

IPA1: A New “Green Revolution” Gene?

Rice (*Oryza sativa*) is not only the staple food for more than half of the world's population but also a model species for plant developmental and genetic studies. To meet the challenge of feeding an increasing population, breeding for high-yield varieties has always been an ultimate goal for rice breeders. Before the era of molecular biology, breeders identified a semi-dwarf mutation (*sd1*) in the 1950s, and its application contributed to the first “green revolution”, which helped to alleviate food shortage in 19 developing countries. In the 1970s, Longping Yuan and his colleagues found and identified a male-sterile wild rice (*O. rufipogon*) variety, which carries a Wild Abortive cytoplasmic male sterility (CMS-WA) gene. Since then, the CMS-WA system has been widely used in the “three-line hybrid” breeding system (male-sterile line, maintainer line, and restorer lines), which increased rice yield by ~20%–30% and contributed significantly to food sufficiency in China. In the 1990s, a more simplified and effective hybrid system, termed the “two-line hybrid” (male-sterile line and male parental line), was developed by Chinese breeders (Mingsong Shi, Longping Yuan, and their colleagues), which utilized photoperiod- and thermo-sensitive genic male-sterile lines (PGMS and TGMS). The annual planting area of hybrid rice has been maintained at about 16 million ha (accounting for about 55% of the total rice cultivation area in China) since the early 1990s, and now hybrid rice has been adopted in many Southeast Asian countries and the United States (Normile, 2008). Thus, hybrid rice was appraised as the second “green revolution” in China.

Taking the advantages of the advanced molecular biology techniques, the causal gene for *SD1* was identified by several research groups and shown to encode GA20ox-2, an enzyme involved in gibberellin biosynthesis (Sasaki et al., 2002; Monna et al., 2002; Spielmeyer et al., 2002). The CMS-WA gene *WA352* utilized in the three-line hybrid was identified by Dr. Yao-Guang Liu's group. *WA352* encodes a protein that interacts with the nucleus-encoded mitochondrial protein COX11 and inhibits COX11 function in peroxide metabolism, thus triggering premature tapetal programmed cell death and consequent pollen abortion (Luo et al., 2013). Recently, several P/TGMS genes widely used in two-line hybrid breeding, including *PMS3*, *P/TMS12-1*, and *TMS5*, have been identified by several groups (Zhou et al., 2016; and references cited therein). The identification and application of *SD1*, *WA352*, *PMS3*, *P/TMS12-1*, and *TMS5* demonstrate the value of “key genes” in crop yield improvement.

A new gene with great value in rice yield improvement, *IPA1/OsSPL14*, was identified a few years ago by both Dr. Jiayang Li and Dr. Motoyuki Ashikari's groups (Jiao et al., 2010; Miura et al., 2010). The concept of “new plant type (NPT)” or “ideal plant architecture (IPA)” with the characteristics of less unproductive tillers, stronger culms, and larger panicles was recently proposed as a new approach to further improve grain yield in rice (Khush, 2001; Wang and Li, 2008). It was found that either a C-to-T SNP in the coding region of *IPA1* that

affects miR156 targeting (named *ipa1-1D* allele) or increased *IPA1* expression in the panicles due to epigenetic regulation in its upstream promoter region (named *IPA1^{WFP}* allele) conferred an ideal plant architecture for rice, including fewer tillers, stronger culms (stalks), larger panicles, and increased grain weight (Jiao et al. 2010; Miura et al. 2010). Further, they demonstrated the utility of specific alleles for improving plant architecture and grain yield through molecular marker-assisted breeding. To further understand the molecular basis of the *IPA1* action, Dr. Jiayang Li's group carried out a genome-wide chromatin immunoprecipitation-sequencing analysis and identified thousands of putative direct target genes of *IPA1* (Lu et al., 2013). Interestingly, they found that *IPA1* could directly bind to the GTAC motif in the promoters of *FINE CULM 1* (orthologous to the maize *TEOSINTE BRANCHED1*, also named *OsTB1*) and *DENSE AND ERECT PANICLE1 (DEP1)*, which are known key regulators of shoot branching (tillering) and panicle morphology, respectively. However, how *IPA1* regulates other downstream targets and their biological effects still remain to be fully elucidated. Addressing this question is important for rational design of optimized ideal plant architecture in rice breeding.

