Chapter 5

Identification and Utilization of Elite Genes from Elite Germplasms for Yield Improvement

Dawei Xue, Qian Qian and Sheng Teng

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1. Introduction

Rice is a major food crop in the world. Half of the world's population relies on rice as their staple food. Due to continuous growth of human population, the area of arable land decreases every year. Therefore, ensuring adequate grain production has become a challenge for many countries. Rice production has an important role in global food security, poverty alleviation and rural employment. The current rate of increase in mean rice yield per annum is only 0.8%, which falls behind the rate of population growth annually. An annual mean increase in rice production of 1.2% is required between 2001 and 2030 in order to catch up with the growing food demand resulting from increase in population[1,2].

Constrained by lack of water resources and arable land, the area for rice cultivation has decreased in the context of economic development and urbanization. It is evident that rice yield cannot be increased by simply expanding the cultivation area. In light of this, it is important to pay attention to increasing rice yield per unit area. There are two major approaches for achieving this goal: improving cultivation conditions and technology, and breeding rice varieties with higher yield potential. In order to improve the cultivation technique, selecting superior cultivars is essential. Practices in rice science and production have shown that high-yield breeding of rice is essential for yield increase, and a breakthrough is usually made through discovery and effective utilization of specific germplasm (gene). The first leap in rice yield per unit area came from breeding semidwarf rice varieties and their popularization. In the 1940s, with rapid development of the chemical industry, chemical fertilizers were applied extensively in rice production. Tall rice varieties showed very low potential for yield increase due to their low tolerance for fertilizers and easily lodging. Chinese rice breeders first proposed the strategy of dwarfing breeding. In the late 1950s, successful breeding of Taichung Native 1 (TN1), Aijiaonante and Guangchangai rice varieties, which were

high-yielding dwarf rice varieties, marked a new epoch in dwarf rice breeding[80]. Later, the International Rice Research Institute cross-bred the Dijiaowujian rice as a dwarf-gene source with tall Pitai rice. The dwarf hybrid IR8, known as miracle rice, was developed in 1964. Compared with tall rice varieties, dwarf rice varieties have several advantages, including fertilizer tolerance, anti-lodging, erect leaves, more panicles and high harvest index. The yield per unit area was increased over 30% by the dwarf rice varieties. The successful breeding of dwarf rice varieties was just the beginning of the global "green revolution" [3]. Academician Yuan Longping discovered wild-abortive cytoplasmic male sterile line, and was the first to realize three-line combination in 1973 with the establishment of hybrid rice seed production system. The success of three line combination directly resulted in yield per mu exceeding 500 kg in many areas of China. The yield increase was over 15%-20% compared with conventional varieties. The discovery of photoperiod-thermo sensitive genic sterile gene facilitates the transition from three-line system to two-line system, which is especially useful for developing hybrids. Their effective utilization enabled the second leap in rice yield, which is also known as the second "Green Revolution" in rice production history.

The global rice yield of current varieties seems to be at a standstill. Reduction of arable land and global warming are also threatening rice production. For this reason, increasing yield per unit area is very important for boosting total yield. Rice yield per unit area heavily depends on the yield potential of the rice variety. In order to achieve the third leap in yield per unit area, many countries have successively put forward the plan of super rice breeding by adopting the technical route of combining ideal plant type with indica-japonica heterosis utilization [4].

In 1918, Japanese rice breeders had suggested super high-yield breeding of rice via indica-japonica cross. The International Rice Research Institute launched a new plant type breeding program based on Javanica rice in 1989. They proppsed morphological indices for higher yield rate were specified as follows: low tillering (3-4 tillers), no unproductive tiller, large panicle, sturdy stem, dark green, thick and erect leaves, vigorous root systems and high harvest index [5]. In 1996, China started the super rice research program which consisted of two parts: conventional super rice employing the technical route of indica-japonica cross, and super hybrid rice breeding with the combination of plant type improvement, intersubspecific heterosis utilization and distant favorable genes. Chinese rice breeders proposed different ideal plant types based on long-term production practice. These ideal plant types included an erect large spike type in north China, an early-growth deep-root type in south China, a sparse-seeding heavy-panicle type in the upper reach of Yangtze River, a functional-leaf erect type and later-stage functional type in middle and lower reaches of Yangtze River [4]. All ideal plant types of rice share some common features: low tiller number, no ineffective tiller, robust stalk, anti-lodging, large panicle, large grain number per spike, high yield per spike, high biological production, high harvest index and vigorous root systems.

Exploitation of F1 hybrid heterosis for the purpose of reaping economic benefits is referred to as hybrid heterosis exploitation. Research on the mechanism of hybrid heterosis is of great significance for the exploitation of heterosis of hybrids in crop genetic breeding. Pei'ai 64S is a low thermo-sensitive dual-purpose genic male sterile line developed by China National

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Hybrid Rice Engineering Research Center, with Nongken 58S as the female parent and Indica Pei'ai 64 as the male parent. Pei'ai 64S was obtained after crossing and backcrossing, as well as multi-generation selection. Because of its stable sterility, seed production security, combination freedom and strong hybrid heterosis, it has been extensively applied in breeding. Pei'ai 64S is China's first indica male sterile line with practical utilization value. Medium maturity indica rice variety 9311 (Yangdao 6) was bred by the Research Institute of Agricultural Sciences in the Lixia River region of Jiangsu Province. It has been extensively applied in rice production for its high quality, high yield, multiple resistance and strong combing ability. Yangdao 6 has served as the male parent for key hybrid varieties including Liangyoupeijiu, Fengliangyou 1, Yangliangyou 6 and Yueyou 938, and it is also the first indica variety used in the sequencing of rice genome framework under the name of 9311.

Effective tiller, grain number per spike and 1000 grain weight are the three major elements used for determining rice yield. These three metrics are also the indicators of hybrid heterosis. Spike number is closely related to rice tillering. Grain number per spike is associated with spike length and grain density. Grain weight depends on grain length, width and thickness. These yield components are considered in ideal plant type. Plant height, tiller, panicle type and grain weight are determined by the interaction between growth, hormone and environment. Generally, these yield-related traits are controlled by quantitative trait loci (QTL) or major genes. At present, some QTL/genes related to yield and heterosis have been cloned and the regulatory mechanism of ideal plant type and hybrid heterosis at the molecular level is being revealed. Knowledge of these mechanisms is especially important in the context of rice breeding.

2. Plant type

The ideal plant type was first proposed in 1968. It is defined as the combination of several traits favorable for photosynthesis, growth and grain yield in one plant type. In such ideal plant type, competition between individuals is reduced and solar energy utilization in the population is maximized, leading to minimal consumption and maximum dry matter accumulation [6]. In a narrow sense, plant type of a crop refers to plant morphology and spatial arrangement of the tillering and leaves. It is also related to other plant traits including plant height, tiller number, tiller angle, panicle type, leaf morphology and leaf angle. In a general sense, plant type may also consist of functional traits related to solar energy utilization of the population, including nitrogen content, photosynthetic efficiency, chlorophyll content, stoma density and extinction coefficient [7].

Apart from rice morphology and spatial arrangement, plant type also covers some functional traits directly related to solar energy utilization of the population. Breeders from the International Rice Research Institute announced a new plant type (NPT) in 1989. It had fewer tillers (5-6 per plant) and no ineffective tiller; large spike, with grain number reaching 150-200 per panicle; plant height of 90-100 cm; anti-lodging robust stalk; thick, erect and dark green leaves; and well-developed root systems [8]. Chinese rice breeders also conducted a series of research

on ideal plant type in different ecological conditions for high-yield breeding and cultivation. Several models of ideal plant type were developed. Prof. Yang Shouren of Shenyang Agricultural University proposed the "short-branch and erect-leaf, large and straight panicle" type for japonica rice in north China [9]. In 1997, Prof. Zhou Kaida of Sichuan Agricultural University proposed "intersubspecific large-spike type", which is adapted to conditions of less wind, high moisture and high temperature in the Sichuan Basin [10]. Similarly, Yuan Longping of China National Hybrid Rice Engineering Research Center proposed "high-canopy and low-spike-layer type", which is also referred to as the "gold hidden in the leaf" type, for the ecological area of middle and lower reaches of the Yangtze River [11]. Huang Yaoxiang of the Research Institute of Guangdong Academy of Agricultural Sciences proposed the "semi-dwarf clustered high-growth super high yield type" for indica rice in south China in 2001 [12]. The China National Rice Research Institute [13], by combining various components of super-high-yield plant type, proposed the "later-stage functional super rice type". In-depth analysis showed that the ideal plant types of China and other countries share the following characteristics: reduced tiller number, fewer ineffective tillers, robust stem, anti-lodging, large spike, large grain number per spike, large grain yield per spike, increased biomass yield, high harvest index and highly developed root system.

Breeding ideal plant types is the goal of super high yield rice breeding in the future. Construction of the ideal plant type in rice has to be done with consideration for traits such as tiller, stem, leaf shape and panicle type. Spike number is used for determining rice yield, and is closely related to tillering. Grain number per spike is associated with spike length and grain density. Grain weight is determined by grain length, width and thickness. These yield components are covered by the factors of the ideal plant type of rice. Plant type, tiller, panicle type and grain weight are determined by interactions between growth hormones and the environment. Successful cloning of QTL/genes related to yield has greatly contributed to our understanding of the regulatory mechanism of ideal plant type at the molecular level.

