

RICE BREEDING IN THE GENOMICS ERA: PERSPECTIVES

D S Brar^{1*} and G S Khush²

¹School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141004, Punjab

²University of California, Davis, CA - 95616 USA

Rice is the most important food crop and a staple food for more than half of the world's population. Worldwide, more than 3.5 billion people depend on rice for more than 20% of their daily calories. Global rice demand is estimated to rise from 723 million tons in 2015 to 852 million tons in 2035, an overall increase of 18% or 129 million tons in the next 20 years. World rice production increased from 256 million tons in 1960 to 680 million tons in 2010. This was achieved primarily through the application of principles of Mendelian genetics and conventional plant breeding coupled with improved production technologies. Achieving the target of an additional 129 million tons in the next 20 years is more challenging, given the continual threat to rice by major diseases such as bacterial blight (BB), blast, tungro, and sheath blight; insects such as brown planthopper (BPH), stem borers, and gall midge; and abiotic stresses such as drought, floods, salinity, cold, heat, etc., particularly in the context of global climate change. The major challenge at present is how to increase rice productivity in an eco-friendly way using fewer chemicals, less water, less land, and less labor (Khush, 2001, 2005). Recent advances in molecular biology and cutting edge science of genomics offer new opportunities to overcome some of these constraints that limit crop productivity and to develop improved germplasm with new genetic properties, accelerate crop breeding programs, and understand through functional genomics function of the genes that govern agronomic traits.

Advances in rice biotechnology and genomics research

During the last few decades, major advances have been made in rice biotechnology and genomics research and more recently with the genome sequencing of indica and japonica rices including wild species of *Oryza*. The first genome to be sequenced was of the bacterium, *Haemophilus influenzae* followed by other organisms such as *Caenorhabditis elegans* (worm), *Drosophila melanogaster* (fruitfly), *Homo sapiens* (human). Among the plant species, *Arabidopsis thaliana* was the first plant whose genome was sequenced in 2000, later in 2005 International Rice Genome Sequencing Project (IRGSP) involving scientists from 10 countries published the genome sequence of rice. Since then, genomes of more than 100 plant species and other organisms have been sequenced. Advances in sequencing technologies and a dramatic drop in

the cost for sequencing have made sequencing a routine technique to generate huge data sets which can be used to determine the genetic architecture of plant species and in exploitation of novel genetic information for breeding superior crop varieties.

In this genomics era, paradigm shift in rice breeding is envisaged particularly with the advances in molecular marker technology and genome sequencing. Several review papers are available on advances in rice breeding and genomics research (Khush, 1995, 2001, 2005; Khush and Brar, 2001; Brar and Khush, 2006, 2013). We have recently reviewed various strategies on frontiers in rice breeding (Brar *et al.*, 2017), a few of which are enumerated in this publication. Some recent developments in rice biotechnology include: (1) construction of dense molecular maps and a new generation of markers such as SNPs, facilitating the mapping of genes/QTLs, (2) tagging and pyramiding of many important genes/QTLs for use in marker-assisted selection (MAS) to accelerate breeding programs for enhancing tolerance of biotic and abiotic stresses and improved quality traits, (3) map-based cloning of agronomically important genes that has facilitated gene-based MAS and allele mining, (4) broadening the gene pool of rice through introgression of novel genes/QTLs from wild species of *Oryza*, (5) availability of large set of new genomics resources such as T-DNA insertion lines (>100,000), 60,000 deletion mutants, and BAC, and EST libraries for functional genomics, (6) high-throughput genotyping (SNP chips, genotyping-by-sequencing) for MAS and gene chips for gene discovery and expression analysis and high throughput phenotyping for biotic and abiotic stresses and other traits, (7) *Agrobacterium*-mediated transformation resulting into large-scale production of transgenics with new genetic properties, including pyramiding of transgenes and (8) availability of whole genome sequence data on indica and japonica rice and bioinformatics for applying forward and reverse genetics in plant breeding. Functional genomics offers new ways to understand the function of genes that govern agronomic traits and to manipulate such genes in rice breeding programs.

