Breeding Rice Hybrids By Design For Future

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Abstract

Hybrid rice technology is one of the promising, sustainable and proven technologies for enhancing the rice productivity with a yield advantage of 15-20% over inbred varieties. Now a targeted approach to increase yield heterosis level to 25-30% is possible with availability of fine mapping and candidate gene information for several important agronomic traits. Hybrid rice offers the unique opportunity of combining many genes from the parental lines to F1 hybrids in a much faster and efficient way. Major loci for fertility restoration of WA-CMS have been fine mapped and marker system based on these loci has been developed for identification of restorer lines and introgression of the restorer loci. Inter sub-specific heterosis between indica and sub-tropical japonica genotypes can be further exploited by varieties with S-5 allele of wide compatibility (WC) gene enhancing development of inter sub-specific hybrids with WC genes along with Rf3 and Rf4 genes for fertility restoration. Attempts are being made to decipher the phenomenon of heterosis through identification of heterotic blocks using markers and gene expression studies. Incorporation of disease resistance genes into parental lines is being done to make resultant hybrids tolerant to biotic stresses like bacterial blight and blast. Several popular parental lines have been incorporated with Xa21, Xa23, Pi1, Pi9, Piz5 and Pi54 in various combinations. Increase of yield in the derived hybrids through introgressions from the wild in the parental lines has been successfully demonstrated in rice. Some yield enhancing QTLs are reported to increase yield in both homozygous and heterozygous states. Candidate genes for the yield components and their alleles associated with increase in yield components are also being identified. Thus, development of high yielding next generation hybrids by careful pyramiding of key traits is envisaged by deploying markers for fertility restorer (Rf3 and Rf4) and wide compatibility genes in choosing restorers, and by incorporation of yield QTLs from wild species and landraces, and genes controlling yield components and biotic stress resistance into parental lines.

Keywords: Hybrid rice, parental lines, yield, biotic stress, markers, candidate genes

Introduction

Rice, as a staple crop for more than half of the world’s population holds the key for the global food security. The introduction of semi-dwarf, fertilizer responsive high yielding varieties, adoption of high input management practices and commercialization of hybrid rice technology have resulted in significant increase in food production in many countries. However, to meet the demand for rice by 2030, our rice production has to be increased from the current production that too in the backdrop of looming water crisis, declining land resources, acute labor shortage and fast changing climate. Among the various options available for increasing the rice production to meet the future targets, hybrid rice technology is one of the feasible and proven strategies as has been convincingly demonstrated in China and other countries including India. Hybrids derived from within indica subspecies with yield advantage of 10-15% have been developed by various national and international programs. Now hybrid rice is widely adopted and is being cultivated across the world occupying about 25m ha. Heterosis at the inter-subspecific level between indica-tropical japonica is also being exploited by overcoming the problems of semi-sterility in the crosses between the subspecies. Currently, the hybrid rice breeding is primarily based on the three-line system consisting of cytoplasmic male sterile (CMS) line (A), a maintainer line (B) and a restorer line (R) with CMS being restored by nuclear fertility restorer (Rf) genes. Two-line hybrids developed by using temperature sensitive genetic male sterility (TGMS) and photoperiod sensitive genetic male sterility (PGMS) are grown in China to a limited extent. Under super hybrid rice project, many F1 hybrids have been developed using the combination of ideotype approach and inter-subspecific heterosis in China (Peng et al 2008).

Conventional plant breeding and heterosis breeding are interlinked and pragmatic combination of the two can
lead us to reach new heights in enhancing rice yields. Heterosis breeding requires excellent material from conventional breeding and there are many examples where the by-products of heterosis breeding have turned out to be excellent varieties thus it is a kind of relay race moving towards the objective of enhancing yields. It would be possible to raise the yield heterosis from the present 10-15% to 25-30% by adopting different approaches of inter-subspecific hybridization using molecular marker technology for pyramiding quantitative trait loci (QTL) for different yield components. Hybrid technology offers the unique opportunity of combining many genes from the parental lines to the F1 hybrids in a much faster and efficient way.