Two recent studies by Dr. Jiayang Li's group and their collaborators have started to shed new light on this important question. In one of the studies, Wang et al. (2017) used yeast two-hybrid screening and identified an *IPA1* interacting protein (named IPI1), which is a RING-finger E3 ligase. They showed that IPI1 promotes *IPA1* degradation in the panicles but stabilizes *IPA1* in shoot apices. This is achieved by the conjunction of different types of polyubiquitin chains (K48-linked in panicles and K63-linked in the shoot apex) to *IPA1* protein. As a result, *ipi1* loss-of-function mutants show more tillers, enlarged panicles, and increased yield per plant. These findings not only add a new layer of regulation to *IPA1*: tissue-specific ubiquitination by IPI1 provides tissue-specific regulation of *IPA1* protein levels and thus plant architecture, but also broaden the practical application of *IPA1* in molecular breeding. For example, knockout of *IPI1* could increase both tiller number and panicle size, leading to high yield potential. This might be especially useful for improving particular hybrids when more tillers are required for high yield.

The second study also provided intriguing new insights into *IPA1* regulation (Zhang et al., 2017). To identify new factors for the *IPA* traits, a group of scientists headed by Dr. Jiayang Li and Dr. Zuhua He explored Yongyou12 (YY12) and related hybrid rice varieties that are widely grown in China. YY12 is a widely commercialized super rice variety, which has achieved a productivity of 14.5 t grains per hectare on average (a national record). YY12 and related cultivars are intersubspecific hybrids

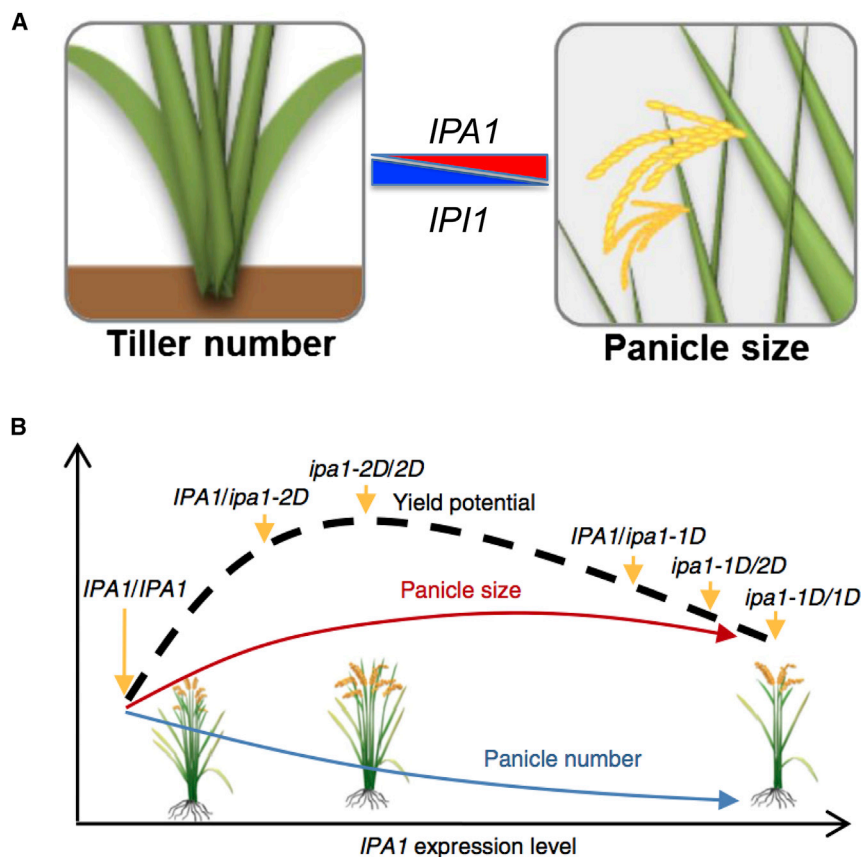


Figure 1. Regulatory Roles of IPA1 and IPI1 in Rice Plant Architecture and Yield Potential

