Prospects of utilization of inter-subspecific heterosis between \textit{indica} and \textit{japonica} rice

ZHANG Gui-quan

Abstract
The Asian cultivated rice (\textit{Oryza sativa} L.) grown worldwide is divided into two subspecies, \textit{indica} and \textit{japonica}. It is well known that the heterosis of inter-subspecies is usually stronger than that of intra-subspecies. Since the 1970s, \textit{indica} hybrid rice, an intra-subspecific hybrid rice, has being widely used in China and even in the world. However, the inter-subspecific hybrid rice between \textit{indica} and \textit{japonica} is still unavailable. The major obstacle is the hybrid sterility of the inter-subspecies. In recent decades, the genetic and molecular basis of \textit{indica-japonica} hybrid sterility was understood more and more clearly. Some breeding approaches for overcoming inter-subspecific hybrid sterility were proposed and used to develop the \textit{indica-japonica} hybrid rice. The updated understanding will offer new approaches for development of breeding lines for overcoming \textit{indica-japonica} hybrid sterility, which facilitates developing of inter-subspecific hybrid rice.

Keywords: heterosis, reproductive isolation, hybrid sterility, genetic basis, molecular mechanism, subspecies, hybrid rice

1. Introduction
The Asian cultivated rice (\textit{Oryza sativa} L.) is an important food crop in the world. The rice is generally divided into two major types or subspecies, \textit{indica} and \textit{japonica} (Garris et al. 2005). In China, “Hsien” or “Xian” rice and “Keng” or “Geng” rice were recorded as early as 2000 years ago in the Han Dynasty literature (Ting 1957). Recently, the two groups of rice were referred to as Xian/\textit{indica} (XI) and Geng/\textit{japonica} (GJ) (Wang et al. 2018). The two subspecies of \textit{indica} and \textit{japonica} are two different ecological types of rice. The rice of \textit{indica} is mainly distributed in the low latitude areas of tropics and subtropics. The rice of \textit{japonica} is mainly distributed in temperate areas.

Rice is a self-pollinating plant. It is generally believed that the heterosis (also known as hybrid vigor) of self-pollinating plant is relatively weak. In the 1970s, \textit{indica} hybrid rice was first used successfully in China. After the 1980s, \textit{indica} hybrid rice has become the main cultivated rice in China, accounting for more than 50% of the rice planting area of China. Practice has proved that \textit{indica} hybrid rice has strong heterosis and plays an important role in improving rice yield per unit area (Yuan and Virmani 1988). At the same time, \textit{japonica} hybrid rice has been continuously studied in China, and a large number of \textit{japonica} hybrid rice varieties have been bred. However, the application of \textit{japonica} hybrid rice is limited because of its weak heterosis. It is generally believed that inter-subspecific crosses have stronger
heterosis than intra-subspecific crosses (Fu et al. 2014; Birchler 2015). However, there are serious hybrid sterility between indica and japonica, which hinders the utilization of inter-subspecific heterosis. Therefore, overcoming the hybrid sterility between indica and japonica becomes a prerequisite for the utilization of inter-subspecific heterosis between indica and japonica rice.

This review summarizes the genetic and molecular basis of indica-japonica hybrid sterility discovered in recent decades. It proposes the breeding approaches for overcoming inter-subspecific hybrid sterility, and presents the perspective of utilization of inter-subspecific heterosis between indica and japonica rice.

2. Reproductive isolation of rice

There are 24 species in the genus Oryza, including nine different genome types. Among them, there are eight species of AA genome, including six wild species, O. nivara, O. rufipogon, O. barthii, O. meridionalis, O. glumaepatula, and O. longistaminata, and two cultivated species, O. sativa and O. glaberrima (Khush 1997; Lu et al. 1999).