In addition, genome selection offers advantage over traditional marker assisted selection for quantitative traits to achieve higher genetic gains by developing superior inbred cultivars and heterotic hybrids. RNAi and genome editing technologies are becoming additional new genomic tools to support plant breeding programs for developing pest resistant varieties and improved grain quality. New tools in biotechnology are being used to engineer the photosynthetic

*Corresponding author : darshanbrar@pau.edu
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system and to introduce C₄ pathway-related genes into C₃ rice. Possibilities are being explored to enhance nitrogen use efficiency (NUE) through manipulation of genes for uptake and transport of nutrients and their interaction with soil microbes. The identification and manipulation of endophytes would be important for enhancing biological nitrogen fixation (BNF). Exploratory research using genomic tools is suggested to develop apomictic rice. Integration of the molecular approaches and genomic tools with conventional breeding is emphasized to accelerate breeding programs, and to increase rice productivity and sustainability.

Rice breeding programmes

Rice is grown under varied ecologies and agro-climatic conditions, which include: irrigated rice (55%), rainfed lowland (25%), upland (12%) and flood prone (8%). It was grown on 158 million hectares with production of 730 million tons in 2015. More than 90% of rice is grown and consumed in Asia. Following are the rice breeding objectives:

1. Increasing the yield potential of inbred varieties and hybrids
2. Maintenance breeding for yield stability with multiple resistance to biotic and abiotic stresses.
3. Enhancing productivity and yield stability with multiple resistance to biotic and abiotic stresses under unfavorable environments
4. Improving grain and nutritional quality
5. Breeding high yielding rice varieties suitable for dry direct seeding

Conventional breeding has been used worldwide over the years for rice improvement. Several success stories in conventional breeding include (i) development of semi-dwarf high yielding rice varieties which ushered into green revolution (ii) utilization of CMS system to develop high yielding hybrid rice varieties (iii) development of high yielding early maturing varieties resulting into increased cropping intensity (iv) development of a series of high yielding varieties with multiple resistance to diseases and insects and tolerance to major abiotic stresses which account for yield stability and reduced use of chemicals and (v) development of series of varieties with improved grain quality characteristics (texture, appearance, cooking and eating quality and aroma). As an example more than 1,000 varieties have been released through conventional breeding in India. Similarly IRRI breeding lines have been released as 900 varieties in 78 countries. Khush (1995, 2001, 2013) has reviewed the strategies used in conventional breeding resulting in the increased rice productivity and stability in yield.

Increasing yield potential of inbreds and hybrids

Conventional breeding involving hybridization between selected parents and selection of desirable genotypes

(recombinants) from the segregating progenies will remain widely used strategies for developing crop varieties with a higher yield potential. The segregating populations are screened for desirable recombinants and selected lines are evaluated in replicated yield trials. Promising lines are finally evaluated in larger plots over different environments and at research stations and at farmer's field for possible release as a variety.

Breeding strategies to increase yield potential

1. Ideotype breeding and exploitation of tropical japonica germplasm
2. Exploitation of heterosis using diverse indica and tropical japonica
3. Identification and introgression of yield enhancing loci/QTLs from traditional and wild species germplasm
4. Allele mining and pyramiding of favorable alleles for key yield related traits
5. Engineering photosynthetic systems to enhance yield potential—transfer of C₄ pathway genes into C₃ rice

Ideotype breeding and exploitation of tropical japonica germplasm: The development of semi-dwarf varieties responsive to fertilizer ushered into green revolution. The so called miracle rice variety "IR8" developed at IRRI in 1966 had biomass around 20 tons/ha with harvest index (HI) of 0.5 accounting for 10t/ha paddy yield. IR8 was short statured had profuse tillering, dark green and erect leaves and sturdy stem. On the other hand, tall and traditional rice varieties could produce a biomass of 13-14 t/ha under most conditions, and their HI was around 0.3. To increase the yield potential of semi-dwarf rice further, IRRI scientists proposed a new plant type (NPT) in 1989 (IRRI, 1989) with characteristics such as reduced tillering (9-10 tillers for transplanted conditions), no nonproductive tillers, 200 - 250 grains per panicle, dark green and erect leaves, a vigorous and deep root system, growth duration of 110-130 days, multiple disease and insect resistance, and higher harvest index.

More than 2,000 crosses were made involving indica x tropical japonica (Bulu) and more than 100,000 pedigree lines were evaluated (Khush, 2001). Breeding lines with desirable ideotype traits were selected and more than 500 NPT-TJ type were selected. Some of these lines showed good agronomic performance in temperate areas as well, where japonica-type grain quality is preferred. (Khush, 2013) Three of these lines were released as varieties in Yunnan Province of China as Diancho-1, Diancho-2, and Diancho-3. To improve the adaptability of these NPT-TJ lines for tropical conditions and to improve their yield potential, they were crossed with elite indica lines and varieties having disease and insect resistance and good grain quality. Several lines designated as NPT- IJ types were evaluated in yield trials. Several of these lines outyielded the best improved indica varieties such as IR72 by as much as 1.0–1.5 t/ha. These breeding lines are being

continuously used in breeding programs and have contributed to increased genetic diversity through the introduction of japonica germplasm into otherwise indica breeding materials.