2.1. Plant height

Plant height is an important component of plant type in rice. Since the end of the 1950s when the first "green revolution" in rice yield was triggered by dwarf breeding, rice grain yield per unit area has increased substantially. This achievement can be attributed to the application of dwarf germplasm, especially semi-dwarf germplasm in breeding. Several studies have shown that dwarfism in many dwarf and semi-dwarf rice varieties is controlled by a recessive major gene and is subject to certain modifier genes [14]. For indica rice, application of the semi-dwarf gene sd-1 is a major contributor to rice yield improvement. The major dwarf cultivars of indica rice are Aijiaonante and Aizizhan. The majority of semi-dwarf indica rice varieties that have been bred are either directly or indirectly derived from the above two cultivars [15]. In 2002, three research groups successively published results of map-based cloning of the sd-1 gene, which supports the first green revolution in molecular level. The sd-1 gene controls plant height in rice. Mutation of sd-1 directly leads to different degrees of dwarfism in rice. The sd-1 protein is involved in the biosynthesis of gibberellin, encoding GA20 oxidase (GA20ox) composed of 389 amino acids. GA20ox is a key enzyme in the gibberellin synthetic pathway,

which catalyzes the conversion of GA53 to GA20. The sd-1 gene is located in chromosome 1 in rice, corresponding to 38381423 - 38384165 map position (5'-3') in Nipponbare. Monna et al. [16] showed that Nipponbare, Sasanishiki and Calrose rice, which are ordinary wild-type rice, contain three exons, with sizes of 558, 318 and 291 bp, and 2 introns with sizes of 105 and 1471 bp. Deo-geo-woo-gen type semi-dwarf varieties IR24, Habataki and Milyang 23 have a 383 bp deletion in the middle of exon 1, covering 278 bp in exon 1 and 2, and 105 bp in the intron. Calrose76 is the outcome of CTC-to-TTC mutation at position 265 in exon 2, which changes a Leu (leucine) residue to Phe (phenyl alanine) residue. Sasaki et al. [17] proposed that the GA20ox-1 gene is independent of sd-1, while the newly discovered GA20-ox-2 is linked to sd-1. Sequences of the sd-1 gene in four cultivars and one wild-type variety were compared. It was found that the length of three exons in SD-1 gene of wild-type variety were 557, 321 and 291 bp respectively while the length of two introns were 103 and 1472 bp. A 383 bp deletion was found in the dwarf Deo-geo-woo-gen variety and its derivative, including 103 bp of intron 1. A GGG-to-GTG mutation at position 94 in Jikkoku rice results in change of glycine to valine. A CTC-to-TTC mutation at position 266 in Calorose 76 rice leads to change of leucine to phenyl alanine. A GAC-to-CAC mutation at position 349 in the dwarf variety Remei leads to a change from aspartic acid to histidine. GA20ox-2 is strongly expressed in the leaves, stem and unbloomed flowers of rice. However, GA20ox-1 is only expressed in unbloomed flowers, which is why the sd-1 gene controls plant height but not the yield. Spielmeyer et al. [18] reported that the 3 sd-1 exons in wild-type variety have lengths of 557, 322 and 291 bp. A 280 bp deletion was found in the GA20ox2 coding region (exon 1 and 2) in the DGWG semi-dwarf indica rice variety. Until now, a total of 5 alleles have been discovered for the sd-1 locus including sd-1 in the wild-type variety, sd-1-d from Deo-geo-woo-gen and its derivative, sd-1-r from Reimei, sd-1-c from Calrose 76 and sd-1-j from Jikkoku. Recently, Asano et al. [77] found that *SD-1* has been subjected to artificial selection in rice evolution and that ancient humans took advantage of functional nucleotide polymorphisms (FNPs) from two SNPs in sd-1 in *japonica* rice.

2.2. Tillering

Rice tillering is an important agronomic character in rice production. Effective tiller number per unit area determines the spike number, which is among the three components of rice yield per unit area, the other two being grain number per spike and 1000 grain weight. Therefore, reducing unproductive tiller is important to improve rice yield. MOC1 was the first gene identified to be related to rice tillering. Li et al. [19] employed map-based cloning to narrow down the location of MOC1 to a 20 kb region in the long arm of Chromosome 6. MOC1 is a member of the GRAS transcription factor family. It is closely related to the LAS gene in Arabidopsis thaliana and the 1s gene in tomato. MOC1 is necessary for initiation of axillary meristem. Loss of MOC1 leads to defects in tiller bud initiation and consequently to complete absence of tillering. Over-expression of MOC1 results in massive tillering in transgenic plants. *OsTB1* and *OSH1* are expressed at lower levels in loss-of-function *MOC1* mutants. Therefore, *MOC1* gene may act as a master regulator in the control of rice tillering. In addition to affecting tillering of rice stem, MOC1 also significantly reduces panicle branches. Cloning of *MOC1* has facilitated our understanding of the regulatory mechanism of rice tillering. However, the

molecular mechanisms regulating *MOC1* remains to be elucidated. Recently, two Chinese research groups found that TAD1/TE directly regulates MOC1, thus revealing an important molecular mechanism of regulation of rice tillers [78,79]. *TAD1/TE* encodes a co-activator of the anaphase-promoting complex (APC/C), a multi-subunit E3 ligase. TAD1 interacts with MOC1, forms a complex with OsAPC10, and functions as a co-activator of APC/C to target MOC1 for degradation in a cell cycle-dependent manner. These findings uncovered a new mechanism underlying shoot branching and found novel determinants of plant architecture and grain yield.

2.3. Panicle type

Grain number per spike of rice is an important agronomic character of rice spike that is directly related to rice yield. Increasing the grain number per spike is part of the goal of high yield breeding. At present, most high yield varieties have significantly increased grain number per spike. Some genes, including Gn1, DEP1 and DEP2, that have a major role in influencing grain number per spike, have been successfully cloned.

QTL-Gn1 was cloned by a Japanese research group [20]. Map-based cloning and sequencing showed that QTL-Gn1 encodes cytokinin oxidase (OsCKX2). Using a cross between the japonica rice cultivar Koshihikari and the indica rice cultivar Habataki, they located QTL-Gn1, a major gene controlling grain number, on the short arm of Chromosome 1. This gene accounts for 44% of phenotypic variation in grain number per panicle. NIL-Gn1 heterozygous plants (Gn1/gn1) were used to generate 96 F2 plants, to decompose Gn1 into two loci, Gn1a and Gn1b, which are of equal effect. Gn1a is located in a region less than 2 cM between R3192 and C12072S, and Gn1b is located upstream of Gn1a. NIL-Gn1a heterozygous plants (Gn1a/gn1a) were used to generate 13000 F2 plants and then finely map the Gn1a gene to a 6.3 kb region between 3A28 and 3A20. There is only one open reading frame in this region, and it belongs to the OsCKX2 gene, which is highly homologous to cytokinin oxidase/dehydrogenase. Sequencing analysis showed that compared with Koshihikari, there are deletions of 16 bases and 6 bases in the 5' untranslated region and exon 1, respectively, of OsCKX2 in Habataki. A 3-base substitution in exon 4 causes changes in the protein product was also found in Habataki. These results suggested the loss of function or deletion of OsCKX2 may lead to increase in rice yield. Complementation tests showed that OsCKX2 is the same gene as Gn1a. OsCKX2 is strongly expressed in leaf, stem, inflorescence meristem and flower, but weakly in apical meristem of rice plants; it is not expressed in the root and embryo. The Gn1a locus is an allele of Habataki, which has increased grain number per spike. Loss of this gene leads to significantly increased content of cytokinin in spikes and increased number of spikelets, i.e. grain number, and consequently increased rice yield. Ashikari et al. [21] obtained NIL-Gn1-Sd-1 by crossing and screening using Koshihikari as the genetic background and NIL-Gn1 and NIL-Sd-1, which are Gn1 alleles from Habataki controlling grain number and Sd-1 allele controlling plant height, respectively. This line has 26% higher grain yield and 18% lower plant height compared to Koshihikari.

Spike characters include spike shape, spike-layer uniformity and grain density. High spike-layer uniformity is conducive to ventilation and photopermeability of the lower part of the

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plant population, which provides a favorable condition for consistent maturity. Developed primary branches and moderate grain density helps reduce spike length, lower the center of gravity of plants, and ensure the consistency of grain maturity. Panicle type is an important agronomic character, depending on the morphology and number of primary and secondary branches. Panicle type can be erect, semi-erect and curved. It is generally believed that erect panicle type has higher solar energy utilization, and is conducive for CO_2 diffusion. It can also modify the biological environment of the population, adjust inter-plant temperature and reduce moisture. The erect-panicle type has higher accumulation of photosynthestic products, better fertilizer tolerance and anti-lodging properties. Yang Shouren et al. first proposed a super high yield japonica rice type with erect panicles. These new lines, which include Shennong 265 and Shennong 89366 that are bred based on this plant type, feature high yield potential [22] and erect panicle, which are desired traits in rice in north China. Therefore, these lines have attracted increasing attention from breeders in that region.

DEP1 is a key pleiotropic gene isolated from Shennong 265 (super rice variety in north China) controlling rice yield. The DEP1 locus is a major QTL controlling yield-related trait of rice, located between SSR markers RM3770 and RM7424 on Chromosome 9. DEP1 corresponds to the 16410553 - 16414701 map position (5'-3') in Nipponbare, as a qPE9-1 allele [24]. The dominant gene at this locus is caused by an acquired mutation, which results in failure to encode a protein similar to phosphatidylethanolamine-binding protein. This mutant DEP1 promotes cell division, reduces the length of neck-panicle node and increases grain density in a panicle. Moreover, a higher branch number and grain number per panicle results in rice yield increase by 15%-20%. Researchers have found that mutation in DEP1 is widely present in erect and semi-erect-panicle high-yield rice that is grown in the middle and lower reaches of the Yangtze River. Thus, it is evident that the DEP1 gene has played an important role in rice yield increase in China [23]. DEP1 not only increases the yield in rice, but also in other crops, such as wheat and barley. Thus, DEP1 is very important in high-yield molecular breeding and for breeding new high-yielding varieties of crops.