Reproductive isolation maintains the integrity of a species over time, reducing or directly impeding gene flow between individuals of different species (Baack et al. 2015). Therefore, reproductive isolation is a common phenomenon in interspecific hybrids and inter-subspecies hybrids of the genus Oryza. Reproductive isolation includes many different ways, which can be divided into two stages: prezygotic reproductive isolation and postzygotic reproductive isolation. Prezygotic reproductive isolation includes pollination patterns, pollen recognition, and environmental adaptation. Postzygotic reproductive isolation includes hybrid lethality, hybrid necrosis/weakness, and hybrid sterility (Ouyang and Zhang 2015). Therefore, reproductive isolation is a common phenomenon between individuals of different species (Baack et al. 2015). Among them, there are eight species in the genus Oryza. Reproductive isolation includes many different ways, which can be divided into two stages: prezygotic reproductive isolation and postzygotic reproductive isolation. Prezygotic reproductive isolation includes pollination patterns, pollen recognition, and environmental adaptation. Postzygotic reproductive isolation includes hybrid lethality, hybrid necrosis/weakness, and hybrid sterility (Ouyang and Zhang 2015).

In interspecific hybrids, more than 20 loci for reproductive isolation were found. Three of single loci, S1 in O. sativa/O. glaberrima (Xie et al. 2017b; Koide et al. 2018), qHMS7 in O. sativa/O. meridionalis (Yu et al. 2018), and S23 in O. sativa/O. glumaepatula (Fang et al. 2019), and three of duplicated gene pairs, S27/S28 in O. sativa/O. glumaepatula (Yamagata et al. 2010), Hwi1/Hwi2 in O. sativa/O. rufipogon (Chen et al. 2014) and DGS1/DGS2 in O. sativa/O. nivara (Nguyen et al. 2017), were cloned. The genetic model and molecular mechanism of reproductive isolation of interspecific hybrids were revealed.

3. Genetic and molecular basis of indica-japonica hybrid sterility

Hybrid sterility is a common phenomenon in indica-japonica crosses, and it is one of the main evidences for classification of indica-japonica subspecies. Because of the abundant genetic resources in the subspecies, the hybrid sterility of different crosses is also quite different (Zhang and Lu 1987). After decades of research, the genetic basis of inter-subspecific hybrid sterility between indica and japonica is more and more clear.

3.1. The loci for indica-japonica hybrid sterility

Although about 50 loci for reproductive isolation have been reported in Oryza (Ouyang and Zhang 2013, 2018; Guo et al. 2016), the sterility of hybrids between indica and japonica subspecies is usually controlled by only six loci (Table 1). Ikehashi and Araki (1986) discovered the S5 locus controlling female sterility in inter-subspecific hybrids between indica and japonica by studying spikelet fertility in a large number of crosses. Their research showed that indica varieties generally carry S5-i, while japonica varieties

Table 1 The loci for hybrid sterility occurred generally in indica-japonica crosses

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sterility</th>
<th>Chromosome</th>
<th>Identification</th>
<th>Molecular mapping</th>
<th>Cloning</th>
<th>Genes in the same regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc</td>
<td>Male</td>
<td>3</td>
<td>Zhang and Lu (2001); Yang et al. (2004)</td>
<td>Shen et al. (2017)</td>
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</table>
generally carry S5-j. In the hybrids of indica/japonica, the female gametes carried S5-j allele are abortive. The S5 locus was located on chromosome 6 by morphological and molecular markers (Ikehashi and Araki 1986; Yanagihara et al. 1995). The S5 gene was then cloned by map-based cloning (Chen et al. 2008; Yang et al. 2012). A large number of studies have shown that female sterility of indica-japonica hybrids is mainly controlled by the S5 locus (Ikehashi and Araki 1986; Song et al. 2005).

For pollen sterility of indica and japonica hybrids, five loci, Sa, Sb, Sc, Sd, and Se, were discovered by studying the pollen sterility of a large number of crosses between indica and japonica (Zhang and Lu 1989, 1993, 1996; Zhang et al. 1993, 1994). Among the five loci, indica rice mainly carries S-i allele, while japonica rice mainly carries S-j allele. In indica-japonica hybrids, the interaction of S-i and S-j alleles resulted in abortion of pollens carrying S-j. The abortive pollens caused by the Sa locus was mainly empty-sterile type, while the abortive pollens caused by the Sb, Sc, Sd, and Se loci was mainly stained-sterile type. These studies indicated that the pollen sterility widely occurring in indica-japonica inter-subspecific crosses was mainly controlled by these five loci. In indica-japonica hybrids, the level of pollen sterility mainly depends on the number of heterozygous loci at the five loci. After that, the five loci were located by molecular markers. Sa was located on chromosome 1 (Zhuang et al. 1999), Sb on chromosome 5 (Zhuang et al. 2002; Li et al. 2006), Sc on chromosome 3 (Zhang and Zhang 2001; Yang et al. 2004), Sd on chromosome 1 (Li et al. 2008), and Se on chromosome 12 (Zhu et al. 2008). Among them, the Sa and Sc genes have been cloned (Long et al. 2008; Shen et al. 2017). In the chromosomal intervals of Sb, Sd, and Se loci, some genes for hybrid sterility were also reported by other researchers. S24 (Zhao et al. 2011) with Sb, S35 (Kubo et al. 2008) with Sd, and S25 (Kubo and Yoshimura 2001) and S36 (Wen et al. 2007) with Se were located in the same regions, which may be the same loci. These studies indicated that the pollen sterility of indica-japonica hybrids was mainly controlled by the five loci.