Like the NPT breeding programme, Chinese scientists proposed another ideotype with attributes such as moderate tillering, heavy panicles (5 g/panicle), slightly taller plant height (about 100 cm), and the top three leaves having characteristics such as flag-leaf length of about 50 and 55 cm for the second and third leaves. All three leaves should be above panicle height and should remain erect until maturity. Leaf area index (LAI) of the top three leaves should be about 6.0 and should have 0.55 harvest index (Yuan, 2001). Rice breeders in China have developed many inbreds and hybrids of this ideotype.

Exploitation of heterosis: Hybrid breeding exploits the increased vigor or heterosis (yield advantage of F₁ hybrids over varieties). In maize, major yield improvement has been associated with the introduction of F₁ hybrids on a commercial scale, average yield advantage being 15-20%. Rice hybrids were introduced in China during the mid-1970s and are now planted on 13–14 million hectares or 50% of the total rice area in that country. The average yield advantage of the current hybrids over varieties is 10–15%. Limited adoption of rice hybrids in other countries is due to the low level of heterosis. Most breeding programs in the tropics have used indica germplasm in hybrid development, which has limited genetic diversity.

To enhance diversity and the level of heterosis, it is suggested to initiate pre-breeding of tropical japonica (TJ) to improve grain quality of japonica lines to match indica type. Once such TJ lines with indica type grain quality become available, only then use such TJ lines in crosses with indica to develop highly heterotic hybrids possessing superior grain quality like indica type. A few steps are listed:

(a) genotype a larger set (~500 accessions) of TJ types following genotyping by sequencing (GBS) approach (b) identify a smaller core set (~100 accessions) of diverse TJ lines (c) cross the core set of TJ with the best available CMS indica lines and evaluate F₁ hybrids for yield and yield components (d) identify highly heterotic hybrids and improve the parental TJ lines involved in these hybrids for grain quality and disease and insect resistance using MAS, ensure that the improved TJ lines possess *Wc* and *Rf* genes (e) use the improved TJ lines as male parent in crosses with CMS lines for the production of commercial F₁ hybrids. Such hybrids are expected to be highly heterotic with increased yield potential.

Identification and introgression of yield enhancing loci/QTLs from wild species germplasm: Wild species of *Oryza* are grass like plants and are weedy and shattering in nature, have low yield possess poor plant type and poor grain characteristics but are important reservoir of useful genes for rice improvement particularly for tolerance to biotic and abiotic stresses and also possess yield enhancing loci/QTLs.

Since the findings of Xiao *et al.* (1996), on identification of two yield enhancing loci, *ylt1* and *ylt2* linked with marker RM 5 on chromosome 1 and RG 256 on chromosome 2 respectively, from *O. rufipogon*, several QTLs for yield related traits from wild species have been identified (Imai *et al.*, 2013; Khush and Brar, 2017).

Future research should focus on the identification of novel QTLs with larger effect for productivity traits from wild species. Introgression and or pyramiding of such QTLs/wild species alleles in the genetic background of high yielding varieties should receive priority. Genomic selection coupled with genotyping by sequencing (GBS) for the simultaneous identification and transfer of QTLs for productivity traits from wild to cultivated species should be undertaken.

