

Harnessing Advances in Rice Genetics and Genomics For Unraveling Genes and QTLs Governing Yield and Yield Components For Developing Designer Rice

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Abstract

Rice (Oryza sativa L.) accounts for over 21 per cent of global calorie intake and in order feed the projected population of 9 billion people in 2050, rice yields have to be increased by 50%. Traditional breeding methods including exploitation of plant type concept and heterosis have been successful in recombining genes or alleles and exploiting their interactions which have contributed to yield gains to achieve food sufficiency in the present world. But rice researchers face a Herculean challenge of improving rice yields per unit area in view of the constraints imposed by changing climate, shrinking land and water resources. Grain yield in rice is a polygenic trait which is highly influenced by environmental factors as well and therefore, rice research over the decades have focused on gaining an understanding on the basis of yield and yield components. The availability of the high quality genome sequence coupled with advances in technological tools, enormous genetic and genomic resources have been made available in rice to accelerate the isolation of agronomically important QTLs or genes governing complex traits such as yield. The present paper focuses on the progress made in mapping QTLs for yield and yield component traits in rice through the development of an array of different mapping populations employing conventional linkage mapping strategy and discusses novel approaches for discovery of new genes and QTLs for yield, mining favourable alleles and designing rice with higher per unit productivity through integration of next generation high throughput genomics and phenomics technologies with rice breeding.

Keywords: Allele mining, genomics, quantitative trait loci, rice, yield, marker assisted selection

Introduction

Rice (*Oryza sativa* L.) forms the staple food of over half of the world's population and accounts for over 21 per cent of global calorie intake. Traditional breeding methods including exploitation of plant type concept and heterosis have been successful in recombining genes or alleles and exploiting their interactions which have contributed to yield gains to meet the world food demand. Global paddy rice production of 696 million tonnes has been achieved in 2010, however to feed the projected population of 9 billion people in 2050, rice yields have to be increased by 50% (FAO 2011). World rice production has doubled since the green revolution but the growth rate in yields has seen a decline since 1980s (Jeon et al 2011). Therefore, breeders' face a Herculean challenge of improving rice yields per unit area in view of the constraints imposed by changing climate, and shrinking land and water resources.

Grain yield in rice is a polygenic trait which is highly influenced by environmental factors as well. Rice yield

is primarily determined by tiller number per plant, grains per panicle and grain weight which are complex in nature. In addition to this, other traits such as plant, leaf and root architecture, biomass yield and other related traits play an important role in determining grain yields. Innovations and developments in molecular marker technology have led to dramatic advances in our understanding of complex traits, which are governed by the expression of several regions of the genome, collectively referred to as QTLs (quantitative trait loci). In the late 1980s, researchers were able to successfully demonstrate that molecular markers can help to identify QTLs to explain a substantial proportion of phenotypic variation of complex traits such as fruit weight in tomato (Paterson et al 1988) and agronomic traits in maize (Stuber et al 1992). Rice genome sequencing and advances in related genomic resources, enormous genetic resources, availability of advanced mapping populations and molecular marker resources has allowed researchers to accelerate the isolation of agronomically important

QTLs governing complex traits such as yield (Mochida and Shinozaki 2010).

We focus here on the progress made in mapping QTLs for yield and yield component traits in rice through the development of an array of different mapping populations employing conventional linkage mapping strategy, novel approaches for discovery of new genes or QTLs for yield, mining favourable alleles and designing rice with higher per unit productivity through integration of the next generation high throughput genomics and phenomics technologies with rice breeding.

a. Conventional technique - Linkage mapping of QTLs for yield and its component traits using biparental populations

(i) Mapping populations

Different types of mapping populations developed through controlled crosses are used for linkage analysis namely F_2 , F_2 derived F_3 ($F_{2:3}$), immortalized F_2 population, backcross inbred lines (BILs), doubled haploids (DHs), recombinant inbred lines (RILs), near-isogenic lines (NILs), chromosomal segment substitution lines (CSSLs), single segment substitution lines (SSSLs) and Introgression Lines (ILs) (Singh and Gopalakrishnan 2012). Detailed discussion on the QTL mapping using different mapping populations in rice has been published earlier (Ashikari and Matsuoka 2006).

Fine mapping of the QTLs in early generation segregating populations such as F_2 and $F_{2:3}$ is difficult as precise estimate of the genetic effect of QTLs cannot be established due to residual heterozygosity in the background. Tanksley (1993) suggested the analysis of QTL effects through the use of NILs or SSSLs. Fine mapping of QTLs using various advanced generation mapping populations consecutive backcross NILs, Trait performance based NILs and heterogenous inbred families have been discussed in detail by Bai et al (2012)

Two RIL populations for mapping yield and yield component traits, and one from the cross between a new plant type (NPT) genotype, Pusa 1266 and a popular Basmati rice cultivar Pusa Basmati 1, and another from the cross between a high yielding *indica* cultivar, Jaya and Pusa 1266 (Balram et al 2007), have been developed at Indian Agricultural Research Institute (IARI). In order to fine map grain number per panicle, a trait performance based NIL population was also developed from a cross between a high grain number RIL and low grain number RIL from the cross Jaya x Pusa1266 (Goel et al 2012).