### 3.2. The allelic variation of the loci for indica-japonica hybrid sterility

Ikehashi and Araki (1986) showed that at the S5 locus, some tropical japonica cultivars carried another S5-n allele besides indica rice carrying S5-i and japonica rice carrying S5-j. Because the interaction of S5-n with both S5-i and S5-j alleles can't produce sterility, S5-n was called wide-compatibility gene (WCG), and the varieties carrying the S5-n gene were called "wide-compatibility varieties (WCVs)". Actually, neutral (n) allele is common in plant hybrid sterility. When two alleles at a locus interact to produce sterility, there may be a third allele, the neutral allele, at that locus. The interaction between the neutral allele and other two alleles didn't produce sterility (Rich 1966). Therefore, S5-n is only a neutral allele of S5 locus. It is ambiguous to call it WCG, especially the varieties carrying S5-n gene to be called WCVs. This is because WCVs refer to the varieties that are compatible with both indica and japonica rice, while S5-n is compatible only at the S5 locus. Studies on hybrid pollen sterility loci of Sa, Sb, Sc, Sd, and Se showed that not only S-i, S-j and S-n alleles were divided, but also effects of the S-i, S-j and S-n alleles had quantitative difference, which resulted in continuous variation of pollen sterility at a single locus (Zhang et al. 1993).

Three of the six genes of hybrid sterility between indica and japonica have been cloned. The molecular basis of allele variation in these three loci is summarized in Table 2. The S5 locus consists of three open reading frames (ORFs). ORF3 encodes a heat shock protein Hsp70, which acts as a protector; ORF4 encodes a membrane protein and acts as a partner; ORF5 encodes aspartate protease, which acts as a killer. It was known that there are two haplotypes or genotypes of S5-j allele: ORF3+ORF4+ORF5+ and ORF3+ORF4–ORF5+. S5-j allele is composed of ORF3–ORF4+ORF5–, and S5-n allele is ORF3+ORF4+ORF5n. Here, ORF5+ differs from ORF5– in only two SNPs on exon2, while ORF5n loses its function due to a major deficiency at the N-terminal. In the S5-i/S5-j heterozygote, ORF5+ binds to ORF4+ and selectively eliminates the

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Haplotypes</th>
<th>Genic model</th>
<th>Molecular mechanism</th>
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<tbody>
<tr>
<td>S5</td>
<td>S5-i</td>
<td>ORF3+/ORF4+/ORF5+, ORF3+/ORF4–/ORF5+</td>
<td>One-locus</td>
<td>A killer-protector system encoded by three tightly linked genes</td>
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<tr>
<td></td>
<td>S5-j</td>
<td>ORF3–/ORF4+ORF5–</td>
<td>One-locus</td>
<td>A two-gene/three component interaction model</td>
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<tr>
<td></td>
<td>S5-n</td>
<td>ORF3+ORF4+ORF5n</td>
<td>One-locus</td>
<td>A model of gene dosage-dependent hybrid male sterility</td>
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<tr>
<td>Sa</td>
<td>Sa-i</td>
<td>SaF+/SaM+</td>
<td>One-locus</td>
<td>A two-gene/three component interaction model</td>
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<td></td>
<td>Sa-j</td>
<td>SaF–/SaM–</td>
<td>One-locus</td>
<td>A model of gene dosage-dependent hybrid male sterility</td>
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<td></td>
<td>Sa-n</td>
<td>SaF–/SaM+, SaF+/SaM–</td>
<td>One-locus</td>
<td>A model of gene dosage-dependent hybrid male sterility</td>
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<tr>
<td>Sc</td>
<td>Sc-i</td>
<td>(Sci)1, (Sci)2</td>
<td>One-locus</td>
<td>A model of gene dosage-dependent hybrid male sterility</td>
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<td></td>
<td>Sc-j</td>
<td>(Scj)1</td>
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female gametophytes of ORF3– (Chen et al. 2008; Yang et al. 2012). The Sa locus is composed of two adjacent genes, SaF and SaM. SaF encodes an F-box protein, while SaM encodes a small ubiquitin-like modified E3 ligase-like protein. The Sa-i allele is composed of SaF+/SaM+, while the Sa-j allele is SaF−/SaM−. Here, there is only one SNP difference between SaF+ and SaF−, and between SaM+ and SaM−. In the Sa-il Sa-j heterozygote, SaF+ selectively killed the male gametes of SaM− with the participation of SaM+.

