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Strategies to manage rice diseases in the post genomic era

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ABSTRACT

Quantitative information on yield losses is vital to the development of sound management strategies for rice diseases. The difference between attainable and actual yield represent the loss due to yield reducing factors of diseases, pests, weeds and calamities. Chronic (brown spot), sporadic but potentially devastating (blast, bacterial blight) and diseases negatively associated with higher attainable yields (sheath blight, false smut) are known to reduce yields up to 10% in different seasons and years. Identification and deployment of qualitative and quantitative resistance to major diseases like blast and bacterial blight and gene/QTL discovery for resistance to diseases such as sheath blight are major challenges. Marker assisted selection/marker assisted backcross breeding has led to the development and releases of a number of improved rice varieties against blast and bacterial blight in the recent past. With the evolution of new races/biotypes it has become necessary to develop broad spectrum, race non-specific resistance to combat the evolution of new virulence. Rice breeding programmes targeting sophisticated signalling pathways against invasion by pathogens, high throughput genotyping associated with large breeding populations for multiple stress tolerance, focused breeding programs involving multi-parent advanced generation inter-crosses (MAGIC) and gene editing technologies are offering new avenues to gene deployment. This paper summarizes achievements in molecular breeding using marker-aided selection and marker assisted backcross breeding (MAS/MABB), and highlights application of new genomic tools to find and deploy novel genes for resistance to multiple diseases.

Key words: *Rice diseases, marker-assisted selection. allele mining, multi-parent population, gene editing*

INTRODUCTION

Large scale adoption of high yielding semi-dwarf rice varieties and increased production technology coupled with increased irrigation infrastructure has contributed to increased production and productivity of rice in Asia in the last 50 years. There are on-going efforts to increase the yield ceiling, stabilize production and reverse the decline in yield in highly productive environments while consolidating the gains of the semi-dwarf era and sustaining the resource base. Though few farmers have obtained attainable yields, actual yields in the vast majority of farms, especially in the rainfed areas are low and the return on additional investment has declined with higher cost of inputs and lower cost of the produce in home markets. The difference between attainable and actual yield represent the loss due to yield reducing factors of diseases, pests, weeds and calamities. Among the diseases chronic diseases like brown spot, sporadic but potentially devastating diseases like leaf and neck blast, bacterial blight and diseases negatively associated with higher attainable yields like sheath blight and false smut are known to reduce yields up to 10% in different seasons and years. In severe epidemics, yield losses ranging from 20% to 40% have been reported in case of bacterial blight (Sonti 1998) and 50% or more in case of blast (Khush et al., 2009). Sheath blight has been reported to cause 20-30% yield loss depending on the severity of infection and approximately 50% yield reduction in test plots of susceptible rice cultivars (Savary and Mew, 1996). All India Coordinated Rice

Project which conducts Production Oriented Survey in different districts of India every year has recorded increase in incidence and intensity of false smut during the last decade. The disease not only reduce yield but also seriously affect grain quality. Increase in disease incidence and intensity is ascribed to the wide-spread cultivation of fertilizer responsive high yielding cultivars and hybrids in recent years and conducive weather during flowering of rice in the monsoon season.

Disease management strategies are now more proactive compared to the reactive regime of disease control practised earlier. Realizing the economic losses caused by the diseases, efforts have been directed to understand the genetic basis of resistance and susceptibility. Host plant resistance is the most important among the multifaceted management strategies as it eliminates the need for additional efforts to reduce damage unless other diseases are additionally present. The studies directed to understand the host-plant interaction in rice have given rise to specialized breeding programs for resistance to diseases and insect-pests. Resistance breeding involves the use of appropriate donors hybridized to the commercial cultivar, subjecting the segregating population to high levels of disease pressure and selection of the surviving plants having resistance and desirable agronomic traits. Although many disease resistance genes were characterised, the conventional breeding approach had limitations in engineering disease resistant varieties due to several obstacles. Long breeding cycles, low selection efficiency, difficulties in hybridization involving distant parents, and lack of additional yield advantage when diseases are not present and/or the inability to keep pace with the yield advantage in other breeding programmes are among the major constraints associated with conventional breeding programmes. In addition, traditional breeding is often negatively affected by linkage drag, which resulted in the transferring of loci conferring potentially undesired agronomic traits due to its close linkage with resistance loci. Pathogen variability, especially in case of blast and bacterial blight added another dimension to the problem and needed to be addressed effectively to ensure stability of resistance. Pyramiding resistance genes against multiple strains of the pathogens using conventional breeding methods was, however, difficult because of their dominance and epistatic effects.

