REVIEW

AGRICULTURAL SCIENCES

Special Topic: Rice Breeding

Breeding high-yield superior quality hybrid super rice by rational design

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ABSTRACT

The challenge of meeting the increasing demand for worldwide rice production has driven a sustained quest for advances in rice breeding for yield. Two breakthroughs that led to quantum leaps in productivity last century were the introduction of semidwarf varieties and of hybrid rice. Subsequent gains in yield have been incremental. The next major leap in rice breeding is now upon us through the application of rational design to create defined ideotypes. The exploitation of wide-cross compatibility and intersubspecific heterosis, combined with rapid genome sequencing and the molecular identification of genes for major yield and quality traits have now unlocked the potential for rational design.

Keywords: *Oryza sativa* L, super rice, hybrids, molecular breeding, grain yield, grain quality

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INTRODUCTION

Food security and sustainable agricultural development are crucial for the wellbeing of all nations and their people. However, food security is threatened by a growing world population, climate change, the loss of arable land to urban development and by increasing demand for animal feed and biofuels. Rice (*Oryza sativa* L.) is one of the most important food crops in the world, supporting 21% of the total calorie intake of the world population and up to 76% of that of Southeast Asia [1,2]. Increases in rice production would therefore have a big impact on world food security. Such increases will require new high-yielding varieties of rice, which will depend in turn on new approaches to produce them.

Rice genetics and functional genomics have been advancing at great pace particularly over the last decade since the first determination of the rice genome sequence [3–7]. However, yields per hectare have effectively plateaued in China, Indonesia, Japan and Korea, and although rising linearly in some key countries such as India and Vietnam, the rates of increase are too slow to meet future demands [8]. This slow rate of increase in yield has come despite sustained initiatives particularly in China, Japan, Korea and at the International Rice Research institute (IRRI) in The Philippines [8–11]. The slow increase in yield potential has reflected the lack of knowledge and tools required to unlock the full potential of the rice genome. Now, we assert that major advances in rice yield potential are not only possible, but are already in the breeding pipeline [3,7,12–15].

The key to this imminent breakthrough is the realization of the 'ideotype' concept of rational design [16] based on modeling using detailed knowledge, and depends on having the genetic diversity and techniques available to achieve the desired outcome. The rice breeding programs of the last few decades have generated such knowledge and resources, including:

- understanding and overcoming the problem of hybrid sterility;
- discovery of male sterility systems and restorer genes for the production of hybrids;
- overcoming problems of wide crosses to exploit heterosis and open up new gene pools; and
- creation of linkage maps for the identification of QTLs.

While this knowledge and these tools are vital, the genomics and molecular genetics revolution
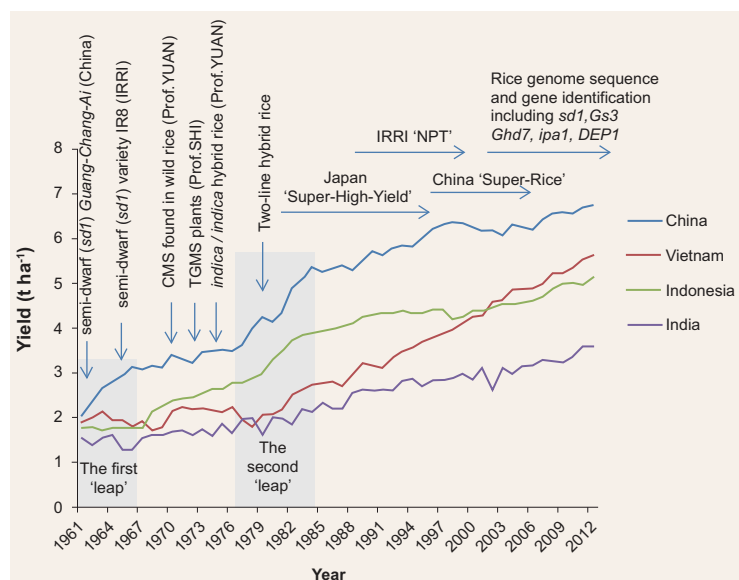
Figure 1. Key developments in rice breeding and historical yields in the major rice-producing countries. Data obtained from the Food and Agriculture Organisation of the United Nations (http://faostat.fao.org/).

of the last decade has provided the additional knowledge and techniques that are required for the ideotype concept to be finally realized, including:

- rapid cheap access to complete genome sequences of diverse rice germplasms;
- thousands of molecular markers for precise marker-assisted breeding;
- map-based cloning of hundreds of genes for major yield, quality and resistance traits; and
- precise editing of genes important for agronomical traits with CRISPR/Cas9 and other technologies.

A key additional component to achieve maximum benefit from the rational design approach is the exploitation of heterosis or hybrid vigor. Heterosis has provided significant yield benefits in hybrids within the *indica* subspecies which is dominant in Southern Asia including China, India, Vietnam and Indonesia. China has therefore played a major role in the breeding and exploitation of hybrid rice [17]. More recently this has extended to include hybrids between *indica* and *japonica* subspecies which have previously suffered with infertility and poor grain quality. Now, the rational design concept and exploitation of heterosis are being simultaneously developed, to create new rice ideotypes designed and produced for specific purposes.

PAST LEAPS IN RICE YIELD

Increasing rice yield has been the main breeding objective for several decades and yield potential of ir-

rigated rice has already experienced two quantum leaps [18]. In 1956, Chinese researchers discovered the semidwarf *sd1* mutant from which they developed the high-yielding variety Guang-Chang-Ai in 1959. In 1966, plant breeders at the IRRI developed a semidwarf, high-yielding tropical *indica* rice variety, IR8 [19,20]. These developments marked the first leap in productivity, particularly in those countries with the resources to exploit this breakthrough, which included China (Fig. 1).

