Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 1788-1793
Received: 15-09-2019
Accepted: 17-10-2019

Maumita Burman
Department of Genetics & Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya., Raipur, Chhattisgarh, India

Sunil Kumar Nair
Department of Genetics & Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya., Raipur, Chhattisgarh, India

Corresponding Author:
Maumita Burman
Department of Genetics & Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya., Raipur, Chhattisgarh, India

Validation of transferred genes and their expression: A review

Maumita Burman and Sunil Kumar Nair

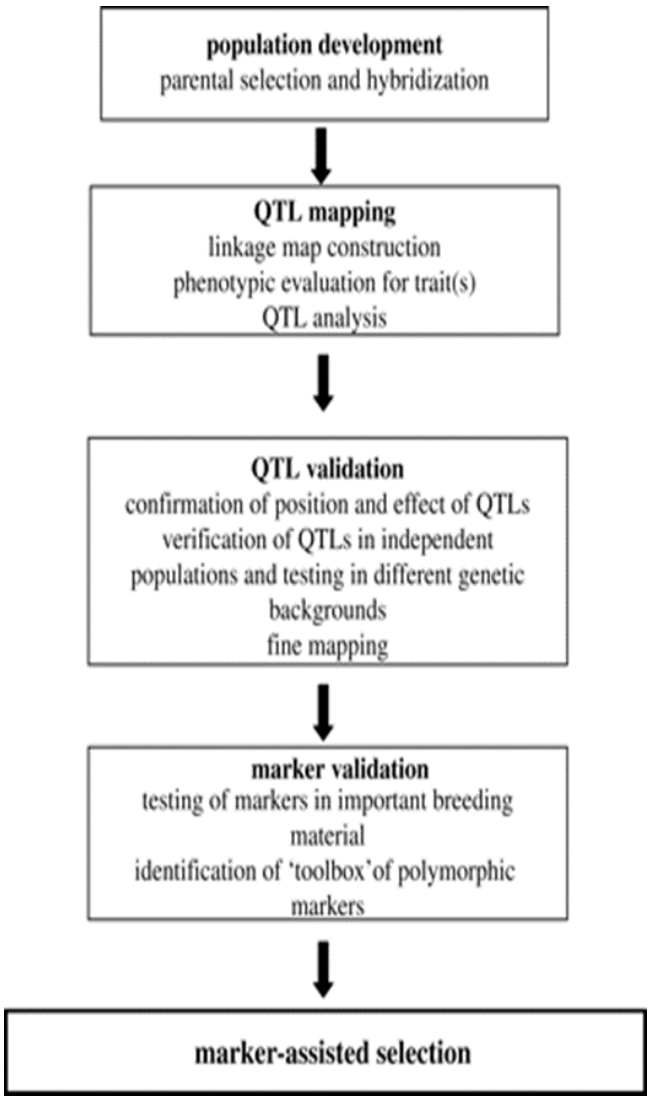
Abstract
Rice (*Oryza sativa*) is one of the most globally important staple food crops. Rice being grown under varied ecologies, confronts several biotic stresses. The key limiting factor in rice production is disease, which leads to annual yield losses of more than 5%. The emergence of new virulent pathotypes has resulted in evolution of novel allelic forms of genes which is necessary for survival of genotypes and vice versa. Incorporation of genetic resistance in cultivars is an effective strategy to manage the disease. More than 70 diseases caused by fungi, bacteria, viruses, and nematodes have been recorded in rice, the most serious of which are rice blast (caused by the hemibiotrophic fungal pathogen *Magnaporthe oryzae*), bacterial leaf blight (*Xanthomonas oryzae pv. oryzae* (Xoo) and sheath blight (*Rhizoctonia solani)*. Resistance to disease and insects is restricted either by major dominant or recessive R genes or by QTLs. To identify resistance genes, DNA markers have been used, and diverse resistance genes have been combined into rice cultivars lacking the desired traits through MAS. To characterize minor resistance QTLs in rice, a validation strategy and functional analysis of QTLs has been proposed based on the hypothesis of the candidate gene. To provide durable resistance, new strategies giving long-term protection over broad geographical areas must therefore be developed.