Figure 1. Variation for panicle size and grain number per panicle in the RILs generated from the cross Pusa 1266 x Jaya.

(ii) Source germplasm for development of mapping populations

The natural genetic variation in cultivated rice can be broadly grouped into (a) intra specific variations which can further be classified into inter-varietal, *indica - indica* and *japonica - japonica*, and inter-specific, *indica - japonica* variations; and (b) inter-specific variations which includes variation within A genome species and between A genomes and other genomes (Vaughan et al 2003). Besides these, genetic stocks from induced mutations developed through physical or chemical mutagenesis or insertional mutations also form useful genetic resource for development of mapping populations in rice (Hirochika et al 2004).

(iii) Mapping and cloning of QTLs for yield and its components

The first report on mapping QTLs for traits of agronomic importance using RILs from an *indica-japonica* cross in rice was published by Xiao et al (1996a). Since then, inter-varietal as well as inter-subspecific populations between *japonica* and *indica* have been widely used for mapping QTLs for yield and yield components in rice (Bai et al 2012). Advanced backcross QTL strategy has been successfully employed in rice for mapping transgressive QTLs with beneficial effects from wild species in populations developed through wide crosses (Xiao et al 1998). ILs derived from advanced backcross populations have also been used for fine mapping of yield associated QTLs (listed in Swamy and Sarla 2008).

QTL mapping for grain number on a set of RILs derived from a cross between Pusa 1266 (high grain number) and Pusa Basmati 1 (low grain number) identified one consistent QTL *qGN4-1* on the long arm of chromosome 4 with major effect on grain number. This QTL was co-localized with major QTLs for primary and secondary branches per panicle, and number of panicles per plant (Deshmukh et al 2010). QTL mapping of yield and yield related traits in new plant type mapping population identified 128 QTLs for 12 yield and yield component traits. To utilize the markers flanking the major QTLs for marker assisted improvement, markers linked to these nine major QTLs were used to find marker QTL association in a set 120 germplasm lines (Balram 2008).

Huge wealth of genomic resources created through the sequencing of the rice genome has helped fine mapping the QTLs and cloning the genes responsible for several agronomically important traits, out of which 20 QTLs or gene are associated with yield related traits such as tiller number, grain number, grain weight, grain size and grain filling (Bai et al 2012). Among these, most of the QTLs have been cloned using progeny derived from a cross between *japonica* and *indica* subspecies, because inter subspecific diversity is significantly larger than the diversity in intra subspecific crosses, which makes it easy to produce molecular markers in the regions of interest in the rice genome (Miura et al 2011).

b. Novel approaches for discovery of new QTLs or genes

QTL mapping for complex traits (such as yield) that involve variants at several loci, each of which contribute small amounts to the overall genetic contribution, linkage studies can identify only those loci with the strongest influence. The advances genome sequencing

and availability of cost-effective sequencing technologies to study genome-wide genetic variation, and advances in statistical methodology and the large complex trait dissection platforms, has greatly facilitated gene identification for various complex traits in crops.

(i) Association mapping

Association mapping exploits historical linkage and recombination accumulated over a large number of generations (Andersson and George 2004). With the advent of high-throughput technologies for re-sequencing and genotyping, association mapping has emerged as a powerful tool to identify marker trait associations in species where it is not easy to create linkage disequilibrium. Thus, it can provide high resolution information that can be used to identify the causative nucleotides underlying phenotypic variability. Association panels for mapping have been developed in several plant species for mapping complex traits in crop plants (see review Zhu et al 2008).

Genome-wide association studies (GWAS) involves correlating allele frequencies at each of several hundred thousand markers spaced throughout the genome with trait variation in a population-based sample (Stranger et al 2011). GWAS mapping makes it possible to simultaneously screen a very large number of accessions for genetic variation underlying diverse complex traits. Rice varieties are mainly homozygous, which makes it possible to employ a 'genotype or sequence once and phenotype many times over' strategy, whereby once the lines are genomically characterized, the genetic data can be reused many times over and across different phenotypes and environments (Zhao et al 2011). GWAS have been utilized to map agronomically important traits in rice (Huang et al 2010) and also to analyze the genetic structure of 413 diverse accessions of *O. sativa* from 82 countries using a rice 44K SNP (Single Nucleotide Polymorphisms) array and phenotypic variation for 34 traits (Zhao et al 2011).

(ii) Nested association mapping (NAM)

(NAM) approach is a novel approach which exploits advantages of both linkage mapping and association mapping and thus, it provides high resolution information that can be used to identify the causative nucleotides underlying phenotypic variability (Yu et al 2008). In general, the strategy of projecting sequence information, nested within informative markers, from the most connected individuals to the remaining individuals is applicable to a wide range of species, including maize, barley and rice. In maize, genome-wide association studies using maize NAM population has been demonstrated as an effective tool for dissecting

QTLs explaining more than 80% genetic variance for complex traits such as flowering time (Buckler et al 2009), leaf architecture (Tian et al 2011), southern leaf blight (Kump et al 2011) and northern leaf blight (Poland et al 2011). These studies in maize have demonstrated that most of the heritability of complex traits can be located within the genome provided the use of appropriate resources and scale of study (Haley, 2011).