DEP2 is responsible for the trait of erect and dense spike in rice. It is located in Chromosome 7, has 10 exons and encodes a protein with 1365 amino acids and unknown function. Sequence analysis of dep2-1 mutant showed that there is a 31-base deletion in exon 6 and a G/A transversion in intron 2. The deletion of 31 bases leads to shift in the reading frame, while the G/A transversion changes the editing position in intron 2, causing another frame shift. DEP2 mainly influences rachis development, promoting the elongation of primary and secondary branches. Mutation in DEP2 causes cell proliferation disorder in spike differentiation, resulting in the phenotype of erect and dense spikes [25]. It has also been shown that DEP2 is at the same locus as the small and round seed 1 (SRS1) gene [26] and EP2 [27]. SRS1/DEP2 not only regulates spike type in rice, but also its seed size. SRS1/DEP2 is mainly expressed in young tissues, such as young spikes. Mutation in srs1/dep2 leads to erect panicle, and small and round seed.

Recently, dense and erect panicle 3 (DEP3) was identified by map-based cloning. It is located in Chromosome 6. *dep3* is an erect and dense panicle mutant of the japonica rice variety. In the wild-type variety, the panicle begins to droop after flowering, which is accompanied by

changes in panicle length, grain shape and grain number per spike. However, dep3 mutants have smaller vascular bundles and thicker stems, which account for the erect-panicle phenotype. Thus, the erect and dense panicle phenotype in rice is controlled by a single recessive gene dep3. It is predicted to encode a patatin-like phospholipase A2 (PLA2) super family domain-containing protein. The mutant allele of dep3 has a deletion of 408 bp at LOC_Os06g46350, which covers 47 bp after the coding region in exon 3 and 361 bp before the 3' untranslated region [28].

2.4. Ideal plant type

Yield of rice is determined by wide diversity of agronomical traits including tiller number, grain number per spike, grain weight, grain-filling rate, plant type, etc.. It is a complex quantitative trait controlled coordinately by multiple genes and environment. In order to improve the yield potential of rice, concept of new plant type is proposed by rice breeders. New plant type is also called as ideal plant type and its key characteristics include decreased tillering, no ineffective tillering, increased grain number per spike, thick and strong stem and developed root system. Theoretical analysis shows that the yield of rice varieties of ideal plant type could increase by 25% than that of the current variety under the equatorial drought conditions. It is commendable to find the favorable mutant of ideal plant type. Using the japonica line "Shaoniejing" possessing characteristics of ideal plant type, Chinese scientists isolated and cloned the major quantitative trait gene IPA1 (Ideal Plant Architecture 1) which controls the ideal plant type of rice in 2010 [29]. Compared with the conventional cultivar such as indica rice TN1, "Shaoniejing" has less tillering, larger spikes, higher grain number per spike, thicker stem and more developed root system with the characteristics of ideal plant type. Backcrossing was performed between Shaoniejing, TN1 (recurrent parent) and conventional japonica rice Hui7 (recurrent parent). It was found that traits including plant height, tiller number stem thickness and panicle type have a co-segregation relationship. Thus, it is indicated that the phenotypic difference between Shaoniejing and conventional variety might be controlled by the same major gene. Analysis of near isogenic lines in Shaoniejing and Hui7 showed that phenotype of plant with IPA1 locus in heterozygote state (IPA1/ipal) was between homozygous wild type (IPA1/IPA1)and homozygous mutant type (ipal/ipal), indicating that IPA1 is a semidominant gene. Using mapping population of BC2F2 constructed by Shaoniejing and TN1, a major QTL—QTL8 was identified and cloned on chromosome 8 using map-based cloning. Sequence analysis found that the third extron in QTL8 of Shaoniejing had a C to A point mutation, causing amino acid to change from leucine to isoleucine. Through constructing plasmid gIPA1 carrying full-length gene and transforming Nipponbare as a receptor, it was found that transgenic plants had decreased tiller, thick stem, increased branch number and grain number per spike. Contrarily, when IPA1 expression was downregulated in RI 22 (it had the same point mutation as Shaoneijing), it was found that the transgenic plant had increased tillering, decreased plant height and thin stem with significant declining in branch and grain numbers. Sequence analysis revealed that IPA1

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encoded the transcription factor OsSPL14 which contained the SBP structural domain. IPA1 was located in the nucleus with the transcriptional activity. Analysis of mRNA *in situ* expression showed that IPA1 had the highest expression in stem tips during vegetative growth period and branch primodium during reproductive growing period. IPA1 contains target site of miR156 and could be regulated by miR156 *in vivo* by means of transcriptional segmentation and translational repression. As point mutation of Shaoniejing occurs at the target site of miR156, the two regulatory channels are influenced, causing simultaneous increase of transcript and protein amount in IPA1. Transgenic study revealed that, although point mutation induced changes in amino acid, it caused no influence on the function of IPA1 protein. Application value of IPA1 gene was explored and ipa1 mutant gene was introduced into rice "Xiushui 11" through backcrossing. Analysis of near isogenic lines of backcross offspring found that strains carrying ipa1 mutant gene had the typical characteristics of ideal plant type and their yield had an increase of over 10% in the field plot experiment compared with their parent strain "Xiushui 11". Therefore, mutation of this gene has induced decreased tillering, thick stem and obvious increase in grain number per spike and 1000 grain weight, and the rice variety had the typical features of ideal plant type. It is a powerful tool for improving the plant type of current rice cultivars and enhancing the rice yield with great application potential in rice breeding[29]. At the same time, this gene was also cloned successfully by the Japanese research group using "Nipponbare" which was widely used in Japan and high-yield rice variety“ST-12”. This allelic gene was introduced into "Nipponbare" whose yield was low with an average production of 2200 grains per plant. Heading number of "Nipponbare" was strengthened after introducing this gene and its yield reached to 3100 grains by about 40% [30]. Using hybrid segregating population of Nipponbare and ST-12, they found gene Gn1a which controlled the grain number per spike on chromosome 1 and WFP gene which controlled the primary branch number on chromosome 8. Through selecting lines with 4 different combinations of Gn1a and WFP genes from BC2F2 population, primary branch number and grain number per spike among different lines were compared. It was found that the pyramiding of Gn1a and WFP from ST-12 could increase the grain number per spike by 40% - 50%, which effectively improved the rice yield [30].

2.5. Summary

Improving yield of rice is an important means to ensure food security in the world today. In order to further strengthen the yield potential of current cultivars to meet people's food demand, super-high-yield breeding based on ideal plant type has become the goal of rice breeders. Studying the control mechanism of ideal plant type to clone the related control gene is of great significance in breeding higher-yield rice variety using genetic engineering. Meanwhile, as model crop of monocotyledon, research of control gene in ideal plant type will contribute to clarifying the molecular mechanism of growth and development of monocotyledon significantly.

3. Hybrid heterosis

The phenomenon of plant heterosis was first described by Shull as the promoting effect of plant development after copulation of gametes of different genotypes[31]. Heterosis of crops was first discovered in tobacco in the middle of the 18th century. Rice heterosis was initially reported by American scientist Jones, who found that some F1 hybrids of rice had increased tillering and higher yield compared with the parents [32]. Later, heterosis in self-pollinated plants was studied and confirmed by more scientists. In the late 1950s, in the context of successful commerical exploitation of corn heterosis in America, rice breeders broaden the exploration channel of heterosis. In 1960s, scientists in India, US, Japan and China began to study the rice heterosis and its application in commercial production successively. For the first time, Xincheng Changyou from Japan achieved three-line combination of japonica rice in 1968. Study on heterosis application of rice began in China when male sterile plant was discovered by Yuan Longping et al. in 1964. Li Bihu in 1970 discovered a wild-type rice with pollen abortion in Nanhong Farm in Yaxian County of Hainan, which was a major breakthrough for the breeding and selection of male sterile rice in China[11]. Three-line combination of hybrid indica rice was achieved successfully in China in 1973. Indica hybrid rice began to receive extensive popularization in China in 1976 and China became the first country in the world to realize the commerical utilization of rice heterosis. Hybrid rice planting resulted in large rice yield increase in China from 1976 to 1995, which was a significant achievement. Cumulative planting area of hybrid rice reached 250 million hm^2 in 1999 with an increased crop production of 370 million tons[11]. Hybrid rice had made great contribution to the food production of China and the world.

3.1. Cytoplasmic male sterility and the fertility-restoring genes

Cytoplasmic male sterility (CMS) refers to the biological phenomena that the male reproductive system of plant cannot develop normally to produce the viable pollen, but the female reproductive system has normal development and vegetative growth. As the major type of hybrid rice combination, cytoplasmic male sterility in rice has attracted more and more attention. Sterile line of rice can be divided into genic male sterility and cytoplasmic male sterility based on sterility mechanism. Genic male sterility can be further divided into dominant male genetic sterility, recessive genic male sterility and environmental sterility. Based on genetic characters of male sterility, cytoplasmic male sterility is classified into sporophyte sterility and gametophyte sterility. Gametophyte sterility mainly consists of Baotai type (BT type), Dian type, Honglian type(HL) and Lide type. Source of sterile cytoplasm in sporophyte is abundant, and wild abortion type (WA), dwarf abortion type (DA), D type, G type, K type, Indonesia paddy type, etc. have large planting area in China (Table 1)[77].

As cytoplasmic male sterility and its fertility restoration are a basis for three-line hybrid rice breeding and production application. Topics on the mechanism of cytoplasmic male sterility and its fertility restoration in rice have attracted attentions of many scientists. Cytoplasmic male sterility is manifested as maternal inheritance and generally related with abnormal open reading frame of mitochondrial genome. In most cases, male sterility could be restored by the

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Types	Sources	CMS varieties
Wild abortion type (WA)	Hainan wild rice	Zhenshan 97A, V20A
Honglian type(HL)	Red awl wild rice	Honglian A, YuetaiA, Yuefeng A
Baotai type (BT)	Boro-Taizhong 65	Fengjin A, Hanfeng A
Dwarf abortion type (DA)	Jiangxi dwarf wild rice	Xieqingzao A
K type	West Africa indica varieties	Chaoyang 1A, Gang46A
D type,	Dish D52	D Shan A, D297A, D62A
Indonesia paddy type	Indonesia varieties	Zhong 9A, II-32A
Dian type	Taipei varieties	Liuqianxin A, Ning 67A

Table 1. The CMS types

fertility-restoring gene (Rf) encoded by nucleus [33]. Therefore, CMS/Rf system is the ideal model for studying the interaction between mitochondrial genome and nuclei genome. It has been widely applied in hybrid breeding in order to improve the yield of crop. Various types of CMS have been found in rice and the main applied types in indica hybrid rice include wild abortion type (CMS-WA), Hongling type(CMS-HL), dwarf abortion type (CMS-DA) and so on. The typical representatives are Zhenshan 97A, Congguang 41A and Xieqingzao A. Main applied types in japonica hybrid rice are Baotai type(BT) and Dian type, representatives being Fengjin A and Liuqianxin A[79].