Keywords: *Oryza sativa* L., Biotic stress, QTL Mapping

Introduction
Biotic stresses significantly limit global crop production. Identification and use of resistant cultivars is currently seen as the best strategy, cheapest, durable and environmentally friendly method to manage biotic stresses. For years, chemicals have been used to control biotic damage of crop plants. Nowadays, interest in the use of chemicals against biotic stress is decreasing because of its various limitations such as the requirement for more than one chemical application, an investment that is not affordable by most small-scale farmers. Besides, using chemical spray may have adverse effects on human health and the environment, including beneficial organisms and may lead to the development of chemical-resistant pathogen races, insects, and weeds (Vincelli, P. 2016; Maidaner *et al*., 2013) [26, 14]. On the other hand, the use of resistant cultivars is currently seen as the best strategy, durable, economical, and environmentally friendly means of biotic stress control (Ragimekula *et al*., 2013; Hansona *et al*., 2016) [18, 8].

Usually, breeding efforts made to incorporate single resistant gene leads to resistance breakdown within a short period. Hence, recent breeding programs have targeted at developing cultivars that can withstand multiple stresses by assembling series of genes from different parents into a single genotype in a phenomenon called gene pyramiding or stacking (Suresh and Malathi, 2013) [24]. Malav *et al*., 2016 [12] stated that gene pyramiding is a breeding method that aimed at assembling multiple desirable genes from multiple parents into a single genotype. The technique is very helpful for developing crops that confer broad spectrum resistance against different races of pathogens or pests or combination of stresses. For several years, traditional breeding has been used to identify and incorporate multiple resistant genes/QTLs into cultivars of interest to develop durable resistance to biotic stresses (Ragimekula *et al*., 2013) [18]. However, conventional method of crop improvement has been complained to be slow, less precise, less flexible, labor-intensive and expensive (Wieczorek, A. 2003; Choudhary *et al*., 2008) [28, 2]. With traditional breeding, breeder's capability to track the presence or absence of the target genes is very slow and limited. This limits the number of genes to be stacked into elite cultivars at any times (Malav *et al*., 2016) [12]. Hence, a technological intervention that can reduce the time and costs necessary to develop and release new cultivars with durable resistance are always welcome. Recently, biotechnological tools like molecular markers and genetic engineering are widely used in crop improvement program for rapid and efficient accumulation of desirable genes from various sources into a single

background to produce broad spectrum/durable resistance. The advent and application of molecular marker technology made it easier to identify, map and efficiently pyramid resistant genes/QTLs into crop plants. DNA markers tightly linked (<5 cM) to the desired gene serve as chromosomal landmark, 'signs' or 'flags' to track the introgression of the desired gene in progenies in a cross (Asad *et al*., 2012) [1]. Hence, identification of resistant genes/ QTLs with closely linked DNA-markers is useful for successful transfer of the gene/QTLs into improved cultivars via marker-assisted selection (MAS). So far, various resistance genes/QTLs of crop plants have been identified and mapped using marker assisted selection. For instance, Yadav *et al*., 2015 [29] identified and mapped nine QTLs associated with sheath blight resistance in rice using MAS. This is the flow chart representation of validation process in brief.



The most widely used markers in major cereals are called simple sequence repeats (SSRs) or microsatellites (Gupta *et al*. 1999; Gupta & Varshney 2000) [7, 6]. They are highly reliable (i.e. reproducible), co-dominant in inheritance, relatively simple and cheap to use and generally highly polymorphic. The only disadvantages of SSRs are that they typically require polyacrylamide gel electrophoresis and generally give information only about a single locus per assay, although multiplexing of several markers is possible. These problems have been overcome in many cases by selecting SSR markers that have large enough size differences for detection in agarose gels, as well as multiplexing several markers in a single reaction. SSR markers also require a substantial investment of time and money to develop, and adequate numbers for high-density mapping are not available in some orphan crop species. Sequence tagged site (STS), sequence characterized amplified region (SCAR) or single nucleotide polymorphism (SNP) markers that are derived from specific DNA sequences of markers (e.g. restriction fragment length polymorphisms: RFLPs) that are linked to a gene or quantitative trait locus (QTL) are also extremely useful for MAS (Shan *et al*. 1999; Sanchez *et al*. 2000; Sharp *et al*. 2001) [22, 20, 23].