(iii) Next generation sequencing (NGS) based approaches for mapping genes and QTLs

Rapid advances in the development of novel high-throughput DNA sequencing have drastically reduced the cost of whole genome sequencing, providing low cost coverage of any genome generating a sequence resource for development of molecular markers and gene discovery, comparative genomics and genome assembly (Gopalakrishnan et al 2012).

MutMap

Recently MutMap, a method which combines DNA sequencing and EMS induced mutagenesis has been developed for rapid gene isolation using a cross of the mutant to wild-type parental line (Abe et al, 2012). MutMap is technically similar to bulked segregant analysis (BSA) of F₂ progeny except that for bulking, only the F₂ plants showing mutant phenotype is considered which helps in identifying causal SNPs with sufficient sequence coverage. MutMap can also be exploited in crop breeding to identify and isolate QTLs, and the SNPs flanking the regions harboring causal mutations for the desired phenotypes which can be used as DNA markers for marker-assisted selection.

BSR-Seq

Taking advantage of high-throughput DNA sequencing methods, another new method for both mapping and quantifying transcriptomes, termed RNA-Seq (RNA sequencing) have been developed, which enables the comparative quantification of gene expression in various genotypes (Wang et al 2009). RNA-Seq relies on the principle that read counts for each transcript from the NGS data reflects relative transcript concentrations and these reads can also be mined for DNA sequence polymorphisms such as SNPs which can be converted into genetic markers (Barbazuk et al 2007). Recently, Liu et al (2012) conceptualized a new genetic mapping strategy called BSR-Seq (bulk segregant RNA-Seq), which combines the power of BSA with the ease of RNA-Seq and appropriate statistical procedures. In BSR-Seq approach, an individual with mutant phenotype is crossed with normal phenotype and in F₂, two RNA bulks, one representing a pool of RNA from individuals showing normal phenotype and another

representing bulk of individuals showing mutant phenotype are constituted and sequenced. The variation in relative transcript concentrations between the pools provides information on potential candidate genes and DNA variations in these genes. BSR-Seq, which is based on RNA sequencing, not only maps the exact position of a gene responsible for a mutant phenotype but also the effects of such a mutant on global patterns of gene expression. Hence, BSR-Seq is not only an efficient strategy for mapping genes, but also provides with other data that facilitate gene cloning. BSR-Seq could be extended to other applications, including the mapping of genes defined by dominant mutants and major QTL loci that influence quantitative traits such as yield and its components.

(iii) Meta QTL analysis

Meta-analysis of QTLs is an approach to identify consensus QTL from the QTLs identified across environments or genetic backgrounds (Goffinet and Gerber 2000). Swamy and Sarla (2011) identified 23 meta-QTLs out of the 76 yield QTLs reported in 10 different studies involving inter-specific crosses. Similarly for yield under drought, Swamy et al (2011) identified 14 meta-QTLs that contain different classes of candidate genes including stress-inducible genes, growth and development-related genes, and sugar transport-related genes.

In our lab, meta-analysis was carried out for QTLs governing yield and yield related traits in rice (*O. sativa*) utilizing the published QTL mapping reports conducted using wide range of mapping populations and environmental conditions. We used a total of 416 QTLs reported in different studies to identify 113 meta-QTLs across 10 yield and yield related traits (Table 1, unpublished data). Thus, Meta-analysis helps to identify Meta QTLs with high effects and reduced class intervals for successful utilization in MAS or fine map to deduce candidate genes for gene discovery.

The analysis indicated that these 113 (27.16%) QTLs were widely distributed across diverse germplasm set as haplotypes with high linkage disequilibrium. Pyramiding such robust QTLs in a common genetic background may help to raise yield ceilings.

c. Mining favourable alleles using next generation sequencing (NGS) techniques

NGS can be effectively integrated with Long Range PCR of pooled DNA samples for maximizing the efficiency of sequencing and reducing the cost of amplification, oligonucleotides and labour (Out et al 2009).

Table 1. MQTLs detected for various yield and yield component traits based on QTLs reported in different QTL mapping studies.