Genetics of resistance

Plants have two types of defense mechanism against attack by pathogenic microbes: one against general microorganisms, and the other against specific pathogen races. The general defense mechanism is known as a pathogen-associated molecular pattern (PAMP) triggered immunity (PTI). PTI is initiated by extracellular surface receptors that recognize general features of microorganisms. As a result of coevolution, plant pathogens have developed various strategies to overcome PTI. One of them is an effector-triggered susceptibility (ETS), which deploys PTI-suppressing pathogen effectors. The more specific defense mechanism against pathogen ETS is known as effector-triggered immunity (ETI), which is stimulated by plant surveillance proteins (R-proteins) that specifically recognize one of the pathogen's effector proteins (Avr proteins). ETI is an accelerated and magnified defense response compared to PTI: in bacterial and fungal pathosystems, the same defense genes are related to both defense mechanisms, but they display stronger and faster activation in ETI than in PTI (Ahn et al., 2005). ETI is accompanied by the active cell death of infected cells, the hypersensitive response (HR), which is known as the ultimate defense mechanism of plants (Bowles, 1990). However, certain pathogens avoid ETI by altering a target effector to prevent the recognition of a particular surveillance protein and/or by deploying other effectors that directly suppress ETI (Abramovitch et al., 2003). Defence response genes are those genes which functions downstream of R- or host pattern recognition receptor (HPRR)-initiated defense signalling pathways.

Disease resistance is generally classified into two types: complete (qualitative) resistance expressed as hypersensitive flecks or no symptoms and partial (quantitative) resistance expressed as low lesion number/area or both. Qualitative resistance conditions incompatibility of the host and pathogen strain, preventing reproduction of the pathogen, while quantitative resistance reduces the extent of pathogen reproduction within the context of a compatible interaction. In most cases, qualitative resistance is modulated by interaction (ETI) between the products of a major disease resistance (R) gene and an a virulence gene; this type of resistance is specific to

pathogen race and its durability limited in a particular cultivar due to the strong selection pressure against the rapid evolution of the pathogen. In contrast, quantitative resistance is conferred by QTLs and is presumably race non-specific and durable. In many cases, qualitative and quantitative resistance genes are co-located on linkage maps and these regions are often rich in genes conferring resistance to multiple pathogens and/or to multiple specificities of the same pathogen. On the integrated map of disease resistance genes in rice, for instance, blast resistance QTLs are co-localized with Pi loci or QTL for resistance to other pathogens (Inukai et al., 2006).