With the advent of molecular markers, fine mapping and availability of rice genome sequence data, the approach for rice improvement has changed for better, especially for hybrid rice technology. Several key traits associated with hybrid rice viz, fertility restoration, wide compatibility and genetic male sterility have been characterized and mapped. Yield QTLs and the allelic variation in the candidate genes related to yield components in cultivated and wild species are being identified. Major loci for biotic stress resistance, abiotic stress tolerance and quality have also been identified in rice. Using several advanced molecular techniques, not only heterosis in rice is being deciphered, but also techniques to predict heterosis are being standardized. A strategy is being proposed in the present review to develop next generation hybrids by careful pyramiding of key traits by deploying both conventional and molecular approaches.

Review of recent advances and innovative aspects related to hybrid rice

A. Molecular tagging and fertility restorer genes for identification of restorers

In rice, though 20 independent CMS systems have been reported, only three CMS systems viz, wild abortive (WA), Boro Tai (BT) and Honglian (HL) are mostly deployed for commercial hybrid seed production (Li and Yuan 2000; Fuji and Toriyama 2009). Of these, the WA system is the most widely used CMS source for indica rice accounting for about 90% of the rice hybrids produced in China and 100% of the hybrids developed outside China (Sattari et al. 2007). Though several research groups have identified chromosomal locations of the Rf genes for various CMS systems in rice, only WA-CMS system is discussed here considering its wider use and economic importance.

The fertility restoration of WA-CMS is reported to be controlled by two major loci viz, Rf3 and Rf4 on chromosomes 1 and 10 and a few minor QTL (Ahmadikhah et al. 2007; Ahmadikhah and Alavi 2009; Bazarkar et al. 2008; Nematzadeh and Kiani 2010; Ngangkham et al. 2010; Sattari et al. 2008; Sheeba et al. 2009). Putative candidate genes have been identified for Rf4 locus (Ngangkham et al. 2010; Balaji et al. 2012). At Directorate of Rice Research (DRR), an attempt was made to fine map, develop candidate gene based markers for Rf3 and Rf4 and develop a marker system to identify restorer lines. Using polymorphic markers developed from microsatellite markers and candidate gene based markers from Rf3 and Rf4 loci, local linkage maps were constructed in two mapping populations of ~1500 F2 progeny from KRH2 (IR58025A/KMR3R) and DRRH2 (IR68897A/DR714-1-2R) hybrids. QTLs and their interactions for fertility restoration in Rf3 and Rf4 loci were identified. Two QTLs were identified on chromosomes 1 and 10 and together they explained ~60 to 70% of phenotypic variance of the fertility restoration trait (Fig. 1).
Sequence comparison of the two candidate genes, from the \( R_f3 \) and \( R_f4 \) regions in male sterile (A) and restorer (R) lines, respectively, showed 2 to 3 bp indels (INsertion/DEletions) and a few substitutions in the \( R_f3 \) region and indels of 327 and 106 bp in the \( R_f4 \) region. The marker system identified in the present study was validated in 212 restorers and 34 maintainers along with earlier reported markers for fertility restoration of WA-CMS. Markers DRCG-RF4-14 and DRCG-RF4-8 for the \( R_f4 \) locus and DRRM-RF3-5 or DRRM-RF3-10 for the \( R_f3 \) locus showed a maximum efficiency of 92% for identification of restorers (Balaji et al 2012).

In order to identify the restorers from the germlasm, a set of 103 breeding lines of unknown restoration status were initially evaluated with molecular markers linked \( R_f4 \) and \( R_f3 \) at DRR. After studies with pollen and spikelet fertility of the lines with APMS6A, about 80% efficiency was observed with the linked markers suggesting their utility in the identification of restorer lines. A detailed screening of restorers in DRR has revealed that those which have both \( R_f3 \) and \( R_f4 \) are effective to result in a high degree of restoration. Therefore, such efficient restorers can be selected using markers tightly linked to \( R_f \) genes for developing new hybrids.