QTL mapping and MAS

The detection of genes or QTLs controlling traits is possible due to genetic linkage analysis, which is based on the principle of genetic recombination during meiosis (Tanksley 1993) [25]. This permits the construction of linkage maps composed of genetic markers for a specific population. Segregating populations such as F_2, F_3 or backcross (BC) populations are frequently used. However, populations that can be maintained and produced permanently, such as recombinant inbreds and doubled haploids, are preferable because they allow replicated and repeated experiments. These types of populations may not be applicable to outbreeding cereals where inbreeding depression can cause non-random changes in gene frequency and loss of vigour of the lines. Using statistical methods such as single-marker analysis or interval mapping to detect associations between DNA markers and phenotypic data, genes or QTLs can be detected in relation to a linkage map (Kearsey 1998) [9]. The identification of QTLs using DNA markers was a major breakthrough in the characterization of quantitative trait.

Previously, it was assumed that most markers associated with QTLs from preliminary mapping studies were directly useful in MAS. However, in recent years it has become widely accepted that QTL confirmation, QTL validation and/or fine (or high resolution) mapping may be required (Langridge *et al*. 2001) [11]. Although there are examples of highly accurate preliminary QTL mapping data as determined by subsequent QTL mapping research (Price 2006) [17], ideally a confirmation step is preferable because QTL positions and effects can be inaccurate due to factors such as sampling bias (Melchinger *et al*. 1998) [13]. QTL validation generally refers to the verification that a QTL is effective in different genetic backgrounds (Langridge *et al*. 2001) [11]. Additional marker-testing steps may involve identifying a 'toolbox' or 'suite' of markers within a 10cM 'window' spanning and flanking a QTL (due to a limited polymorphism of individual markers in different genotypes) and converting markers into a form that requires simpler methods of detection. Once tightly linked markers that reliably predict a trait phenotype have been identified, they may be used for MAS.

The fundamental advantages of MAS over conventional phenotypic selection are as follows

a. It may be simpler than phenotypic screening, which can save time, resources and effort. Classical examples of traits that are difficult and laborious to measure are cereal cyst nematode and root lesion nematode resistance in wheat (Eastwood *et al*. 1991; Eagles *et al*. 2001; Zwart *et al*. 2004) [5, 4, 30]. Other examples are quality traits which generally require expensive screening procedures.
b. Selection can be carried out at the seedling stage. This may be useful for many traits, but especially for traits that are expressed at later developmental stages. Therefore,

undesirable plant genotypes can be quickly eliminated. This may have tremendous benefits in rice breeding because typical rice production practices involve sowing pre-germinated seeds and transplanting seedlings into rice paddies, making it easy to transplant only selected seedlings to the main field.

c. Single plants can be selected. Using conventional screening methods for many traits, plant families or plots are grown because single-plant selection is unreliable due to environmental factors. With MAS, individual plants can be selected based on their genotype. For most trait homozygous and heterozygous plants cannot be distinguished by conventional phenotypic screening.

These advantages can be exploited by breeders to accelerate the breeding process (Ribaut & Hoisington 1998; Morris *et al*. 2003) [19, 15]. Target genotypes can be more effectively selected, which may enable certain traits to be 'fast-tracked', resulting in quicker line development and variety release. Markers can also be used as a replacement for phenotyping, which allows selection in off-season nurseries making it more cost-effective to grow more generations per year (Ribaut & Hoisington 1998) [19]. Another benefit from using MAS is that the total number of lines that need to be tested can be reduced. Since many lines can be discarded after MAS early in a breeding scheme, this permits more efficient use of glasshouse and/or field space-which is often limited-because only important breeding material is maintained.