Nearly 100 blast resistance (R) genes and over 350 quantitative trait loci (QTLs) have been identified to date, of which 21 have been cloned and characterized in detail (Sharma et al., 2012). Structural and functional analyses of many major R genes have shown that they encode proteins with similar structural motifs- nucleotide binding site, kinase domains, leucine-rich repeats- that are responsible for ligand recognition and signal transduction. About 42 bacterial blight resistance (R) genes, designated from *Xa1* to *Xa42*, conferring resistance against various strains of Xoo, have also been identified from cultivated, mutant population, and wild rice species (Vikal and Bhatia, 2017). Among these R genes, 14 are recessive;nine R genes have been cloned and characterised encoding different types of proteins. Resistance to rice sheath blight is a complex, quantitative trait controlled by polygenes (Pinson et al., 2005). About 50 sheath blight resistance quantitative trait loci have been detected on all the 12 rice chromosomes (Jia et al., 2009; Zuo et al., 2010; Xu et al., 2011; Wang et al., 2012). Although no rice variety has yet been identified to have complete or high level of resistance to false smut, cultivars do exhibit significant differences in quantitative resistance to U. virens. (Biswas, 2001, Li et al., 2008, Xu et al., 2001, Zhou et al., 2014). It was reported that the rice cultivar IR28 has a relatively high resistance to the disease, which was controlled by two major and multiple minor resistance genes (Li et al., 2008). Eight QTLs controlling false smut resistance were also found in the resistant rice variety Lemont (Zhou et al., 2014). However, QTL for false smut resistance in rice has not yet been isolated and resistance mechanisms are largely unknown.A QTL for resistance to brown spot was identified from a cross between the land race Denorado (R) and IR 36 (S) which was located on chromosome 12. The identified R haplotype was later found to be present in most of the land races, suggesting that farmers would have selected varieties that perform well under soil stress tolerance. The region on chromosome 12 harbouring QTL to brown spot also has QTLs for soil stress tolerance, blast resistance and 1000 grain weight (Leung et al., 2015).

Marker assisted selection (MAS) and marker assisted backcross breeding (MABB)

Development of molecular techniques to detect variations in DNA has made it possible to track the genes using a set of closely linked or gene based markers and specifically select for the desirable gene(s) in a background of the parent, reducing or eliminating the need for repeated phenotyping. Variations in DNA sequences are the result of substitutions (point mutations), re-arrangements (insertions or deletions or indels) or errors in the replication of tandemly repeated DNA. DNA markers are accepted widely as potentially valuable tools for crop breeding. Marker assisted selection involves the use of genetic/DNA markers to follow regions of the genome that encode specific traits like disease resistance. Markers that co-segregate with the target trait are reliable which depends on the closeness of the marker to the linked gene. While early work focussed on the use of restriction fragment length polymorphisms (RFLP) detected via southern hybridization, polymerase chain reaction (PCR) based marker systems (AFLP, SSR) are now more widely used. New techniques not requiring gel electrophoresis are also gaining in importance.

Pyramiding of disease resistance genes is often complicated by the masking effect of major R genes. The use of markers in disease resistance breeding is not only for improving efficiency but a necessity for accumulating different defense mechanisms against different races of pathogen or multiple pathogens.PCR based allele specific markers provide an efficient marker system for marker assisted selection (MAS). MAS led to the development and release of a number of improved rice varieties against blast and bacterial blight in the recent past. Marker Assisted Backcross Breeding (MABB) was used for incorporating bacterial

