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Review Paper

QTL mapping and candidate gene sequencing for disease resistance in rice - review

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ABSTRACT

Rice serves as the major staple food for more than half of the world's population and is being cultivated across the globe. However, huge production losses in rice crop occurs due to the biotic stresses such as fungal blast caused by *Magnaporthe grisea.* Rice blast is endemic to most of the rice-growing areas. Loss due to rice blast may range up to 90% depending upon the part of plant infected and severity of the disease. Development of resistant varieties against the pathogen is the most economical and coherent approaches to cope up with the disease and increase the production of rice crop. Identification of QTLs for disease resistance is the important step for the development of resistant cultivars. To identify the resistance QTLs, a candidate gene strategy has been established. This strategy is very effective for identification of the disease resistance QTLs in rice and other cereals. After identification, a single minor QTL can be utilized in rice improvement programmes by modulating the expression of the gene underlying the QTL. Pyramiding of two to three minor QTLs, whose expression can be managed and that function in several defence signal transduction pathways, is beneficial for breeding of rice cultivars that are resistant to major diseases. Thus, identification and subsequent utilization of QTLs can serve as economically importance approach in crop improvement programmes for imparting resistance towards the diseases.

Keywords: *Candidate genes, diseases, QTLs, resistant varieties, rice.*

Rice (*Oryza sativa* L.) is one of the most important food crops, which feeds more than half of the world's population (Sravan *et al.,* 2016; Dar *et al.,* 2021). Due to the importance of rice in global food supply, it is important to enhance its production through improvements in agronomic practices and the crop varieties. Development of rice varieties showing resistance against common diseases can be an effective strategy that ultimately affects the quantitative and qualitative performance of rice.

Many agriculturally important traits such as yield, grain quality, tolerance to environmental stresses and various forms of disease resistance are controlled by large number of genes which have been termed as polygenes by Mather (1949). A quantitative trait locus (QTL) is a region of DNA that is associated with the quantitative trait in the phenotype of a population (Miles and Wayne, 2008). Quantitative characters have been considered as a major area of study in genetics, as these are known to be a source of natural variation existing in all the crop plants (Majid *et al.,* 2022). In order to exploit agronomically important QTLs in crop improvement programmes, the first and the foremost step is the identification and mapping of these QTLs in genomes of crop species. Identification of QTLs with genetically linked DNA-markers is useful for incorporation of genes into improved cultivars via marker-assisted selection (MAS), map-based cloning of the tagged genes and for better understanding of the genetics of complex traits (Asins, 2002; Jaiswal *et al.,* 2019). Linkage analysis and association mapping are the two most commonly used approaches for QTL mapping. Association mapping has three advantages compared to conventional linkage mapping. Firstly, it

saves time and cost of construction of suitable segregating populations and by using existing populations there can be a wide diversity of materials, secondly association mapping is able to detect multi-allelic variation, and thirdly it helps to identify the most favourable alleles contributing to a target trait in a single analysis. Further, its higher resolution is more powerful for fine mapping of quantitative trait loci. Rabion *et al.* (2016) have studied three chromosomal regions associated with resistance to rice blast through genome wide association mapping. Mgonja *et al.* (2016) reported that the Genome-Wide Association Mapping identified 31 genomic regions associated with blast resistance (RABR) in the rice genome.

Principle of QTL Analysis: QTL analysis is based on the principle of determining linkage of phenotypic markers and genotypic markers. Markers involve the partitioning of mapping population into various genotypic clusters on the basis of presence or absence of a specific marker locus and determine the significant differences between the groups with respect to the trait being measured. A significant difference between phenotypic measurement of the groups, depending on the marker system and the type of population, indicating that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait of interest.

Steps of QTL Mapping: The different steps associated with QTL mapping have been presented in Fig. 1 and elaborated as under:

(1) Selection of two parental strains possessing different alleles that affect variation in a trait.
(2) Development of a suitable mapping population by crossing the selected parents.
(3) Phenotyping of the mapping population for the trait of interest under greenhouse, screen-house and field conditions over years and locations.
(4) Generation of the molecular data on the basis of population with adequate number of uniformly spaced polymorphic markers.
(5) Construction of a genetic map.
(6) Identification of genetic markers associated with the trait of interest using various statistical procedures.

