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Molecular approaches for selection and identification of blast resistance genes in rice

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Abstract
As the population is increasing day by day, there is a need to increase the food grain production especially rice. So, it is a need to increase the yield and also to minimize the yield loss caused by various diseases and insect pests. Among the diseases of rice, Blast (caused by *Magnaporthe oryzae)* is the major cause for reduction of rice yield ie., about 30 to 35%. It can infect rice plant right from seedlings to adult plant stages affecting leaves, nodes, collar, panicles and roots. Many management practices such as chemical control, biological control, disease forecasting and conventional breeding can be adopted to overcome the loss due to blast disease but, they solely can't control it because of high labour cost and more time consumption. To overcome these problems, Blast resistance genetics in rice is getting strengthened these days because of the progress in the molecular marker development and functional genomics. By using molecular breeding, the identification of R genes with the help of biotechnological/molecular tools will be helpful in modern plant breeding to improve blast resistance and its durability. Marker-assisted selection (MAS) and conventional breeding together have facilitated R genes to be combined (or 'pyramided') in elite rice varieties. This paper contains overviews of R genes of blast pathogen and molecular approaches for their identification, selection and management.

Keywords: Conventional breeding, Resistance genes, MAS, MABB, Molecular markers.

Introduction
For more than half of the world's population, Rice (*Oryzasativa* L.) is the staple food. It provides more than 19% of the calories consumed by the world population (Suzuki, H., 1967) [39]. As the global population is raising day by day, the demand for global rice is estimated to rise from 6.76 × 108 t in 2010 to 8.52 × 108 t in 2035 (Khush, 2013) [23]. To produce that additional quantity of rice i.e., 1.76 × 108 t, there is a need to increase the yield and also to minimize the yield loss caused by various diseases and insect pests. The major constraint for sustainable rice production is, Rice blast disease, caused by *Magnaporthe oryzae.* This has been reported to occur in more than 85 countries with an yield loss of about 30–50% under conducive environmental conditions (Katsuya, *et al.* 1969) [22]. It can infect rice plant right from seedlings to adult plant stages affecting leaves, nodes, collar, panicles and roots. Many management practices such as chemical control, biological control, disease forecasting and conventional breeding (recurrent selection, pedigree method and mutation breeding) can be adopted to overcome the loss due to blast disease but, they solely can't control it because of high labour cost and more time consumption. Conventional breeding is generally affected by linkage drag, due to which undesired trait closely linked with resistance gene were also transferred. Complete resistance conferred by major blast resistance (R) gene was revealed with the analysis of rice germplasm with different races. Due to single R gene locus, the resistance may be broken down with some race specific characteristics. Blast resistance genetics in rice is getting strengthened these days because of the progress in the molecular marker development and functional genomics, (Hayashi *et al.*, 2006) [17]. Resistance for blast can be improved in elite rice varieties with resistant germplasm carrying both major and minor R genes, which became an important genetic resource for rice breeders. By the utilization of genetic and genomic resources, the identification of these R genes with the help of genomic or

biotechnological tools will be helpful in modern plant breeding. About 100 quantitative blast R genes have been detected in rice, and about 22 R genes have been successfully cloned and characterized (Pb1, Pia, Pib, Pid2, Pid3, Pik, Pik-h/Pi54, Pik-m, Pik-p, Pish, Pit, Pita, Piz-t, Pi1, Pi2/Piz-5, Pi5, Pi9, Pi21, Pi25, Pi36, Pi37, Pi35, Pi64, Pi56, Pi63 and PiCO39) (Devanna *et al.*, 2014) [9]. To improve their blast resistance and durability, Marker-assisted selection (MAS) and conventional breeding together have facilitated R genes to be combined (or 'pyramided') in elite rice varieties.