Considering the potential advantages of MAS over conventional breeding, one rarely discussed point is that markers will not necessarily be useful or more effective for every trait, despite the substantial investment in time, money and resources required for their development. For many traits, effective phenotypic screening methods already exist and these will often be less expensive for selection in large populations. However, when whole-genome scans are being used, even these traits can be selected for if the genetic control is understood.

Identification and Linkage Mapping of Resistance Genes/QTLs in Crop Plants

Gene mapping describes the methods used to identify the locus of a gene and the distances between genes. There are two distinctive types of "maps" used in the field of genome mapping: genetic maps and physical maps. They differ in techniques used to construct them and in the degree of resolution. Genetic map distances are constructed based on the genetic linkage information while physical maps use actual physical distances (has high resolution) usually measured in number of base pairs (Parlevliet, 1978) [16]. QTL map is a type of genetic map, which indicates the approximate location of a quantitative trait locus (QTL) within an interval delineated by two or more markers on a genetic map.

Genetic mapping/linkage mapping of genes/QTLs

Genetic mapping can be defined as the process of determining the linear order of molecular markers or genes (generally, loci) along a stretch of DNA or chromosome (Dixit *et al.*, 2014) [3]. Linkage map indicate the relative position of markers on chromosome or linkage groups (LGs) based on the frequencies of recombination that occur between markers on homologous chromosomes during meiosis. Recombination frequency between two markers is proportional to the distance separating the markers. The greater the frequency of recombination, the greater the distance between two genetic markers; conversely, the smaller the recombination frequency, the closer the markers are to one another. The distance between markers on a genetic map is given as Morgan (M) or centimorgan (cM), where one cM is the distance that separates two markers (or genes), between which a 1% chance of recombination exists (corresponding to one recombination event in 100 meioses). That means 99% of the times these two markers (genes) co-segregate, and hence MAS can be applied to select progenies with desired traits during crossing. The following steps are prerequisites for a successful linkage or genetic mapping of a target genome (Dixit *et al*, 2014) [3].

Selection of parent plants

The first step in linkage mapping is the selection of genetically divergent parents that exhibit sufficient polymorphisms for the trait of interest, but are not so distant as to cause sterility of the progeny (Dixit *et al.*, 2014) [3]. Accordingly, in determining the chromosomal position of resistant genes/QTLs toward a particular pathogen, parental lines with sufficient polymorphism (pure resistant and pure susceptible parental liens) should be selected phenotypically in the field and/or using marker system (Dixit *et al.*, 2014) [3].

Developing mapping population

Following the selection of parental lines, the next key step is developing a mapping polymorphic population (Dixit *et al.*, 2014) [3]. Several types of mapping populations may be suitable for a particular project (Weising *et al*., 2005) [27] including:

1. Double haploid lines (DHLs): Regenerated plants from pollen (which is haploid) of the F1 plants and treated to restore diploid condition in which every locus is homozygous.

2. Backcross (BC) population: The F1 plants are backcrossed to one of the parents.

3. F2 population: F1 plants are selfed.

4. Recombinant inbred lines (RILs)

Inbred generation derived by selfing individual F2 plants and further single seed descent. A population of RILs represents an 'immortal' or permanent mapping population. Each of the above mapping populations has both advantages and disadvantages, and the choice of the type of mapping population depends on many factors such as the plant species, type of marker system used, and the trait to be mapped (Weising *et al*., 2005) [27]. Accordingly, F2 populations and BC populations are simple and can be developed in short period for self-pollinating species. While RIL population takes six to eight generations. Although development of a DH population takes much less time than RIL; it is only possible in species that are amenable to tissue culture. RIL and DH populations are good in that theyproduce homozygous or 'true-breeding' lines that can be multiplied and reproduced without genetic change occurring. This allows undertaking replicated trials across different locations and years. With regard to the marker choice, co-dominant markers are best informative in F2 population, while information obtained by dominant marker systems can be maximized by using RILs or DHLs. Double haploids, F2 families, or RILs are advantageous if the trait to be mapped cannot be accurately measured on a single-plant basis but must be assessed in replicated field experiments (Weising *et al*., 2005) [27].

