heterotic *japonica* hybrids by two-line method, which would very likely break the deadlock of stagnant yield and area in hybrid rice. For example, Xiangliangyou 68, an early-cropping two-line hybrid rice combination with high yield, fine grain quality and early maturity, was successfully released to commercial production in 1998. It shows a very promising prospect in overcoming the great difficult long existing in developing high-yield, good-quality and early-maturity hybrid rice in China.

However, it should be pointed out that two-line hybrid rice also faces the risk of seed purity in case of lower temperature occurred in thermo-sensitive stage of TGMS lines in hybrid rice seed production. Because the fertility alteration of TGMS lines is conditioned by temperature, even in hot season like summer, low temperature may occur in rainy days and last for a few days. Therefore, to guarantee the seed purity in two-line hybrid seed production is essentially important for developing practical TGMS lines with their Critical Sterility Inducing Temperature (CSIT) low enough, generally 23 C for temperate zones and 24 C for subtropical zone.

5.4. 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 Annong S-1, 5460 S and Hengnong S-1 are also identified. Up to now, tens of practical PTGMS lines in rice which possess the characteristics of low CSIT to secure hybrid seed production have been technically identified and approved. At present, the PTGMS lines used in rice production mainly derive from either PGMS Nongken 58S or TGMS Annong S. More attention should be paid to the following areas in order to improve screening and utilizing efficiency of photo-thermo sensitive male sterility (Lu and Zou, 2000).

1. Seed production safety: Because the fertility of photo-thermo sensitive male sterile lines is regulated by light and temperature, safely producing hybrid seed in the target region must be taken into account during the selection of sterile lines. The window for sterility to stably occur must be more than 30 days. The stable length of sterility period mainly depends on the critical temperature and critical photo-period length for fertility transition in the selected PTGMS lines. In South China, we generally select temperature-sensitive sterile lines because inter-annual light changes are minor and inter-annual temperature

changes are major, where the suitable critical temperature for fertility transition is 24-25°C in this region. In Central China, PGMS, TGMS and P-TGMS are all applicable, but the suitable critical temperature of fertility transition should be 23°C ~ 24°C, and the day length of critical light should be around 13.5 h. We should select the sterile lines with relatively stronger photo-sensitivity but weaker temperature-sensitivity for fertility transition in North and Northeast rice region, because there are longer day length and relatively lower temperature there.

2. Easy to multiply: Because the TGMS lines are fertile when the temperature is below the critical temperature, photo-thermo sensitive male sterile lines must have strong cold tolerance, which makes them survive from the cold water irrigation to have high grain output in multiplication.

3. High combining ability: A successful hybrid with high vigor is directly determined by the combining ability level of sterile lines. Combining ability (CA) includes specific CA (SCA) and general CA (GCA), the former is for crossing with a specific parent and the latter is for crossing with many parents. The GCA is usually highly related with its comprehensive characters, advantages and disadvantages, so the sterile lines with high GCA have high chances to produce high yielding combinations.

According to above principles, the breeders have bred many elite two-line hybrid varieties with good quality, high yield and good resistance, such as Fengliangyou Xiang1, Zhunliangyou 527, Yangliangyou 6, Liangyou 288, Zhuliangyou 02, Zhuliangyou 120, et al. After more than twenty years for nationwide collaborative studies, important progress has been made in two-line hybrid rice in both theoretical mechanism and practical application, which has resulted in a yield advantage of 10 percent over three-line hybrids. Along with improvements of techniques on daily bases, two-line hybrid rice is becoming more and more popular in large-scale application. The area planted to two-line hybrid rice increases year after year from 4, 300 ha in 1991 to 704, 400 ha in 1999 in China. In 2000, the growing area of two-line hybrid rice in China reached 1.5 million ha, and total yield reached 109.3 million tons with average yield of 7287kg/ha which was 4.16% more than that of three-line hybrid rice. Currently, the main combinations in commercial production are Liangyou Peijiu, PZS7, Peiliangyou 288, Xiangliangyou 288 and Fengliangyou 1. In 2002, a two-line hybrid rice Liangyou Peijiu took up the first place of planting areas from Shanyou 63, a three-line hybrid rice that had maintained the leading place for more than ten year in China.