In 1971, the potential for hybrid rice production was opened up by discovery of a male sterile plant of wild rice *O. rufipogon* in Hainan Province of China by the team of Prof. Longping Yuan [21], paving a way for the development of F_1 hybrid rice which was introduced in China in 1976. The exploitation of heterosis by introduction of hybrid rice marked the second breakthrough and a second leap in production. The achievements of the 1960s and 1970s led to increases in rice production of ∼50% over a 10 year period in many countries, as exemplified by China, Indonesia and Vietnam (Fig. 1). Hybrids have been at the heart of rice breeding in China ever since.

THEORY AND PRACTICE OF BREEDING HYBRID SUPER-RICE

Revolutionized methodology of heterosis breeding

Hybrids are formed by cross-fertilization between different strains or species of plants, and the resultant increased vigor of hybrids compared to both parents is known as heterosis or hybrid vigor [22,23]. Rice is predominantly a self-fertilizing plant, so heterosis is not achieved naturally. However, the exploitation of the male sterility gene from *O. rufipogon* in the1970s marked the beginning of the development and exploitation of hybrid rice. Heterosis in rice has revolutionized its breeding and production. Since the use of hybrids started in the 1970s, the yield of rice steadily increased to achieve a further gain of 20% or more, but gains have slowed in recent times (Fig. 1). The development of hybrid rice breeding has occurred in three phases, each using a particular methodology: the three-line method or Cytoplasmic Male Sterility (CMS) system; the two-line method or Thermosensitive or Photoperiod-sensitive Genic Male Sterile (TGMS or PGMS) system; the one-line method or apomixis system [21,24,25]. The former two approaches have been most successfully applied in hybrid breeding (Fig. 2) as discussed below.

Genetic studies demonstrated that the sterility of the *O. Rufipogon* wild rice discovered in Hainan Province in southern China was due to CMS and
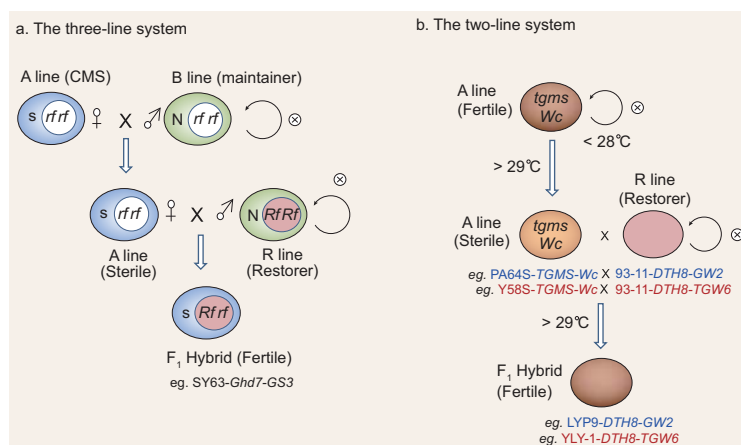
Figure 2. Systems of hybrid rice production. (a) The three-line system requires a CMS (Cytoplasmic Male Sterile) line which is maintained by a fertile B line. Crossing sterile (S) progeny as female parent with a Restorer line (R) containing nuclear Restorer of Fertility (*Rf*) genes, produces fertile F_1 hybrid progeny. (b) In the two-line system, an A-line with thermosensitive genic male sterility (*tgms*) is maintained at permissive temperature (below 28°C), and subsequently elevated above 28°C to cross as female parent with a restorer line (R) as male parent, to produce fertile F_1 hybrids.

was named *Wild Abortion* (*WA*) [22,26,27]. To broaden the genetic diversity of hybrid rice, Chinese researchers have identified other sources of *WA* and exploited them together with other newly discovered CMS lines such as HL (Honglian, from a red-awned wild rice donor plant), G (variety Gambiaca), D (variety Dissi d52/57), IP (variety Indonesia Paddy), DA (a variant of *O. rufipogon*), K (variety K52) and BT (variety Chinsurah Boro II and Taizhong 1). Many CMS lines were developed and the three-line system of CMS established for hybrid seed production on a commercial scale. The system includes a CMS (A) line, a maintainer (B) line and a restorer (R) line [28]. Hybrid (F_1) rice is produced using the A-line as the female parent and R-line with *Restorer of fertility* (*Rf*) gene and yield-enhancing genes from both parents. Large-scale screening led to the identification of diverse germplasms containing *Rf* gene(s) which were used to develop superior restorer lines. The successful development of restorer lines such as *Minghui63, 93-11, R1128* and *R9308* led to the release of a series of three-line hybrid rice varieties such as *Shanyou63* and *Xieyou9308,* which were widely adopted and increased yield by at least 20% (Fig. 2A) [15,29]. For instance, *Shanyou63* reached an average yield of 7.5 t ha^{-1} with a maximum of 9.82 t ha^{-1} on a 6.67-ha field test. In 1973, some TGMS lines in which fertility is controlled by temperature and photoperiod, were discovered in *japonica* rice *NK58S*, which led to the development of two-line hybrid rice [28,30,31] (Fig. 2B) for large-scale grain production in China.

However, full exploitation of hybrid rice potential required yet another breakthrough.