blight resistance genes (*xa13* and *Xa21*) into the genetic background of Pusa Basmati1, which led to development of Improved Pusa Basmati 1 (Pusa 1460) as one of the first products of molecular breeding (Singh et al., 2016). Improved Samba Mahsuri (ISM) was developed by MABB with three bacterial blight resistance genes, *xa5*, *xa13* and *Xa21* (Sundaram et al., 2008). ISMwas released in 2008 as a replacement of Samba Mahsuri in the southern states of India. A survey conducted on the adoption of ISM in Andhra Pradesh, revealed that the trait value, which represents the value that farmers have obtained by cultivating ISM instead of Samba Mahsuri, was Rs. 245 Crores (Reddy, 2017). This was based on farmer feedback on the cumulative production of 7 lakh tons of ISMat 5.7 t/ha during 2011-2016. This represents the reduction in loss that was prevented due to the adoption of ISM, owing to its resistance to BLB. Improved Lalat and Improved Tapaswini with *xa5*, *xa13* and *Xa21* genes were developed at the National Rice Research Institute (Doku et al., 2013a,b). Lalat was further improved with resistance to blast (*Pi2, Pi9*), gall midge (*Gm1, Gm4*), submergence (Sub1) and salinity (Saltol) (Das and Rao, 2015). Marker-assisted transfer of genes conferring resistance to three different diseases in rice was also accomplished (Singh et al., 2012, Singh and Gopalakrishnan, 2016) wherein genes *xa13* and *Xa21* for BB resistance, *Pi54* for blast resistance, and a major QTL qSBR11-1 against sheath blight were combined through marker-assisted backcross breeding to improve basmati type varieties.Two bacterial blight resistant varieties (Pusa 1592, and Punjab Basmati-3) and one blast resistant variety (Pusa 1609) were among the 68 new rice varieties released in the country during 2016-17. Pusa 1592 was developed derived from Pusa Sugandh 5 through MABB using *xa13, Xa21* and Punjab Basmati-3 was derived by MABB using Basmati 386 as the recurrent parent and the previously developed semi-dwarf rice line IET 19498 harbouring sd1 in addition to *xa13* and *Xa21*. Pusa 1609 has blast resistance genes *Piz5* and *Pi54* (Table 1). *xa5*, *xa13* and *Xa21* were pyramided on Swarna (Swarna MAS, CR Dhan 800) and a deep water rice variety Jalmagna using MABB (Pradhan et al., 2017). The strategy was to add stable resistance to popular varieties that are renowned for their wide adaptation and production stability across environments. Although breeding and deployment of resistance cultivars using R genes have been an effective approach to managing rice resistance against bacterial blight and blast diseases, this resistance can be rapidly overcome due to the strong selection pressure against and the rapid evolution of the pathogens.

Widening the spectrum of resistance to match pathogen population dynamics

Though many R genes and QTLs have been identified and many of them characterized, few among them have been utilised in commercial varieties. However, the diversity of resistance genes has been put to good use in developing differential varieties for pathogen characterization. Near-isogenic lines (NILs) for both bacterial blight resistance genes and blast resistance genes have been produced and used for differentiating pathogen races, evaluating effectiveness of individual resistance genes and molecular cloning of disease resistance genes. Development of NILs and molecular markers has provided the essential tools for understanding pathogen diversity and deploying disease resistant varieties. The NILs carrying individual resistance genes has been widely used in characterizing the bacterial blight and blast pathogen populations with the objective of determining the appropriate resistance genes to use in resistance breeding. Understanding of pathogen population genetics and evolution is essential for effective utilization of host resistance genes. Systematic monitoring of the pathogen populations that incorporate current understanding of effector biology is a key aspect to drive pathogen-informed gene deployment (Dossa et al., 2015). As knowledge of pathogen population accumulated, the research also gradually shifted from pathogen population characterization to experimenting with various deployment strategies.

Pyramiding R genes, instead of quantitative resistance genes which are difficult to accumulate, has been the breeding strategy in case of bacterial blight and blast. However, with the evolution of new races/ biotypes it has become necessary to develop broad spectrum, race non-specific resistance to combat the evolution of new virulences. Selection pressure towards virulence is a given whenever managing pests and diseases. Breeders therefore need a wide array of genetic options in order to diversify the arsenal of resistance traits deployed in crops, thereby reducing this selection pressure (Vincelli, 2016). Some cultivars

Table 1. Blast and bacterial blight resistant varieties developed by MABB and released in India

Variety	Parent variety	Disease targeted	Genes used	Reference
Improved Pusa Basmati 1	Pusa Basmati 1	BB	Xa 13, Xa 21	Singh et al., 2011
Improved Samba Mahsuri	Samba Mahsuri	BB	Xa 5, xa 13, Xa 21	Sundaram et al., 2008
Pusa 1592	Pusa Sugandh5	BB	Xa 13, Xa 21	DARE-ICAR Ann. Rep 2016-17
Pusa 1609 and Pusa 1612	Pusa Sugandh5	Bl	Piz5, Pi54	DARE-ICAR Ann. Rep 2016-17
Punjab Basmati-3	Basmati 386	BB	Xa 13, Xa 21	Singh et al. (2014); DARE-ICAR Ann.Rep 2016-17
Improved Lalat	Lalat	BB	Xa 5, xa 13, Xa 21 (xa 4 present in Lalat)	Dokku et al. (2013a.)
Improved Tapaswini	Tapaswini	BB	Xa 5, xa 13, Xa 21	Dokku et al. (2013b).
CR dhan 800 (Swarna MAS)	Swarna	BB	Xa 5, xa 13, Xa 21	Minutes 78th meeting Central Sub-committee on crop standards Aug 2017