Determining mapping population size

In linkage mapping, the resolution of a map and the ability to determine marker order largely depend on population size (Dixit *et al*, 2014) [3]. A vague lower threshold that can localize quantitative trait loci (QTL) is a size of 100 individuals. However, high resolution maps for map-based cloning of target genes ideally require population sizes of more than 500 or even 1000 individuals. They (Yadav *et al*., 2015) [29] used 210 F2 and 150 BC1 F2 mapping population to map QTLs governing the sheath blight resistance in rice. Hence, it is important to decide the appropriate mapping population size required in locating chromosomal position of trait of interest, and generally the larger (>100) the mapping population, the better the map resolution would be (Dixit *et al*, 2014) [3].

Phenotype evaluation

Once a population segregating traits of interest is obtained, mapping the trait typically involves measuring the phenotype. Phenotypic evaluation can be undertaken in the field under natural condition (where high disease pressure can be expected) or in greenhouse/growth room in which the plants are inoculated with specific pathogen strains. Compared to the field evaluation, a greenhouse seedling inoculation can assess disease reactions quickly, reduce some sources of environmental variation by use of characterized pathogen strains and defined inoculum concentrations, and avoid confounding effects from other pests or diseases (Hansona *et al.*, 2016) [8].

Genotype profiling

Generation of genotypic data for the mapping population involve two steps. First, DNA samples from the parental lines are screened for polymorphisms, using markers that span the chromosome(s) of interest. To scan the whole genome, polymorphic markers spaced approximately every 25 cM to 30 cM are needed. The second step is genotyping the mapping population with the selected polymorphic markers (Dixit *et al*, 2014) [3]. It is important to include many markers as much as possible (Weising *et al*., 2005) [27].

Construction of linkage maps

The marker data collected through genotyping of the mapping population are used to construct the linkage map. Linkage analysis is based on the fact that two marker loci that are close to each other on the same chromosome tend to co-segregate; i.e., will be inherited together (Dixit *et al*, 2014) [3]. The frequency of recombinant (non-parental) genotypes is used to calculate recombination frequency, which is then used to infer the genetic distance between markers. By analyzing the segregation of markers, the relative order and distances between markers can be determined. The lower the frequency of recombination between two markers, the closer they are situated on a chromosome; conversely, the higher the frequency of recombination between two markers, the further away they are situated on a chromosome (Weising *et al*., 2005) [27].

Simple statistical tests such as a $\chi 2$ analysis will test the independent assortment of two loci and hence linkage. For two loci, a recombination frequency <50% indicates linkage. Usually, Kosambi's mapping function is used to derive genetic distances (cM) between linked loci from their recombination frequency. Linkage between two loci is usually calculated with an odds ratio (i.e., the ratio of linkage versus no linkage). This ratio is more conveniently expressed as the logarithm of the ratio and is called a logarithm of odds (LOD) value or LOD score. A LOD score of 3 is normally accepted as a lower significance threshold to assert linkage (Dixit *et al*, 2014) [3] and the QTLs of interest are thought to exist at positions where an LOD score exceeded the corresponding significant threshold. Linked markers are grouped together into linkage groups (LG). In QTL analysis, the proportion of phenotypic variation explained by each QTL is calculated as R2 value, and the degree of dominance of a QTL is estimated as the ratio of dominance effect to additive effect. A number of mapping computer programs are available for mapping traits controlled by single genes as well as quantitative traits like Mapmaker/EXP (Sehgal *et al*., 2016) [21] and Join Map v.4.0 (Lander *et al*., 1987) [10].