Allele mining and pyramiding of favorable alleles for key yield related traits: So far most genetic research has focused on identifying and pyramiding genes/QTLs for tolerance of biotic and abiotic stresses. However, with new tools of genomics and the availability of whole-genome sequence, it has now become easier to clone genes/QTLs even for yield-related traits. A map-based cloning strategy and specialized genetic stocks such as near-isogenic lines (NILs) and chromosome segment substitution lines (CSSLs) have been used to clone genes/QTLs for key yield components such as (i) number of grains per panicle, (ii) grain size and weight, and (iii) number of tillers per plant, including grain filling. So far, more than 20 yield-related QTLs have been cloned and their biology well understood (Ashikari and Matsuoka, 2006; Miura *et al.*, 2011; Bai *et al.*, 2012). As an example, for grain number alone, five genes (*Gn1*, *Dep3*, *Ghd7*, *DTH8*, and *Dep1*) located on chromosomes 1, 6, 7, 8, and 9, respectively, have been cloned. The nature of the alleles desirable for breeding programs is also varied, including loss of function (*dep1*), high expression (*WFP*), or low expression (*Gn1a*). A major challenge in stacking these QTLs is not technological but the biology, regarding how these genes will interact with each other when desirable alleles at all the loci are pyramided. A second challenge to the pyramiding of these QTLs is the identification of alleles present in the recipient line. Many times, the functional marker or the markers closely linked to the QTL/gene are not associated with the functional domains of the gene. Hence, before initiating pyramiding of QTLs for the same trait, it is advisable to sequence the allele in the recipient parent and compare it with the donor parent allele. If the two alleles are functionally different, then only the pyramiding should be continued, or else one may not find any superiority in performance of the pyramided lines.

The strategy comprises of (i) identification of desirable alleles for the target yield related traits from the available germplasm based on the information on the cloned genes/QTLs (ii) developing isogenic lines for the selected allele in a common genetic background using MAS, (iii) pyramiding these genes and (iv) evaluation of pyramided lines for possible increase in yield potential over the high yielding

recurrent parent. The above strategy looks quite promising but needs validation on priority.

Engineering photosynthetic systems to enhance yield potential—transfer of C4 pathway genes into C3 rice: The C4 photosynthetic system is more efficient and offers several advantages over C3. The C4 rice could yield 25% more than the existing C3 rice and in addition could have higher nutrient- and water-use efficiency. Converting C3 rice to C4 is a long-term (15–20 years) option. Some approaches for converting C3 to C4 involve improving radiation-use efficiency (RUE) through the regulation of Rubisco, the introduction of C4-like traits such as CO₂ concentrating mechanisms, and improvement in light interception. Thus improvement of photosynthesis at the whole-canopy level is being explored as an option to increase yield potential of rice. IRRI in collaboration with partner institutes in USA, UK and others are using genomic and other transgenic approaches to transfer genes from C4 system into C3 rice. It is a long term project would involve extensive research to fine tune anatomy, biochemistry and physiological attributes before fully functional C4 rice could become available.

Maintenance breeding for yield stability with multiple resistance to biotic and abiotic stresses

Several biotic and abiotic stresses adversely affect rice production and yield stability particularly in the context of climate change. In addition, new diseases and pests are emerging with the change in pathogen races and insect biotypes. On an average, yield losses could be as high as 20–30%. To cope up with these losses and maintain yield stability, maintenance breeding is an important option.

Plant breeders and geneticists have identified several donors from diverse sources (primitive cultivars, landraces, wild species) for tolerance to disease and insects. Similarly several donors for tolerance to abiotic stresses such as drought, submergence, salinity, soil toxicities, heat and cold have been identified. These donors have been used to develop varieties with multiple resistance to biotic and abiotic stress through conventional breeding.

A number of genes for resistance to diseases and insects have been identified using classical genetic analysis. Some examples on resistance genes identified include 30 genes for BB, 75 for blast, 25 for BPH, 10 each for GLH and gall midge. These genes have been successfully used in resistance breeding and numerous varieties with multiple resistance to diseases and insects released worldwide. Among numerous examples, IR 36 and IR 64 varieties are the classical examples developed through conventional breeding which have multiple resistance to several biotic and abiotic stresses.

Some genes from wild species with wide spectrum of resistance have also been introgressed into rice cultivars. Examples include *Xa21*, *Xa25*, *Xa27*, *Xa29*, *Xa38* for bacterial blight; *Pi9*, *Pi40* for blast; *Gs* for grassy stunt; *Bph10*, *bph11*, *bph12*, *Bph13*, *Bph14*, *Bph18*, *Bph20*, *Bph21* for BPH. *Wbph7*,

Wbph8 for resistance to white backed planthopper (Khush and Brar, 2001, 2017). Transfer of these genes into improved rice varieties has resulted into yield stability of rice and saved million of dollars by reducing use of pesticides.