Traits	QTLs reported	No. of MQTLs detected through meta-QTL analysis
(i) Primary yield traits		
1. Number of productive tillers	30	8
2. Number of grain per panicle	41	14
3. Test grain weight	121	25
4. Grain yield	27	10
(ii) Secondary yield traits		
5. Plant height	57	13
6. Heading date	34	9
7. Number of tillers	8	4
8. Panicle length	36	11
9. Spikelets per panicle	54	16
10. Spikelet fertility	8	3
Total	416	113

This technology has the power to detect new mutations and discover rare alleles as a fragment of 10-kb may be read 100,000 times or more (Druley et al 2009). Amplicon sequencing, in which amplicons generated by PCR provides the basis for large scale analysis of allelic variations (SNPs and InDels) in genes, gene families and specific sequence regions of interest for population studies and marker development (Henry et al 2012). This technique has been used to characterize genetic variations, including 501 SNP and 113 InDels in 17 genes involved in starch biosynthesis genes in a set of 233 rice genotypes (Kharabian-Masouleh et al 2011). Once the QTLs governing yield and yield component traits are cloned, superior alleles at the particular locus can be identified through amplicon sequencing approach.

d. Innovative breeding strategies for identifying and combining favourable alleles across the genome

A total of 22 QTLs/ genes affecting traits of agronomic importance in rice have been cloned and gene based/ linked markers have been successfully utilized for MAS to accumulate beneficial genes for yield improvement of *japonica* rice (Ashikari and Matsuoka 2006; Ando et al 2008). However, it has been frequently observed that the phenotypic effects of QTL transferred by MAS tend to vary from one genetic background to other (Ohsumi

et al 2011). Genetic background effects have been reported in most cases of MAS for QTL (Hospital 2009). Wang et al (2012) developed NILs containing one or more target genes by marker-assisted transfer of '93-11' alleles at *qHD8*, *qHD7*, and *qHD6.1*, and the *GS3* gene for grain size into cv. Zhenshan97. Comparison of the NILs showed existence of epistasis between alleles at different loci and background effect on *qHD8*, which are very important for pyramiding of desirable alleles at the target QTL. Therefore, it is very important to assess the interaction and genetic background effect, in order to transfer and pyramid target QTL by marker-assisted selection. Similarly in transgenic studies results have shown that altered expression of one major gene may affect functioning of other related genes. Over expression of miRNA *OsmiR393* resulted in increased tiller number and early flowering by downregulation of a tillering inhibitor gene *OsTBI*, but was found to impair tolerance to abiotic stress such as salt and drought (Xia et al 2012). In another study, it was shown that suppression of the expression of *OsBAK1* gene increased grain yield by altering rice architecture producing dwarf plants with erect leaves (Li et al 2009) but affects normal leaf development and increased the susceptibility to blast disease (Park et al 2011). These examples from rice present a dilemma for molecular breeding of major QTLs for improving rice yields.

(i) Association breeding

In a breeding program, the germplasm is in a constant state of flux, and to improve complex traits it is desirable to identify favourable combinations of alleles at multiple loci. This approach is termed as association breeding which integrates of association analysis into breeding programs (Sorrells et al 2009). In association breeding, genotypic data (providing whole genome coverage) and the appropriate analyses are incorporated to validate previously mapped marker or trait associations and potentially identify new ones to facilitate marker-assisted selection of parents and segregating populations. This information is used to estimate allelic value at selected loci (or all loci in genome-wide selection) and then create a genotypic value index for each genotype and trait (Christopher et al 2007).

(ii) Breeding by design

Conceptually, with the availability of huge genomic and genetic resources, high throughput genotyping in rice, it seems that through breeding by design, the *in silico* designing of superior genotypes by combining favourable allelic combinations at multiple loci governing yield should be possible (Peleman and van der Voort 2003).

(iii) Genomic selection

Other attractive concept for improving complex traits is genomic selection (GS) is an approach (Meuwissen et al 2001) that uses all the available molecular markers across the genome and allows calculation of genomic estimated breeding values (GEBVs). GS uses a 'training population' of individuals that have been both genotyped and phenotyped to develop a model that takes genotypic data from a 'candidate population' of untested individuals and produces genomic estimated breeding values (Jannink et al 2010). However, phenotyping still remains the weakest link in the application of these novel breeding strategies and further refinements would be needed in optimizing these techniques for cost effective implementation in the application of these futuristic concepts for developing designer crops for crop improvement.

e. Strategies to identify and utilize novel alleles to improve yield in rice**(i) Identification and pyramiding of QTLs from mega rice varieties**

Mega varieties of such as Swarna, Sambha Mahsuri, IR8 and IR64 are adapted to varied ecosystems and occupy a huge area under rice cultivation. Therefore, identification and pyramiding of QTLs from these mega varieties could be another effective strategy for harnessing the cumulative or interactive benefits of multiple genetic loci for yield and its components into proven yield advantage. Mapping of such genetic loci from diverse lines and combining them can be accomplished through a strategy such as developing NAM population discussed earlier in this chapter.

(ii) Pyramiding heterotic QTLs from widely grown commercial hybrids

A total of 60 rice hybrids have been released for commercial cultivation in India. Some of the most widely grown hybrids include PA6444, PHB71, Pusa RH10 and PA6129. Most of the released hybrids are half sibs as they are based on a common seed parent, IR58025A. In spite of the limitation of narrow genetic diversity from the female side, these hybrids are adapted to varied ecosystems. Mapping of heterotic QTLs in the F_2 or immortalized F_2 population developed from crosses involving B line into respective restorer and selective introgression of these QTLs into opposite parental lines may lead to development of super inbreds which may result into enhanced level of heterosis. Use of immortalized F_2 population (Hua et al 2003) is of special significance in identifying heterotic QTL as this approach permits multiplication of all possible heterozygotes in desired quantity for conducting multilocation or replicated trials.