Some indica varieties carry SaF−/SaM+. This genotype has affinity for both Sa-i and Sa-j, so it is considered as Sa-n allele. By inducing SaF or SaM mutation with RNAi and RISPR/Cas9 techniques, the genotypes of SaF−/SaM+ and SaF+/SaM− were produced and proved to have the characteristics of Sa-n (Long et al. 2008; Xie et al. 2017a). Sc gene encodes the domain protein of DUF1618, which is essential for pollen development. Unlike S5 and Sa, Sc-j carries only one copy of the gene (Sc-j), while Sc-i carries two (Sc-b1/Sc-b2) or three (Sc-iα/Sc-b1/Sc-b2) copies in tandem. In Sc-il Sc-j heterozygote, Sc-i allele is highly expressed in sporophyte, which inhibits the expression of Sc-j allele in pollen and results in abortion of male gametes carrying Sc-j. By using CRISPR/Cas9 technology to induce inactivation of 1–2 copies of the three copies of Sc-i in the Sc-il Sc-j heterozygote, the expression of Sc-j gene can be increased and the pollen fertility can be improved. Therefore, the inhibition of Sc-j allele expression is regulated by gene dosage of Sc-i. By reducing the copies of Sc-i, the inhibition of Sc-j can be eliminated. When the Sc-i gene with only one copy, it becomes the Sc-n allele (Shen et al. 2017).

These studies showed that the molecular basis of S5, Sa, and Sc loci varied greatly. The S5 locus consists of three ORFs, the Sa locus consists of two genes, and the Sc locus consists of one to three copies of a gene. However, the differences in haplotype didn’t change the genetic behavior of alleles. According to their genetic behavior, the alleles of these loci are still divided into S-i, S-j, and S-n. In addition, the S-i, S-j, and S-n alleles of these loci may have multiple different haplotypes, and their functions may vary quantitatively. For example, the S5-i allele includes ORF3+/ ORF4+ORF5+ and ORF3+ORF4−ORF5+ haplotypes, the Sa-n allele includes haplotypes of SaF−/SaM+ and SaF+/ SaM−, while the Sc-i allele includes two (Sc-b1/Sc-b2) and three (Sc-iα/Sc-b1/Sc-b2) copies (Table 2). These results support the results of early genetic analysis that there are quantitative variations in the effects of S-i, S-j, and S-n alleles at the loci (Zhang et al. 1993).