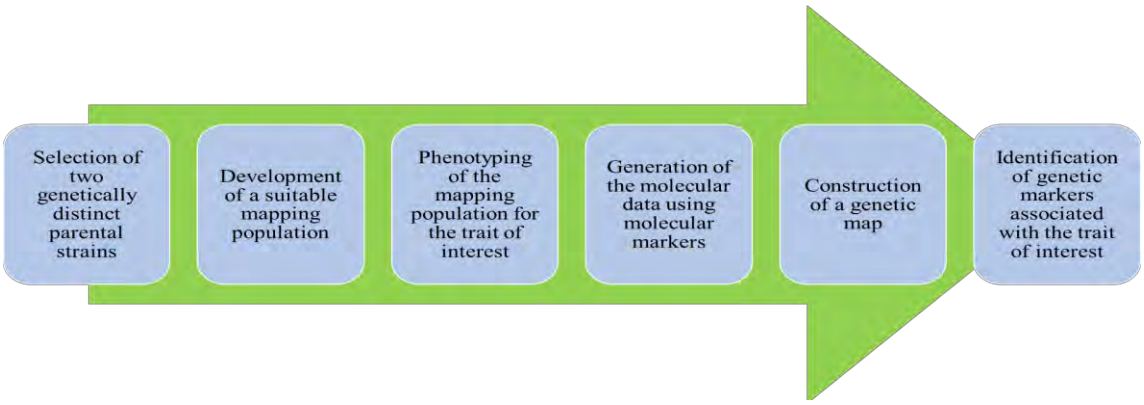

Fig 1: Different steps associated with QTL mapping

Different Mapping Populations: Different types of mapping populations that are commonly used in a QTL Mapping have been presented in Fig 2 and may be given as follows:

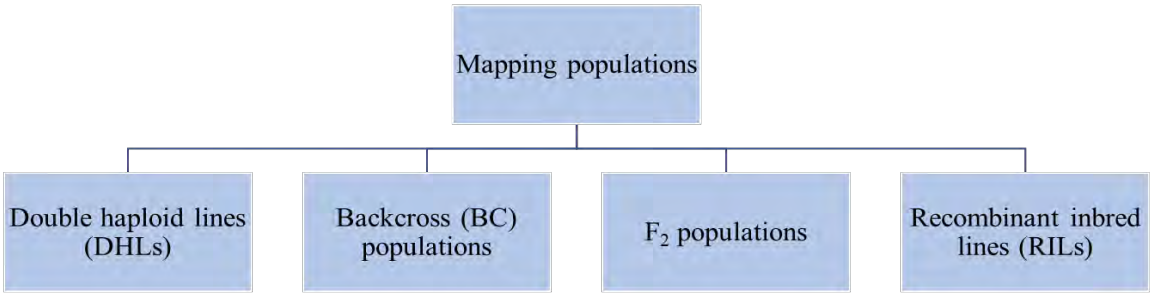

Fig 2: Different types of mapping populations

Double haploid lines (DHLs): Double haploids are produced from the haploid pollen of F_1 species which are then treated to restore diploid condition. Here every locus is homozygous.

Advantages:

- Immortal population.
- Used in mapping of both quantitative and qualitative characters.
- The ability to produce homozygous lines after a single round recombination saves a lot of time for plant breeders.

Disadvantages:

- The doubled haploids are developed from haploids. It is difficult to obtain haploid in all crops.
- Expensive method
- The over-usage of doubled haploidy may reduce genetic variation in breeding germplasm, hence one has to take several factors into consideration before deploying doubled haploidy in breeding programmes.

Backcross (BC) populations: Backcross populations are produced by repeatedly crossing F_1 plants to one of the parents.