At present, fertility-restoring gene in cytoplasmic male sterility has been positioned and cloned in *Zea mays*, *Petunia hybrida*, *Daucus carota* and other plants [34, 35, 36]. For rice, two research groups in Japan [37, 38] have reported the fine positioning and cloning of fertility-restoring gene Rf-1 for BT type. The results showed that Rf-1 (PRR791) gene also encodes a mitochondial positioning protein which contains PPR. Recently, fertility-restoring gene Rf5 which could restore the cytoplasmic male sterile line of Honglian type was obtained by Hu et al. [39] through map-based cloning and proved to be consistent with Rf1a. Results by Akagi et al. [38] also indicated that a PRR homologous gene (Rf-1b) exists beside this Rf-1 gene (also called as Rf-1a). However, it was supposed that Rf-1b has no restoring function. Study of cytoplasmic male sterility mechanism and functional analysis of fertility-restoring gene in rice CMS-BT was published by Chinese research group in 2006[40]. Results revealed that cytoplasmic male sterile line of BT type contains an abnormal mitochondrial open reading frame—orf79. There is a cotranscription with atp6 gene to encode a cytotoxic peptide. Using transgenic plant, it was proved that the expression of orf79 in rice caused male gametophyte sterility of pollen. A polygene cluster encoding PPR protein was found in Rf-1 loci of chromosome 10. At least 2 members including Rfla (it was reported as PRR791 by the Japanese research group) and Rf1b were proved to have fertility restoring function for BT type.

Studies showed that Rf1 could restore the fertility of cytoplasmic male sterile line of Baotai type. Rf5 could also restore the fertility of cytoplasmic male sterile line of Honglian type. For the near isogenic line with cytoplasmic male sterility, the pollen is fertile if it carries Rf-1 gene,

and otherwise, sterile. Varieties carrying Rf-1 gene such as IR24, IR36 and MTC-18R could correct the cytoplasmic male sterility of BT type but varieties carrying recessive gene rf-1 could not, such as Nipponbare. Rf-1 cDNA has a full length of 2760bp in MTC-10R. It only contains 1 exon which encodes a protein product composed of 791 amino acids. The product contains 16 trigonous pentapeptide repeat sequence motifs and mitochondrial targeting peptides. The near isogenic line MTC-10R(Rf-1/Rf-1)could restore the cytoplasmic male sterility, but the near isogenic line MTC-10A(rf-1/rf-1)with 1bp and 547bp deletion in Rf-1A locus could not restore the cytoplasmic male sterility [38]. With a full length of 3870bp, Rf1b cDNA only has one exon in restoring line Minghui 63. The exon encodes a protein product composed of 506 amino acids containing PPR motif and mitochondrial targeting peptide. The proteins encoded by fertility-restoring allele of 6 restorer lines (male sterile line or maintainer line) are different on 9 amino acids from non-fertility restoring allele of 6 non-restorer lines. The shared difference is that the base A at position 1235 in the fertility-restoring allele Rf1b is replaced by G in the non-fertility restoring allele, causing the changing of asparaginate into serine at position 412 [40]. Fertility-restoring allele Rf1a in the restorer line could encode the complete protein. However, due to frameshift mutation, allele of non-restorer line of japonica rice encodes a truncated protein which only contains 266 amino acids. Protein encoded by allele of non-restorer line of indica rice has a transformation of 55 amino acids [40].

Two open reading frames of Rf-1A and Rf-1B are found in isogenic line MTC-10R which could correct the cytoplasmic male sterility. Due to the presence of Rf-1B, the terminator codon occurs in advance, causing the formation of a short protein with no mitochondrial targeting peptide, Rf-1A is exactly the Rf-1 gene. Rf-1A encodes a protein which contains 16 trigonous pentapeptide repeat (PPR) motifs and is targeted to mitochondrion. Rf-1A is expressed in inflorescence during booting stage and PPR motif with tandem duplication is considered to be capable of having specific binding with RNA and DNA. Therefore, Rf-1A is a fertility-restoring gene through processing the atp6/orf79 transcript from mitochondrial genome in BT type. Cytoplasmic male sterility of BT type could be corrected by rice varieties carrying Rf-1 gene, which has no effect on WA type [38]. In Boro II rice, abnormal mitochondrial open reading frame orf79 has co-transcription with doubled atp6 gene, encoding a cytotoxic peptide. Specific accumulation of this toxic polypeptide causes male sterility of gametophyte. The two related fertility-restoring genes Rf1a and Rf1b are located in the typical Rf-1 locus as members of polygenic cluster, encoding the trigonous pentapeptide repeat protein. RF1A and RF1B are both targeted to the mitochondrion and they prevent the formation of toxic peptide to restore the fertility by restriction and decomposition of B-atp6/orf79 mRNA. For decomposing mRNA, RF1A is epistatic over RF1B. Besides, RF1A could not only degrade B-atp6/orf79 mRNA but also promote the editing of atp6 mRNAs [40].

Rf1a and Rf1b are both fertility restoring-gene with expression in spikes, leaves and roots. Proteins RF1A and RF1B encoded by them are both targeted at mitochondrion. Via restriction, B-atp6/orf79 mRNA is blocked by RF1A to prevent the generation of ORF79 protein to restore the fertility. And fertility is restored by RF1B via degrading B-atp6/orf79 mRNA. When RF1A and RF1B are present simultaneously, RFIA functions with preference, that is, RF1A is epistatic over RF1B in mRNA processing. In addition to the function of dissecting B-atp6/orf79 mRNA,

RF1A could also improve atp6 mRNA editing. It is presumed that the latter is the basic function of RF1A and the former the new function developed during the evolution [40].

Through forming the complex with GRP_162-rich glycine protein, trigonous pentapeptide protein RF5 restores the fertility of cytoplasmic male sterile line of Honglian type [39]. Two non-allelic nuclear restorer genes including Rf5 and Rf6 are involved in the gametophyte fertility restoring model of Honglian type (Rf6 is a new restorer gene locus located in the short arm on chromosome 8). Half of the pollens in F_1 plants carrying either Rf5 or Rf6 are fertile and fertility of 75% pollens is normal in hybrid carrying both Rf5 and Rf6. Seed setting rate of F_1 plants carrying 2 non-allelic genes is higher than that of F1 carrying only 1 restorer gene under adverse environment [41].

3.2. Photoperiod (thermo)-sensitive male sterile

For the first time in 1973, natural nuclear male sterile line Nongken 58S which was mediated by photoperiod and thermal was discovered by Shi Mingsong in a late japonica rice field in Hubei province. The discovery and effective utilization of photoperiod (thermo)-sensitive genic male sterile (PTGMS) line Nongken 58S opened a new chapter in China's hybrid rice research. Because the PTGMS line could be dually used as sterile and maintainer lines, the maintainer line is no longer needed in the two-line hybrid rice cultivation. Under different thermal and photoperoid conditions, the PTGMS line could be used not only as sterile line for hybrid seed production, but also as maintainer line for self reproduction. Thus, process of seed reproduction and breeding are simplified, reducing the production cost of hybrid seeds. Besides, it is not restricted by restoring and maintaining relationship. Therefore, it could strengthen the degree of genetic complexity of breeding parents in the rice hybrid breeding and expand the genetic distance between the 2 parents. So it is favorable for selecting and breeding strong and optical combination with higher heterosis. However, study of fertility transition mechanism of PTGMS line is still weak and could not adapt to the development of application studies on two-line hybrid rice. Especially, the studies on genetic mechanism and regulatory mechanism in photoperiod thermo-sensitive genic male sterile line are not very intensive. Therefore, strengthening the studies on fertility transition mechanism of photoperoid thermo-sensitive genic male sterile line of rice, especially the studies on the genetics and molecular biology, and finding the gene and protein closely related with fertility transition regulation are important. The achievements made in these respect will promote the breeding, selection and mating of photoperiod thermo-sensitive genic male sterile lines and the utilization of heterosis in other crops in future. Photoperiod thermo-sensitive genic male sterile lines show diversity in genetics. This is because sterility is a kind of biological phenomenon related t photoperoid and thermal ecological conditions and expression of sterile gene requires optimal light and temperature conditions. Researchers have already carried out a great number of studies on the sterility inheritance rules of all kinds of photoperiod thermo-sensitive male sterile resources including Nongken 58S, Annong S-1, Hengnong S-1 and 546OS.Some basic inheritance rules have been clarified.