Applications

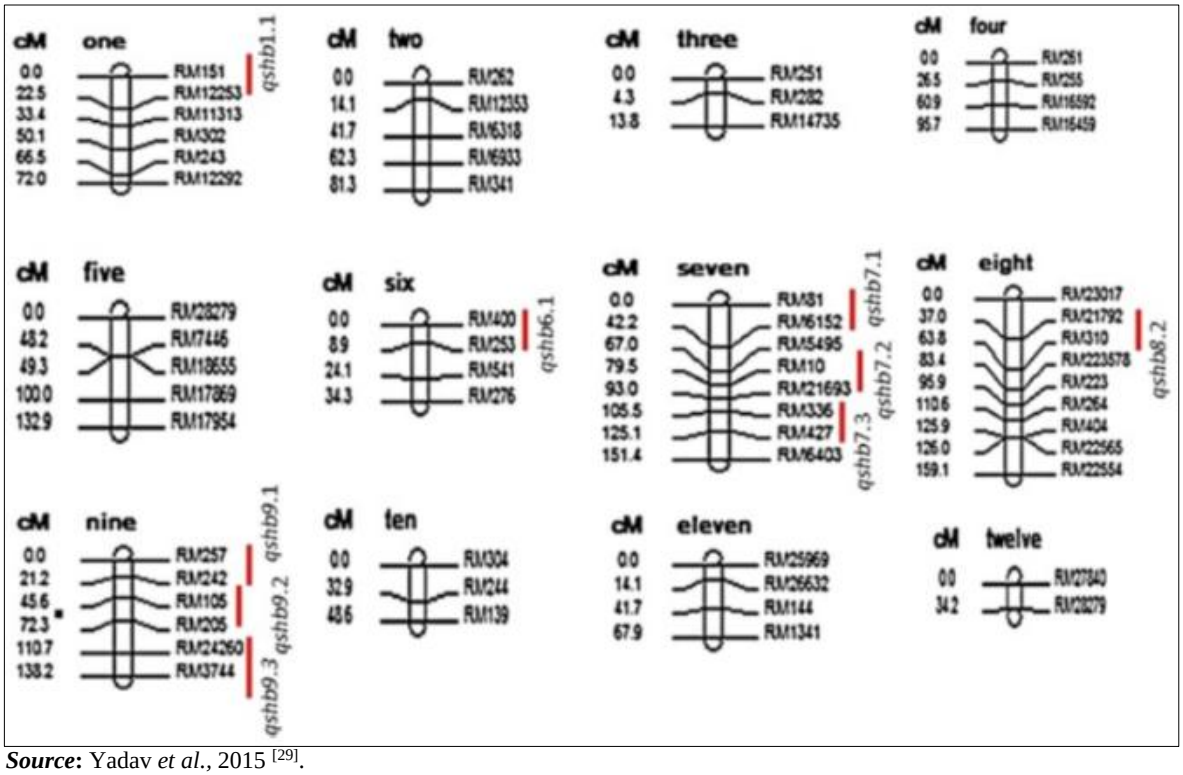
Identification and mapping of resistant genes/QTLs in two selected crops

Identification of QTLs and possible candidate genes conferring sheath blight resistance in rice (*Oryza sativa* L.) Sheath blight is one of the most devastating diseases of rice caused by the fungus Rhizoctonia solani Kühn. Pyramiding of diverse Sheath blight resistant (ShBR) QTLs could help to achieve higher levels of resistance to ShB. In line with this, (Yadav *et al*., 2015) [29] aimed at identifying and mapping QTLs and candidate genes associated with sheath blight resistance in rice. As a procedure, two mapping populations namely 210 F2 (derived from the cross between the susceptible BPT-5204 and moderately resistant ARC10531) and 151 BC1F2 populations (derived from the same cross) were developed. After greenhouse phenotypic evaluation in the presence of the pathogen R. solani, the F2 population was genotyped using 70 polymorphic SSR markers. A linkage map was constructed using Mapmaker 3.0 and significance threshold of >3 was considered for linkage grouping. Finally, 9 ShBR QTLs have been identified and mapped to five chromosomes (1, 6, 7, 8 and 9) with phenotypic variance ranging from 8.40% to 21.76%. They identified new markers linked to the ShB resistances QTLs on chromosome 1, 6 and 8. The study also identified two major ShBR-QTLs: qshb7.3 (explained 21.76% of the total phenotypic variance) and qshb9.2 (explained 19.81% of the phenotypic variance) that can be transferred using MAS into elite cultivars.