3.3. The genic model of indica-japonica hybrid sterility

Oka (1974) summarized four possible genic models in hybrid sterility of cultivated rice: duplicate gametic-lethal, single locus sporoph-sporophytic interaction, single locus sporophytic sterility, and complementary sporophytic sterility. He developed a series of near-isogenic F1 sterile lines with japonica variety Taichung 65 as recurrent parents. The genic model of hybrid sterility between the Taichung 65 near-isogenic F1 sterile lines (TISLs) was believed to be the duplicate gametic-lethal model. Ikehashi and Araki (1986) showed that the genic model of the S5 locus was the one-locus spor-sporophytic interaction model, or one-locus model. At this locus, the interaction of S5-i and S5-j in sporophyte resulted in the abortion of female gametes carrying S5-j. Zhang and Lu (1993) used the TISLs provided by Dr. Hika-Ichi Oka (National Institute of Genetics, Misirnu, Japan) to analyze the genic model of hybrid sterility. The results showed that the pollen sterility of hybrids didn’t conform to the duplicate gametic-lethal model proposed by Oka (1974), but to the one-locus spor-sporophytic interaction model. Subsequently, the results of molecular mapping of the S5 female sterile locus and the Sa, Sb, Sc, Sd, and Se male sterile loci showed that the genic models of these loci conformed to the one-locus spor-sporophytic interaction model. At these loci, the interaction between S-i and S-j in sporophytes results in abortion of female or male gametes carrying S-j, which leads to distorted segregation of molecular marker genotypes (Yanagihara et al. 1995; Zhuang et al. 1999; Yang et al. 2004; Li et al. 2006, 2008; Zhu et al. 2008). The genic models of indica-japonica hybrid sterility are shown in Fig. 1.

By cloning S5, Sa, and Sc genes, the molecular mechanism of these genes has been further understood. It is known that the molecular mechanism is a killer-protector system encoded by three tightly linked genes at the S5 locus, a two-gene/three component interaction model at the Sa locus, and the model of gene dosage-dependent hybrid male sterility at the Sc locus (Long et al. 2008; Yang et al. 2012; Shen et al. 2017). Although the three loci conformed to the one-locus spor-sporophytic interaction model, their molecular mechanisms are quite different, which indicates that the same genic model has different molecular mechanisms (Table 2).

3.4. The loci for hybrid sterility in specific cross-combinations

Rice is rich in genetic resources. The sterility of intersubspecific hybrid between indica and japonica is a complex phenomenon. In the loci controlling hybrid sterility of indica-japonica subspecies, some loci may be common, such as S5, Sa, Sb, Sc, Sd, and Se, while other loci may only appear in specific combinations, such as S7, hsa1, and DPL1/DPL2.

The S7 locus was first identified causing hybrid sterility
but also wild rice, and Ingra is closely related to rice. Phylogenetic tree analysis showed that ORF3 exists in not only Asian rice (O. sativa L.) but also wild rice, and Ingra closely related to O. barthii and O. meridionalis and distanced from O. sativa (Yu et al. 2016). It is noted that the Aus variety Ingra showed red pericarp. Compared with other varieties, Ingra showed more specific amino acid sites in ORF3, the protein of candidate gene for sterility. During the formation of female gametophytes in heterozygotes S7\textsuperscript{i}/S7\textsuperscript{p} (in Ingra/Cpslo17 cross) and S7\textsuperscript{i}/S7\textsuperscript{p} (in Ingra/IR36 cross), gametes carrying S7\textsuperscript{p} and controls Aus-type sterility could not resist the effect of S7\textsuperscript{i} from sporophyte, thus leading to embryo sac abortion (Yu et al. 2016). It is noted that the Aus variety Ingra showed red pericarp. Compared with other varieties, Ingra showed more specific amino acid sites in ORF3, the protein of candidate gene for sterility. Phylogenetic tree analysis showed that ORF3 exists in not only Asian rice (O. sativa L.) but also wild rice, and Ingra is closely related to O. barthii and O. meridionalis and distanced from O. sativa (Yu et al. 2016). So far, allele interactions of S7 causing female sterility have been only found in Ingra hybrids.