Advantages:

- The elite combination is not lost.
- Less time require to develop.
- Elite genotype of recurrent parent will produce elite genotype at the end of backcrossing programme.
- Frequently used in resistance breeding.

Disadvantages:

- Process is not used to develop quantitative traits.
- Population is not immortal.
- It requires many seasons to get new cultivar.
- More restricted for recessive traits.

F_2 populations: F_2 population are generated by selfing of F_1 plants

Advantages:

- Development of F_2 populations requires only two generations, which is minimum for any bi-parental population to develop.
- It takes minimum efforts for development.
- They are ideal for identifying heterosis QTLs.

Disadvantages:

- Limited use in fine mapping.
- F_2 populations cannot be easily preserved because F_2 plants are frequently immortal.

Recombinant inbred lines (RILs): These are generated by continuous inbreeding or selfing of F_2 plants, and further Single Seed Descent method is adopted. A population of RILs represents an 'immortal' mapping population.

Advantages

- Used for QTL mapping.
- Immortal Population.

Disadvantages

- Requires many seasons to develop.

Detection of QTLs: Different methods for QTL detection are single-marker analysis, interval mapping by maximum likelihood, interval mapping by regression and composite interval mapping (Fig 3). These are elaborated as under:

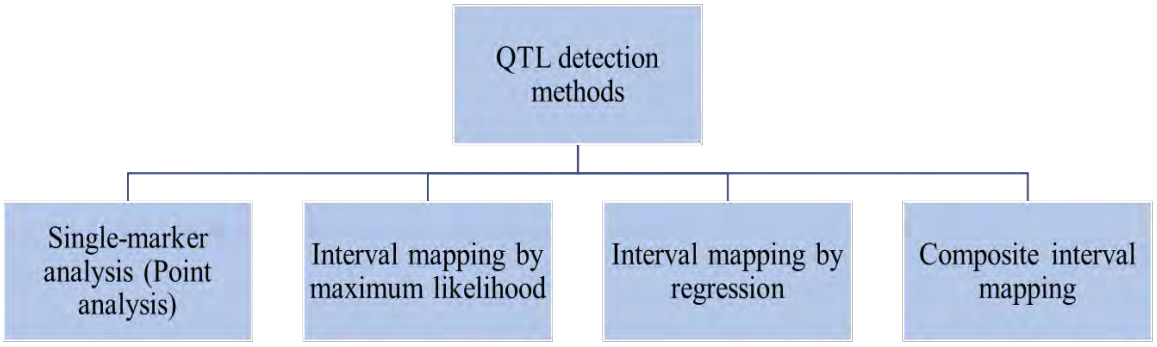

Fig. 3: Different methods involved in the detection of QTLs

Single-marker analysis (Point analysis): One of the important methods for determining a QTL in the presence of a marker is to study single-genetic markers one at a time. The phenotypic means for progeny of each marker class are then compared (e.g., means of the marker classes AA, Aa, aa). The difference between two means provide an estimate of the phenotypic effect of substituting an "A" allele by an "a" allele. To determine whether the inferred phenotypic effect is significantly different, statistical test *viz.,* t-test or F-test, is being carried out. A significant value indicates that a QTL is located in the vicinity of the marker. Single-point analysis does not require a complete molecular linkage map. The farther a QTL is from the marker, the less likely it is to be detected statistically because of the crossover events between the marker and the gene.

Interval mapping by maximum likelihood: Interval mapping method is widely used for QTL analysis. This method utilizes the information of two flanking markers to estimate the position of QTL.

Interval mapping by regression: Interval mapping by regression (Haley and Knott, 1992) was developed for simplifying the maximum likelihood method. It is mostly similar to the method of basic QTL analysis (regression on coded marker genotypes) except that phenotypes are regressed on QTL genotypes. Since the QTL genotypes are not known, they are being replaced by the probabilities estimated from the nearest flanking markers.