Utilization of strong intersubspecific heterosis

Many studies indicated that the degree of heterosis in intrasubspecies *japonica/japonica* hybrids is less than that of *indica/indica* hybrids, which is in turn less than intersubspecies *indica/japonica* hybrids [15]. The latter hybrids possess a very strong source and large sink, with a yield potential 30% higher than that of intrasubspecies *indica* hybrids. Although hybrid rice production technology was developed in China and applied in major rice growing countries [15], the narrow genetic diversity of parental varieties became a major cause of the yield plateau that has been evident for nearly 20 years. A recent study on the genetic diversity based on genome-sequencing of more than 1529 germplasms demonstrated that there is a ∼40% genetic diversity of single nucleotile polymorphism (SNP) differentiation between *japonica* and *indica*, but during domestication the diversity index of SNPs was reduced from 0.55 in *O. rufipogon* to 0.18 in *O. sativa* [12,32]. To broaden genetic diversity, crosses between *japonica* and *indica* subspecies have gradually been introduced into hybrid breeding methodology since the 1980s. The resulting intersubspecific heterosis can be very strong, but the sterility of the F_1 generation caused by the inharmonious genetic backgrounds of *indica* and *japonica* posed the main barrier to its application. This problem has been addressed following the discovery of the *Wide compatibility* (*Wc*) genes [33] encoding an aspartly protease. This gene normally causes embryo abortion or pollen sterility in the hybrids, but alleles such as *S5-n* or *Sa* allow fully fertile hybrids [34–36].

To increase F_1 fertility, an approach was developed to harness heterosis through the two-line system using the *Wc* gene. A TGMS line containing the *Wc* gene, *Peiai64S* (*PA64S*), was developed by two rounds of crosses between *indica* (*NK58S*) and a *japonica* breeding line, and so obviously carries some *japonica* genetic traits. Because of the combination of the *Wc* and *TGMS* genes, *PA64S* has been widely used as a female parent for breeding two-line hybrid rice in China. Hybrids between *PA64S* and many restorers such as *93-11, E32, 18–26* and *R1128* all exhibited very strong heterosis and extremely high yield potential. In fact, *PA64S* was the female parent of the majority of the pioneering hybrid super-rice varieties in China. For instance, the hybrid super-rice variety *Liang-You-Pei-Jiu (LYP9)* (F_1) was produced using *PA64S* and the *indica* line *93-11*. *LYP9*

and subsequently *YLY1* (*Y Liangyou1*), which has a tall erect-leaf canopy and larger panicle size, were developed in the 1990s and 2000s and reached 8.25 t and 9.0 t ha^{-1} with a maximum yield of 12.11 and 13.5 t ha^{-1}, respectively.

The development of restorer lines by intersubspecific hybridization with the help of the *Wc* gene is known as 'intermediate subspecies differential selection' or '*indica*-compatible *japonica*' [37,38]. Marker assisted selection (MAS) was applied to select individuals with the *Wc* gene. Many outstanding restorer lines, including *93-11, E32, 18–26* and *C418* [38,39], have been developed using this method. A good example is R900, which is the restorer line of the hybrid super-rice variety YLY 900 with a recorded maximum yield of 15 t ha^{-1} in China in 2014. Recently, restorer line *R1128* was developed using intersubspecific cross *SH527/R855//10-33*, producing a maximum of up to 980 grains per panicle.

Thus, major increases in yield have been achieved through the development of hybrid rice largely by exploiting the relatively simple concept of hybrid vigor, but it became increasingly apparent that for subsequent gains, a combination of heterosis with specific genes for 'ideal plant type' or 'ideotype' would be required to provide the much-needed breakthrough in yield.

IMPROVEMENT OF RICE PLANT TYPES

The architecture of a particular plant variety directly affects the production capacity. This is not simply a function of the capacity of an individual plant to capture light and other resources, but of the capacity of a population of plants. The model for expression of the expected traits in a particular environment, or the theory of minimal competition and the ideotype concept, was first proposed by Donald [16]. Yang *et al.* [40] later proposed that an ideal individual plant possesses a combination of plant morphological and physiological traits for improving the utilization efficiency of solar energy by the plant community as a whole. At the population level, this refers to an ideally spaced arrangement of the photosynthetic units to allow the biological and economic yield to reach an optimal level.

In 1982, the Japanese government formally started a 15-year rice breeding program aimed at producing 'super-high-yield' [9] (Fig. 1). As a result, several high-yielding cultivars such as Akenohoshi and Akichikara with large-panicle traits were developed. An increase in rice potential yield appeared to occur in the early 1990s. The cultivar Takanari, released in 1990, reached an average yield of 10.5 t ha^{-1}, 36% more than Nipponbare, a landmark

cultivar released in 1963 [41,42]. Recently, breeders further produced new high-yield good-quality varieties such as Hokuriku 193 in 2007 [43] and Momiroman in 2008 [44], as well as a fairly good-quality variety, Akita 63, which had a recorded high average yield of 11.1 t ha^{-1} over 3 years [45]. However, owing to more attention to better quality and reduced N-fertilizer levels, only a slow rate of increase in yields has been achieved in farmer's fields in Japan, from 6 t ha^{-1} in 1975 to approach 6.5 t ha^{-1} at present.