carrying resistance QTLs, such as the *Pi21* locus, have maintained resistance throughout a century of cultivation in Japan but co-introduction of resistance and undesirable agricultural traits, including grain characteristics from donors, has prevented the use of potential genetic resources for the development of elite cultivars (Fukuoka et al., 2009). Map based cloning of the recessive allele of *pi21*, conferring resistance, was used to successfully break the linkage drag associated with the gene (poor flavor) in a cross involving Sensho (resistant donor) and Koshihikari (the recipient parent).

Rice has evolved to utilize a network of sophisticated signaling pathways against invasion by phytopathogens, for example pathogen-associated molecular patterns (PAMPs), systemic acquired resistance (SAR) and hypersensitive response. Plant hormones such as SA, JA and IAA mediate broad-spectrum disease resistance in rice. Recent studies demonstrate that IAA acts as a negative regulator in the plant immune response. *X. oryzaepvoryzae*, *X. oryzaepvoryzicola*, and *M. grisea* secrete IAA in planta and also induce rice to synthesize its own IAA at the infection site. IAA induces the production of expansins, the cell wall loosening proteins, and makes rice vulnerable to infection. GH3-2 (Glycoside Hydrolase family 3), encoding an IAA-amido synthetase, confers broad-spectrum quantitative resistance against these pathogens by suppressing auxin signalling. It is expected that, controlled under a pathogen-induced strong promoter, GH3-2 could be a candidate for rice breeding programs (Fu et al., 2011).

Use of candidate genes involved in plant defense have been advocated to accumulate race non-specific resistance in commercial cultivars susceptible to the disease. An advanced backcross population derived from the cross Vandana/Moroberekan (Wu et al., 2004), was genotyped for defense response gene markers and phenotyped for blast resistance. Six candidate genes were found associated with partial resistance in this population. Fifteen BC_3F_5 lines were inter-crossed to accumulate the candidate genes at IRRI, Philippines using recurrent selection (Carrillo et al., 2005). Rice lines containing none to six defense response (DR) genes (thaumatin, oxalate oxidase, oxalate oxidase-like proteins, chitinase, peroxidase, HSP90) were evaluated in three blast endemic locations in India during 2004-2006 and their performance compared with the level of resistance in monogenic lines having different Pi genes. Disease progress curves in lines carrying five and six DR genes were comparable to the monogenic lines carrying R genes *Piz* and *Pi9* effective at all three locations. While the monogenic lines generally exhibited an 'all or nothing effect' with high or low disease, the introgressed population had a range of disease intensities that declined progressively with the addition of each DR gene (Variar et al., 2009). Although the application of candidate gene-aided selection has proven successful in accumulating quantitative disease resistance in rice, a limitation of this approach has been the relatively low level of variation of candidate genes based on restriction site polymorphism. Genome-wide association studies and QTL mapping, on the other hand, can examine common variation across the entire genome, and as such can detect a new region of interest that is in or near a potential candidate gene.

Allele and data mining

Wild relatives and local landraces of rice harbour a large

store of valuable genes that can be used to develop varieties with improved tolerance to stresses and other agronomic traits. Resistance genes are generally identified in germplasm collections using differential physiological races of pathogens. Fine mapping and cloning of many blast resistance genes and development of PCR-based markers have enabled faster screening and identification of such genes using allele mining approaches. Allele mining has been used to identify novel alleles or allelic variants of a gene/or candidate genes of interest, based on the available information about the genes, from a wide range of germplasm (Imam, et al., 2014 a,b; Singh et al., 2015).