In recent years, more and more two-line hybrid rice varieties are released. In 2011, six and 51 two-line hybrid combinations were released from national and provincial institutions, respectively (Fig. 3). The planting area of two-line hybrid rice reached 2.7 million ha, about 9.0% of total rice cultivation area and 18.6% of hybrid rice planting area. In 2010, the top three hybrid varieties with the most planting area were all two-line combinations. The cultivation area of two-line hybrid rice will further expend with the progress of research on seed production.

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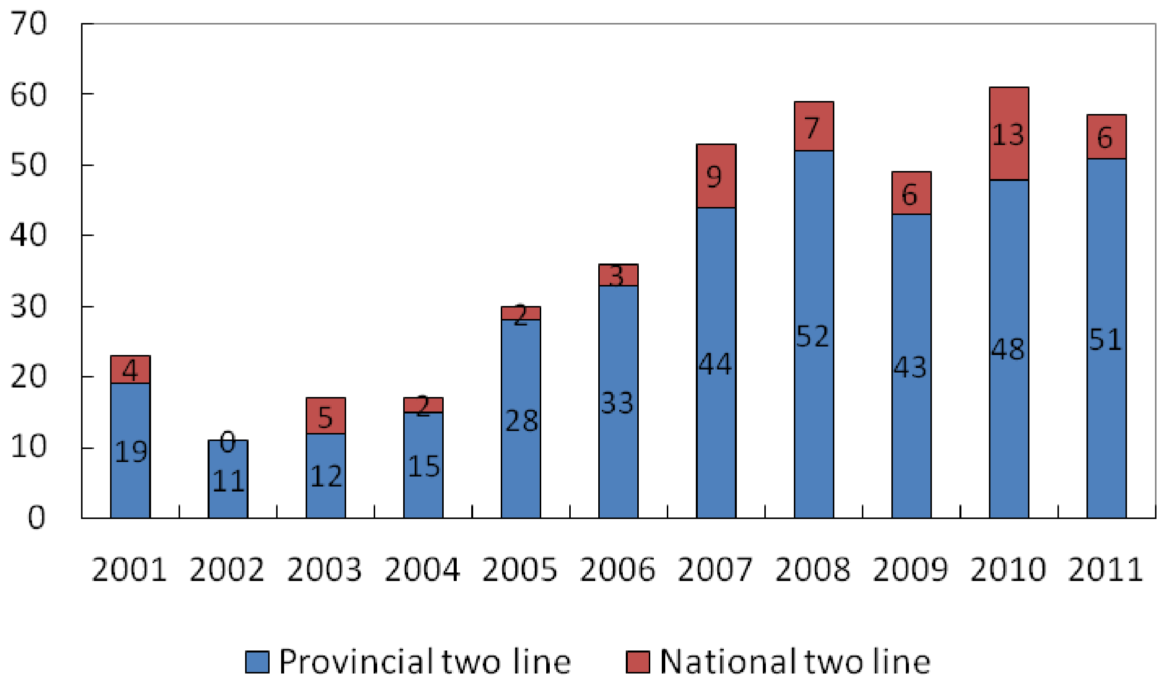

Figure 3. Two-line hybrid rice varieties released in China (2001-2011).

6. Super hybrid rice breeding

In order to achieve another leap of rice yield and secure food supply in China, after summarizing the experiences and lessons at home and abroad, Chinese scientists put forward a national program to breed super rice in 1996. A primary goal of this program is to combine ideal plant type with heterosis of *indica*/*japonica*, and improve rice yield, quality and resistance. Through joint research, a series of new super rice varieties have been approved for release from national and provincial institutions. The super rice varieties have demonstrated the yield of 12 t/ha in a scale of 100 mu or 6.7 ha model trial. From 1998 to 2004, the accumulative demonstration and extension areas of super hybrid rice have 10 million ha. Practices show that developing super hybrid rice is a necessary choice to increase rice yield, stabilize total production of rice, improve the efficiency of the rice planting, and ensure food security in China.