In 1989, IRRI launched a 'New Plant Type' (NPT; also known as 'Super Rice') breeding program [18,46,47] (Fig. 1). The original NPT was proposed to have few but fully effective tillers (5–6 per plant); 200–250 grains per panicle; plant heights of 90–100 cm; thick and sturdy stems; thick, erect and dark green leaves; vigorous root systems; 100–130 days' growth duration; and increased harvest index [20]. Because the first NPT rice varieties showed low production of biomass, poor grain filling and susceptibility to diseases and insects, the NPT indicators were adjusted in 1995. The second phase NPT varieties were intended to have an increase in plant height and panicle numbers, and reduced number of grains per panicle. However, Peng *et al.* [18] and Yang *et al.* [48] found that there was no significant difference in grain yield between the second-generation NPT lines and the original *indica* varieties in field comparisons. The main reasons were that rapid growth in the tropical environment is not suitable for growing rice varieties with few tillers, and that poor grain filling caused low biomass production. Therefore, based on these outcomes, it was proposed that to achieve a 10% increase in the rice yield potential in the dry season of the tropics, the NPT rice should be revised to produce 150 spikelets per panicle, ∼10 panicles, together with high grain-filling percentage and harvest index. A few NPT lines (such as IR72967-12-2-3) yielded 8% –15% higher than the established *indica* variety, *IR72*. Its higher yield was associated with the production of higher aboveground total biomass and greater grain weight [18]. Although the NPT program achieved only modest gains in yield, it did highlight some of the pitfalls and helped to redefine the characteristics needed to create an ideal plant type for yield.

China established a nationwide mega project entitled 'Breeding and cultivation system of super rice in China' in 1996 [29]. The 'super rice' program aimed to create new varieties with yield increases at least 15% greater than the main contemporary cultivars in two pilot sites in two successive years. The combined use of heterosis and 'ideal plant type' was adopted as the main strategy. The Chinese Ministry of Agriculture organized efforts to formulate the
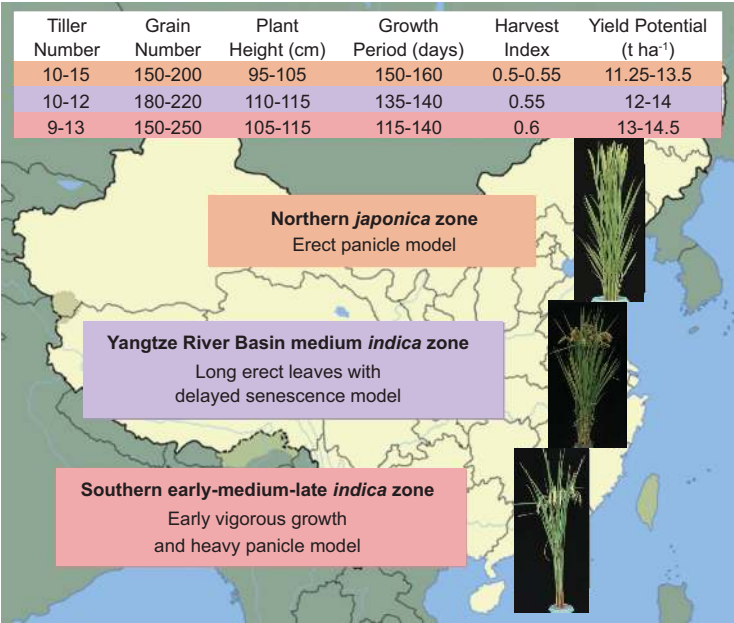
Tiller Number	Grain Number	Plant Height (cm)	Growth Period (days)	Harvest Index	Yield Potential (t ha⁻¹)
10-15	150-200	95-105	150-160	0.5-0.55	11.25-13.5
10-12	180-220	110-115	135-140	0.55	12-14
9-13	150-250	105-115	115-140	0.6	13-14.5

Northern *japonica* zone
Erect panicle model

Yangtze River Basin medium *indica* zone
Long erect leaves with
delayed senescence model

Southern early-medium-late *indica* zone
Early vigorous growth
and heavy panicle model

Figure 3. Ideotypes of super rice in different geographical zones in China. In addition to the characters that distinguish ideotypes, each should have common characters such as strong roots and resistance to disease and insects.

standards for ideal plant type of super rice suitable for the three major rice production areas in China, including Northern, Southern and the Yangtze River regions (Fig. 3). Five models of ideal plants were originally proposed: the erect panicle model; the early vigorous growth and heavy panicle model; the weighted panicle model; the functional canopy leaf model; and the vigorous late stage model. The latter three were subsequently combined into a single model characterized by long erect leaves with delayed senescence.

From project initiation in 1996 up to 2013, 67 hybrids (46 three-line hybrids and 21 two-line hybrids) and 34 inbred rice varieties that met the super rice criteria of the Chinese Ministry of Agriculture, were released by provincial or national seed boards (http://www.agri.gov.cn/V20/ZX/nyyw/201304/t20130408_3426784.htm). Therefore, significant advances have been achieved in China and elsewhere [49], although yield increases have been modest compared to the quantum leaps in production rates seen last century. While hybrid rice technology and breeding for plant type clearly led to major advances in breeding and production, the limitation to exploiting hybrid rice to its full potential has been the lack of detailed knowledge of the genes underlying yield. Now, we believe that the super rice program is set to deliver another transformation in rice breeding to achieve a new 'quantum leap' in productivity.

EMERGENCE OF THE UNDERLYING GENETIC BASIS OF HYBRID SUPER-RICE

Grain yield is a complex agronomic trait that is controlled by multiple genes, identification of which has been the objective of QTL analysis in rice. Hundreds of QTLs related to yield traits have been documented using F_2, doubled haploid and recombinant inbred line (RIL) populations (http://www.gramene.org) [50]. Forty two QTLs controlling yield potential have been cloned [2,51]. Some of these QTL mapping and cloning studies were conducted using genetic populations derived from parental lines of the hybrid super rice varieties.