B. Molecular mapping and marker-assisted introgression of wide-compatibility genes for exploitation of inter-subspecific heterosis

Most of the hybrids grown outside China belong to the intra sub-specific hybrids, which involve the parents from indica subspecies only. Exploitation of inter-subspecific heterosis by using indica and sub tropical japonica genotypes would be the best way to further enhance the levels of heterosis by 10-15%. In hybrids derived from such inter-subspecific crosses, a serious problem of semi-sterility in the hybrids was invariably encountered. The constraint of hybrid sterility could be overcome by deploying wide compatible varieties possessing sterility neutralizing wide compatibility gene (WC) loci (Ikehashi and Araki, 1986). Out of several genes reported to be involved in hybrid sterility, the \( S_5 \) locus on chromosome 6 is considered to be the major. Three alleles at the \( S_5 \) locus have been identified: an indica allele (\( S_5-i \)), a japonica allele (\( S_5-j \)), and a neutral allele (\( S_5-n \)) known as a wide-compatibility gene (WC). The \( S_5 \) locus has been fine mapped. The candidate gene controlling \( S_5 \)-locus was identified as a gene encoding aspartyl protease and a 90-bp deletion is associated with the neutral allele; in addition, many SNPs are responsible for indica and japonica specific alleles at the locus (Qui et al 2005; Ji et al 2005; Singh et al 2006; Chen et al 2008). Sundaram et al (2010) developed a functional marker which can distinguish all the three allelic states (i.e. indica, japonica and neutral) at \( S_5 \) (Fig. 2). The study also revealed that \( S_5 \) is the most important wide compatibility locus and \( S_8 \) together with \( S_5 \) explain more than 90% of the trait phenotype in most crosses. At DRR, Sundaram et al (2010) and Revathi et al (2010) have characterized more than 250 parental lines and other genotypes with respect to their haplotype at \( S_5 \) by using the developed marker system and identified many wide compatible genotypes to obtain heterotic inter-subspecific hybrids. Preliminary screening of 100 restorer lines for the presence of \( R_f3 \), \( R_f4 \) and WC genes at DRR using markers lead to identification of 23 genotypes with all the three genes, and their potential as super restorers is being tested. Therefore, incorporation of WC genes along with the \( R_f3 \) and \( R_f4 \) genes would make the restorers suitable parental lines for developing inter sub-specific hybrids.

c. Prediction of heterosis with the help of molecular markers and genomic tools

Ever since the exploitation of hybrid vigour assumed importance as the means to raise genetic yield ceiling in rice, attempts have been to identify beforehand the cross combinations that would result in heterotic hybrids. With all conventional means and methods failing to establish a consistent relationship between the parental diversity with heterosis, efforts have been made to study the phenomenon at molecular marker level. Molecular markers have been used by different research groups to characterize parental lines and for prediction of heterosis of the cross combinations based on their molecular genetic distance (Zhang et al 1994; Zhang et al 1995; Zhang et al 1996). However, these studies were inconclusive and could reveal only a low to moderate level of correlation between marker data and heterosis levels. Later, Liu and Wu (1998) identified favorable and unfavorable SSR alleles significantly affecting yield heterosis and suggested their possible use in hybrid rice breeding. Another study revealed that the loci may have positive or negative effects, and they counteracted each other if the total loci were utilized to predict heterosis performance (Hua et al 2002).

Zhang et al (2008) reported that specific heterozygosity of positive markers (markers with significant effects on the target traits) display high correlation with heterosis for grain yield in rice. In line with this, the concept of ‘effect-increasing’ loci was proposed recently for the prediction of heterotic grain yield in indica rice (Renning et al 2008). At DRR, in a recent study a set of nine cytoplasmic male sterile (CMS) lines and 32 restorer lines were surveyed with EST based SSRs and genomic SSRs to identify hypervariable EST (expressed sequence tag) markers associated with heterosis.
The set of 10 key-informative EST-SSR markers have shown positive correlation ($r=0.75$) with heterosis for grain yield of six public bred rice hybrids were validated in a new set of 14 experimental hybrids and the EST-SSR markers showed higher correlation ($r=0.79$) with grain yield heterosis and per day productivity indicating the predictive value of these EST SSRs (Table 1) (Jaikishen et al 2010).