6.1. Model of super hybrid rice

Backgrounds are confirmed to be unique based on the results of RFLP variations among *indica* hybrid varieties and their parents. The yield ceiling has remained in hybrid rice for nearly 10 years because of insufficient genetic diversity. Optimal combination means that the hybrid rice combination has a reasonable genetic difference between its parents, such as 1) lowland rice with upland rice varieties, 2) geographically different varieties, 3) ecologically different varieties, 4) dominantly different varieties, and 5) *indica* and *japonica* subspecies (Chen et al., 2007). However, we can only exploit part of the heterosis between *indica* and *japonica* subspecies, but not the heterosis between typical *indica* and *japonica* rice or excessive *indica* and *japonica* ingredients. Cheng et al. (2007) indicated that when *indica* or indicalinous cytoplasmic male sterile (CMS) lines are crossed with restorer lines having different *indica* and *japonica* genetic backgrounds, the hybrids from indicalinous or japonicalinous restorer lines (*indica-japonica* differentiation index 11-15) have the highest yield. Therefore, breeding high yielding hybrids by crossing *indica* with *japonica* with aid of wide compatibility gene has been paid great attentions. In the *indica* rice growing regions, breeders strategically introgress *japonica* consanguinity into *indica* rice, and in the *japonica* rice growing regions, introgress *indica* consanguinity into *japonica* rice, instead. So far, a set of indicalinous or japonicalinous germplasms for super rice breeding have been intentionally developed. Some of such germplasms have been successfully used in breeding of super inbred and hybrid rice (Table 3). For instance, Shennong 89366 is one of the core parents for IRRI to develop new plant type super rice. ed by Shennong 89366 is bred by Shenyang Agricultural University, China and has served as a donor for short sturdy stems and long-big panicles (Chen et al., 2003). R9308, an indica restorer line from a cross of C57//No. 300/IR26, has been successfully used in the breeding program for super hybrid rice by the China National Rice Research Institute (CNRRI). Xieyou 9308 (Xieqingzao A/R9308), a hybrid rice combination with super high yielding, multi-resistance to diseases and good grain quality, was registered in Zhejiang Province, China in 1999. It is estimated that there are 25% *japonica* and 75% *indica* genetic components in R9308. The hybrid Xieyou 9308 has super high yielding potential with harmonious plant type (Cheng et al., 2005). Another example is Liangyou peijiu, a two-line super *indica* hybrid developed by the Jiangsu Academy of Agricultural Sciences collaboratively with the Hunan Hybrid Rice Research Center, China (Lu et al., 2000). Its female parent is Pei'ai 64S, a thermo-sensitive male sterile line with tropic japonica in its pedigree.

Combination name	Parental cross	Pedigree of major parent
Xieyou 9308	Xieqingzao A/R9308	R9308: C57 (*j*) //No.300 (*j*) /IR26 (*i*)
Liangyou Peijiu	Pei'ai 64 S/R9311	Pei'ai 64S: Nongken 58S (*j*) /Peiai 64 (*i*) // Peiai 64 (*i*) /
II you 602	II-32A/Luhui 602	Luhui602: 02428 (*j*) /Gui 630 (*i*) //IR24 (*i*)
II you 7954	II-32A/Zhehui 7954	Zhehui 7954: R9516/M105 R9516: Peiai'64S/Teqing M105: Miyang 46 (*i*) /Lunhui 422 (*m*)
Guodao 1	Zhong 9A/R8006	R8006: IRBB60//T2070/Duoxi 1 T2070: WL1312 (*j*) /Lunhui 422 (*m*) /Minghui 63 (*i*)
Guodao 3	Zhong 8A/R8006	
Guodao 6	Neixiang 2A/R8006	
Liaoyou 5218	Liao 5216A/C418	C418: Lunhui422 (*m*) /Miyang 23 (*i*)

Table 3. Some super rice hybrids derived from gene introgression of *indica* (*i*), *japonica* (*j*) and medium (m) type

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6.2. Strategies to breed super hybrid rice