Kubo and Yoshimura (2005) showed that F\textsubscript{1} female sterility is controlled by three complementary loci, hybrid sterility-a\textsubscript{1} (hsa1), hsa2, and hsa3 in an indica-japonica rice cross. The indica variety IR24 has a recessive sterility factor termed hsa1\textsuperscript{a} on the long arm of chromosome 12 and that hsa1\textsuperscript{a} causes female sterility by interacting with two complementary factors, hsa2 and hsa3, located on chromosomes 8 and 9, respectively. Thereafter, positional cloning of the hsa1 region showed that the hsa1 locus was composed of two interacting genes, HSA1a and HSA1b. The HSA1a encodes a monoco-specific DUF1618 protein. The sterile allele of HSA1b (HSA1b−) was an ancestral allele, whereas the fertile japonica allele (HSA1b+) was a derived one. There are three subgroups of haplotypes at the hsa1 locus: subgroup I, the "japonica type"; with HSA1a−/HSA1b−; subgroup II, the "ancestral type", with HSA1a−/HSA1b+; and subgroup III, the "indica type", with HSA1a+/HSA1b−. The HSA1a−/HSA1a− heterozygosity caused semi-sterility due to selective abortion of the HSA1a− female gamete in HSA1b− homozygous plants. Therefore, the HSA1a−/HSA1b− haplotype causes sterility in F\textsubscript{1} and F\textsubscript{2} hybrids. The cross-combination of indica/ancestral haplotypes caused F\textsubscript{1} sterility, whereas the cross-combination of indica/japonica haplotypes caused F\textsubscript{2} sterility (Kubo et al. 2016). Obviously, F\textsubscript{1} sterility causing by hsa1 only occurs in the cross-combinations of indica/ancestral haplotypes, but not in the cross-combinations of indica/japonica haplotypes.

Mizuta et al. (2010) identified a pair of genes, DOPPELGANGER1 (DPL1) on chromosome 1 and DOPPELGANGER2 (DPL2) on chromosome 6 in F\textsubscript{2} population from a cross between japonica cultivar Nipponbare and indica Aus cultivar Kasalath. Alleles on Nipponbare chromosome 1 (DPL1-N\textsuperscript{1}) and Kasalath chromosome 6 (DPL2-K\textsuperscript{1}) had the same coding sequence structure. DPL1-N\textsuperscript{1} and DPL2-K\textsuperscript{1} encode highly similar, small, unknown proteins of 94 and 95 amino acids, respectively. In contrast, alleles on Kasalath chromosome 1 (DPL1-K\textsuperscript{1}) and Nipponbare chromosome 6 (DPL2-N\textsuperscript{1}) had structural differences from the above two alleles. In near isogenic lines (NILs) having heterozygous DPL1 (DPL1-K\textsuperscript{1}/DPL1-N\textsuperscript{1}) in Nipponbare background (DPL2-N\textsuperscript{1}/DPL2-K\textsuperscript{1}), pollen having DPL1-K\textsuperscript{1}/DPL2-N\textsuperscript{1} failed to germinate, but could be rescued by transformation with functional DPLs. In O. sativa cultivars, the genotypes of DPL1/DPL2 could be classified into three groups: group I, the japonica type of DPL1-N\textsuperscript{1}/DPL2-N\textsuperscript{1}; group II, the indica type of DPL1-N\textsuperscript{1}/DPL2-K\textsuperscript{1}; and group III, the indica Aus type of DPL1-K\textsuperscript{1}/DPL2-K\textsuperscript{1}. DPL1-K\textsuperscript{1}-type disruption occurred in a common ancestor of Aus and O. rufipogon in group III. The results indicated that the hybrid pollen sterility controlled by DPL1/DPL2 only occurs in the hybrids between Nipponbare and its NILs having the DPL1-K\textsuperscript{1} gene from Kasalath, but not in any cross-combinations among the three groups of DPL1/DPL2.

The molecular basis of the S7, hsa1 and DPL1/DPL2 loci is summarized in Table 3. The three loci control hybrid sterility...
sterility through different molecular mechanisms. The hybrid sterility controlled by these loci occurred in the specific cross combinations, which may not exist in most of the indica-japonica hybrids. For the female sterility or embryo sac abortion of indica-japonica hybrid, in addition to S5, S7, and hsa1 mentioned above, other loci such as S8 (Wan et al. 1993; Singh et al. 2006), S9 (Wan et al. 1996; Zhao et al. 2006), and S31 (Zhao et al. 2006, 2007) were also identified. However, it was found that the female sterility of indica-japonica hybrid was mainly controlled by S5 locus (Ikehashi and Araki 1986; Song et al. 2005). This suggests that other loci may only occur in specific cross combinations.

4. Overcoming indica-japonica hybrid sterility

Through a large number of studies, the genetic basis of inter-subspecific hybrid sterility between indica and japonica has been clearly understood. These understandings provide a theoretical basis for overcoming inter-subspecific hybrid sterility between indica and japonica. Because of the interaction between S-i and S-j in these hybrid sterile loci resulting in the gametes carrying S-j aborted, it is possible that the S-i genes of indica rice at these loci could be transferred to japonica rice by backcrossing. The new japonica lines with S-i/S-i genotypes and japonica genetic background lines are called “indica-compatible japonica lines” (ICJLs) (Zhang et al. 1994; Zhang and Lu 1999).