Identification of genetic variation in the pathogen

The extent of genetic variation and instability in *M. grisea* has been explained by theories like mitotic recombination (Suzuki, 1967) [39], Parasexual recombination (Genovesi, *et al.* 1976, Zeigler, *et al.*, 1997) [13, 44], hyphal fusion (Shen, *et al.*, 1998.) [33] etc. were advanced to explain the high levels of variation encountered in the blast pathogen. Considerable effort has indeed gone into designing new strategies to understand and document genetic variation in *M.grisea* (Biju-Duvall, *et al.* 1998) [3] these are:-

Resistance genes:

Resistance to blast may be conditioned by major genes or by quantitative trait loci (QTLs) (McCouch, *et al.*, 2007) [26]. The deployment of major gene resistance will minimize selection pressure and thereby prevent evolution of resistance in the pathogen population (Bonman, *et al.*, 1992) [4]. The resistant genes are clustered in several regions from wild & cultivated varieties of several regions in to the rice genome. Nine loci have been reported on chromosome 11, five blast resistance genes on chromosome 6 and 11 loci have been reported on chromosome 12. More recently, a number of resistance genes have also been tagged to molecular markers, facilitating their identification and selection in segregating populations after hybridization.

Table 1: List of some major resistance genes located on different chromosome in rice

Gene	Chromosome	Markers	Reference
Pi28(t)	10	RZ500	Sallaud *et al.*, 2003 [31]
Pi-a	11	-	Kiyosawa, 1967 [24].
Pi-f	11	-	Shinoda *et al.*,1971 [34]
Pi-k	11	-	Shinoda *et al.*,1971 [34]
Pik^h	11	RM2191	Sharma *et al.*, 2005,
Pi-is-1	11	-	Goto *et al.*,1970 [14]
Pi-1	11	RG303-G181	Causse *et al.*,1994 [6]
Pi-7 *(t)*	11	RG103A-RG16	Wang *et al.*,1994 [40]
Pi-18	11	RZ536	Sang *et al.*,1996 [30]
Pi30(t)	11	OPZ11-f	Sallaud *et al.*, 2003 [31]
Pita	12	RG869	Bryan *et al.*,2000 [5], cloned
Pi-4*(t)*	12	RG869	Yu *et al.*,1991[43]
Pi-6*(t)*	12	RG81	Causse *et al.*,1994 [6]
*Pita*2	12	RG869	Jia *et al.*, 2003 [20]
Pi12(t)	12	RG869	Zhen *et al.*,1996
Pi-19*(t)*	12	RG241	Shinoda *et al.*,1971 [34]
*Pi*20	12	Xnpb 88	Imbe *et al.* 1997
Pi62(t)	12	Rz 816	Imbe *et al.* 1997
*Pi*157	12	RG341	Naqui and Chattou, 1996
Pi31(t)	12	-	Sallaud *et al.*, 2003 [31]
Pi32(t)	12	-	Sallaud *et al.*, 2003 [31]

Partial resistance

Parlevliet (1988) [28] describes partial resistance as an incomplete quantitative resistance based on minor genes. It is characterized by compatibility between the pathogen and the plant with reduced development of disease compared to plants with no partial resistance (Bonman, *et al.*, 1990). This form of resistance is suitable for low to moderately blast-conducive environments (Bonman, *et al.*, 1990). Genetic studies indicate that partial resistance is under oligo or polygenic control and can be affected by the environment. Several reserachers have said that there are minor genes that play an important role in maintaining an acceptable level of disease under field conditions (Sirithunya, *et al.*, 1999) [36]. Such genes would be difficult to identify and characterize in the presence of major genes as these have epistatic interactions among themselves. Their presence could also affect the accuracy of classification of lines for complete resistance to blast.

Gene pyramiding

Johnson (1984) [21] describes durable resistance as that which remains effective while a cultivar possessing it, is widely cultivated. it is one of the most efficient strategy for achieving durable resistance against rice blast (Koide *et al.*, 2009) and it accumulates many different blast resistance genes such as Pi1, Pi5, Piz-5 and Pita (Liu *et al*., 2002; Lee *et al*., 2009), Pish and Pib (Hittalmani *et al*., 2000) [19]; Pi-d(t)1, Pi-b, and Pi-ta2 (Chen *et al*., 2004) [7]; Pi1 and Pi2genes (Fu *et al*., 2012), Piz-5 and Pi54 (Singh *et al*., 2012). This term refers to the combining of two or more major genes for resistance in a single plant genotype (Mundt, 1990). While the use of single major genes limits the useful life span of resistant cultivars to few years, gene pyramiding could delay resistance breakdown by conferring 'horizontal resistance' effective against all prevalent pathotypes of a pathogen. Combinations of resistance genes are thought to provide broader spectra of resistance through both ordinary gene action and quantitative complementation that results in durable resistance. Two QTLs for blast resistance were transferred from Jao Hom Nin (JHN) into RD6 (Wongsaprom *et al*., 2010) [41]. Xa23 for bacterial blight resistance and Pi9 for blast resistance were pyramided in Guangzhan 63S (GZ63S) (Ni *et al*., 2015) [27]. Similarly, Xa21, Xa13 and Pi54 were pyramided in Indian rice varieties

(Arunakumari *et al*., 2016) [2].