6.2.1. Construction of harmonious plant type based on substantial biomass production

Evolutionary change of rice variety in China indicates that increasing yield through dwarfing is due to the increase of harvest index, whereas through hybrid rice is due to the increase of biological production or biomass. In the experiment under the special ecological conditions at Yongsheng county, Yunnan Province, China, the plot grain yield, biological yield and harvest index of an *indica* hybrid Shanyou 63 were 15.27 t/ha, 28.29 t/ha and 0.54, respectively. Its harvest index under normal ecological conditions is almost the same (about 0.5) (Yang et al., 2006). We think that the key to further increase grain yield is the increase of biomass with a stable harvest index. Undoubtedly, proper increase of plant height is beneficial for increasing biomass, but the lodging resistance should be increased as well. Currently, leaf area index (LAI) in some high yielding varieties is 8-10, which seems to be the maximum. In order to increase the spikelet number and filled grain number per panicle, an indirect strategy is to properly raise plant height, ameliorate leaf stature and leaf quality, and strengthen root system vigor. It is known that the erect and slight rolling uppermost three leaves favor the full utilization of light energy after heading, by promoting CO_2 diffusion, increasing photosynthetic rate on the back face of leaves, accelerating the increase in biological yield, mitigating the conflict between panicle number and size, and improving the lodging resistance of rice plants (Cheng et al., 2007). Now, a set of super hybrids with slight rolling leaves have been developed and used in production. They generally have more than 12 t/ha of yield potential in combination with erect and late senescent leaves and lodging resistant culms.

6.2.2. Utilization of intersubspecific heterosis

It is known that the heterosis of inter-subspecific hybrids is much stronger than that of intervarietal hybrids. Therefore, utilization of inter-subspecific hybrids is the most feasible approach for realizing super high yield. At present efforts have been focused on using Pei'ai 64S as a major female parent in the selection of super high-yielding combinations. Because Pei'ai 64S is an intermediate type between *indica* and *japonica*, it has a very wide compatibility. To exploit the heterosis of inter-subspecific hybrids and improve the efficiency of super high-yielding hybrid breeding, the emphasis is on the development of various widely compatible lines, especially those that have a broad spectrum of compatibility, including restorer lines and male sterile lines of *indica* type, *japonica* type and the intermediate type with different growth durations. This emphasis will create abundant parental lines for breeding various super high-yielding hybrids to well adapt different ecological environments in China.

6.2.3. Improvement of important agronomic traits by molecular breeding techniques

Genetic engineering techniques, such as anther culture, marker-assisted selection and gene transformation, offer reliable opportunities to accelerate breeding progress, increase selection efficiency and overcome genetic barriers to transfer genes across species. These techniques have played important roles in breeding super hybrid rice as well. For example, dramatic progress has been made in the development of transgenic rice plants with a high level of resistance to insects (stem borer, brown planthopper), diseases (tungro virus, rice yellow stunt virus, blast, bacterial blight), herbicide (glufosate), and abiotic stresses (salinity and drought), as well as better nutritional value (e.g. glutelin, vitamin A) and higher yield. Some transgenic rice plants have already been subjected to evaluation under field conditions. In 1995, based on molecular analysis and field experiments carried out as part of a cooperative research program with Cornell University, China National Hybrid Rice Research and Development Center (CNHRRDC) identified two favourable quantitative trait loci (QTLs) (yld1 and yld2) from wild rice (*O. rufipogon* L.). Each of the QTL genes contributed a yield advantage of 18% over the high-yielding hybrid V64 (one of the most elite hybrids in China, with a yield potential of 80 kg/ha per day). By means of molecular marker-assisted backcrossing and selection, the development of near-isogenic lines carrying these two QTL genes is under way (Xiao et al., 1996). If biotechnology can be used to transfer apomixis to rice from grass species, hybrid rice production will be revolutionized and reach even higher levels.

6.3. Achievements of super rice breeding in China

In recent years, super rice breeding and extension for heterosis demonstration in China have made outstanding progress. On the bases to actively use conventional hybrid rice technology for new variety breeding, more and more attention is paid to strengthen the breeding technology innovation by combining molecular breeding technology with the conventional breeding technology. Bacterial blight broad-spectrum resistance gene *Xa21* has been introgressed into restorer lines by marker-assisted selection technology to develop restorer line R8006 with disease-resistance, good quality and high combining ability. As a result, R8006 has produced a series of successful combinations such as Guodao 1, Guodao 3, II you 8006 and Guodao 6 in China National Rice Research Institute. Among them, Guodao 6 has tall and erect plant type as obvious high-yield characteristics, so that the record in southern area was broken by it. In 2004, Guodao 6 had the average yield of 12.08 t/ha in a 100 mu or 6.7 ha model trial. It was released by National Crop Variety Approval Committee in 2006. Similarly, bacterial blight broad-spectrum resistance gene *Xa4* and *Xa21* are introgressed into restorer lines by marker-assisted selection technology in Rice Research Institute of Sichuan Agricultural University. This introgression has resulted in developing restorer line Shuhui527 with disease-resistance and high combining ability, from which a series of combinations such as superior two-line hybrid rice combination Zhunliangyou 527 and three-line hybrid rice Dyou 527, Gangyou 527, Xieyou 527, Guyou 527 have been bred. Some of the 527 hybrids reached record of high yield frequently. A comprehensive review paper "Super Hybrid Rice Molecular Breeding Research" published on China Rice Science has been downloaded more than 10, 000 times from China rice information network during last two years.