(iii) *indica-japonica* hybridization

The *indica-japonica* hybridization was started in early 1950s on a large scale with a view to combine the desirable attributes of *japonica* sub species such as strong culm, dark-green erect leaves, high photosynthetic efficiency with high tillering and better quality of *indica* rice. However, it could not result into a major breakthrough owing to cryptic structural hybridity. With the discovery of wide compatibility locus, the interest in *indica/japonica* hybridization has been reinvigorated. The new plant type (NPT) and hybrids derived thereof, have huge potential for breaking the yield barrier. However, the efforts on these lines are limited outside China and IRRI. Developing mapping population utilizing NPT as one parent in crosses with mega-*indica* rice varieties may help in discovering novel allele from *japonica* with favourable effect in *indica* background (Balram et al 2007).

(iv) Utilization of land races, wild and weedy relatives

The value of germplasm including land races, wild and weedy relatives in terms of the latent traits (traits that are expressed only in favourable genetic background) has been demonstrated beyond doubt in rice and tomato (Xiao et al 1996b; Tanksley and McCouch, 1997). However, very few concerted efforts have been made for utilization of the huge genetic wealth. With the phenomenal developments in rice genomics and understanding of genome organization/ complexity of wild and weedy relatives of rice (Wing et al 2005), it should be possible to overcome the domestication bottlenecks and transfer favourable alleles from exotic sources (McCouch et al 2007).

From achieving yield gains through conventional breeding, advances in rice genomics have helped in gaining knowledge on the genetic and molecular basis of grain yield. This has helped to improve grain yields through introgressions of major QTL and in the near future it would possible to combine novel alleles from landraces or wild relatives at multiple loci which interact favourably to increase yield through improvement of component traits (Fig. 2). To achieve the breakthrough in yield gains, some of the pertinent issues that need to be addressed are:

- How many QTLs to pyramid in order to achieve the theoretical maximum yields considering the fact that every introgression of a new allele happens at cost of the replacement of the corresponding native allele, which has its own contribution in a given background?

➤ What is the favourable combination of alleles at QTLs which would help in maximizing the gain because QTLs expression is context dependent to a large extent resulting in variable expression in different genetic background?

➤ Biological potential of the crop as influenced by factors such as carrying capacity, photosynthetic rate, translocation efficiency and partitioning of assimilates etc.?

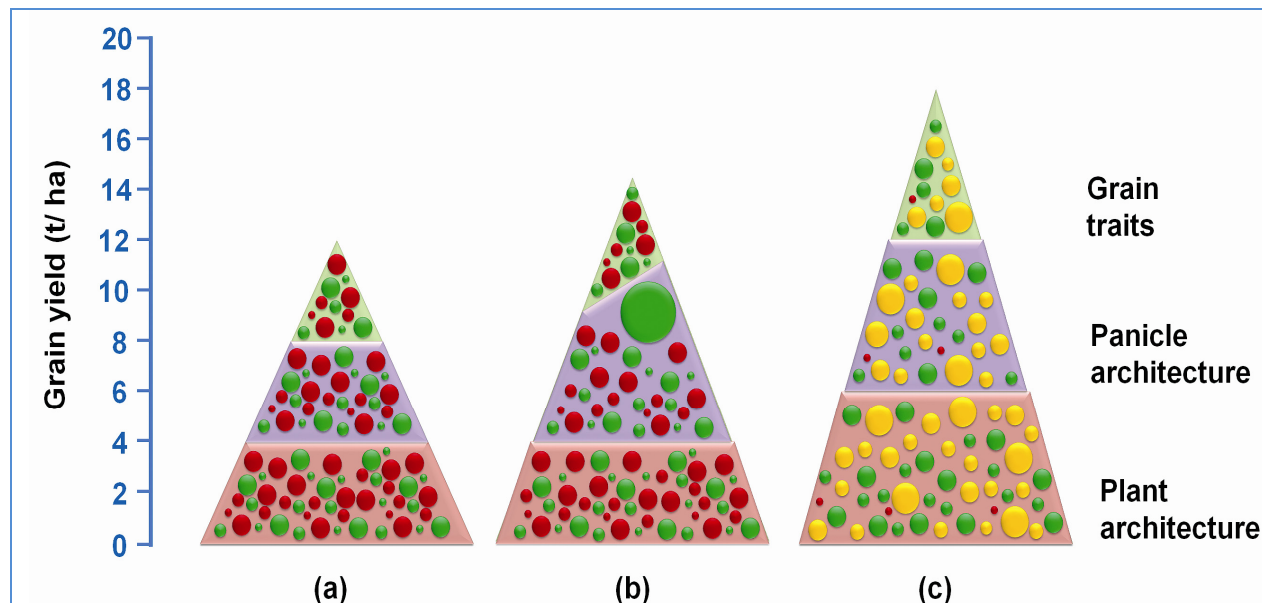


Figure 2. A model showing various strategies for improving grain yield in rice utilizing the present understanding on QTLs influencing yield and yield components. Green bead – indicates desirable allele at a locus, red bead – indicates undesirable allele and the size of the bead indicates the magnitude of contribution of respective locus towards realization of trait phenotype.