Another recent study by Zhang et al (2010) has revealed a significant correlation between genetic distance ascertained from functional markers and grain yield heterosis. A study involving marker-based categorization of rice germplasm (including parental lines) into heterotic groups is being attempted at Indian Agricultural Research Institute, India. In addition to molecular markers, gene expression studies involving heterotic hybrids and their parental lines have helped to identify several candidate genes associated with heterosis. Huang et al (2006) analyzed gene expression profiles of an elite rice hybrid and its parents at three stages of panicle development, using a cDNA microarray consisting of 9198 ESTs and genes associated with positive and negative heterosis were identified. Garcia et al (2008) analyzed the expression of candidate genes in the genomic regions where yield QTLs were localized and observed a significant upregulation of genes. A combination of parental genetic and metabolic markers identified through molecular marker and metabolomic studies has been suggested to improve the prediction of heterosis (Gartner et al 2009). Wei et al (2009) performed a transcriptomic analysis of super hybrid rice LYP9 and its parents and concluded that genes associated with energy metabolism and transport, are significantly upregulated in the heterotic hybrid.
Table 1. Grain yield, duration and standard heterosis of public bred Indian rice hybrids and the correlation between standard heterosis and coefficient of marker polymorphism

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Derived from the cross</th>
<th>Grain Yield (kg/ha)</th>
<th>Duration (days)</th>
<th>Standard heterosis (%)</th>
<th>Coefficient of marker polymorphism</th>
<th>Coefficient of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMS line</td>
<td>Restorer line</td>
<td></td>
<td></td>
<td>EST SSRs Genomic SSRs EST SSRs Genomic SSRs</td>
<td></td>
</tr>
<tr>
<td>DRRH2</td>
<td>IR68897A</td>
<td>DR714-2-1R</td>
<td>4834</td>
<td>115</td>
<td>5.09</td>
<td>0.6 0.8 0.75* 0.09</td>
</tr>
<tr>
<td>PSD1</td>
<td>IR58025A</td>
<td>UPRI 92-133</td>
<td>4401</td>
<td>121</td>
<td>-4.33</td>
<td>0.5 0.8</td>
</tr>
<tr>
<td>KRH2</td>
<td>IR58025A</td>
<td>KMR3R</td>
<td>5948</td>
<td>130</td>
<td>29.3</td>
<td>0.7 0.8</td>
</tr>
<tr>
<td>NSD2</td>
<td>IR58025A</td>
<td>NDR3026</td>
<td>4851</td>
<td>134</td>
<td>5.46</td>
<td>0.5 0.6</td>
</tr>
<tr>
<td>DRRH1</td>
<td>IR58025A</td>
<td>IR40750R</td>
<td>4880</td>
<td>135</td>
<td>6.09</td>
<td>0.6 0.8</td>
</tr>
<tr>
<td>Sahyadri</td>
<td>IR58025A</td>
<td>BR 827-35</td>
<td>5115</td>
<td>136</td>
<td>11.2</td>
<td>0.5 0.7</td>
</tr>
<tr>
<td>Standard check (Jaya)</td>
<td></td>
<td></td>
<td>4600</td>
<td>135</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at $P < 0.05$

Later, another study involving serial analysis of gene expression (SAGE) in the same rice hybrid by Song et al (2010) indicated that genes associated with photosynthesis and carbon fixation are in general upregulated in hybrids as compared to their parents. Some of the methylation patterns analyzed in elite Indian rice hybrid have been shown to be consistent across heterotic hybrids (Sakthivel et al 2010).

The research group at Yale University, USA in collaboration with scientists at National Institute of Biological Sciences, China has initiated studies related to global epigenetic and transcriptional trends among parental lines and reported unique patterns associated with heterosis. Qian et al (2011) showed the role of genes associated with gibberellin response pathway in heterosis in seedlings. Genome-wide DNA polymorphisms in a total of six A and R lines suggested role of non-synonymous single nucleotide polymorphisms (SNPs) and indels in 2625 genes in heterosis (Subbaiyan 2012).