Over last 16 years, super rice research in China has gained significant advances in the aspects of breeding methodology, creation of breeding materials and selection and promotion of elite rice varieties. New pathway is proposed to utilize inter-subspecies heterosis between *indica* and *japonica* and harmonious plant type construction. Under the guidance of breeding methods, the super rice breeding program has been successively conducted and a series of new super rice varieties have been commercially released, such as the three-line super hybrid rice combinations Xieyou 9308, II youming 86, II youhang 1, II you 162, D you 527, Zhong 9 you 8012 and II you 602; the two-line super hybrid rice combinations Liangyoupeijiu, Fengliangyou 1, Xinliangyou 6 and Zhunliangyou 527; and super inbred rice varieties Jijing 88, Shennong 265 and Shennong 606. Hitherto, a total of 101 new inbred rice varieties or hybrid rice combinations have been identified and nominated as super rice by the Chinese Ministry of Agriculture (CMOA), about half of which are the three-line hybrid rice combinations from South China. The demonstration and promotion of super rice have resulted in an increase of rice yield. According to the CMOA statistics, the accumulative planting area of super rice has increased to 23.85 million hectares (Fig. 4). The average yield of super rice is over 9 t/ha, 0.75 t/ha higher than that of traditional rice varieties. Totally, super rice yield has increased by 17.7 million tons since 1998. These super rice varieties cover rice regions in the Yangzte River Valley, South China and Northeast of China. In 2011, the yield of super hybrid rice Y Liangyou 2 was up to 13.9 t/ha in 6.6 ha demonstration area in Hunan province, which passed, for the first time, the yield target of the third phase in National Super RiceProgram.

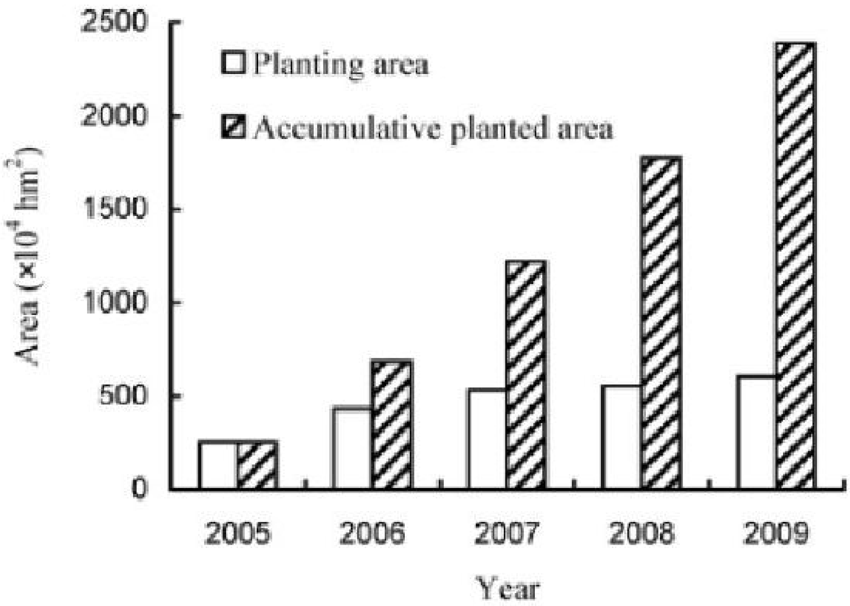

Figure 4. Planting area of super hybrid rice from 2005 to 2009 (the Ministry of Agriculture, P. R. China)

6.4. Outstanding elite hybrid rice varieties in China

Guodao 6 was released in 2007, which elite traits include high yielding and good quality with a yield potential of 12.5 t/ha and planting area of 95, 000 ha in 2010 (Table 4). Y Liangyou 1 was released in 2006, which elite traits include high yielding, good quality, and wide adaptability with a yield potential of 12.50 t/ha and planting area of 353, 000 ha in 2010. Xin Liangyou 6 was released in 2005, which elite traits include good quality and high yielding with a yield potential of 12.5 t/ha and planting area of 271, 000 ha in 2010. Zhongzheyou 1 was released in 2004, which elite traits include high yielding, good quality, and ideotype with a yield potential of 12.3 t/ha and planting area of 245, 000 ha in 2010.