Broadening gene pool of rice through introgression of genes from wild species

Wide hybridization involves hybridization between cultivated species and their wild relatives. It is an important plant breeding method to broaden the gene pool for tolerance of major biotic and abiotic stresses. The genus *Oryza* to which cultivated rice (*O. sativa* 2n = 24, AA) belongs has 24 species (2n = 24, 48) representing different genomes (AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, and HHKK). These wild species are an important reservoir of useful genes for resistance to diseases and insects and tolerance of abiotic stresses. Khush (1977) transferred grassy stunt resistance from *O. nivara* into IR24. Since then several genes have been transferred from different wild species. Some examples include transfer of resistance to tungro disease, BB, Blast, BPH and WBPH (Brar and Khush, 1997). Some of the IRRI breeding lines from wide crosses have been released as varieties in different countries (MTL 98, MTL 103, MTL 105, MTL 114, Matatag 9, AS 996, NSIC Rc 112) for commercial cultivation (Brar and Singh. 2011).

Molecular mapping of genes/QTLs and MAS

Advances in molecular marker technology and genomics have facilitated mapping and cloning of genes/QTLs for resistance to major diseases and insects. These genes/QTLs have been pyramided and transferred through MAS into elite breeding lines. One of the classical example is transfer of *Xa21* gene through MAS singly or in combination with other BB resistance genes *Xa4*, *xa5*, *xa13*. More than dozen resistant varieties with pyramided genes have been released in Philippines, India, China and others. Such BB resistance genes have also been transferred through MAS into several parental lines used in hybrid rice breeding (Brar and Singh, 2011). Pyramiding of genes for resistance to leaf blast have also been reported, however, progress in neck blast resistance breeding is slow, where high throughput screening protocols are lacking. Some QTLs have been identified for resistance to sheath blight. However large effect QTLs are not available. Hence priority should be given to pyramid QTLs with small effects, and also to identify large effect QTLs from diverse sources. Among insects, BPH is a major pest, and 30 genes for resistance are known. However, level of resistance of individual gene is not high. Thus pyramiding of different genes is suggested to develop BPH resistant varieties with wide spectrum of resistance to different biotypes.

RNAi is being used to develop stem borer and sheath blight resistant lines where genetic variability is limited in the germplasm. Priority should be given to develop pest resistant varieties with wide spectrum of resistance preferably with different mechanisms of resistance.

For many diseases and insects such as sheath blight (ShB), brown spot, and leaf folder, no major resistance genes are known in the cultivated or wild germplasm. However, germplasm lines with partial resistance are known for all these biotic stresses. For ShB, partial resistance has been reported in landraces and wild species. There is a need for pyramiding minor-effect QTL for ShB and other such biotic stresses. Emphasis should be given to map new QTLs from diverse genetic backgrounds followed by pyramiding of the minor-effect QTL into elite varieties.

Molecular characterization of pathogen populations for temporal and spatial deployment of genes for resistance

It is well known that pest populations, including insects, fungi, bacteria, viruses, and nematodes, are highly variable in morphological, physiological, and pathological (virulence) characteristics. Effective deployment of the genes could be achieved provided information on pathogen variability/virulence is known. Traditionally, differential sets are used for analyzing variability within pathogen/insect populations. With DNA sequencing technologies becoming cheaper and high-throughput, the whole genome of several insect and other pathogenic populations has become available. In rice, for example, whole-genome sequences of bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*, blast fungus *Pyricularia oryzae*, and brown plant hopper *Nilaparvata lugens* are available and in-depth understanding of the *vir/avr* genes is available for these pathogens. It is thus important to precisely characterize the pathogen population structure in different areas and hot spots so as to deploy relevant resistance genes in such areas based on the actual pathogen population and race/biotype of the pest. NILs carrying a series of known genes for resistance to BB, blast including pyramided lines are available which need to be tested in hot spots in different rice growing areas across different states, regions and countries. Based on the pathogen populations and disease reaction, deploy only those genes or combination of genes which confer resistance in specific locations.

Enhancing productivity and yield stability with multiple resistance to biotic and abiotic stresses under unfavorable environments

Majority of the rices in rainfed and upland ecologies are grown under unfavorable environments which include poor, infertile and toxic soils adversely affected by severe drought, salinity and floods, cold and heat. Besides several diseases and insects such as blast, brown spot and borers also attack rice crop in these ecologies. A number of donors for tolerance to abiotic stresses have been identified and used in conventional breeding. As a result, a number of varieties tolerant to these stresses have been developed. However, level of tolerance is low and also these varieties in general lack tolerance to abiotic stresses at reproductive stage, which is the most critical stage causing significant losses in yield.