The traits contributing to grain yield are grouped into three major categories: (i) plant architecture such as tiller number, culm strength, leaf angle, plant height, etc, (ii) panicle architecture such as grain number per panicle, panicle length, primary and secondary branches, spikelet fertility, etc, and (iii) grain traits such as grain weight, grain size, grain filling, etc.

a = Conventional breeding approach to produce thousands of recombinants through intercrossing of genotypes and identify productive recombinants based on phenotypic performance. The best genotype possesses desirable alleles at some loci and undesirable alleles at other loci for a particular trait that limit the extent a trait can be improved through this approach. The yield pyramid is well balanced but the maximum yield under optimum growth conditions through this approach is only 12 t/ha.

b = Though marker assisted breeding approach, a major QTL governing a trait is incorporated into a popular variety. The incorporation of a major QTL such as *Gn1a* in this case can typically produce yield gains of 15 - 20% by improving grain number per panicle but the undesirable alleles at other loci governing grain number trait will still limit the yield gain. The yield pyramid is also slightly imbalanced due to changes resulting from incorporation of a major QTL.

c = Innovative breeding approach which entails mapping of QTLs governing yield traits and identifying novel elite alleles (shown as yellow beads) through introgression of novel alleles from landraces and wild species and combining the elite synergistic alleles into a genotype. This approach utilizes the advances in understanding the genetic and molecular bases of traits and precision offered by high-throughput genotyping and phenotyping. It aids accurate characterization of genotype with high density markers such as SNPs and phenotyping agronomic trait using high throughput phenomics facility. This enables to identify and pyramid favourable complimentary alleles to maximize the expression of component traits to achieve the best gains in yield improvement. The yield pyramid is completely balanced with a significant increase in expression of alleles (shown by increase in size of beads) and the genotype can produce grain yield of 18 t/ha.

Advances in sequencing technology has played a vital role in creating resources which has enhanced our capability to resolve genetic variants which in turn has helped in understanding of the molecular basis underlying various traits in crop plants. The combination of advanced sequencing technologies, genome-wide association studies, novel mapping populations (Morrell et al 2012) and tools to analyse the huge volume of data generated using these techniques will improve our ability to connect phenotypes and genotypes, and apply of these techniques for crop improvement.

REFERENCES

- Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H, Matsumura H, Yoshida K, Mitsuoka C, Tamiru M, Innan H, Cano L, Kamoun S and Terauchi R.** 2012. Genome sequencing reveals agronomically important loci in rice using MutMap. *Nat Biotech* doi:10.1038/nbt.2095.
- Andersson L and Georges M.** 2004. Domestic-animal genomics: deciphering the genetics of complex traits. *Nat Rev Genet* 5:202–212.
- Ashikari M and Matsuoka M.** 2006. Identification, isolation and pyramiding of quantitative trait loci for rice breeding. *Trends Plant Sci* 11(7): 344-50.
- Bai XF, Wu B and Xing YZ.** 2012. Yield-related QTLs and their applications in rice genetic improvement. *J Integr Plant Biol* doi:10.1111/j.1744-7909.2012.01117.x.
- Balram M, Richa S, Anand S, Nagarajan M, Singh NK and Singh AK.** 2007. Development and characterization of a Recombinant Inbred Line (RIL) mapping population in rice (*Oryza sativa* L.). *Pusa Agri Sci* 29: 16-21.
- Balram M.** 2008. *Molecular mapping of yield and yield related QTLs using recombinant inbred line population in rice (Oryza sativa L.)* Ph.D.Thesis Indian Agric Res Inst, New Delhi.
- Barbazuk WB, Emrich SJ, Chen HD, Li L and Schnable PS.** 2007. SNP discovery via 454 transcriptome sequencing. *Plant J* 51: 910-918.
- Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ, Browne C, Ersoz E, Flint-Garcia S, Garcia A, Glaubitz JC, Goodman MM, Harjes C, Guill K, Kroon DE, Larsson S, Lepak NK, Li H, Mitchell SE, Pressoir G, Peiffer JA, Oropeza Rosas M, Rocheford TR, Romay MC, Romero S, Salvo S, Sanchez Villeda H, da Silva HS, Sun Q, Tian F, Upadaya N, Ware D, Yates H, Yu J, Zhang Z, Kresovich S and McMullen MD.** 2009. The genetic architecture of maize flowering time. *Science* 325: 714-718.
- Christopher M, Mace E, Jordan D, Rodgers D, McGowan P, Delacy I, Banks P, Sheppard J, Butler D and Poulsen D.** 2007. Applications of pedigree-based genome mapping in wheat and barley breeding programs. *Euphytica* 154: 307-316.
- Deshmukh R, Singh A, Jain N, Anand S, Gacche R, Singh A, Gaikwad K, Sharma TR, Mohapatra T and Singh NK.** 2010. Identification of candidate genes for grain number in rice (*Oryza sativa* L.). *Funct Integr Genomics* 10:339-347.
- Druley TE, Vallania FLM, Wegner DJ, Varley KE, Knowles OL, Bonds JA, Robison SW, Doniger SW, Hamvas A and Cole FS.** 2009. Quantification of rare allelic variants from pooled genomic DNA. *Nat Methods* 6: 263-265.
- FAO.** 2011. *Crop prospects and food situation 2*: 6.
- Goel N, Musarrat J, Balram M, Gopalakrishnan S, Bhowmick PK, Singh D, Nagarajan M, Vinod KK, Singh NK, Prabhu KV and Singh AK.** 2012. Fine mapping of a major QTL controlling grain number and related traits in rice (*Oryza sativa* L.). Abs. *Int Conf Plant Biotechnology for Food Security: New Frontiers*, Feb 21-24, 2012, National Agricultural Science Centre, New Delhi, India.
- Goffinet B and Gerber S.** 2000. Quantitative trait loci: a meta-analysis. *Genetics* 155: 463-473.
- Gopalakrishnan S, Waters DLE, Katiyar SK, Sadananda AR, Satyadev V and Henry R.** 2012. Genome-wide DNA polymorphisms in elite indica rice inbreds discovered by whole-genome sequencing. *Pl Biotech J* doi: 10.1111/j.1467-7652.2011.00676.x.
- Haley C.** 2011. A cornucopia of maize genes. *Nat Genet* 43: 87-88.
- Henry RJ, Edwards M, Waters DLE, Gopalakrishnan S, Bundock P, Sexton TR, Masouleh AR, Nock CJ and Pattimore J.** 2012. Molecular markers for plants derived from large scale sequencing. *J Biosci* 37(4): *in press*.
- Hirochika H, Guiderdoni E, An G, Hsing Y, Eun MY, Han C, Upadhyaya N, Ramachandran S, Zhang Q, Pereira A, Sundaresan V and Leung H.** 2004. Rice mutant resources for gene discovery. *Plant Mol Biol* 54: 325-334.
- Hospital F.** 2009. Challenges for effective marker-assisted selection in plants. *Genetica* 136:303-310.
- Hua J, Xing Y, Wu W, Xu C, Sun X, Yu S and Zhang Q.** 2003. Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. *Proc Natl Acad Sci USA* 100: 2574-2579.

- Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y, Li C, Zhu C, Lu T, Zhang Z, Li M, Fan D, Guo Y, Wang A, Wang L, Deng L, Li W, Lu Y, Weng Q, Liu K, Huang T, Zhou T, Jing Y, Li W, Lin Z, Buckler ES, Qian Q, Zhang Q, Li J and Han B. 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. *Nat Genet* 42: 926–927.
- Jannink J, Lorenz AJ and Iwata H. 2010. Genomic selection in plant breeding: from theory to practice. *Brief Funct Genomics* 9:166-177.
- Jeon JS, Jung KH, Kim HB, Suh JP and Khush GS. 2011. Genetic and molecular insights into the enhancement of rice yield potential. *J Plant Biol* 54:1–9.
- Kharabian-Masouleh A, Waters DLE, Reinke RF and Henry RJ. 2011. Discovery of polymorphisms in starch related genes in rice germplasm by amplification of pooled DNA and deeply parallel sequencing. *PL Biotech J* 9: 1074-1085.
- Kump KL, Bradbury PJ, Wissner RJ, Buckler ES, Belcher AR, Oropeza-Rosas MA, Zwonitzer JC, Kresovich S, McMullen MD, Ware D, Balint-Kurti PJ and Holland JB. 2011. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nat Genet* 43: 163-168.
- Li D, Wang L, Wang M, Xu YY, Luo W, Liu YJ, Xu ZH, Li J and Chong K. 2009. Engineering *OsBAK1* gene as a molecular tool to improve rice architecture for high yield. *Plant Biotechnol J* 7(8):791-806.
- Liu S, Yeh CT, Tang HM, Nettleton D and Schnable PS. 2012. Gene mapping via Bulk Segregant RNA-Seq (BSR-Seq). *PLoS ONE* 7: e36406.
- McCouch SR, Sweeney M, Li J, Jiang H, Thomson M, Septiningsih E, Edwards J, Moncada P, Xiao J, Garris A, Tai T, Martinez C, Tohme J, Sugiono M, McClung A, Yuan LP and Ahn SN. 2007. Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa*. *Euphytica*, 154:317-339.
- Meuwissen THE, Hayes BJ and Goddard ME. 2001. Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157: 1819-1829.
- Miura K, Ashikari M and Matsuoka M. 2011. The role of QTLs in the breeding of high-yielding rice, *Trends Plant Sci* 16: 319-326.
- Mochida K and Shinozaki K. 2010. Genomics and bioinformatics resources for crop improvement. *Plant Cell Physiol* 51: 497-523.
- Morrell PL, Buckler ES and Ross-Ibarra J. 2012. Crop genomics: advances and applications. *Nat Rev Genet* 13: 85-96.
- Ohsumi A, Takai T, Ida M, Yamamoto T, Sanoh YA, Yano M, Ando T and Kondo M. 2011. Evaluation of yield performance in rice near-isogenic lines with increased spikelet number. *Field Crops Res* 120:68-75.
- Out AA, van Minderhout I, Goeman JJ, Ariyurek Y, Ossowski S, Schneeberger K, Weigel D, van Galen M, Taschner PEM and Tops CMJ. 2009. Deep sequencing to reveal new variants in pooled DNA samples. *Hum Mut* 30: 1703-1712.
- Park HS, Ryu HY, Kim BH, Kim SY, Yoon IS and Nam KH. 2011. A subset of *OsSERK* genes, including *OsBAK1*, affects normal growth and leaf development of rice. *Mol Cells* 32(6): 561-569.
- Paterson A, Lander ES, Hewitt JD, Peterson S, Lincoln SE and Tanksley SD. 1988. Resolution of quantitative traits into Mendelian factors using a complete linkage map of restriction fragment length polymorphisms. *Nature* 335: 721-726.
- Peleman JD and van der Voort JR. 2003. Breeding by design. *Trends Pl Sci* 8: 330-334.
- Poland JA, Bradbury PJ, Buckler ES and Nelson RJ. 2011. Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize *Proc Natl Acad Sci USA* 108: 6893-6898.
- Singh AK and Gopalakrishnan S. 2012. Development and use of mapping populations in crops: genetic considerations. In: Training Manual. *Winter School on molecular approaches for allele mining and crop improvement*, Indian Agricultural Research Institute, New Delhi, 18 p.
- Sorrells ME, Heffner EL and Jannink JL. 2009. Association breeding strategies for improvement of self pollinated crops. In: *Genomics-Assisted Breeding: W232*, Plant and Animal Genome XX Conf, January 10-14, 2009., San Diego, USA, 3081.
- Stranger BE, Stahl EA and Raj T. 2011. Progress and promise of genome-wide association studies for human complex trait genetics. *Genetics* 187: 367-383.
- Stuber CW, Lincoln SE, Wolff DW, Helentjaris T and Lander ES. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* 132(3): 823- 839.
- Swamy BPM and Sarla N. 2008. Yield-enhancing quantitative trait loci (QTLs) from wild species, *Biotech Adv* 26: 106-120.
- Swamy BPM and Sarla N. 2011. Meta-analysis of yield QTLs derived from inter-specific crosses of rice reveals consensus regions and candidate genes. *Plant Mol Biol Rep* 29: 663-680.