Composite interval mapping: One of the factors that weakens interval mapping is fitting the model for a QTL at only one location. The problems associated with this approach are that the effects of additional QTL will contribute to sampling variance and the combined effect of two QTLs will lead to biased estimates. The method of composite interval mapping (CIM) can serve as solution. CIM will perform the analysis in the usual way, except that the variance from other QTLs is accounted for by including partial regression coefficients from markers ('cofactors') in other regions of the genome. CIM gives more power and accuracy than simple interval mapping (SIM) because the effects of other QTLs are not included as residual variance. CIM can remove the bias that can be caused by QTLs that are linked to the position being tested.

Candidate Gene Approaches:

A glance of traditional candidate gene approaches: The candidate gene approach is based on the rationale that a major component of quantitative genetic variation of phenotype under investigation is caused by functional mutation of the putative gene. Candidate genes are basically the genes with known biological function that regulates the developmental processes directly or indirectly of the investigated traits, which can be confirmed by evaluating the effects of the causative gene variants in an association analysis. Candidate gene approach has been widely applied for gene-disease research, genetic association studies, biomarker and drug target selection in many organisms from animals to humans (Tabor *et al*., 2002; Waza *et al.,* 2013; Majid *et al.,* 2020). To date, many candidate genes of economic traits were primarily or even repeatedly detected, although the total number of the publicly accepted genes are yet absolutely small. Moreover, candidate gene analysis is usually an indispensable procedure for subsequent positional cloning of QTLs controlling the major genetic variation of interested traits after initial genome scans.

Resistance QTLs are valuable resources for rice improvement: Quantitative resistance is generally considered as partial resistance in a particular cultivar (Parlevliet, 1979). Multiple loci control this type of disease resistance which are referred to as QTLs, and differs from that of simple Mendelian inheritance. Thus, selection of these QTLs is cumbersome. However, various reports revealed that pyramiding resistance QTLs can achieve the same level or even a better resistance than R gene (Castro *et al*., 2003a, 2003b; Richardson *et al.,* 2006; Majid *et al.,* 2017). The population of rice possessing 241 recombinant inbred lines (RILs) and obtained from a cross between Zhenshan 97 (susceptible) and Minghui 63 (resistant) by following single-seed descent method was used to study quantitative resistance against *Xoo* and *M. grisea* (Chen 2001; Chen *et al*., 2003). The resistance of Minghui 63 to *M. grisea* was controlled by the R gene rbr2, which encodes a nucleotide- binding site-leucine-rich repeat type protein, and eight minor QTLs, which accounted for 1.6–6.1% of the phenotypic variation of the resistance (Chen *et al*., 2003; Yang *et al*., 2008). In case of susceptible variety Zhenshan 97, six resistance QTLs, which explained 2.1–11.7% of the phenotypic variation of the resistance to *M. grisea*, have been identified (Chen *et al*., 2003). Resistance assessment of each line in this population (Chen *et al*., 2003) showed that at least nine genotypes which were free of rbr2 but accumulated the QTLs from both the resistant and susceptible parents, possessed the same or even enhanced level of resistance to *M. grisea* isolate F1366 in comparison to control Minghui 63. Four rice lines which carried rbr2 and most of the QTLs from both the parents, showed markedly enhanced resistance to F1366 as compared to Minghui 63.

Chen (2001) also examined the resistance of rice lines against bacterial blight using the RIL population. It was observed that both Minghui 63 and Zhenshan 97 were susceptible to the *Xoo* strain KS-1–21. There was not any R gene in the population, however six minor QTLs, which explained 4.1–9.2% of the phenotypic variation for resistance to KS-1–21, were seen from Minghui 63 and only one minor QTL, explaining 4.9% of the phenotypic variation, was identified from Zhenshan 97. Analysis of the data by Chen (2001) showed the three RILs *viz.,* R96, R117, and R240 that possessed most of the resistance QTLs from both parents were highly resistant to KS-1–21. Rest of the RILs (R30, R34, R54, R92, R143, R180, and R209) that possessed only one or two of the QTLs from the parents revealed increased susceptibility to KS-1–21 as compared to their parents, Minghui 63 and Zhenshan 97. The polygenic control is much more durable than qualitative resistance, because each gene involved in it has a small effect on the resistance of host. The addition of these small gene effects shows longer life span than the resistance conferred by a single gene.