Identification and introgression of genes/QTLs for tolerance to abiotic stresses

Molecular markers have facilitated tagging of genes / QTLs and to apply MAS for transferring and pyramiding of such QTLs. One of the most successful examples is the transfer of *Sub1* gene into several mega varieties. Following MAS, more than 12 varieties carrying *Sub1* which are tolerant to submergence have been released in India, Philippines, Nepal, Bangladesh and Indonesia (Brar and Singh, 2011). Allele mining for submergence is being explored to identify additional favorable alleles for submergence tolerance.

Many donors for tolerance to drought such as N22, Apo, Aday Sel, Moroberekan, Vandana and Way Rarem have been identified. In collaboration with partner institutes, IRRI has identified as many as 14 QTLs with large and consistent effects on grain yield under drought in the back ground of lowland and upland cultivars (Kumar *et al.*, 2014). These QTLs include *qDTY1.1*, *qDTY1.2*, *qDTY2.1*, *qDTY2.2*, *qDTY2.3*, *qDTY3.1*, *qDTY3.2*, *qDTY4.1*, *qDTY6.1*, *qDTY6.2*, *qDTY8.1*, *qDTY9.1*, *qDTY10.1*, and *qDTY12.1*. MAS was practiced to transfer these QTLs into mega varieties such as Swarna, IR64, MTU1010, Sabitri and TDK1. Some of these QTLs have been pyramided where these have shown increased tolerance to drought in combinations rather than single QTL. Future research should focus on introgression of QTLs for grain yield under drought stress at reproductive stage tolerance. A few lines combining tolerance to submergence and drought through introgression of *qDTY_{1,1'}*, *qDTY_{2,1'}* and *qDTY_{3,1'}* in Swarna Sub 1 have also been developed. To increase productivity and yield stability, priority should be given on developing rice varieties with multiple tolerance to different abiotic stresses at reproductive stage with different mechanisms of tolerance.

Several QTL/genes have been cloned for submergence tolerance (*SUB1A*), drought tolerance (*DRO1*), phosphorus-use efficiency (*PSTOLI*) etc. It would be interesting to look for allelic variation for these loci and evaluate the performance of various functional haplotypes for specific stresses and use the most efficient alleles in breeding programs. Alternatively, a combination of alleles could be stacked through the transgenic approach and their efficiency evaluated.

Enhancing nutrient use efficiency

Nitrogen (N), phosphorus (P), and potassium (K) are the three key nutrients required in higher quantities. On an average, the rice plant uses 30–40% of the applied N, and there is a considerable loss because of denitrification and nitrification that leaches into the lower strata of the soil, leading to contamination of groundwater. Rock phosphate, which is a source for P fertilizer, is concentrated in just five countries globally and it is expected to last for not more than a century. The same is true for K. Thus, breeding for N, P, and K efficiency should be a priority so as to save farmers' costs and environmental sustainability. Major constraints to

the identification of germplasm that uses nutrients efficiently are the screening techniques and the quantifiable phenotype. There is a need to develop efficient screening techniques and quantifiable phenotype for the mapping and cloning of genes/QTLs that can use N, P, K, and other nutrients more efficiently. A protein kinase gene conferring phosphorus starvation tolerance (*PSTOL1*) has been cloned from an upland rice variety (Kasalath) and it is invariably absent in varieties developed for lowland irrigated conditions. This gene is present invariably in upland cultivars and in some accessions of *O. glaberrima* in truncated form. Allelic variations, if any, need to be identified and used in enhancing nutrient uptake.

Overexpression of native genes, especially transcription factors, through the transgenic approach has been reported in rice and many other crops for a series of traits, including induction of tolerance of biotic and abiotic stress, and nutrient-use efficiency. One such recent example is overexpression of a pH-sensitive nitrate transporter gene in rice (Fan *et al.*, 2016). Overexpression of native nitrate transporter gene *OsNRT2.3b* in rice enhanced the pH-buffering capacity of the plant, thus increasing N, Fe, and P uptake. In field trials, increased expression of *OsNRT2.3b* was shown to increase grain yield and nitrogen-use efficiency (NUE) by 40%. Emphasis should be given to identify such genes for enhancing nutrient use efficiency of rice grown under irrigated, rain fed and other ecologies.