- Swamy BPM, Vikram P, Dixit S, Ahmed HU and Kumar A.** 2011. Meta-analysis of grain yield QTL identified during agricultural drought in grasses showed consensus. *BMC Genomics* 12: 319.
- Tanksley S.** 1993. Mapping polygenes. *Annu Rev Genet* 27:205-233.
- Tanksley S and McCouch S.** 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277:1063-1066
- Tian F, Bradbury PJ, Brown PJ, Hung H, Sun Q, FlinGarcia S, Rocheford TR, McMullen MD, Holland JB and Buckler ES.** 2011. Genome-wide association study of leaf architecture in the maize nested association mapping population. *Nat Genet* 43: 159-162.
- Vaughan D, Morishima H and Kadowaki K.** 2003. Diversity in the *Oryza* genus. *Curr Opin Plant Biol* 6:139-146.
- Wang P, Xing YZ, Li ZK and Yu S.** 2012. Improving rice yield and quality by QTL pyramiding. *Mol Breed* 29: 903-913.
- Wang Z, Gerstein M and Snyder M.** 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10: 57-63.
- Wing RA, Ammiraju JS, Luo M, Kim H, Yu Y, Kudrna D, et al.** 2005. The *Oryza* map alignment project: the golden path to unlocking the genetic potential of wild rice species. *Plant Mol Biol* 59: 53-62.
- Xia K, Wang R, Ou X, Fang Z, Tian C, Duan J, Wang Y and Zhang M.** 2012. *OsTIR1* and *OsAFB2* downregulation via *OsmiR393* overexpression leads to more tillers, early flowering and less tolerance to salt and drought in rice. *PLoS ONE* 7: e30039.
- Xiao J, Li J, Yuan L and Tanksley SD.** 1996a. Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. *Theor Appl Genet* 92: 230-244.
- Xiao J, Grandillo S, Sang N, McCouch S and Tanksley S.** 1996b. Genes from wild rice improve yield. *Nature* 384:223-224
- Xiao JH, Li JM, Crandillo S, Ahn SN, Yuan LP, Tanksley SD and McCouch SR.** 1998. Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. *Genetics* 150: 899-909.
- Yu J, Holland JB, McMullen MD and Buckler ES.** 2008. Genetic design and statistical power of nested association mapping. *Genetics* 178: 539-551.
- Zhao K, Tung CW, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Bustamante CD and McCouch SR.** 2011. Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nat Comm* 2:467.
- Zhu C, Gore M, Yu J and Buckler ES.** 2008. Status and prospects of association mapping in plants. *Plant Genome* 1(1): 5-20.
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