Re-sequencing of 3,000 genebank accessions (The 3000 Rice Genomes Project, 2014) has provided the opportunity to identify new diversity for disease resistance by data mining. An understanding of the evolutionary differentiation of resistance genes is important for exploring additional diversity in the rice gene pool (Leung et al., 2015). Blast R genes from different donors tend to be located within the same genomic locus; for example, *Pi2/Pi9/Piz-t*, *Pi5/Pii*, and *Pik/Pi1/Pikm/Pikh/Pik*. The Pi alleles in the same locus from different donor plants are located either in the same genomic position (orthologues) or in different positions (paralogues). By comparing the differences in sequence and structure in resistant and susceptible haplotypes, blast R-gene loci can be grouped into two types. A type I locus refers to the one in which high sequence similarity and conserved genomic organization are maintained between resistant and susceptible haplotypes. For this type, the differentiation between R and S alleles is primarily caused by localized mutations, including nucleotide substitutions, small insertions/ deletions (InDels), and insertion of transposable elements. In contrast, a type II locus refers to one in which genomic organization or sequence similarity or both in resistant haplotypes are significantly different from that in susceptible haplotypes. It is therefore more feasible to develop R-gene specific markers to distinguish functional from non-functional alleles for a type II locus than for a type I locus. Using three type II R-gene loci, *Pi9, Pi5*, and *Pikm*, as targets, approximately 23.6 %, 31.3 %, and 33.4 % of the 3,000 genomes were found to carry the putative functional Pi9, Pi5, and Pikm alleles, respectively (Leung, 2015). Furthermore, 75 % of the lines bearing the predicted Pi9-allele belong to the *indica* type, a frequency almost four times higher than in japonica type. The association of R genes within rice types is of interest for the exploration and deployment of R gene diversity in different geographic regions.

Multi-parent populations

Although potentially useful landraces can now be identified, their use in breeding is often hindered by unfavourable linkages. Efficient breeding designs are needed to transfer the useful diversity to breeding. Multi-parent Advanced Generation InterCross (MAGIC) is a breeding design to produce highly recombined populations. MAGIC involves several cycles of inter-mating among multiple parental lines (Cavanagh et al., 2008). In a standard MAGIC design, founder lines (from 4 to 16) are inter-crossed in a half-diallele design. The F_1 plants are then inter-crossed to produce new F_1s with four different genomes (four-way cross). The resulting F_1 is crossed with other F_1s in eight-way crosses. The F_1 plants from 8-ways are then selfed by single seed decent for several generations to produce Advanced Inbred Lines (AIL). The populations resulting from such a breeding design are expected to have increased recombination and generate pre - breeding materials with new genotypic diversity (Bandillo et al., 2013). Breeders have adopted variations of this design in an attempt to maximise recombinations. MAGIC populations will have greater genotypic diversity, a higher level of recombination, and reduced linkage drag. Because of these advantages, the MAGIC approach has been applied to many crop and plant species for genetic research and breeding (Huang et al., 2015).

With the availability of whole genome sequences (WGS), the perspective of identification of DNA markers has shifted from fragment based polymorphism identification to sequence based single nucleotide polymorphism (SNP). The advent of next generation sequencing (NGS) technologies and powerful computational pipelines has reduced the cost of whole genome sequencing by many folds allowing discovery, sequencing and genotyping of thousands of markers in a single step. Multi-parent populations are now attractive for researchers due to the development of high-throughput SNP genotyping platforms and advances in statistical methods to analyze data from

such populations. Two multi-parent advanced generation intercross (MAGIC) populations were developed by IRRI (Bandillo et al., 2013), inter-crossing eight elite lines from the Asia indica pool (*indica* MAGIC) and the *japonica* group (japonica MAGIC). Each population is comprised of eight founder lines that include elite and modern varieties known to exhibit high yield potential, good grain quality, and tolerance to a range of biotic and abiotic stresses. The MAGIC lines were disseminated, genetically characterized and evaluated to identify lines adapted to a range of production constraints in Asia and Africa.