Pathogenicity tests

For a long time, to assess variation in natural pathogen populations, differences in pathogenicity between individual isolates have been used. These were primarily based on (Flor, 1956) 'gene-for-gene' concept. For these, Avirulence genes provide an important source of markers. Depending on the rice cultivars they successfully infect, races of *M. grisea* have been distinguished among the pathogen isolates. Strains with different virulence on standard sets of cultivars are considered to represent different pathotypes (Kiyosawa, 1972) [25]. An important parameter that influences estimates of variability, is the set of differentials used in pathotype assays. Standard sets of differential cultivars have been developed by blast researchers (Qinghua *et al.* 1999) [29] in order to classify local isolates into pathotypes. Also, the fact that traditional varieties used as differentials may harbour more than one gene for blast resistance complicates analysis of host–pathogen interactions. At International Rice Research Institute (IRRI) several near isogenic lines (NILs) carrying blast resistance genes in different backgrounds have been established.

Molecular markers for identification of blast resistance genes:

There are many different types of molecular markers(DNA based and PCR based) has been developed in molecular breeding. these markers are Random amplified polymorphic DNAs (RAPD), fragment-length polymorphism (RFLP), amplified fragment length polymorphisms(AFLPs), simple sequence repeats(SSRs) and cleaved amplified polymorphic sequences(CAPs). Among all the markers, only CAPs and SSRs are used for genotyping because it requires small quantity of DNA samples from tissue samples. So these two markers are very suitable for identification of resistance genes in seedling stage and these markers are cost effective and beneficial for applied breeding program. Hayashi *et al.*, (2006) [17], developed PCR based marker system for 9 rice blast resistance genes basing on SNPs and InDels and the genotypes can be assessed by the presence of amplified products using allele specific primers. SNPs and InDels are easily available in rice genome and can be used for developing a sufficient number of markers within target genomics region. At IRRI, one PCR based marker(RG64) has been developed for the blast resistance gene Pi-2 (Hittalmani *et al*. 1995) [18]. Rice blast resistance genes Pi-1, Pi-2 and Pi-4 have been introgressed into 10 agronomically and commercially accepted rice varieties such as IR36, IR50, IR64 and IR72 etc. possibility of selection for these genes are only their close linkage to RFLP markers (Hittalmani *et al*. 2000) [19].

Molecular approaches for management of blast pathogen

Nowadays different types of molecular approaches are use for the management of blast disease. Molecular markers are highly precise and reduce the selection time which is the major limitation of conventional breeding approach. They are widely used for the selection of desired traits and identification of genomic regions associated with different important disease including blast resistance against broad-spectrum isolates. Blast fungi infects the rice plant and invades the epidermal cells **(**Skamnioti and Gurr, 2009) [37]. Effector-triggered immunity (ETI) arises when M. oryzae Avr (avirulence) proteins were determined by rice R (resistance) proteins. These effector proteins were cloned and characterized, including Avr effectors (Avr-Pita 1, PWL1, PWL2, ACE1, Avr-CO39, AvrPiz-t, AvrPia, AvrPii, AvrPik/km/kp, AvrPib), secreted LysM proteins (SLP1 and MC69) and biotrophy-associated secreted (BAS) proteins (BAS1, BAS2, BAS3 and BAS4) Selin *et al.*, 2016) [32]. The understanding of the fungal infection mechanisms was improved with the availability of new molecular tools. At the time of disease development, the available genome sequences of *M. oryzae* and rice help us in explaining the molecular mechanism and their interactions. Molecular marker information and the host-pathogen interaction made MAS programme possible for the introgression of blast resistance genes into new breeding programmes of rice. Molecular markers are widely used for the selection of desired traits and identification of resistance gene by using molecular approaches. Some approaches are discussed below.