At present, gene pms3 which controls the photoperoid-sensitive male sterility in japonica rice Nongken 58S [42] and gene p/tms12-1 which controls the thermo-sensitive male sterility in

indica rice Peiai 64S [43] already have been cloned. Studies proved that located at the same locus, they are a non-coding RNA. Researchers in Huazhong Agricultural University successfully cloned gene pms3 controlling the photoperoid-sensitive genetic sterile line of rice in 2012. They found that it is a long non-coding RNA that controls the sterility of Nongken 58S; pms3 is the transcript 1 of LOC_12g36030. Studies indicated that it is a RNA molecule associated with male sterility specific to long-time lighting with a length of 1236bp (LDMAR). For normal rice under long-day condition, the expression of this gene could ensure normal pollen development and male fertility. However, for photoperoid-sensitive genic male sterile line of rice, base mutation of pms3 interval causes methylation of promotor interval in this gene with decreased expression. As a result, it could not meet the requirement of pollen development. Thus, this causes the male sterility under long-day condition [42]. Gene p/tms12-1 which controls the thermo-sensitive male sterility was cloned from Peiai 64S, which was the parent of thermo-sensitive genic male sterile line for two-line hybrid indica rice with the largest planting area by researches from South China Agricultural University. This gene is a non-coding RNA gene and its original transcript produces a small RNA after processing at least 2 times. Compared with the normal rice variety, thermo-sensitive male sterile line of rice had a single base mutation in this small RNA. It was further revealed by the studies that Nongken 58S also has the same gene mutation and this single base mutation is the common cause for thermo-sensitive male sterility of indica rice and photoperoid-sensitive male sterility of japonica rice. In normal rice, the expression of wild-type P/TMS12-1 restrains the occurrence of thermo-sensitive or photoperoid-sensitive male sterility. However, for thermo-sensitive and photoperoid-sensitive male sterile line of rice, the expression level of small RNA and its interaction with target gene are influenced by mutation of p/tms12-1, causing male sterility [43]. Successful cloning of pms3(p/tms12-1)gene had a very great significance for accelerating the breeding of two-line male sterile varieties of rice and promoting the research and development of crop heterosis utilization.

3.3. Wide and specific compatibility genes and subspecies heterosis

Making full utilization of heterosis between subspecies of indica and japonica rice is a major and effective means to increase the rice yield per unit area. However, this utilization is restricted by the low fertility of indica-japonica hybrid F1. Asian cultivated rice is divided into 2 subspecies, *indica* and *japonica*. Heterosis of intersubspecific indica-japonica hybrid is far greater than that of intrasubspecific hybrid. However, because reproductive isolation exists widely between subspecies in nature, hybrid fertility of the intersubspecific hybrid declines, which results in low seed setting rate. Breeding of hybrid rice had been limited within the subspecies for a long time because of this restriction because of the difficulty to utilize the stronger intersubspecies heterosis. Later, rice resources which could break the reproductive isolation are discovered by scientists, and known as wide compatibility varieties. Using indica rice varieties IR36 and IR50 and japonica rice varieties Qiuguang and Ribenyou as testers, Japanese scientist IKehashi, et al. [44] performed the hybrid fertility identification for 74 intermediate varieties. Six varieties including Ketan NangKa, Cpslo-17, etc. and hybrid F1 of indica rice and japonica rice all had high seed setting rate. They were believed to have wide compatibility gene (WCG), and named as wide compatibility varieties (WCV). After extensive

testing by Chinese researchers, Balila, Qiuguang, Nantehao and IR36 were officially assigned as the testers for wide compatibility in China in 1989[78]. At present, a great number of wide compatibility lines are selected for hybrid rice breeding through different ways. For example, WA type cytoplasmic male sterile line 02428A, Reyan 1A, Peiai 64S and other wide compatible sterile lines were bred through backcrossing between wide compatible materials and gene-cytoplasmic male sterile lines. Wide compatibility restorer lines including H108, H64, H921, D069, P26, JM-2, Zhong 413, T2070, 9308 were bred with japonica-indica rice cross.

Sterility of indica-japonica hybrid is the key obstacle to taking advantage of hybrid vigor, and its mechanism has for a long time remained as one of the research hotspots for rice breeding and molecular genetics. For the past decades, genetic analysis has already located a host of loci related to the sterility of hybrid rice, but still little is known about the molecular mechanism for the reproductive segregation between the two rice subspecies. In 1984, Japan's rice breeding expert Ikehashi argued that the sterility of indica-japonica hybrid is mainly controlled by the allele at S5 locus on Chromosome 6. S5-n is known as a WCG, and the rice variety containing S5-n gene is a WCV, whose hybrids with indica and japonica show normal fertility [45]. In 2008 after many years of extensive research, Chinese scientists successfully cloned S5 gene and preliminarily illuminated the molecular mechanism for S5 to regulate the sterility of hybrid [46]. The research shows that S5-j is located on Chromosome 6, cDNA having a total length of 2495 bp and containing three exons. It encodes aspartyl protease made up of 472 amino acids and the product contains signal peptides, central domain, N terminal and C terminal. S5 is not expressed in leaves, but in the developing panicle. *In-situ* hybridization shows that S5 is expressed in various organs of ovule, including nucleus, integument, macrospore mother cell and embryo sac. S5 gene regulates seed setting percentage by controlling the sterility of female gamete. Protein s5-i and s5-j of indica and japonica are different on two amino acids. Located in the central domain, both two amino acids may have an effect on the activity or stability of aspartyl protease. Just like Nipponbare and Balilla, in indica rice-japonica hybrid, locus 273 is lLeucine and locus 471 is valine; for indica Nanjing 11, locus 273 is phenylalanine and locus 471 is alanine. At locus 172 bp in the downstream of terminator coden, there is deletion of an A; wide-compatibility variety 02428: deletion appears at 67 pb before ATG and 69 bp after ATG transcription start site, totaling 136bp, resulting in the deletion of 115 amino acids at N terminal of signal peptides and rendering it unable to be located on the cell wall. Therefore, the deletion of large segment on S5 gene of wide-compatibility variety has led to loss of function. Neither the hybrid with indica nor with japonica can affect hybrid fertility. Sequencing of 16 different varieties (including indica and japonica and wide-compatibility variety) has further confirmed the above results. At locus S5-i and S5-j of indica and japonica, indica-japonica differentiation occurs due to the infertility of indica-japonica hybrid, thus creating rich diversity of rice varieties and leading to reproductive segregation. However, the existence of wide-compatibility gene S5-n has provided a bridge for the gene exchange between sub-species of indica-japonica hybrid, maintaining the integrity of rice variety. Wide-compatibility gene S5-n enjoys bright prospect for application in the breed improvement of rice variety, for it can be directly used to develop other wide-compatibility genes and also in breeding wide-compatibility varieties as molecular marker. Effective application of wide-compatibility genes can help

overcome the infertility of the hybrid between indica and japonica rice subspecies so as to improve rice yield by relying on the strong hybrid vigor of indica-japonica sub-species[47].

It's worth noting that the research findings of Aradidopsis indicate that aspartyl protease is mainly involved in the transduction of disease resistant signal and the programmed cell death of regenerative tissues. Although the current research has failed to fully reveal the functional mechanism of S5, they can be sure that S5 has close ties with the emergence and survival of macrospore. According to the analysis of crystalline structure, aspartyl protease has three structural domains, namely, central structural domain, ring structure of N-terminal and ring structure of C-terminal. Sequence alignment and analysis show that the two mutational sites amino acid 273 and 471 in S5 are located in the central domain. However, the problems of the decreasing extent in the activity of aspartyl protease are connected with the fertility of female gamete (embryo sac), and the reason forthe functionally deficient S5-n not to affect the fertility of female gamete (embryo sac) in homozygosity and heterozygosity need further research. Recently, researchers discover a "killer-protector" system encoded by three closely interlocked open reading frames (ORF3, ORF4 and ORF5), which controls the fertility of indica-hybrid hybrid. ORF5 gene plays the "killer" role, assisted by ORF4. Conversely, ORF3, as the protector, has the opposite function. In the forming process of gynospore, the action of ORF5+ ("killer") and ORF4+ ("partner") can cause the stress response of endoplasmic reticulum (ER), while ORF3+ ("protector") blocks the ER stress response in cells and facilitates cells to produce normal gamete. But ORF3- cannot block ER stress response, thus causing programmed cell death and embryo sac abortion to happen in advance [48]. This research has given a relatively complete elaboration of the molecular mechanism of S5, and revealed the molecular mechanism for controlling the fertility of indica-japonica hybrid. It provides reference for studying the sterility of indica-japonica hybrid, molecular mechanism for reproductive segregation and biological evolution. This killer-protector system regulates the sterility of a hybrid from two subspecies. The non-fatal combination of ORF4 and ORF5 allows the indica-japonica hybrid to pass its genes to the next generation, thus overcoming the hybrid sterility and laying the foundation for the development of ideal rice varieties. This finding has vast application potential in improving rice varieties. The relevant information can be directly used to develop other wide-compatibility genes and breed wide-compatibility varieties. It will help fix reproductive segregation, overcome sterility of hybrid between indica-japonica sub-species and make use of hybrid vigor of indica-japonica sub-species to increase rice yield.

Besides wide-compatibility genes, there are also some specific-compatibility genes present in rice. Based on the systematic research on pollen fertility, Zhang Guiquan et al. [49] put forward the theory of specific compatibility genes, holding that the pollen fertility of indica-japonica hybrid is controlled by at least six loci, namely, S-a, s-b, S-c, S-d, S-e and S-f. The pollen sterility of hybrid is mainly determined by the number of heterozygous loci and the differentiation distance of alleles. Heterozygous alleles lead to sterility, while homozygous alleles lead to compatibility. Such gene is called specific compatibility gene. On these loci, indica variety often carries S^i/S^i, while japonica carries S^j/S^j. In their hybrid, the interaction of S^i gene and S^j gene causes the abortion of S^j-carrying male gamete. [50]. Sa gene locus affects the fertility of F_1 hybrid between indica-japonica subspecies and the interaction of indica-japonica alleles leads

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to the abortion of male gamete and reduces the seed setting percentage. Using cultivar Taichung 65 and isogenic F_1 sterile line TISL4 as the materials, Zhuang Chuxiong et al (51) employed such technologies as RFLP and RAPD to locate S-a locus on Chromosome 1 and the genetic distance from CDO548 is 6.4 cM.