(A). Antagonistic interaction between *IPA1* and *IPI1*. *IPI1* regulates rice plant architecture (tiller number and panicle size) by modulating *IPA1* protein levels in a tissue-specific manner. Modified from Wang et al. (2017) with permission.

(B). A model of yield potential shaped by different combinations of *IPA1* alleles (*IPA1*, *ipa1-1D*, and *ipa1-2D*). Adapted from Zhang et al. (2017) with permission.

(*indica* × *japonica*) with IPA characteristics, but no *ipa1* alleles have been identified in these varieties. Using an F_2 mapping population of YYP1 (the original IPA breeding stock of YY12 with *indica* background) and *japonica* Nipponbare (NIP), they identified a major QTL, named *qWS8* (QTL for wide stem on chromosome 8, also named *ipa1-2D*) that has pleiotropic effects in promoting stem diameter and panicle primary branch but reducing tiller number, similar to the original *ipa1-1D* allele. Further detailed genetic and molecular analyses identified the causal functional variation, YYP1, contains three tandem repeats of a 3137-bp sequence about 4 kb upstream of *IPA1*, while NIP only contains a single copy of this sequence in the corresponding region. They further showed that the repeat structure in NIL^{*ipa1-2D*} somehow alters the chromatin structure of the upstream region of *IPA1*, which attenuates the epigenetic repression from nearby heterochromatin, making it less methylated and thus increasing the expression of *IPA1*.

These findings greatly facilitate the optimization of IPA traits through fine-tuning tissue-specific expression of *IPA1*. *IPA1* is known to have opposite effects on tiller number and panicle branch, two key components of grain yield. Balancing the tiller number and panicle branch number is critical for achieving the optimal ideal plant architecture for highest grain potential. The finding that *IPA1* regulates tiller number, stem diameter, and panicle primary branch number in a dosage-dependent manner now offers a realistic solution to achieving this breeding goal via optimizing the expression levels of *IPA1*. This was exemplified by the authors who *de novo* designed and developed four hybrid varieties to compare the allelic effects of *ipa1-1D* and *ipa1-2D*.

Compared with *ipa1-1D*, *ipa1-2D* is a weak allele for upregulation of *IPA1* expression, which confers a large effect on panicle size (a desired trait) but less deleterious effect on over-reduction of tiller number. Haplotype analysis with 2464 accessions found ten haplotypes, but only five *indica* varieties from China share the same SNPs as YYP1, suggesting that *qWS8* is a rare allele that has recently arisen in China. Thus, *ipa1-2D* holds great potential for improving grain yield and is expected to be applied as a primary target in future breeding of super hybrid rice. Interestingly, a notable feature of *ipa1-1D* and *ipa1-2D* alleles is strong culms (stalks), which might be useful for increasing rice planting density. This trait is also particularly advantageous for mechanical harvesting due to increased lodging resistance. Different combinations of *ipa1-1D*, *IPA1^{WFP}*, and *ipa1-2D* alleles can be created and pyramided with *IPI1* and *qPL6* (a major QTL for panicle length) or other traits (such as herbicide resistance) to provide a repertoire of genetic resources for breeding of IPA super rice that fits different environmental conditions and genetic backgrounds (Figure 1). In addition, novel alleles with better *IPA1* expression can be generated through the genome editing approach. Indeed, *IPA1* may turn out to be a new “green revolution” gene in rice!

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