At DRR, expression profile of some key reported candidate genes is being studied in hybrids showing either positive grain yield heterosis or negative grain yield heterosis and derived from a common set of parental lines. However, none of these studies are able to propose a set of validated candidate genes which can be used by the breeders to choose potential parents even without crossing them. The strategy of identification of set of EST-SSR markers targeting the heterotic blocks as shown in DRR experimental studies appears to be promising for prediction of heterosis.

d. Introggression of major biotic stress resistance genes into hybrid rice parental lines

Biotic stresses such bacterial blight (BB), blast, stem borer, leaf folder, brown plant hopper (BPH) and gall midge seriously affect the rice crop including rice hybrids. Therefore, concerted efforts are needed to insulate the parental lines with resistance genes. With the advent of molecular breeding and availability of markers tagged to most of the biotic stresses, it has become easy to incorporate resistance genes into parental lines so that the resultant hybrids can withstand the pressure of biotic stresses.

A significant progress has already been made to improvement of resistance to bacterial blight. This is primarily due to availability of effective dominant resistance genes like $Xa21$ and $Xa7$. The inherent heterozygous nature of rice hybrids limits deployment of recessive resistance genes, since they have to be introgressed into both male and female parents. $Xa21$ has been introgressed into hybrid rice parental lines by a few research groups, principally into restorer lines (Chen et al 2000; 2001). Chen et al (2000) and Zhang et al (2006) introgressed $Xa21$ into the genetic background of an elite restorer line, Minghui 63 with the help of marker assisted selection (MAS) to improve the BB resistance of the derived hybrid Shanyou 63. In addition to $Xa21$, another dominant BB resistance gene, $Xa23$ has been introduced into restorer lines (Deng et al 2006). Chen et al (2008), improved BB resistance of 6078, an elite restorer line by MAS of $Xa21$. Basavaraj et al (2010) introduced $Xa21$ into the male and female parents of the hybrid Pusa RH10.
Recently, Hari et al (2011) introduced Xa21 into the elite restorer line KMR-3R and the hybrid KRH2 through marker assisted backcrossing (MABC), while simultaneously improving grain and cooking quality through phenotypic selection. For introgressing rice blast resistance into hybrid rice parental lines, Jin et al (2006) introduced the major blast resistance gene Pil into the genetic background of a TGMS line GD-8S with MAS. Wen and Gao (2011) introduced Pit9 into the restorer line Luhui17 through marker-assisted breeding. Singh et al (2011) have introduced Pit5 and Pit34 into the restorer line PRR78 through MABC. At DRR, both BB and blast resistance genes have been successfully introduced into IR28025B, the widely used CMS maintainer (Hari et al 2011). So far, there is no published report on marker-assisted introgression of any pest resistance gene into hybrid rice.

e. Identification of yield related QTLs from wild relatives of Oryza and targeted introgression into parental lines

A large amount of genetic variation in the genus Oryza lies unexploited in wild progenitors (Wang et al 1992). Wild relatives were used initially to transfer disease and insect resistance traits (Brar et al 1995). Now, the attention has shifted to map yield enhancing QTLs from wild species and enrich the cultivated gene pool by introgression of favourable genes or gene complexes (Xiao et al 1996; Swamy and Sarla 2008). O. rufipogon (Marri et al 2005; Moncada et al 2001; Septiningsih et al 2003; Thomson et al 2003; Fu et al 2010), O. glaberrima (Aluko et al 2004), O. glumaepatula (Brondani et al 2002), O. grandiglumis (Yoon et al 2006) and O. nivara (Swamy et al 2011) have been exploited for yield and grain quality traits. This enables the efficient use of wild species to broaden the genetic base of the existing cultivars and also to improve complex traits by marker aided introgression of superior wild alleles.