Further research found that Sa locus is actually made up of two adjacent gene loci SaM and SaF, encoding ubiquitin-like modifier E3 ligase and F-box proteins[52]. Allele SaM^+ encodes an ubiquitin-like modifier E3 ligase made up of 257 amino acids, while a G→T single site mutation at intron 5 in SaM^-, causing premature termination of translation and the end product, is only made of 217 amino acids. SaF encodes a F-box protein composed of 476 amino acids. Compared with SaF^+, a single nucleotide mutation occurs in SaF^-, resulting in phenylalanine for serine substitution at position 287[52]. The haplotype in most indica varieties is SaM^+SaF^+, while SaM^-SaF^- in all japonica varieties. The semi-sterility of indica-japonica hybrid is due to SaF^+'s direct interaction with SaM^- and indirect interaction with SaM^+, which has led to the abortion of pollen that carries SaM^-. Due to the existence of repression domain, SaM^+ does not directly interact with SaF^+, but SaM^+ will inevitably cause male sterility. Male sterility would be impossible if any of SaM^+, SaM^- or SaF^+ is lacking. This "two pairs of alleles/three elements" interaction model has provided a satisfactory explanation for the incompatibility of indica-japonica hybrid [52].

In F_1 plant, combinations of alleles at adjacent positions (SaM^+SaF^+ or SaM^-SaF^-) separate in the haploid microspore. Therefore, only the protein migration between spores can result in the concurrence of SaM^+, SaM^- and SaF^+. It may be impossible for SaM^- to migrate due to the deletion of a domain in its truncated proteins, so SaF^+ and SaM protein need transport from its own microspore to the microspore that carries SaM^- for the interaction to happen. SaF^+SaM^- complex further interacts with SaM^+, leading to male sterility by resulting in killing the microspore that carriers SaM^-. Since the male developmental defect of hybrid occurs in the early period of microspores, the transport of these proteins may occur via the cytoplasmic channel during the tetrad period. The SNPs analysis of SaF and SaM shows that the functional variation on SaF has already existed before the evolution and seperation of most rice varieties. The mutation on SaM occurs in the population of ordinary wild-type rice (Oryza rufipogon) that carries SuM^+SuF^- in south China, thus creating SuM^-SaF haplotype. Through analysis, the authors conclude that their research data agree with the recently presented assumption that indica and japonica originate from different wild rice populations [53]. Some varieties containing SaM^+SaF haplotype have also been found in indica. Since its hybrid with indica or japonica lacks SaM^+ or SaF^+, it is fertile. Therefore, $SaM^+$and SaF can be defined as compatibility locus Sa^-n. Sa^-n (SaM^+SaF^-), Sa-i (SaM^+SaF^+) and Sa-j (SaM^-SaF^-) are similar to S5 locus, thus forming a three-allele system to control rice hybrid's male sterility and fertility (compatibility). The molecular mechanisms for the sterility of rice hybrid are thus unified.

Considering that indica-japonica hybrid has great application prospect in improving rice variety, the obtaining of relevant information about Sa and S5 genes can facilitate its use as molecular marker in large-scale screening for compatible germplasm of rice varieties. Or people can also use transgenic technology to create new compatibility hybrid lines. The breakthrough in the research of relevant molecular mechanism for the sterility of indica-

japonica rice hybrid has laid a solid foundation for making use of the strong hybrid vigor of inica-japonica subspecies to increase rice yield.

4. Grain shape

Rice's grain shape traits are important agronomic characters directly related to yield, so to reveal the genetic and development mechanism of grain shape and apply it in breeding is an important means to increase the per unit yield of rice. Since grain shape in rice is closely connected with its appearance, processing quality, cooking and edible qualities, grain shape traits affect not only rice yield, but also rice qualities, playing an important role in the forming of yield and quality in rice [54]. The grain shape of the world's rice varieties can be divided into several types: coarse grain, fine grain, short grain, long grain and ultra-large grain. Grain shape traits mainly include grain's length, width, length/width ratio and length/thickness ratio. Many tests show that the inheritance of grain length is controlled by single gene, double gene, polygene and minorgene. Grain width and thickness are mostly in normal distribution, indicating that this trait is controlled by polygene; grain weight is one of the important factors to constitute yield-related trait as well as the integrated indicator of grain length, grain width and grain thickness. It is generally believed that grain weight is controlled by polygene. Therefore, grain's length, width, thickness, length/width ratio and weight belong to quantitative traits controlled by polygene. Meanwhile, there is correlation between different traits [55]. QTL positioning is an important means to analyze the inheritance of quantitative traits. Up to now, the number of already positioned QTL for controlling rice's grain shape has exceeded 200 [56]. The positioning, cloning and functional analysis of the important genes that control rice's yield-related traits can help improve the molecular genetics of rice's yield-related traits and increase per unit yield of rice. Grain size is an important determinant factor of the yield of rice grain as well as the objective trait for crop domestication and artificial breeding. At present, some grain shape-related genes have been cloned by means of map-based cloning, such as GS3, GW2, GW5, GS5 and GW8.

4.1. GS3

GS3 is the first cloned major QTL controlling grain length and weight and also the minor QTL controlling grain width and plumpness. Fan et al [57] used Minghui 63 (large grain) as the recurrent parent in continuous cross breeding and backcrossing with Chuan 7 (small grain) and constructed the near-isogenic lines for positioning GS3. Through analysis of 201 random samples in the offsprings of BC_3F_2, it is found that GS3 has accounted for the 80-90% variation in the grain weight and length of the population. They built advanced backcross population BC_3F_1 and selected recombinants for the target zones. They conducted fine mapping in the Minghui 63-based BC3F2 (GS3-NIL) plant population, and selected single plants that display recessive phenotype for recombinant screen, positioning GS3 within the range of 7.9 kb. Spanning over a length of 956 bp, GS3 cDNA contains 5 exons, encoding a transmembrane protein made up of 232 amino acids. The protein product consists of the following four structural domains: a structural domain for adjusting the size of organs unique to plants (OSR),

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a transmembrane domain, the cysteine-rich homologous region of tumor necrosis factor recipient/nerve growth factor receptor (TNFR/NGFR) and von willebrand factor type C at C terminal (VWFC module). OSR domain was previously called PEBP domain. Sequence analysis shows that compared with small grain varieties, the coden IGC encoding cysteine at position 55 in the second exon in large grain varieties mutates to stop coden TGA and causes the advance termination of protein translation (deletion of 178 animo acids). Finally, this results in the deletion of PEBP-like domains and other three domains. Apparently, GS3-encoded protein can negatively regulate grain weight [57]. It is found in the latest database software analysis that GS3 does not belong to the PEBP family. By comparison, they found that the predicted GS3 PEBP is only about 1/3 of the actual PEBP, with 20.3%-28.4% similarity. By comparing to a database sequence, it is shown that the N terminal of GS3 has a highly similar and conserved 66 aa structural domain in most angiosperm, e.g. DEP 1 for controlling panicle type. The author temporarily re-names the domain as OSR [58]. GS3 acts as negative regulator for the size of rice grain and organ. In-situ hybridization shows that GS3 is expressed in young panicles and decreased as the panicles grow. It is also slightly expressed in other tissues like embryo, apical meristem, leaves and stalk, but largely expressed in roots and crowns. Real-time PCR has also proved the above results. Wild-type allele contains four presumed structural domains: OSR domain at N terminal, a transmembrane domain, the TNFR/NGFR family cysteine-rich domain and VWFC at C terminal. It is found that the protein encoded by this gene consists of two confrontational parts and the "gaming" of the two parts at the beginning and end of GS3 protein finally determines the size of grains. The rice varieties without GS3 protein (or the protein is non-functional) is long-grain type (about 10 mm long); the rice varieties containing complete GS3 protein belongs to medium-grain type (about 8mm); the rice varieties containing only ORS belongs to short-grain type (about 6 mm) [58]. The research also found that almost all the excellent indica rice varieties contain complete GS3 protein, and therefore are medium-grain type. The GS3 protein is not functional in long-grain-type indica varieties. Gene transfer and substitution can effectively change the grain shape of rice variety, indicating that GS3 plays a decisive role in the yield and quality of rice and also in the mutation and evolution of grain shape. Homologous gene to GS3 is also found in other species, including corn, barley and soybean, while OSR exits in all these homologous genes, indicating that these genes may also control the seed size of corresponding species. Therefore, this finding will have significant prospect of application. First of all, genetic variation can be directly used in breeding varieties for desired grain size and improving the yield of rice. Secondly, based on the research information about rice, the GS3 homologous genes of other species can be cloned so as to guide the breed improvement of corresponding species. Researchers have already been done on how to apply GS3 gene in rice breeding design. Yang et al [59] used the single-segment substitution line developed from indica variety " Huaxian 74" carrying GS3 gene, to perform pyramiding breeding with single-segment substitution line carrying other favorable genes. Twenty-six homozygous pyramided lines containing GS3 and other favorable genes were obtained in F4. The measurement of grain length confirmed that these pyramided lines have desired long grain length, with much improved appearance qualities. This indicates that using single-segment substitution lines and GS3 can help realize rice breeding design aim to modify the grain length.

4.2. GW2

GW2 is a major gene controlling grain width and weight. Song et al [60] used the offsprings of F2 between WY3 and FAZ1 for preliminary QTL positioning. It is found that the allele coming from WY3 has significantly increased the grain width and weight. Advanced backcross population and the screened recessive single plants were used to position GW2 within the range of 8.2 kb. GW2 in FAZ1 contains eight exons. With a total length of 1634 bp, cDNA encodes the protein composed of 425 amino acids of 47kDa. The deletion of a base on exon 4 causes GW2 allele to terminate the translation in advance, and the product is only composed of 115 amino acids. GW2 encodes a ring-type E3 ubiquitin ligase in the cytoplasm and performs negative regulation on cell division by anchoring the substrate to the proteasome for degradation. The absence of GW2 function renders it impossible to transfer ubiquitin to target protein, making the supposedly degradable substrate hard for specific recognition, activating cell division in spikelet and increasing the width of husk. On the other hand, the grain filling rate is also raised, followed by the growth in the size of endosperm. As a result, the width of the husk, the weight of the grain and yield all increased. The histological analysis of AZ1 and NIL (GW2) indicates that larger rice husk of NIL (GW2) is mainly due to the growth in the number rather than the size of cells. The growth in the endosperm of NIL (GW2) is mainly caused by the growth in the size rather than the number of cells. Compared with FAZ1 (recurrent parents), near isogenic line NIL(GW2) can significantly increase the width and tiller number of grains. GW2 (WY3) allele has significantly increased grain width and 1000 seed weight, thus raising single plant yield. This allele can also increase panicles per plant and prolong the growth period while greatly reducing the seeds per panicle and the length of main panicle, indicating pleiotropism of GW2. Through molecular-marker-assisted selection, researchers transferred the GW2 gene in large-grain varieties to small-grain variety FAZ1 to breed new line. By comparison of the yield of NIL (GW2) and small-grain parent FAZ1, it is found that plant yield of NIL (GW2) increased by 19.7% and plot yield increased by 15.9% over small-grain parent, indicating that this gene is valuable in high-yield breeding. In order to prove whether the growth in grain size and yield in NIL (GW2) can affect rice quality, the rice quality was compared between NIL (GW2) and small-grain parent (FAZ1). It turned out that GW2 large-grains allele had an effect on the appearance of rice grains, while the physical and chemical indicators remained unchanged. It is speculated that the edible and cooking qualities are not much affected. Meanwhile, it is also found that both corns and wheat contain GW2 allele, so the discovery of this gene will greatly advance the research on high-yield breeding of crops [60].