Resequencing of *PA64S* and *93-11*, the parents of the hybrid super rice *LYP9*, together with 132 RILs derived from the cross, has been accomplished using the Solexa sequencing technology. A high-resolution linkage map was constructed with an average interval of 0.454 cM between recombinant blocks. The genome sequence of *PA64S* reached 382 Mb with 92% quality sequence. Similarly, the published *93-11* genome sequence was updated to reach 423.0 Mb, of which 369.8 Mb is high quality sequence [52]. Therefore, the reference-guided and improved assemblies of *PA64S* and *93-11* provided an important reference for *indica*, and an ideal platform for functional genomics research and molecular breeding in hybrid rice (http://rice.genomics.org.cn/rice).

Using this high-density SNP map, 12 QTLs underlying four yield-component traits from the *LYP9* RIL population were identified [52]. Some of these QTLs were also detected in RIL populations derived using parents of hybrids *Xieyou9308* and *Shanyou63*. In another study, 781 F_2 plants derived from a cross between a hybrid rice restorer line 'Giant Spike Rice' *R1128*, and the *japonica* cultivar *Nipponbare* were resequenced [39] and forty-nine QTLs for five yield factors were identified, revealing that *R1128* carries multiple yield-enhancing QTLs, including s*d1*, *Gn1a* and *IPA1*. The restorer line 9311-*GS2* could enhance yield 15% relative to the parent [53]. At least eleven QTLs including *Ghd7*, *DTH8*, *GS3*, *GW2*, *sd1*, *TGW6*, *HTD1*, *IPA1*, *GL7(GW7)*, *GS2* and *Gn1a* [19,53–66] have been identified to play an important role in these hybrid rice varieties (Table 1).

Thus, hybrid rice varieties have played a key role in the identification of major QTLs, which in turn have helped us to understand how increased yield has been achieved in some hybrids. For example, the *GS3* gene, which plays a key role in controlling grain shape and grain length, was first cloned from *Shangyou63* [55]. The *GW2* gene for grain width and weight, which encodes a previously unknown RING-type protein with E3 ubiquitin

Table 1. Eleven major QTLs controlling yield confirmed in hybrid rice.

QTL	Trait	Character of QTL	Nature of allele	Chr	Ref
Gn1a	Grain number	Encoding cytokinin oxidase. High yield with increased spikelets	Low expression	1	19
GS2	1000-grain weight	Encoding growth-regulating factor 4(OsGRF4).High yield with increasing grain size	Gain of function	2	53
sd1	Plant height	Encoding a gibberellin 20-oxidase, high yield with reduced plant height	Loss function	1	54
gs3	Grain length and weight	Encoding a transmembrane protein. high yield with a greater grain length and weight	Loss function	3	55 56 57
HTD1	High tillers and dwarf	Encoding plastidic dioxygenase MAX3. High yield with increasing tillers	Loss function	4	58
ipa1 WFP	Ideal plant architecture	Encoding a transcription factor containing SBP-box under control of microRNA OsmiR156; high yield with an ideal plant type	High and ectopic expression	8	59 60
DTH8 Ghd8	Flowering repressor	Encoding a CCAAT-box-binding transcription factor. High yield with delayed heading date and bigger panicle	Functional allele	8	61
TGW6	1000-grain weight	Encoding an IAA-glucose hydrolase. High yield with increasing grain	Loss function	6	62
GW2	Grain width and weight	Encoding a RING-type ubiquitin E3 ligase. High yield with increased grain width and weight	Loss function	2	63
Ghd7	Grains, plant height and heading date	Encoding a CCT domain protein. High yield with delayed heading date and bigger panicle	Functional allele	7	64
GL7 GW7	Grain length Grain weight	Encoding a homologus protein of Arabidopsis thaliana LONGIFOLIA. High quality with increasing grain length	High expression	7	65 66

ligase activity, was detected in restorer lines *93-11* and *R9308* [63]. The *Gn1a* allele from Habataki which increased grain number per plant by ∼21% and grain yield by 10% [19] was subsequently detected in restorer line *R1128*, a parental line of hybrid rice that has giant panicles.

Plant architecture, such as tiller number, plant height and panicle type, is another important yield trait for which QTLs have been cloned recently. For example, *Ghd7*, a pleiotropic gene controlling grain number, plant height and heading date, was cloned using $F_{2:3}$ and RIL populations derived from the *indica* hybrid rice parents *Zhenshan97* and *Minghui63*. The *Ghd7* gene is regulated by day length, and directs delayed heading date and increased plant height, panicle size and yield (50% higher in NIL-*Ghd7*) [64]. In addition, a major QTL *IPA1* (or *WEALTHY FARMER'S PANICLE, WFP*) was identified using the map-based cloning approach from a *japonica* rice variety *Shaonie Jing* [59]. The *IPA1* gene modulates development-related genes leading to the for-

mation of plants with few tillers, strong stems, increased grain number and grain weight and a significant enhancement in yield (Fig. 4) [67]. An *IPA1* (*WFP*) allele was also detected in the restorer line *R1128* with giant panicle and hybrid super rice variety *Yongyou12*. This finding will certainly facilitate the development of ideotypes in hybrid super rice varieties.