After the studies involving wild and hybrid rice parental lines by Xiao et al (1996a, b; 1998), the focus was on introgression of agronomically useful genes from wild species. Introgressions from the wild which increase yield in either B line or R line will also increase yield in the derived hybrids. Some yield enhancing QTLs are reported to enhance yield in both homozygous and heterozygous state. At DRR, the popular restorer line KMR3 is targeted for yield enhancement with introgressions from O. rufipogon. Around 500 KMR3-O. rufipogon introgression lines (ILs) were evaluated for yield and salinity tolerance. Four high yielding lines are in the third year of multilocation testing in coastal and inland saline and alkaline areas. These KMR3/O. rufipogon ILs as restorer lines can help to produce hybrids with increased salinity tolerance. ILs with increased resistance to bacterial leaf blight or blast have also been identified. DRR in collaboration with Barwale Foundation, Hyderabad is also involved in identification of useful introgressions from O. meridionalis in the maintainer line IR58025B. This line IR58025B was used for mapping QTLs for yield and the BC2F3 introgression lines will be converted to CMS lines. Such CMS lines with well exserted stigma will also be useful in improving percent seed set in hybrid production thus helping to reduce the cost of seed production. A recent study (Sudhakar et al 2012) has revealed that a novel allele of Os11Gsk gene from O. rufipogon is significantly associated with increased yield of introgression lines of KMR3R (Figure 3). As a proven strategy, the hybrid rice parental lines can be improved with introgressions from wild species for yield and other traits with systematic approach.

f. Incorporation of yield component genes into the parental lines

Grain yield of rice is multiplicatively determined by number of panicles per plant, number of grains per panicle and grain weight. Number of grains per panicle is in turn controlled by number of spikelets and grain filling. Substantial variability exists for all the yield component traits in rice leading to different combinations of the component traits for differential yields. Several thousands of QTLs were reported for yield and its components in rice (www.gramene.org) and their biological significance is under validation. Major QTLs for yield components have been identified through positional cloning approach (Table 2). Molecular cloning and analysis of a QTL for grain number GRAIN NUMBER1 (Gn1a) showed that it encodes cytokinin oxidase/dehydrogenase (OsCKX2) and the reduction in its expression increases cytokinin and in turn increases the grain number (Ashikari et al 2005). From wild species, PROG1, a key pleiotropic gene controlling plant architecture and yield-related traits was identified in rice and its recessive allele was found to control the number of primary and secondary branches, grain number, grain yield and transition from prostrate to erect growth (Tan et al 2008).
Table 2. Cloned yield component genes/QTLs