Improving grain and nutritional quality

Rice grain quality is highly variable depending upon the consumers preference. Grain size, shape, and appearance and cooking characteristics are important parameters of grain quality. Amylose content of grain determines its cooking quality. High amylose rice when cooked is dry and fluffy, low amylose rice is sticky. In Asia, rices with high (25 %), intermediate (20 %) and low (15 %) amylose are grown. Breeding programs focus to improve grain quality according to the needs of consumers. A large number of varieties with different quality characteristics have been released worldwide. Plant breeders are looking for new varieties with high head rice recovery, free from chalkiness and with improved cooking and eating quality characteristics. Precise and high through put phenotyping for these traits is needed which can be used in breeding programs in segregating generations. Also molecular markers need to be developed to apply MAS to transfer desirable quality traits into varieties with different quality preferences.

Varieties with high protein content are needed to reduce malnutrition among the rice consumers. A large number of consumers suffers from Fe and Zn deficiency. Wide variability exists for these nutrients in both cultivated and wild species of *Oryza*. *Oryza glumaepatula*, for example, has higher grain Zn concentration and *O. meridionalis* has higher protein content. QTLs for these traits need to be mapped and transferred from landraces and wild species into elite lines. In wheat, QTL associated with increased grain

protein content has been cloned and designated as *Gpc-B1* (Uauy *et al.*, 2006). With the clear demonstration of *Gpc-B1* being associated with an increase in grain protein content and the availability of functional markers, this gene is now being transferred into several elite varieties of wheat through MAS. Similar strategy needs to be developed and tested in rice for improving protein content of high yielding varieties.

Improving nutritional quality through transgenic approaches

Several research groups are working on developing biofortified rice with high zinc, high iron and beta carotene precursor of vitamin A. Amongst these traits, zinc can be enhanced using conventional breeding as there is sufficient variability in the rice germplasm and breeding process has been accelerated using MAS. Since iron is mainly localized in aleurone layer of rice grain and after polishing, only little iron is left in the grain. Transgenic approaches are being used to increase iron content using endosperm specific promoters. To overcome vitamin A deficiency, Ye *et al.* (2000) produced transgenic rice (golden rice) by introducing three genes: phytoene synthase (*psy*), phytoene desaturase (*crtl*) and lycopene β -carotene (*lcy*). *Psy* and *lcy* originated from daffodil and *crtl* from bacterium *Erwinia uredovera*. Later in Golden rice-2, *psy* from maize has been used in combination with *crt 1*, which led to as high as 25 μ g/g carotenoid content accumulation in the rice grains (Paine *et al.*, 2005). The carotenoid locus from the leading GR2 event has been introgressed at IRRI in collaboration with collaborating scientists into mega-varieties of rice (IR64, PSBRc18, and BR 29) using marker assisted breeding.

Future priorities in transgenic research should focus on the use of high-throughput transformation protocols, the production of marker-free transgenics, the use of organ-specific promoters, promoters for inducible gene expression, and pyramiding of transgenes. Emphasis should be given to identifying new transgenes, particularly for those traits for which conventional approaches are not successful. Focus should be on tolerance to abiotic stresses and improving nutritional value. Although transgenics have been generated by various laboratories for a series of traits, no commercial release has been made so far. It is suggested that all the available transgenic events be collected at key institutes within a country and be evaluated thoroughly for agronomic performance and the target traits. Reliable events should be thoroughly characterized for the expression and stability of the target trait at the phenotypic and molecular level. The promising transgenic events should be evaluated as a priority in multi-location trials along with biosafety studies.

Breeding high yielding varieties suitable for dry direct seeding

Rice is grown by different methods (1) transplanting 2-4 week old seedlings in puddled soils (2) broadcasting freshly

sprouted seedlings in puddled soils (3) direct seeding under wet conditions and (4) direct seeding under dry conditions. In North-Western India, comprising states of Punjab, Haryana and Eastern Uttar Pradesh, rice is grown under irrigated conditions. However, due to shortage of labor for manual transplanting, there is urgent need to develop high yielding varieties suitable for dry direct seeding. At present, only a few farmers grow rice under dry direct seeding, however the major constraint is lack of improved germplasm which can yield at least close to transplanted rice.

Focused research on breeding varieties suitable for direct dry seeding is suggested. This will require identifying donors with appropriate plant type, selection and evaluation of segregating populations and breeding lines under dry direct seeded conditions. Whole breeding process should be carried out under targeted environments. Furthermore, there is a need to search for traits such as (i) tolerance to anaerobic germination, (ii) weed competitive ability with early seedling vigor and growth to suppress weeds (iii) tolerance to iron deficiency particularly when grown in lighter soils and (iv) tolerance to nematodes. Besides, variety must have resistance to diseases, insects, improved grain quality and higher yield.