MARKER-BASED VARIETAL IMPROVEMENT OF HYBRID SUPER RICE

Yield improvement

Progress in functional genomics in rice including identification of key functional genes and updated genome sequences is greatly accelerating the rational design and molecular breeding of hybrid super rice by MAS, marker assisted backcross (MABC) and marker assisted gene pyramiding (MAGP) [68]. A series of high-yield genes cloned recently,
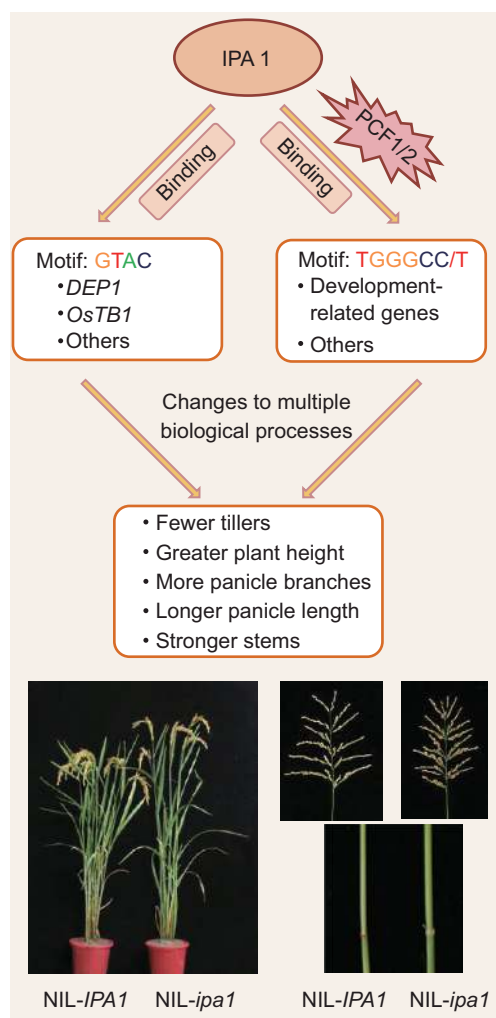
Figure 4. The role of rice *Ideal Plant Architecture1* gene *IPA1*. The *IPA1* (or *WEALTHY FARMER'S PANICLE, WFP*) gene encodes a transcription factor containing an SBP (*Squamosa-Binding Protein*)-box which directly binds to the GTAC motif to regulate key plant architecture regulators, including *DEP1* and *OsTB1*, whereas it indirectly binds to the TGGGCC/T motif through interaction with PCF1 and PCF2 (*Proliferating Cell Factor*) to modulate development-related genes [67]. The expression of genes involved in multiple biological processes, such as apoptosis, cell cycle, development, stress response and plant hormone signaling, is altered, leading to the formation of 'ideal plant architecture'. Plants with the *ipa1* allele have few tillers, strong stems, increased grain number and grain weight and a significant enhancement in yield. [NIL, near-isogenic line].

including *IPA1*, *Ghd7*, *DEP1* and *GW8*, have been applied in rational design of super rice by molecular breeding. This refers to the breeding of desirable new cultivars using a combination of precise chromosome haplotyping and extensive phenotyping based on manipulating all allelic variations, which increases the speed and efficiency of QTL-based breeding.

The use of 'Next-Generation Sequencing' technology is beneficial not only to bridge the knowledge gap between genotypes and phenotypes but also for identifying genetic variation in the ever-growing number of sequenced genomes, which can then be used in rational design-based molecular breeding. Combining multiple defined genes ('gene pyramiding') has been successfully developed and applied in breeding super rice combinations with greatly increased yields [15,48].

Gn1a encoding a cytokinin oxidase directly influences rice yield but the plants carrying this allele are typically prone to lodging. To solve this problem, Koshihikari-*Gn1a-sd1* lines were produced by successive backcrossing. Compared to Koshihikari, Koshihikari-*Gn1a-sd1* lines showed an 18% decrease in plant height and retained the characteristic of high grain number. The positive influence of *Gn1a* on the grain yield exceeds the negative influence of *sd1* because the yield of the NIL-*Gn1a-sd1* lines was 15% higher than that of Koshihikari [19]. *Gn1a* and *IPA1* have been introgressed into hybrid rice restorer lines such as 93-11 and R1128 for the improvement of super rice [39].

To explore the feasibility of altering grain length through rational design, Yang *et al.* [56] used Chromosome Segment Substitution Lines (CSSLs) of *Huajingxian 74* (*HJX74*), which carry the grain length gene *GS3* from hybrid rice *Shanyou63*, and other CSSLs, which carry superior traits related to amylose content (AC; *Wx*), fragrance (*fgr-8*), grain width (*gw8*), grain weight (*GWT*), and precociousness (*Hd-1*), to produce a hybrid line. In the F_4 population, 26 homozygous lines that contain *GS3* and other beneficial genes were identified. The phenotypes in these lines confirmed that the genetic design not only retained the desired grain length, but also effectively improved the quality of *HJX74* [69] by reducing grain width and hence its 'chalkiness'. Thus, it seems that the *gw8* allele resulted in higher quality grains, while the HJX74-*GW8* allele enhanced grain yield.

Dense and Erect Panicle (*DEP1*) is an important agronomic trait for carbon partitioning into grain development. A dominant gain-of-function mutation at the *DEP1* locus caused a truncation in a protein which contains a phosphatidylethanolamine-binding-protein-like domain. This allele enhances meristem activity, resulting in a reduced length of the inflorescence internode, an increased number of grains per panicle, and consequently a 15%–20% increase in grain yield [70]. The *DEP1* and *Gn1* genes were introduced into the restorer line *93-11* and backcrossed three times to establish *DEP1*-9311 and *Gn1*-9311 single-gene introgression lines (ILs) [71]. The resulting lines harboring the two target genes

were then generated by crossing single-gene introgression lines. Due to improved resource allocation, the yield of the *DEP1/Gn1*-9311 lines was significantly increased, reaching as high as 12 t ha^{-1} [71].

Qian *et al.* [72] introduced nine tillering genes, including *IPA1*, into the *indica* rice restorer line 93-11 to construct ILs and CSSLs. The resultant CSSLs carry two to nine tillering genes through crossing various CSSLs carrying single-tillering genes. The effects of the individual tillering genes were evaluated by analyzing their phenotypic contributions to generate a genotype–phenotype (G–P) database. Therefore, CSSLs can be selected to generate a specific number of tillers. For example, a tiller number ranging from 28 to 31 was developed in the line CSSL21, which contains five tillering genes that can be traced and manipulated by markers linked to each. Thus, by using proper combinations of tillering genes, a rice variety can be rationally designed and created to have the desired phenotype [71,73].