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL/gene</th>
<th>Chromosome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain number</td>
<td>Gn1a</td>
<td>1</td>
<td>Ashikari et al 2005</td>
</tr>
<tr>
<td>Grain number</td>
<td>Dep3</td>
<td>6</td>
<td>Qiao et al 2011</td>
</tr>
<tr>
<td>Grain number</td>
<td>Ghd7</td>
<td>7</td>
<td>Xue et al 2008</td>
</tr>
<tr>
<td>Grain number</td>
<td>Ghd8</td>
<td>8</td>
<td>Wei et al 2010</td>
</tr>
<tr>
<td>Grain number</td>
<td>Dep1</td>
<td>9</td>
<td>Huang et al 2009</td>
</tr>
<tr>
<td>Grain filling</td>
<td>GIF1</td>
<td>4</td>
<td>Wang et al 2008</td>
</tr>
<tr>
<td>Grain filling</td>
<td>FLO</td>
<td>4</td>
<td>Qiao et al 2010</td>
</tr>
<tr>
<td>Grain filling</td>
<td>SPS</td>
<td>2</td>
<td>Rao et al 2011</td>
</tr>
<tr>
<td>Tiller and grain number</td>
<td>EP3</td>
<td>2</td>
<td>Piao et al 2009</td>
</tr>
<tr>
<td>Tiller and grain number</td>
<td>LRK1</td>
<td>2</td>
<td>Zha et al 2009</td>
</tr>
<tr>
<td>Tiller and grain number</td>
<td>MOC1</td>
<td>6</td>
<td>Li et al 2003</td>
</tr>
<tr>
<td>Tiller and grain number</td>
<td>PROG1</td>
<td>7</td>
<td>Jin et al 2008; Tan et al 2008</td>
</tr>
<tr>
<td>Tiller and grain number</td>
<td>WFP</td>
<td>8</td>
<td>Jiao et al 2010; Miura et al 2010</td>
</tr>
<tr>
<td>Grain weight and size</td>
<td>GW2</td>
<td>2</td>
<td>Song et al 2007</td>
</tr>
<tr>
<td>Grain weight and size</td>
<td>GS3</td>
<td>3</td>
<td>Fan et al 2006</td>
</tr>
<tr>
<td>Grain weight and size</td>
<td>SRS3</td>
<td>5</td>
<td>Kitagawa et al 2010</td>
</tr>
<tr>
<td>Grain weight and size</td>
<td>GS5</td>
<td>5</td>
<td>Li et al 2011</td>
</tr>
<tr>
<td>Grain weight and size</td>
<td>GW5</td>
<td>5</td>
<td>Shomura et al 2008; Weng et al 2008</td>
</tr>
<tr>
<td>Grain number and HI</td>
<td>DEP2</td>
<td>7</td>
<td>Zhu et al 2010; Li et al 2010</td>
</tr>
<tr>
<td>Grain number and HI</td>
<td>APO1</td>
<td>6</td>
<td>Terao et al 2010</td>
</tr>
<tr>
<td>Grain number and size</td>
<td>SP1</td>
<td>11</td>
<td>Li et al 2009</td>
</tr>
</tbody>
</table>

Two candidate genes DEP1 and DEP3 enhancing the meristematic activity were also identified which reduced length of inflorescence internode, increased number of grains and yield (Huang et al 2009; Qiao et al 2011). Two more QTLs influencing the grain productivity and heading date were also fine mapped and candidate genes were identified Ghd7 and Ghd8 (Xue et al 2008; Yan et al 2011).

A gene for strong culm and increase in spikelet number SCM2 was cloned and identified to be a weak allele of APO1 gene, which encodes an F-box protein. This gene increases the secondary branch number of the panicle (Ookawa et al 2010). At DRR, to identify genes associated with grain filling process across the panicle, candidate gene (CG) based mapping approach was attempted in F2 population derived from the cross between Rasi, a rice variety known for its good grain filling and IC114927, a local landrace. Analysis of 444 F2 mapping population showed CG marker based on sucrose phosphate synthase gene on chromosome 2 to be significantly associated with filling of grains on primary branches of upper half of the panicle and another CG marker based on transporter gene on chromosome 11 to be associated with filling of grains on primary branches of lower half; secondary branches of upper half and lower half of the panicle. Both the positive alleles were contributed by Rasi (Fig. 4) (Rao et al 2011).

With the availability of a wealth of information and markers related to major yield components, now it is possible to combine the favourable alleles with the help of candidate gene markers. Unlike in conventional breeding which takes longer time to pyramid the genes of interest, this can be done faster by incorporating different sets of genes into parental lines and combining them through the F1 hybrid.
Prospects of achieving the breeding goal

Hybrid rice has been proved to be a practical and feasible approach to enhance 15-20% yield over the conventional bred varieties. Further enhancement of yield heterosis is a challenging task but not impossible, given the kind of recently available techniques of biotechnology. It is possible to design the next generation hybrids by deploying time tested conventional breeding tools and molecular tools. Use of strategies such as MAS, genomics, expression analysis, proteomics and metabolomics would not only bring in much needed precision but also expedite the process considerably. Use of markers for fertility restorer genes (Rf3 and Rf4) and wide compatibility in choosing restorers, incorporation of yield QTLs from wild species and landraces, pyramiding of genes controlling yield components and biotic stress resistance into parental lines would enable the breeders to develop high yielding next generation hybrids. Understanding the molecular basis of heterosis and deploying the molecular tools to predict heterosis would enable breeders to save considerable time and labor in generating high yielding hybrids.

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