Aariety name	Year of release	Elite traits	Yield potential (t/ha)	2010 planting area (ha)
Guodao 6	2007	high yielding, good quality	12.50	95, 000
Y liangyou 1	2006	high yielding, good quality, wide adaptability	12.50	353, 000
Xin liangyou 6	2005	good quality, high yielding	12.50	271, 000
Zhongzhe you 1	2004	high yielding, good quality, ideotype	12.30	245, 000

Table 4. Outstanding elite hybrid rice varieties.

6.5. Future directions for super rice breeding

Although great achievements have been resulted from past 13 years in super rice breeding and the yields of some hybrids have approached to the designed target, Chinese scientists are consistently making their efforts to further increase grain yield of rice, regardless of more difficulties and more constraints than even before.

6.6. Strengthen the exploitation and utilization of favorable genes

The core of super rice breeding is an effective use of germplasms and favorable genes. Because the genetic diversity in rice variety gene pool is limited, we must extensively utilize exogenous genes for variety improvements. We can exploit valuable genes from not only the cultivated rice, but also wild rice species, and even other crops to increase yield, quality and resistance to diseases and insects, and tolerance to adverse circumstances. The molecular marker-assisted selection techniques should be effectively used for transformation of high-yielding genes and other important agronomic trait genes from various sources into current variety genetic background. Especially for some complicated traits that lack in rice such as high photosynthetic efficiency gene in maize, stem borer resistant gene (*Bt*) and herbicide-resistant gene in microorganism, the transgenic technology is the best choice to improve rice in the future.

6.7. Strengthen the evaluation on root system (including physiological traits)

In recent years of super hybrid rice breeding practices, a conflict of large panicle with premature senescence becomes more and more troublesome to rice breeders and scientists. The large panicle needs longer time for the gains to fully fill and the premature senescence closes the sink before the completion of filling process. Therefore, studying root system to delay senescence of rice plant has become a hot subject in rice community (Wu and Cheng, 2005). Rice root system is not only a vital organ to stand the plant, absorb water and minerals, but also an important place where bioactive substances (hormones, amino acids, etc.) are synthesized. Root senescence at the late developmental stage directly affects the life span of functional leaves, grain filling and root vigor, especially during the grain-filling period. Obviously, the root vigor is the guarantee for high-yielding of super rice. So far, the genetic research on the root-related morphological traits such as root length, thickness, number, dry weight, density, volume, penetration depth, and root/shoot ratio, as well as other physiological traits such as absorption ability to N, P and K, and root vigor, etc. has achieved great progress (Wu, 2006). However, these studies are still at preliminary stage, and further systematical study is required to propose the key indicator and the appraisal methods for rice breeding, and to explore the gene regulation of the root system and the relationship with the plant organs above ground.

6.8. Strengthen the seed production with high security and efficiency

As we know, super hybrid rice could not be commercially and successfully utilized if hybrid seed production costs too much, seed yield is too low and seed purity is not high enough. In the future, we should strengthen the research on the characteristics of flowering time, stigma exsertion and out-crossing rate of the female parents for super hybrid rice, and establish a new system for super high-yielding seed reproduction. The efficiency of seed production will bring seed price down. The security of seed production will not only yield good quantity and quality of hybrid seed, but also reduce production risk. Therefore, improving seed production will promote the rapid and stable extension of super hybrid rice.

6.9. Strengthen the combination of super rice with suitable cultivation management

China plays a leading role in global rice production, and the key from Chinese experience is the integration of superior varieties with suitable cultivation management. Research and promotion of the cultivation technology have played an important role in the two break-throughs of rice yield in China. Besides high yielding, super rice should have high grain quality, and high efficiency to utilize resources with low environmental pollution. The superiority of varieties and suitability of cultivation practices jointly determine the production scale for a super hybrid rice to be promoted in commercialization.