Quality improvement

Multiple factors contribute to grain quality including appearance, fragrance, cooking and eating properties, and nutritional value. Currently in China, strong emphasis is given to improving eating, cooking and appearance qualities of hybrid rice, especially *indica* hybrids. Appearance is mainly specified by grain length and width (and the ratio), and translucency or chalkiness of the endosperm. Cooking and eating qualities are mostly determined by AC, which influences the gelatinization temperature (GT) and gel consistency (GC) of the rice [74].

Molecular marker-based genetic analysis in the last decade identified major loci for these quality traits. The *Wx* locus plays major roles in AC and GC, and a minor role in GT [74–76], which is affected predominantly by *ALK* (Alkali digestion) linked to *Wx* [76,77]. Several genes for grain quality QTLs, such as *GS3* for grain length, *GW8* for grain width, *Chalk5* for chalkiness, *Badh2* for rice fragrance, *Wx* and *ALK*, have been cloned [57,63,69,78–81]. The single-locus inheritance clearly indicated that MAS can play a major role in quality improvement. Indeed, Zhou *et al.* [82] were able to improve the quality of *Zhenshan97*, the female parent of a number of widely used hybrids in China with poor quality because of high AC, low GC and low GT, together with a chalky endosperm. MAS was applied to introgress the *Wx* gene region from *Minghui63* that has medium AC, low GC and high GT to *Zhenshan97*. The selected lines and their hybrids with *Minghui 63* or *Shanyou 63* (*wx-MH*) showed reduced AC and increased GC and GT, coupled with

reduced grain chalkiness, representing a significant improvement in cooking, eating and appearance qualities. Moreover, the *wx* gene was used to develop new rice lines (D154, D156 and D174) with low AC (13.36%, 14.28% and 13.13%, respectively) from *Tianfeng B* (26.80% amylose) [83,84]. Such approaches can also potentially increase the amount of 'resistant' starch to reduce glycemic index and improve digestive health.

Tolerance to environmental stresses, disease and pests

Rice is susceptible to many abiotic stresses including high and low temperature, salinity, drought and flooding. Major resistance genes have been identified at the molecular level for each of these characters [83]. For example, the *submergence1* (*Sub1*) gene has been introgressed into a series of Indian rice varieties, thus providing good yields even after submergence for two weeks [85]. Nutrient use efficiency is another target for rice improvement, to reduce consumption of nitrogen and phosphate fertilizers. For example, the plant G protein complex from a *DEP1* allelic gene regulates nitrogen signaling, and modulation of heterotrimeric G protein activity provides a strategy for environmentally sustainable increases in nitrogen use efficiency and rice grain yield [86].

Two major diseases of rice are bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and fungal blast (*Magnaporthe grisea*). Resistance genes for both have been identified and have been introgressed into restorer lines for variety improvement. For example a hybrid super rice *Guodao 6* with multiple tolerance and high-yield traits is now cultivated widely in Southern China [73]. Two restorer lines carrying the bacterial blight-resistant gene *Xa21*, *Zhonghui8006* and *Zhonghui218*, were bred, and a series of hybrid rice combinations were made, such as *Guodao 1* [87], *Guodao 3* [88] and *II You8006* [89]. Researchers from Guangdong Academy of Agricultural Sciences [90,91] successfully transferred the genes *Pi-1* and *Pi-2* into the sterile lines GD-7S and GD-8S to produce new sterile lines RGD-7S and RGD-8S with high resistance to rice blast. From these they screened out new combinations of two-line hybrid rice, *Yueza 746*, *Yueza 751*, *Yueza 4206* and *Yueza 750*, with high resistance to rice blast.

Major insect pests include stemborers (e.g. *Chilo suppressalis*) and leaffolders (e.g. *Cnaphalocrocis medinalis*). Considerable success has been achieved in providing resistance to these insects using the *Cry* genes from *Bacillus thuringiensis* [92] in restorer lines *MH63-Bt* and TGMS line *T16s*. Another important

insect pest is the brown planthopper (BPH; *Nilaparvata lugens*). At least 19 BPH resistance genes have been identified from cultivated and wild rice species and molecular mapping of these genes has facilitated introgression into hybrid rice varieties [93,94].

FUTURE MODEL OF HYBRID SUPER RICE BREEDING BY RATIONAL DESIGN

The potential

Rice plants can theoretically transform more than 5% of the energy of physiologically useful solar radiation into accumulated organic matter (biomass) [95]. If we employ a conservative figure of 2.5%, the yield of single-season rice in southern China can theoretically reach 22.5 t ha^{-1}. Thus, achieving yields higher than 15.0 t ha^{-1} is theoretically possible [29,72]. To reach this target using the combined advantages of ideal plant architecture and the intersubspecies heterosis of *indica* and *japonica*, the proposed ideal varieties of hybrid super rice should have 7–9 tillers, 260 grains per panicle at 27 g per 1000 grains and be 110–130 cm high (Fig 3) [15]. The creation of varieties with such an ideotype is achievable using combinations of genes to produce plants with good morphology, suitable compact type, moderate tillering ability, and small differences in size between main panicle and tiller panicles.