Genome/gene editing technologies

Genome editing is a relatively new technology that is gaining importance as a tool for crop improvement because of its advantages over routinely used methods of genetic engineering. Gene editing uses site directed mutagenesis (as opposed to random mutagenesis) to delete, insert or replace a DNA sequence. Development of engineered site specific nucleases (SSNs) has paved the way for single nucleotide excision mechanism for crop improvement. These genome editing technologies (GETs) use programmable nucleases to increase the specificity of the target locus (Arora and Narula, 2017). Genome-editing technology is precise and efficient. It involves the induction of double-stranded breaks (DSBs) at specific sites of DNA, using molecular scissors that include engineered and programmable site-specific nucleases (SSNs), such as meganucleases (MNs), zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and RNA-guided nuclease (RGN) systems, the most widely used RGN being the clustered regularly interspaced short palindromic repeat (CRISPR)/ CRISPR-associated system 9 (CRISPR/Cas9), and DNA-guided nuclease (DGN) system, *i.e.,* NgAgo (an acronym for *Natronobacteriumgregoryi Argonaute*). The programmable nuclease is used to induce a double-strand break in the DNA, while the repair is left to the plant cell itself, and mistakes are introduced, while the cell is repairing the double-strand break using the relatively error-prone NHEJ pathway. From a biological point of view, it could be considered as a form of targeted mutagenesis.

In recent years, sequence-specific nucleases (SSNs) have been demonstrated to be powerful tools for the improvement of crops via gene-specific genome editing, and CRISPR/Cas9 is thought to be the most effective SSN.The gene-specific DNA double-strand breaks (DSBs) caused by the SSNs are repaired primarily by the high-fidelity homologous recombination (HR) or error-prone non-homologous end joining (NHEJ) pathways. NHEJ often introduces small insertion or deletion (InDel) mutations at the cut site that lead to the loss of gene function. Compared with RNAi technology, SSN-based genome editing can achieve complete knockout without incorporating exogenous DNA. (Wang et al., 2016) reported improvement of rice blast resistance via CRISPR/Cas9-targeted knockout of the ethylene responsive factor (ERF) gene OsERF922 in Kuiku131, a *japonica* rice variety widely cultivated in northern China. OsEFF922 acts as a negative regulator of blast resistance in rice as the knockdown of its expression by RNA interference (RNAi) is known to enhance rice resistance to *M. oryzae*. (Liu et al., 2012).

A pC-ERF922 construct with CRISPR, exhibiting gene editing activity in rice protoplasts, was used to transform the rice variety Kuiku131 by Agrobacterium mediated transformation, with the goal of enhancing its blast resistance by gene-specific editing (Wang et al., 2016).Sanger sequencing of the C-ERF922-induced mutant plants revealed that theyharboured various insertion or deletion (InDel) mutations at the target site. All of theC-ERF922-induced allele mutations were transmitted to subsequent generations. Mutant plants harbouring the desired gene modification but not containing the transferred DNA were obtained by segregation in the T_1 and T_2 generations. The homozygous mutant lines were further examined for a blast resistance and agronomic traits. The results revealed that the number of blast lesions formed following pathogen infection was significantly less in the mutant lines compared with wild-type plants at both the seedling and tillering stages. Furthermore, there were no significant differences between any of the mutant lines and the wild-type plants with regard to the agronomic traits tested. Gene editing was used earlier to mutate OsSWEET14, which aids in pathogen survival and virulence, to produce disease-resistant rice with normal phenotypes (Li et al., 2012). Another gene, OsBADH2 was also targeted using TALENs to produce a generation of fragrant rice that contain 2-

Table 2. Gene edited using SSNs to improve disease resistance in rice.