4.3. GW5

Another cloned gene for controlling gain width is GW5, which affects the grain width and weight of rice. The allele coming from Asominori significantly increases grain width and weight [61]. GW5 is preliminarily located between SSR marker RM3328 and RMw513 on the short arm of Chromosome 5 at 2.33 cM and 0.37 cM, respectively. By expanding the population and developing CAPS marker, GW5 was narrowed down to OJ1097-A12 and between CAPS markers Cw5 and Cw6. Within this region, compared with wide-type rice with slender grains,

1212 bp nucleotide is deleted in wide-grain variety. GW5 for controlling grain width is exactly in this deleted sequence [61]. GW5 encodes a nuclear-localised protein made up of 144 amino acids, which contains a nuclear localization signal and a histidine-rich domain. The yeast two-hybrid experiment proved that GW5 interacts with polyubiquitin chain, indicating that GW5 may regulate grain width and weight through ubiquitin proteasome. The lack of GW5 function renders it impossible to transfer ubiquitin to target protein, making the supposedly degradable substrate hard for specific recognition and thus activating cell division in spikelet. As a result, the husk width, grain weight and yield all increase [61].

4.4. GS5

The already cloned GS3, GW2 and GW5 grain shape-related genes are all in negative correlation with grain shape. That is, a high gene expression level corresponds to the decrease in seed size. The cloned GS5 is a positive regulator for grain width, seed setting percentage and thousand seed weight. High GS5 expression level can help accelerate cell cycle and facilitate the transverse cell division of spikelet, thus increasing husk width and speeding up the filling of rice grains and the development of endosperm. This will finally lead to larger and heavier seeds and higher single plant yield [62]. A lot of researches show that besides the difference in grain size, in the two genetic materials with identical genetic background, large-grain materials have higher GS5 expression than small-grain ones. The grain width, thousand seed weight and single plant yield also increase by 8.7%, 7.0% and 7.4% respectively. GS5 is located between RM593 and RM574 on the short arm of Chromosome 5, encoding serine carboxypeptidase. The sequencing of recombinant single plant and the transformation test of GS5 show that GS5's influence on grain size comes from promoter variation. The comparative sequencing of GS5 promoter for 51 rice varieties from different parts of Asia shows that GS5 has three different combinations in nature: GS5 large-grain haplotype, GS5 medium-grain haplotype and GS5 small-grain haplotype. They perfectly correspond to the three grain widths of different varieties: wide, medium and narrow shape. Of the above three types, GS5 small-grain haplotype is wild type, while GS5 large-grain haplotype is the mutant with acquired functions in rice domestication and breeding. The mosaic transformation of promoter further shows that the forming of these mutants relies on the natural variation of GS5 promoter. Therefore, GS5 plays an important role in the artificial domestication and breeding of rice and contributes greatly to the diversity of genes controlling the grain size of rice. These results indicate that the mutation of GS5 gene is related to the grain size of rice. The discovery of this mutation can help boost rice yield and may also help increase the yield of other crops [62].

4.5. GW8

Chinese researchers discover a key functional gene GW8 which can simultaneously affect rice quality and yield. By making use of QTL mapping and advanced backcross population, it is located at the distance of about 7.5 Kb on Chromosome 8 between marker RM502 and PSM711. The sequencing shows that a SBP transcription factor OsSPL16 encoded by this gene can

simultaneously control grain size, grain shape and rice quality [63]. In the Pakistan's Basmati rice including the most delicious varieties in the world, the variation of OsSPL16 promoter is observed, which results in decreased expression. Moreover, the gene overexpression can promote cell division, broaden the grain, improve grain filling rate and increase thousand-grain weight. All these will contribute to rice yield increase. The research also finds that GW8 gene is present in high-yield rice which is grown over large areas in China at present. GW8 gene has been discovered to play an important role in the China's rice yield increase. Later in the experimental fields in Beijing, Guangzhou and Hainan, the researchers discover a variant type of GW8 gene in high-yield rice. The key site mutation not only improves rice quality, but increases grain number per spike. If the new variation site of GW8 gene is introduced into Basmati rice, the yield will increase by 14% with high quality; if it is introduced into China's high-yield rice variety, the rice quality will be remarkably enhanced with unchanged yield. In the meantime, GW8 gene has been used in molecular breeding program, and new variety Huabiao No. 1 containing excellent genes such as GW8 was successfully bred in 2009. Huabiao No. 1 has passed variety certification in Guangdong [63]. Therefore, successful cloning of GW8 gene and expounding of molecular mechanism provide new genes with important application value for high-yield and good-quality molecular breeding of hybrid rice. These achievements help reveal molecular mechanism of product synergy for rice quality.

4.6. Utilization of grains shape genes

Those cloned genes controlling rice grain shape related to yield trait are not only favorable to reveal the complex genetic mechanism of rice yield-related trait, but also provide theoretical and technical basis for molecular marker-assisted selection in rice. Through researches on primary core collection of 170 rice varieties and 10 oversea rice varieties, Fan et al [64] found that C-A single base mutation (SNP) on the second exon of GS3 is highly associated with grain length. On this basis, they developed a functional marker SF28, which can be applied to molecular marker-assisted selection of GS3 gene to improve rice appearance and yield. Song et al [60] also make in-depth researches on yield and quality in rice breeding, and discovered that NIL (*GW2*) increased single plant grain yield by 19.7% compared to FAZ1. However, the grain number per spike decreased by 29.9%, and *GW2* alleles from WY3 had no influence on leaf morphology, grain filling and edible quality of FAZ1.

In the breeding practice, cloning of the important genes controlling rice grain shape gives some revelation to its molecular mechanism, but its application in breeding is still difficult. For example, some gene resources may originate from natural selection before Indica-Japonica differentiation or artificial selection after Indica-Japonica differentiation. The fulfillment of some gene functions requires specific genetic factors under different genetic backgrounds. Quality declining due to enlarging grain size and increasing grain weight is another problem that needs to be solved. Therefore, in addition to making use of single gene, the genetic improvement of important agronomic characters of rice is also necessary. Mining key genes with pleiotropism or gene pyramiding is an effective and quick means to breed super-high-yield rice varieties.

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5. Genes with pleiotropic effect to yield related traits

High and stable yield has always been considered as one of the most important objectives in crop research. The genes related to rice yield are the key object of the research on rice breeding and molecular biology. Rice yield per unit area depends on grain number per spike, effective panicle number per plant, thousand-grain weight and seed setting percentage. Meanwhile, plant height and growth period exert huge influences on rice plant morphology and adaptability. The research also finds that many pleiotropic genes are present in rice and involved in regulating multiple growth and development processes, as well as rice vegetative growth and reproduction. Pleiotropic genes are crucial in regulating rice morphogenesis and flower organ development, directly associated with rice yield. Yield and heading date are the basic properties to evaluate practical value of rice. The former reflects income, while the latter decides rice adaption area and season. At present, some pleiotropic genes affecting yield, composition factors and heading date have been cloned.

5.1. Ghd7

Ghd7 is the first reported pleiotropic gene which exerts major influence on rice heading date and yield-related trait [65]. It also can control grain number per spike, plant height and heading date simultaneously. Among the $F_{2:3}$ and recombinant inbred line population constructed by Zhenshan 97 and Minghui 63, *Ghd7* is located between marker R1440 and C1023 on Chromosome 7, and further accurately located on 79kb between RM5436 and RM2256. Through backcrossing, the fragment containing *Ghd7* in Minghui 63 is introduced into Zhenshan 97, and near isogenic line is obtained based on Zhenshan 97. Compared to the recurrent parent Zhenshan 97, NIL heading date is late by 21.2 days, and plant height higher by 33cm, main stem spikelets increased from 130 to 216, and yield per plant also increased by 50%. Through classical map-based cloning, three NIL-F2 macro-populations are adopted to locate Ghd7 to the range of 0.28 cM. In comparison with Zhenshan 97, Minghui 63 allele at the genetic locus delays heading, and enhances plant height, grain number per panicle and yield. In fact, the pleiotropism of Ghd7 region has been generally revealed in the preliminary mapping. Among Zhenshan 97/Minghui 63RIL populations, Minghui 63 allele delays heading, and enhances plant height, grain number per panicle or thousand-grain weight [66]; while in F2:3 population, Minghui 63 allele delays heading, and enhances plant height, grain number per panicle, thousand-grain weight and rice yield [67]. Through map-based cloning, this Minghui 63 *Ghd7* cDNA has a total length of 1013bp, and encodes a nucleoprotein composed of 257 amino acids; the product is CCT (CO, CO-LIKE and TIMING OF CAB1) structural protein, which is similar to CCT domain of Arabidopsis thaliana CO protein, but is obviously different from the latter. Ghd7 protein has no obvious zinc finger, and no homology relation with the Arabidopsis thaliana CO protein. Ghd7 expression is mainly in tender leaves, apical meristem, secondary branch differentiation primordium and phloem of vascular bundle in mature leaves. In view of microscopic structures of plants with Ghd7 expression, cell number obviously increases, so it is supposed that Ghd7 accelerates cell division. More secondary branches are differentiated in the development of immature spike, which become an anatomical base of improving grain number per spike. At the same time, thickening of stems also is in favor of keeping good plant