Regulation of Disease Resistance QTLs: In plants, resistance to diseases has been regulated by two classes of genes *viz.,* R genes and pathogen-induced defence-responsive or defence related genes. The signal transduction network in plants gets activated by pathogen recognition which is composed of the products from the two classes of genes. The expression of defence responsive genes is being induced or suppressed by pathogen infection, which provide the researchers a sign of identifying genes putatively involved in the interaction of host and the pathogens. Large numbers of defence-responsive genes have been identified in rice by using differential expression analysis (Kim *et al*., 2001). In rice resistance to bacterial blight and fungal blast, defence responsive genes have been classified into three types (Wen *et al*., 2003):

(1) Pathogen non-specific: in which the expression of these genes has been affected by infection of both *Xoo* and *M. grisea.*
(2) Pathogen specific but race non-specific: here the expression has been affected by infection with different races of *Xoo* or different isolates of *M. grisea,* but not both; and
(3) Both pathogen and race specific: here the expression has been influenced only by the infection of one *Xoo* race or one *M. grisea* isolate.

Most of the defence-responsive genes belong to the pathogen non-specific type, and these may be the sources for broad-spectrum resistance in breeding programs. These results also suggest that bacterial blight and fungal blast resistance share common pathways, however they are also regulated by unique defence paths. Mapping of these defence-responsive genes on rice molecular linkage maps has showed that some of the genes co-localize with disease resistance QTLs (Wang *et al*., 2001). Thus, some of the defence-responsive genes involving the resistance QTLs, can be used as candidates for characterization of QTLs.

Genetic studies of the rice-*M. oryzae* interaction have identified almost 100 race-specific resistance genes and over 350 QTLs on the entire rice genome, except chromosome 3 (Ashkani *et al.,* 2015). Some of the QTLs that are associated with the quantitative resistance to blast in rice are presented in Table 1. More than 200 QTLs associated with sheath blight resistance have been identified using different populations, such as double haploid lines (DHLs), recombinant inbred lines (RILs), near isogenic introgression lines (NILs) and backcross populations (Sharma *et al.,* 2009).

Table 1: Some important Quantitative Trait Loci for resistance to blast in rice

Gene/QTL	Chromosome	Reference
Pi-b	2	Wang *et al.*, 1999
Pi-63	4	Fukuoka *et al.*, 2001
Pi-t	1	Hayashi *et al.*, 2006
Pi-z	6	Zhou *et al.*, 2006
Pi-37	1	Lin *et al.*, 2007
Pi-36	8	Liu *et al.*, 2007
Pi-sh	1	Takahashi *et al.*, 2010
Pi-k	11	Zhai *et al.*, 2011
Pik-h/Pi-54	11	Rai *et al.*, 2011
Pi-1	11	Hua *et al.*, 2012
Pi60(t) and Pi61(t)	12	Lei *et al.*, 2013
Pi-h2(t)	1	Xiao *et al.*, 2015
Pi-h1(t)	11	Xiao *et al.*, 2015
Pi-h3(t)	12	Xiao *et al.*, 2015
Pi-65(t)	11	Zheng *et al.*, 2016
Pi-57(t)	12	Dong *et al.*, 2017
Pi-67	12	Joshi *et al.*, 2019
Pb2	11	Yu *et al.*, 2022