To test the assumption, a set of chromosomal single-segment substitution lines (SSSLs) carrying S-i genes at Sa, Sb, Sc, Sd and Se loci were developed using a japonica variety Taichung 65 as a recipient, and a set of indica varieties as S-i donors. Then, the S-i genes of Sa, Sb, Sc, Sd, and Se loci were pyramided together by crossing these SSLs, and a series of TISLs with different genotypes at the loci were developed. The hybrid pollen sterility of TISLs with different genotypes were tested with indica and japonica rice. The results showed that with the increase of S-i genes carried by TISLs, their hybrid sterility with japonica rice increased, while those with indica rice decreased (Zhang and Lu 1996; Guo et al. 2016). Furthermore, by crossing japonica rice carrying S5-n gene with TISLs carrying S-i gene at Sb, Sc, Sd, and Se loci, several ICJLs carrying Sb-i, Sc-i, Sd-i, Se-i, and S5-n genes with different japonica backgrounds were developed. The ICJLs showed serious pollen sterility and spikelet sterility when crossed with japonica rice, while the pollen fertility and spikelet fertility were normal or near normal when crossed with indica rice. Therefore, in the crosses of ICJLs with indica varieties, the hybrid sterility could be overcome effectively, and the pollen fertility and spikelet fertility in the indica-japonica hybrids were normal (Guo et al. 2016). The similar characteristic of ICJLs was also found in marker-assisted breeding of a photoperiod-sensitive male sterile japonica rice with high cross-compatibility with indica rice (Chen et al. 2011).

Based on the genetic basis of hybrid sterility between indica and japonica rice, there are two breeding approaches for overcoming inter-subspecific hybrid sterility. (1) To develop ICJLs by transferring S-i genes of S5, S6, S7, Sc, Sd, and Se loci from indica to japonica rice, and using the ICJLs crossing with indica varieties to develop indica-japonica hybrid rice. (2) To develop indica wide compatibility lines (IWCLs) by pyramiding the S-n genes of S5, S6, S7, Sc, Sd, and Se loci in the indica genetic background, and crossing of IWCLs with japonica varieties to develop indica-japonica hybrid rice; or to develop japonica wide compatibility lines (JWCLs) by pyramiding the S-n genes of S5, Sa, Sb, Sc, Sd, and Se loci in the japonica genetic background, and crossing of JWCLs with indica varieties to develop indica-japonica hybrid rice (Fig. 2).

5. Perspective

In the past century, rice has experienced the evolution of four generations of varieties. The first generation (1G) was the high-stalk rice commonly used before the 1960s. The second generation (2G) is the semi-dwarf rice commonly used since the 1960s. The third generation (3G) is the