Some progress has been made to identify QTLs for the above traits, however, more work is needed to develop high yielding varieties suitable for commercial cultivation.

Future outlook

Pre-breeding and trait development

Pre-breeding should be given priority for key agronomic traits. Good progress has been made in trait development for resistance to biotic stresses such as BB, blast, BPH, and gall midge. However, well-defined genes/QTLs are needed for resistance to sheath blight, neck blast and stemborers. Emphasis should be given for enhancing tolerance to abiotic stresses such as drought, salinity, heat and cold particularly for reproductive stage tolerance, enhanced nutrient uptake and superior grain quality. Intensify pre-breeding efforts to increase the yield potential of basmati rice.

Milling is affected by several factors, of which chalkiness is important resulting in heavy grain breakage and poor grain quality. A number of QTLs for chalkiness have been identified which should be used to improve milling and head rice recovery. Efforts should be made to identify diverse genes/QTLs with different mechanisms for all these traits. There is a need to develop NILs for each of these traits, map them, and make them available to breeders for stacking through MAS. So far, emphasis has been primarily on varietal development with limited emphasis on pre-breeding and trait development. Equally important is trait development for newly emerging diseases such as false smut.

Mapping of QTLs

QTLs have been identified and mapped for only a few

selected traits. With technological advances in genome sequencing, it is now easier and faster to identify and map QTLs. We suggest undertaking precise mapping of QTLs for various agronomic traits and biotic and abiotic stresses on a larger scale. Breeders in collaboration with biotechnologists should seek to develop various specialized genetic stocks such as NILs, RILs, AILs (alien introgression lines) NAM (nested association mapping), MAGIC (multi-parent advanced generation inter-cross), and CSSLs (chromosome segment substitution lines) for the mapping of QTLs.

Integrating MAS in breeding programs

MAS is becoming a powerful tool for accelerating breeding not only for incorporating major genes but also for QTLs. With the availability of high-throughput genotyping platforms, MAS should become an integral part of breeding programs. Emphasis should be given to the pyramiding of genes/QTLs with different mechanisms for resistance/tolerance of biotic and abiotic stresses. Since several genes/QTLs have been cloned for yield component traits, priority should be given to the pyramiding of yield component QTLs for enhancing yield potential in inbred as well as in hybrids.

Enhancing nutrient-use efficiency

Molecular approaches should be used on priority to exploit natural variation and identification of genes for nutrient uptake and transport. Emphasis should be given to understand the role of soil microbes and their interaction with rice roots in promoting NUE.

Developing varieties with low in-put requirement

Efforts should be made to develop varieties with low in-put (less nutrients, less water) requirement. Selection of donors, segregating progenies and evaluation of breeding lines should be carried out under low in-put environments.

Developing special purpose rice

There is need to develop special purpose rices having medicinal value such as those with low glycemic index, good for diabetic people, and others having anti-oxidant properties helpful in protecting the body from damage caused by free radicals and to reduce the incidence of diseases, such as atherosclerosis and cancer. Such projects may be initiated in a few selected institutes involving team of breeders, biotechnologists, nutritionists and medical scientists.

Developing haploid induction systems in indica rice

There is urgent need to develop high throughput methods for production of double-haploids to accelerate breeding process. Various approaches to enhance anther culturability, production of haploids in indica rice through chromosome elimination involving wide crosses, and the search for haploid inducer genes/stocks and exploitation of natural and or induced allelic variation in centromere specific histone (CENH3) should be given priority.

Biological nitrogen fixation

There is a need to explore use of genomic tools for enhancing BNF through endophytes.

RNAi and genome editing

These two newly emerging technologies hold promise and should be explored to enhance pest resistance and in improving quality characteristics of rice cultivars.

GM technology

Efforts must continue to develop transgenics with value added traits particularly for those traits where conventional breeding is not very successful. Though at present some policy and regulatory constraints exist for the commercialization of GM rice, this should not impede research on and investment in transgenics.

Training in rice genomics

Since genomics technologies are expanding at a faster rate, training of young breeders should be strengthened. A repeat of the 1990s model in rice biotechnology as adopted by the Rockefeller Foundation is required for training in rice genomics. Also refresher courses(3-5 weeks) at regular intervals should be introduced for training newly recruited genomics teams in conventional breeding and in field oriented research .

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