Candidate Gene strategy for Isolation of minor disease resistance QTLs: A candidate gene strategy has been established to isolate disease resistance QTLs in rice. Hu *et al.* (2008) established a candidate gene strategy that integrates linkage map, expression profile and functional complementation analyses to isolate disease resistance QTLs in rice. The strategy has proven to be applicable for identifying the genes underlying minor resistance QTLs in rice for blast and bacterial leaf blight. Yadav *et al.* (2015) utilized the composite interval mapping analysis and identified 9 QTLs mapped to five different chromosomes with phenotypic variance ranging from 8.40 to 21.76%. A hypothetical β 1-3 glucanase with other 31 candidate genes were identified in silico utilizing rice database RAP-DB within the identified QTL region qshb9.2. A detailed insight into these candidate genes has facilitated to study intricate nature of sheath blight. Devi *et al.* (2020) used the combination of mapping and microarray analysis approaches to identify a candidate gene Os03g0281466 (malectin-serine threonine kinase) in the fine mapped region. This gene was named as Pi68 (t) and confers field resistance to leaf as well as neck blast in rice. Carrillo *et al.* (2021) used candidate gene approach to accumulate different disease QTL from a blast-resistant rice variety (Moroberekan) into a drought-tolerant variety (Vandana). It was observed that the level of resistance effective in multiple locations was proportional to the number of CG alleles accumulated in advanced breeding lines. This strategy for isolation of disease resistance QTLs in rice has four steps (Fig 4).

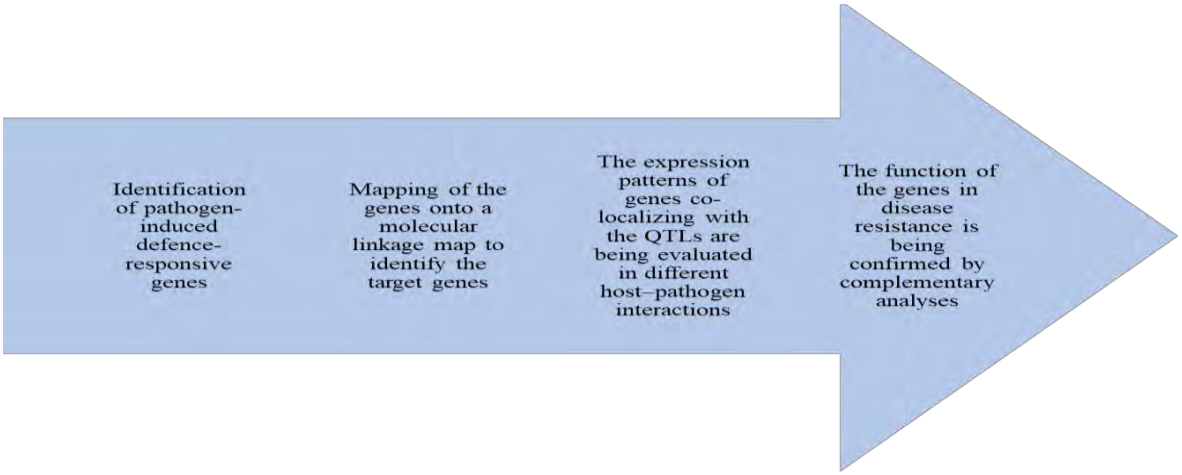

Fig 4: Steps involved in the isolation of disease resistance QTLs in rice

1. Pathogen-induced defence-responsive genes are being identified by differential expression analysis.
2. The genes are mapped onto a molecular linkage map to identify those genes showing chromosomal locations with respect to the known disease resistance QTLs by bioinformatics analysis.
3. The expression patterns of genes co-localizing with the QTLs are checked in different host–pathogen interactions in-order to identify the one whose expression is being influenced by a wide range of pathogens.
4. The function of the genes in disease resistance is confirmed by complementary analyses via overexpressing or suppressing of the target gene.

CONCLUSION

The polygenic control is considered as much more durable, as each gene involved has a small effect on host resistance. Characterization of the genes underlying resistance QTLs is helpful for rice improvement programmes. Quantitative resistance is putatively race nonspecific and thus forms a valuable source for broad spectrum resistance. The candidate gene strategy has proven to be most efficient approach to isolate minor QTLs. Recent advances in molecular marker technology, coupled with development of better theoretical models and high-throughput strategies, are expected to enable greater power and precision in detection of QTL and their utilization for crop improvement including resistance to diseases.

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