7. Challenges and prospects

7.1. Challenges

Although tremendous achievements have been made in hybrid rice breeding, there also are some constraints and challenges in its development. To sum up, the major problems are as follows.

7.1.1. Planting area has not been at a standstill for years

In 1991, the acreage of hybrid rice reached its peak at 17.6 million ha, but after that the acreage decreased and remained at about 14.2 million ha in 2011. The main reasons are considered to be the cease and even decrease in the acreage of double cropping early hybrid rice and japonica hybrid rice. Recently, only 20% of early cropping rice area in South China are covered by hybrid rice, while over 90% of late cropping rice area are under hybrid rice in the same region. The availability of early cropping hybrid rice varieties is very limited to growers because it is very difficult to integrate short growth duration and acceptable grain quality into elite high yielding combinations.

7.1.2. Grain quality of hybrid rice needs improving

With the increase of living standards for rice consumers in China, grain quality of rice is required to be improved. In comparison with conventional rice, hybrid rice usually has poorer grain quality measured mainly by the traits of head rice recovery and chalkiness. How to develop rice hybrids with both high yield and good grain quality is still a challenge for breeders.

7.1.3. Limited sources of male sterile cytoplasm to develop better CMS lines

Currently, more than 75% of the CMS lines used in commercial production belong to WA types. This dominant cytoplasm creates a great uniformity of WA cytoplasm, and genetic uniformity has been responsible for an epidemic of a destructive pest. Therefore, more efforts should be paid to diversify male sterile cytoplasms.

7.2. Prospcects

Conventional breeding has played an essential role in rice cultivar innovation for decades. Large-area application of three-line hybrid rice has showed that hybrid rice technology brings rice yield up to its potential level of physiological yield. With advanced root system and heterosis of seedling and nutrition in early stage, the application of hybrid rice to not only irrigated areas, but also low-lying fields, rain-fed fields and upland fields should be equally important. To commercialize the hybrid rice worldwide, studies on mechanical operation of hybrid seed production and male sterile line regeneration will also be an important subject in hybrid rice research.

In the past dozen years, we have made great progress in rice genome researches, such as establishing a dense molecular linkage map, locating a large amount of major and minor genes underlying important traits including resistance to bacterial blight and rice blast, plant height, reproductive period and tillers, and fully sequencing both *indica* and *japonica* DNA and subsequent functional genomic studies. With research advancement on rice genome, molecular breeding technology has become a new breeding technology to screen and breed new cultivars according to both phenotype and genotype, thus has been applied to rice breeding. Marker-assisted selection (MAS), quantitative trait locus (QTL) analysis and genetic transformation techniques are the most useful tools for rice molecular breeding, and have been used to identify new germplasms and elite rice cultivars. Chinese rice geneticists and breeders have made great progress in identifying QTLs responsible for important agronomic traits such as grain yield and quality, growth and development, disease and pest resistance and abiotic tolerance (Wang et al., 2005). MAS is a method to use molecular markers closely linked to a target gene as a molecular tag, so that the target gene can be quickly identified from breeding populations in the lab. In China, MAS is widely used to pyramid functional genes into popular hybrid rice cultivars to improve important agronomic traits of hybrid rice, such as resistance and grain quality.

In summary, hybrid rice has made a great contribution to safeguarding the food supply in China and is still a major source of elite rice cultivars. However, hybrid rice production is rather time-consuming and the limited available genetic resources leave little room for the continued improvement of rice. With the completion of rice genome sequence, scientists are better equipped to unravel rice gene functions on a genome-wide scale, providing breeders with abundant genetic resources for continued generation of elite rice varieties to maintain a sustainable food supply in China. We expect that the successful implementation of a combinatorial approach using hybrid rice technology will play a crucial role in our effort to improve rice cultivars in China. The immediate goal is to breed varieties with a further improved yield potential, enhanced stress resistance and good grain quality by using molecular and genomic information to break the rice yield plateau in the future.

Author details

Liyong Cao* and Xiaodeng Zhan

*Address all correspondence to: caolycgf@mail.hz.zj.cn

China National Center for Rice Improvement, China National Rice Research Institute, Zhejiang,, China

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