Marker-assisted selection (MAS) for blast resistance in rice

Emergence of new virulent race of pathogen cannot be easily identified by conventional breeding because it is usually depends upon the phenotype of artificial identification field performance of resistance (Zhang *et al.,* 2006) [45]. So we can say that MAS is very useful in identification of blast resistance genes because phenotypes of blast resistance are encoded by single or few genes (Young, 1996). specific interaction of R gene and avirulence (Avr) from host-pathogen interaction was exploited by using MAS for control of blast pathogen. By using Molecular markers, the efficiency of conventional breeding is improved for a desired traits. Blast resistance SSR markers (RM168, RM8225, RM1233, RM6836, RM5961 and RM413) have been reported and these markers is used in MAS. Availability of molecular markers with MAS strategies is necessary for development of long lasting blast resistant variety (Ashkani *et al.*, 2012) [1]. Important factors for less using of molecular markers for identification of phenotypes are high cost of marker and less reliable for varietal development. MAS is very useful for the identification of R genes which is used to develop the blast resistant rice varieties (Yan *et al.*, 2017) [42].

Marker-assisted backcross breeding (MABB) for blast resistance in rice improvement

Marker assisted backcrossing is the process for minimizing the size of donor parent where target genes are present and enhancing the recovery of recurrent parent by using molecular markers (Hasan *et al.*, 2015) [16]. To transfer the targeted gene along with minimizing the donor part and recovering the recurrent parent genes are the main principle of MABB (Sundaram *et al.*, 2009) [38]. Molecular markers which are closely with specific characters are used in MABB (Collard and Mackill, 2008; Hasan *et al.,* 2015) [8, 16]. it is very useful as compare to conventional backcrossing because of less time consumption. So that this strategy is used to transfer many other resistance genes in different commercial varieties all over the world (Gouda *et al*, 2013) [15]. Blast resistance genes Pi1, Pi2 and Pi33 has been introgressed into ADT43 (rice variety) by using MABB (Divya *et al.*, 2014) [10]. For development of the new rice varieties there are so many studies of MAS have been reported (Fu *et al.*, 2012) [12].

Advantages of molecular markers for selecting resistance genes:

Marker assisted selection (MAS) is a process by which indirect selection of the genes for targeted traits can be done

by the use of DNA marker. With the fast development of molecular biotechnologies, MAS is gaining more importance with the presence of some advantage like efficiency and effectiveness as compared to conventional phenotypic selection (Collard *et al*.; Xu and Crouch). some advantages of molecular markers are:-

1. Helps in saving time and reducing the cost of breeding which cant be seen in conventional approaches.
2. Selection based on DNA markers may be more reliable as the influence of environmental factors is not seen which is not possible in case of conventional approaches as they are held in field.
3. MAS is useful for improving the complete resistance to rice blast by backcross breeding as it is often controlled by a major gene.
4. Helps in pyramiding two or more genes affecting blast resistance.
5. Helps in monitoring the presence of multiple resistance genes.
6. Through MAS, about 90% of the recurrent parental genotype can be recovered within two generations.7Helps in deeper understanding of the traits we are selecting and can help in efficient selection in the future.

Future perspectives of molecular marker for blast resistance genes

MAS uses DNA markers for indirect selection of phenotype and its efficiency is highly dependent on the strength of association between using DNA markers and genes responsible for the phenotypes. A marker has strong association with the phenotype (McCouch *et al*. 2007) [26] if it can distinguish the polymorphism underlying the phenotypic effect of the gene (i.e., gene specific marker or functional marker), almost all reported markers for rice blast are linked to the resistance genes and very few gene specific markers were reported. Since such linkage markers have, a very close relationship with phenotype than gene specific markers, there are a few limitations on applying them to MAS. Thus these markers help the breeders in supporting the conventional approaches indirectly and their usage should be increased further for getting better achievements.

Conclusion

It can be concluded that the use of molecular approaches for identification of blast resistance genes in breeding program can benefit in terms of time saving as compared to the conventional breeding method and other management practices. Blast is most destructive disease which leads to significant reduction in yield. The most efficient method to reduce disease incidence is the use of resistant cultivars. Developing these resistant cultivars through conventional approaches is time taking. Because of this reason, the vision of the breeders focused on the use of molecular and biological tools by which the efficiency and effectiveness of the developed cultivar can be increased. These resistant cultivars are developed by the use of some recent molecular techniques like MAS, MABB and genome editing. However, due to the variable nature of pathogen, the need of regular research on the advancement of sustainable resistant cultivars will always be a never ending process due to co-evolution of pathogens.

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