Disease	Pathogen	Gene targeted	Method	Reference
Blast	*Xanthomonas oryzae*	OsSWEET14	TALEN	Li et al 2012
Bacterial blight	*Xanthomonas oryzae*	OsSWEET11, OsSWEET14	CRISPR/Cas9	Jiang et al., 2013
Blast	*Magnaporthe oryzae*	OsERF922 ethylene responsive mediated transformation factor	CRISPR/Agrobacterium transcription factor	Wang et al., 2016

acetyl-1-pyrroline (2AP), a major fragrance compound (Shan et al., 2015) (Table 2). Genome editing can thus produce defined genetic changes in targeted genes with high efficiency and limited off-target changes. Furthermore, it can be done in ways that leave no trace in the plant of foreign DNA such as antibiotic resistance genes and plasmid fragments. Interestingly, a complex trait par excellence, yield, was also shown to be amenable to CRISPR-Cas9 technology (Li et al., 2017) used CRISPR-Cas9 to mutate the regulatory genes *Gna1, DEP1*, and *GS3* and obtained rice plants with increased grain numbers, dense erect panicles plus semidwarf phenotype, and larger grains, respectively.

Perspectives

Substantial progress has been made in understanding resistance to pathogens and developing more efficient means to deploy resistance to control rice diseases. The most important development in understanding molecular mechanisms of disease resistance has been the cloning of R genes against blast and bacterial blight diseases. As a model crop with a fully sequenced genome, rice provides good opportunities to delve deeper into the molecular mechanisms governing disease resistance and engineer the development of rice varieties with diversified arsenal of resistance with broad spectrum efficacy against several diseases. An important consideration for successful development, diffusion and impact for new rice varieties is the need to constantly improve yield, grain quality, multiple stress tolerance and hence fitness in the targeted ecosystem. Multi-parent populations are considered an advance over bi-parental populations and association mapping as the former focuses only on difference in the genomic regions of two individuals and the latter, even though it captures far greater diversity, requires very large samples to detect genomic regions of interest. MAGIC is an attractivealternative from both theoretical and practical standpoints. Rice MAGIC lines developed at IRRI are presently being extensively phenotyped in South and South East Asia, presenting new challenges for dissection of complex traits such as yield,drought tolerance, and quantitative disease resistance. Multi-parent Advanced Generation Recurrent Selection (MAGRes) combines MAGIC for the development of recombinant lines, withmarker-assisted recurrent selection (MARS) for identificationof superior lines for intercrossing, to develop lines possessing elite alleles from diverse parents. The MAGReS lines will be highly diverse and can be usedas donors for different breeding lines, leading to directrelease of superior lines as new varieties for commercial cultivation (Huang et al., 2015).

Induction of specific mutations by means of site specific nucleases would allow direct modification of effector targets leading to resistant mutants. If the process of gene editing does not involve heterologous integration of DNA, (unlike insertions and deletions induced by non-homologous end joining mechanisms), the product may not come in the ambit of GMOs. However, possibilities offered by genome editing also haveramifications to IP, access, and benefit issues, even whenit concerns deletions and loss-of-function mutations (Van de Wiel et al., 2017).

Diffusion of the newly released rice varieties are often slow for several reasons. Availability of quality seed, lack of awareness among the farmers about disease and pest resistant varieties, flaws in input delivery mechanisms and problems of non-synchronous flowering, earliness etc., are reasons for slow adoption. Improved Samba Mahsuri was reported to be early by about 10 days compared to the parent variety and the flowering was non-synchronous leading to poor grain quality and low pricesoffered by millers. Varietal replacement is higher in areas where disease problems recur every year compared to others where the environment does not favour disease. More focus should, therefore, be given to vertical expansion of disease resistant varieties in identified epidemic areas rather than horizontal expansion into areas that are not seriously affected by the disease (Reddy, 2017). Reducing selection pressure towards overcoming

resistance traits by integrated disease management will help to extend the life of resistance genes in a particular cultivar/region. Strategic gene deployment integrated with crop and nutrient management can contribute to sustainability through reduced fungicide use and reduced production costs.

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