shape to facilitate stable yield. Ghd7 expression is controlled by photoperiod, and mRNA expression is characterized by day and night rhythm. The expression is inhibited in short days; while in long days, Ghd7 inhibits Hd3a and Ehd1 expression, and this mechanism of flowering control may be unique to rice. As this gene significantly prolong the plant growth period, the panicles have a longer time for development which results in higher grain number and spikes become larger. Moreover, this gene also affects flowering time, plant height and other traits, showing obvious pleiotropic feature. Under the long-day conditions, *Ghd7* over-expression can delay heading, increase plant height and grain number per panicle. The natural mutants with weakened functions can be grown in temperate or even colder regions. Therefore, *Ghd7* plays an important role in yield potential and adaptability on global scale of rice [65]. The subsequent researches discover that single phyA or combination of phyB and phyC can induce accumulation of Ghd7 mRNA, and phyB can independently reduce Ghd7m RNA to a certain extent. Furthermore, phyB and phyA can separately affect the activity of Ghd7 and Hd1 [68]. However, Shibaya et al. [69] indicated genetic interaction between *Hd2* and H*d16* or *Hd2* and *Ghd7*. *Hd2* and the related genetic interaction play an important role in controlling heading date under the long-day conditions.

Researches prove that most of high-yield rice varieties contain *Ghd7* gene. Wild-type *Ghd7* gene can delay heading, and improve plant height and grain number per panicle. Comparative sequencing of *Ghd7* alleles among 19 rice cultivers over Asia found that five genotypes had the proteins encoded by various *Ghd7* alleles. Minghui 63 is the representative of the first genotype, and alleles for this genotype have stronger functions. The varieties with this genotype are mainly distributed in rice production areas in the south of China as well as tropical and sub-tropical region, with longer growth time. Cultivar Nipponbare represents the second genotype, and alleles for this genotype have weakened functions. The varieties with this genotype are mainly distributed in North China and the same latitude regions. Hejiang 19 and Mudanjiang 8 are the representatives of the third genotype, and the alleles totally lose the functions due to terminator mutation. The varieties with this genotype are mainly distributed in Heilongjiang province in the north of China, and rice growth period is adaptable to shorter-summer condition. The fourth genotype is only discovered in Teqing varieties, and this genotype also has stronger functions. The distributed geographical regions are similar to the first one. The last genotype is deficiency of *Ghd7*, and the varieties with this genotype are mainly distributed in China's double cropping rice areas. Based on the above-mentioned researches, it is known that different genotypes of *Ghd7* are associated with rice variety distribution. These genes are not only involved in development regulation, but also are related to plant geographical distribution. The successful cloning of rice *Ghd7* gene greatly deepens the understanding of genetic and molecular basis of complex quantitative traits. This also constitutes a good illustration of pleiotropism rarely discovered in traditional genetics. The isolation of *Ghd7* gene demonstrates that complex quantitative traits such as yield can be improved through biotechnology like qualitative traits. Relevant information of this gene can be directly used in the mining of genes that are significant to improving yield and ecological adaptability to realize genetic improvement. *Ghd7* gene, which has been isolated with confirmed function in yield increase, can greatly shorten the screening time of high-yielding rice variety. This is a key step taken towards improving crop yield in the global range.

5.2. DTH8 (Ghd8)

The effect of *DTH8 (Ghd8)* is similar to that of *Ghd7* in delaying rice heading and improving yield-related traits. Other functions include enhancing plant height, grain number per panicle, total grain number per panicle and yield [70,71]. The pleiotropism is also observed in preliminary mapping. In the Zhenshan 97/HR5 RIL population, HR5 allele delays heading, and improves plant height and total grain number per spike [72]. Through construction of near isogenic lines containing target genes, *Ghd8* is isolated and cloned by virtue of preliminary mapping, comparative sequencing and genetic transformation. It encodes a HAP3 subunit containing CCAAT-box-binding transcription factor [70, 71]. HAP complex consists of three subunits, HAP2/NF-YA/CBF-B, HAP3/NF-YB/CBF-A and HAP5/NF-YC/CBF-C. Moreover, HAP complex can bind to CCAAT sequence in the promoter, and regulate expression of target genes. OsHAP3H is a HAP3 subunit of HAP complex [73]. *DTH8*/*Ghd8*/*LHD1* is proved to be the HAP3H subunit encoding "CCAAT box binding protein" of the transcription factor. It can simultaneously regulate rice yield, plant height and heading date [70, 71, 74]. It is reported that *DTH8* can be expressed in the multiple tissues, down-regulate the transcription of *Ehd1* and *Hd3a* under long-day conditions, and is independent of *Ghd7* and *Hd1.* Under long-day condition, the introduction of *DTH8* allele in Asominori can obviously prolong heading date, and increase plant height and grain number per panicle of *CSSL61 [72]. Ghd8* can delay rice flowering by regulating Ehd1, RFT1 and Hd3a under long-day condition, but promote rice flowering in short-day condition. Also Ghd 8 can up-regulate the expression of MOC1 gene controlling rice tillering and lateral branches to increase tillering number, primary and secondary branch numbers [71]. However, some variations in LHD1 (Ghd8) coding area are related to late panicles. LHD1 can down-regulate the expression of some flowering transcription activators such as Ehd1, Hd3a and RFT1 in long-day condition, but not inhibit these genes in short-day condition. This indicates that LHD1 can delay flowering through inhibiting their expression in long-day condition [74]. By main variation sites and character association analysis of Ghd8 and also cluster analysis of monoploidy of different protein sequences, near-isogenic lines of different allelotypes are constructed to obtain four favorable Ghd8 allelotypes. Among them, Ghd8-9311 and Ghd8-ruf allelotypes can increase yield but not delay flowering, so these alleles are suitable for varieties grown in areas with good sunlight and temperature. Ghd8-MH63 and Ghd8-Nip allelotypes are photostable, so they are applicable to increase yield of varieties in short-day areas.

5.3. APO1

APO1 is also a pleiotropic gene, and can simultaneously affect vegetative growth and reproductive development. During vegetative growth stage, apo1 mutant can promote blade growth and blade number more than wild type. During reproductive growth stage, apo1 mutant can lead to smaller panicle, and smaller primary branch and spikelet numbers than wild type. apo1 mutant transforms flower stamens into lodicules, causing carpel to abnormally stretch and carpellody in glumes. Thus, lodicule number increases and stamen reduces. APO1 encodes an F-box protein, which is mainly expressed in apical meristem and lateral organ primordium. APO1 plays an important role in regulation of meristem destiny, and positively regulates the

primary branch and spikelet numbers. Spikelet meristems of apo1 mutant form early and the formation period of lodicules and carpel is also prolonged. This inferred infer that APO1 participates in time regulation of meristem attributes. APO1 positively regulates the expression of C-class gene related to homoetic transformation, and affects flower organ attributes [75]. In 2010, Japanese scholars made use of chromosomal segment substitution lines (CSSL) to identify a QTL effectively controlling stalk thickness, which is named as SCM2. SCM2 is equivalent to previously reported APO1 gene controlling panicle structure through map-based cloning. The near isogenic lines containing SCM2 have phenotypes of reinforced stalk strength and increased spike number, showing that this gene has pleiotropic effects. Although SCM2 is the functionally acquired mutant of APO1, it has no negative effects associated with over-expression of APO1 mutant as have been previously, including decreasing spike number and abnormal spikelets. SCM2 can prevent yield reduction from lodging due to application of chemical fertilizer in high-plant varieties [76].

6. Perspective

Discovery of semi-dwarf gene sd1 from DGWG and Aizizhan triggers a revolution in global rice production. Cytoplasmic male sterile gene found in wild rice enables the rice heterosis to be fully shown and realized through three line system, followed by another leap in rice yield. The application of excellent germplasm resources and their genes plays a key role in enhancing rice yield. With the development of new technology, germplasm will find more applications. In face of new requirements for current world's production development, the following respects should be considered in the researches on rice yield increase: (1) select and breed high yield, high quality new rice variety with endurable storage and stress tolerance; (2) select and breed new rice variety for special purposes; (3) trans-breed a series of cytoplasmic male sterile lines and restorer lines with stable, high quality and combining fertility; (4) clone and isolate genes controlling important agronomic characters. In general, core collection, backbone parents, excellent medium material and excellent variety are mainly considered as the basis to systematically create and construct saturated mutant library and genotype-phenotype database. Gene cloning, association analysis and gene regulatory network analysis should be adopted to study the molecular mechanism of good character formation, and explore allelism difference and genetic effect of relevant characters such as high yield, high quality and stress resistance of super rice. High-throughput genotyping, favorable gene pyramiding and improvement, conventional and molecular breeding are proper techniques to breed the new lines and variety of super rice with advantages in yield, quality and stress resistance. To breed the above-mentioned varieties, the key is to further develop and utilize new gene resources based on the existing varieties, including gene resources contained in indica and japonica subspecies, *Oryza* species and mutant species. At the same time, we need to improve breeding method and identification technology, including combined adoption of transgene technology, composite hybridization and rice molecular marker-assisted selection. In particular, the conventional breeding method should be combined with biotechnology, which will be the important way to breed super rice of super-high yield.

Author details

Dawei Xue[1], Qian Qian[2] and Sheng Teng[3*]

*Address all correspondence to: steng@sibs.ac.cn

1 College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang Province, P.R.China

2 State Key Laboratory of Rice Biology, China National Rice Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, P.R.China

3 Shanghai Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, The Chinese Academy of Sciences, Shanghai, P.R.China

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