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</tr>
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<tr>
<td>S7</td>
<td>S7&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Ingra-ORF3</td>
<td>One-locus</td>
<td>During the formation of female gametophytes in heterozygotes S7&lt;sup&gt;+&lt;/sup&gt;/S7&lt;sup&gt;−&lt;/sup&gt;, S7&lt;sup&gt;−&lt;/sup&gt;/S7&lt;sup&gt;−&lt;/sup&gt; or S7&lt;sup&gt;−&lt;/sup&gt;/S7&lt;sup&gt;−&lt;/sup&gt; gametes carrying S7&lt;sup&gt;−&lt;/sup&gt; could not resist the effect of S7&lt;sup&gt;+&lt;/sup&gt; from sporophyte, thus leading to embryo sac abortion.</td>
</tr>
<tr>
<td>hsa1</td>
<td>HSA1a-&lt;i&gt;j&lt;/i&gt;/HSA1b-&lt;i&gt;j&lt;/i&gt;</td>
<td>japonica-type</td>
<td>Unclear</td>
<td>The HSA1a-&lt;i&gt;j&lt;/i&gt;/HSA1a-&lt;i&gt;p&lt;/i&gt; heterozygosity caused semi-sterility due to selective abortion of the HSA1a-&lt;i&gt;j&lt;/i&gt; female gametes in HSA1b-&lt;i&gt;p&lt;/i&gt; homozygous plants.</td>
</tr>
<tr>
<td>DPL1</td>
<td>DPL1-N&lt;sup&gt;−&lt;/sup&gt;/DPL1-K&lt;sup&gt;−&lt;/sup&gt;</td>
<td>DPL1-N&lt;sup&gt;−&lt;/sup&gt;/DPL2-K&lt;sup&gt;−&lt;/sup&gt;/DPL2-K&lt;sup&gt;−&lt;/sup&gt;</td>
<td>Duplicated genes</td>
<td>In near isogenic lines (NILs) having heterozygous DPL1 (DPL1-K&lt;sup&gt;−&lt;/sup&gt;/DPL1-N&lt;sup&gt;−&lt;/sup&gt;) in Nipponbare background (DPL2-N&lt;sup&gt;−&lt;/sup&gt;/DPL2-N&lt;sup&gt;−&lt;/sup&gt;), pollens having DPL1-K&lt;sup&gt;−&lt;/sup&gt;/DPL2-N&lt;sup&gt;−&lt;/sup&gt; failed to germinate.</td>
</tr>
</tbody>
</table>
intra-subspecific hybrid rice since the 1970s, including indica hybrid rice and japonica hybrid rice. The fourth generation (4G) is the inter-subspecific introgression rice developed gradually since the end of last century, including japonica-introgressive indica, indica-introgressive japonica, japonica-introgresive indica hybrid rice, and indica-introgressive japonica hybrid rice. The inter-subspecific hybrid rice between indica and japonica will be the fifth generation (5G) of rice (Zhang 2019b).

The understanding of genetic basis of inter-subspecific hybrid sterility between indica and japonica provides a theoretical basis for overcoming inter-subspecific hybrid sterility. By the development of ICJLs, it is confirmed that the inter-subspecific hybrid sterility could be overcome by using the $S_5$, $Sa$, $Sb$, $Sc$, $Sd$, and $Se$ loci; $L$, line; $H$, hybrid.

In the past 20 years, our laboratory has been engaged in the exploration of breeding by design in rice. A chromosomal single-segment substitution line (SSSL) library of AA genome of Oryza was constructed by using indica rice variety Huajingxian 74 (HJX74) as recipient. At present, 2360 SSSLs were obtained from 43 donors of seven species of rice AA genome. The genes in substitution fragments of SSSLs was analyzed and a lot of gene information was obtained. Target genes were selected from the SSSL library to carry out rice design. A platform for rice breeding by design was established based on the SSSL library (Zhang 2019a). Recently, a series of IWCRs were developed by pyramiding $S$-n genes of the $S_5$, $Sa$, $Sb$, $Sc$, $Sd$, and $Se$ loci as well as $Rf1$ gene on the HJX74-SSSL platform.

Using the IWCRs crossed with more than 70 japonica cytoplasmic male sterility (CMS) lines collected from various japonica rice regions of China, about 1000 combinations of indica-japonica hybrid rice were developed. Through widely testing in various rice regions of China for several years, the fertility of the hybrid combinations was mostly normal or close to normal. Some combinations were tested in production experiments in different rice regions of China. The results showed that these combinations generally had strong heterosis and great potential of grain yield. It can be expected that through further efforts, the industrialization of indica-japonica hybrid rice can be realized. As the fifth generation of rice, the inter-subspecific hybrid rice between

![Fig. 2 Overcoming of indica-japonica hybrid sterility. A, the hybrid of indica/japonica, being sterile due to the interaction of $Sn$-$i/Sn$-$j$. B, the hybrid of indica/ICJL (indica-compatible japonica line), being fertile due to the homozygous genotype of $Sn$-$i/Sn$-$i$. C, the hybrid of IWCL (indica wide compatibility line)/japonica, being fertile due to the role of $Sn$-$n$ from IWCL. D, the hybrid of indica/JWCL (japonica wide compatibility line), being fertile due to the role of $Sn$-$n$ from JWCL. $Sn$, the $S_5$, $Sa$, $Sb$, $Sc$, $Sd$, and $Se$ loci; $L$, line; $H$, hybrid.](image-url)
indica and japonica will be coming.

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