Increasing plant height and lodging tolerance

An appropriate increase in plant height will be a priority, enabling such an enhanced rice biomass and grain yield to be achieved, and this can be accomplished using ideal plant architecture genes, such as the *IPA1* gene. The *ipa1* gene from *japonica* rice with solid stem and short, thick and heavy basal internodes could be used to develop an intersubspecific combination to solve the lodging problems in super-high-yielding high-height varieties. Indeed, the newly released intersubspecific hybrid super rice varieties *YLY2* and *Yongyou12* with high yield and lodging tolerance contain a beneficial allele of the *IPA1* gene (QQ, LG and JL, data unpublished). Introduction of the *ipa1* allele into *Xiushui 11* (*XS11*), a *japonica* rice variety, has produced a variety with a 60% increase in grain number and ∼10% increase in grain yield [59]. Using MAGP, lines harboring a desirable allele of the *IPA1* gene will facilitate the rational design of elite super rice varieties or the parental restorer line of hybrid rice, as demonstrated by the lines *R1128* and *9311-ipa1* [39].

New erect and large panicle super rice sterility lines

To solve the contradiction between large panicles and multiple panicles in super-high-yield rice, a panicle trait optimization strategy was proposed by introducing the erect panicle-type indicators into the super rice plant-type design [29]. Among super rice varieties with an erect panicle, *Shennong265* showed not only erect panicles, but also strong root activity and high yield in a range of growing environments. When rice varieties with erect or semierect panicles due to mutations of the *DEP1* gene were planted in Northeast China and the Yangtze River area, their yields were significantly greater than genotypes lacking the appropriate *DEP1* allele. Interestingly, the newly released intersubspecific hybrid super rice variety *Yongyou12* has the desirable allele of *DEP1*. In the future, the desirable *DEP1* allele from *japonica* cultivars could be transferred to male sterile lines commonly used in hybrid super rice breeding for subtropical and tropical regions to create introgression lines such as PA64S-*dep1* and *Zhong*9A-*dep1*.

The future for hybrid super rice

A very promising MAS method, known as genomic selection (GS) or genome-wide selection, uses genome-wide markers to predict the breeding (genotypic) values of the selection candidates to improve quantitative traits [96]. The currently available genome information from the global rice germplasm will be beneficial for developing elite super rice varieties such as new idiotype super rice with *indica–japonica* heterosis [97], and through genome selection or molecular breeding via rational design. Rational design of ideotypes and their creation using defined genes, MAS and GS will transform the breeding of hybrid super rice, exploiting strong intersubspecific heterosis together with ideal plant type genes, providing predetermined core morphological and functional characters. For example, a hybrid super rice has been designed involving crossing a *japonica* sterile line with desired alleles of *DEP1*, *Ghd7* and *GS3* with an *indica* restorer line carrying favorable alleles of *Gn1a*, *IPA1*, *Ghd8*, *Hd1* and *Dro1* (Fig. 5). The resultant hybrid rice with these elite yield QTLs is expected to show an optimized relationship between large source-sink with strong functional leaves and vigorous root system [71,73,98]. On top of this foundation, specific genes will be included for key physiological, quality or resistance traits. Rice productivity will depend increasingly on resilience to biotic and abiotic stresses, and ability to achieve good yields under conditions of nutrient limitation. Already genes for such traits have been
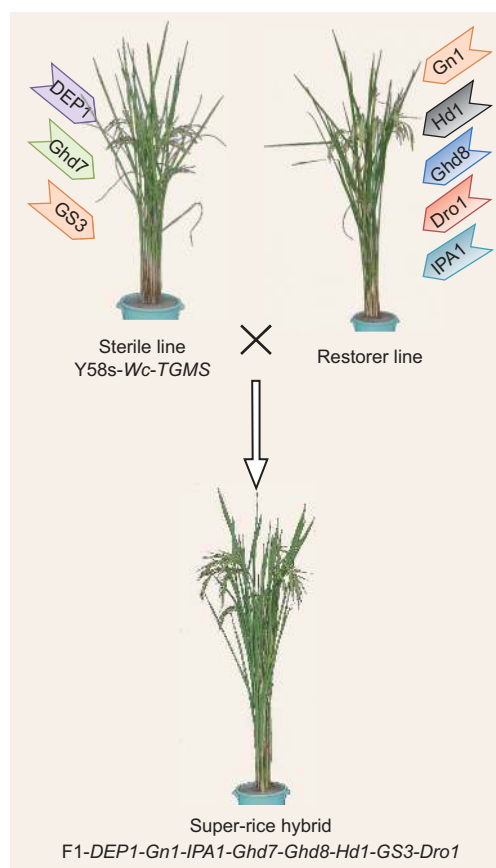
Figure 5. A model for breeding hybrid super rice ideotypes by rational genetic design. Sterile and Restorer parental lines are generated each with rationally prescribed genes so that the F_1 hybrids are provided with the desired characters for specific conditions and with specific quality traits.

identified, with the expectation that many more will be discovered. The ability to readily incorporate such genes into rice varieties will become increasingly important in the future as climate change will not only bring more severe and unpredictable weather events, but will also change the distribution of plant pests.

Where appropriate, transgenic approaches will be used, such as for insecticidal Cry proteins or for grain biofortification with provitamin A [99,100], iron and zinc (www.harvestplus.org/pdfs/rice.pdf). In the long term, research programs aimed at introducing some characteristics of C4 photosynthesis into rice may become realistic [101], but this can only achieve yield benefits if appropriate attention is paid to plant type. Future rice production can potentially be improved by direct seeding of paddy fields, which will depend on breeding of varieties for improved seedling vigor. Productivity in many developing nations is still far below its potential and can be increased with improved genetic resources and agro-

nomic practices. The knowledge, genetic resources and techniques are now in place to drive another quantum leap in rice production.

FUNDING

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