Validation of linked microsatellite markers associated with sheath blight resistance in rice

Another crucial step in linkage mapping is validation of the co-segregation of the identified marker and the trait. Usually, Bulk Segregant Analysis (BSA) has been employed to identify the DNA markers linked to the sheath blight resistance gene. Accordingly, in their validation analysis (Yadav *et al*., 2015) [29] pooled the DNA from 10 extremes resistant and 10 extreme susceptible plants of the BC1F2 separately. And then, amplified along with both parents using the same SSR markers: RM336 and RM205. Finally, it was found that the resistance alleles show co-segregation among the parents ARC10531and BPT-5204 i.e., presence of the markers confirm presence of the resistant genes. Moreover, an in-silico analysis using rice data base RAP-DB for search of defense responsive gene identified 32 genes within QTL region near to the marker RM205 on chromosome 9. Functional annotation of predicted genes by blastp revealed one defense responsive gene ß 1-3 glucanase like protein present in a single copy within the cluster and it may be responsible for sheath blight resistance in the rice line ARC-

10531. This shows that the identified markers are very efficient and helpful to select progenies carrying the desired genes/ QTLs in crop breeding program. Hence, genetic mapping is helpful to identify and map markers linked to desired agronomic traits to be used in genome-assisted crop improvement.



***Source*:** Yadav *et al*., 2015 [29].

Fig 1: Molecular genetics map of rice along with positions of QTLs for sheath blight resistance.

Conclusion

Biotic factors such as viruses, bacteria, fungi, nematodes, insect pests cause significant yield loss across the world. The use of resistant cultivars is seen as the best strategy, economical, durable and environmentally friendly to control these biotic stresses. As single gene-based resistance breakdown within a short period, current breeding programs targeted at stacking multiple resistance genes/QTLs into a single genotype to develop durable biotic stress resistant cultivars. The present seminar paper reviewed the types of genetic resistance (major and minor genes) in plants and the methodologies involved in identification, mapping and then pyramiding of genes/QTLs into crop plants to develop durable/broad-spectrum resistance to biotic stresses. Usually gene mapping is the starting point of many important downstream studies. Herein, linkage map construction procedures are reviewed in detail and supported with practical examples of mapping QTLs conferring resistance to different diseases.

References

1. Asad MA, Xia XC, Wang CS, He ZH. Molecular mapping of stripe rust resistance gene YrSN104 in Chinese wheat line Shaannong104. Hereditas. 2012; 149:146-152.
2. Choudhary K, Choudhary OP, Shekhawat NS. Marker assisted selection: A novel approach for crop improvement. American-Eurasian J Agronomy. 2008; 1:26-30.
3. Dixit R, Rai DV, Agarwal R, Pundhir A. Physical Mapping of genome and genes. J. Biol. Eng. Res. Rev. 2014; 1:06-11.
4. Eagles H, Bariana H, Ogbonnaya F, Rebetzke G, Hollamby G, Henry R *et al*. Implementation of markers in Australian wheat breeding. Aust. J Agric. Res. 2001; 52:1349-1356.
5. Eastwood RF, Lagudah ES, Appels R, Hannah M, Kollmorgen JF. Triticum tauschii-a novel source of resistance to cereal cyst nematode (*Heterodera avenae*). Aust. J Agric. Res. 1991; 42:69-77.
6. Gupta PK, Varshney RK. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica. 2000; 113:163-185.
7. Gupta PK, Varshney RK, Sharma PC, Ramesh B. Molecular markers and their applications in wheat breeding. Plant Breed. 1999; 118:369-390.
8. Hansona P, Lua SF, Wanga JF, Chena W, Kenyona L. Conventional and molecular marker-assisted selection and pyramiding of genes for multiple disease resistance in tomato. Sci. Hort. 2016; 201:346-354.
9. Kearsey MJ. The principles of QTL analysis (a minimal mathematics approach). J Exp. Bot. 1998; 49:1619-1623.
10. Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics. 1987; 1:174-181.
11. Langridge P, Lagudah E, Holton T, Appels R, Sharp P, Chalmers K. *et al*. Trends in genetic and genome analyses in wheat: a review. Aust. J Agric. Res. 2001; 52:1043-1077. (doi:10.1071/AR01082)
12. Malav AK, Kuldeep I, Chandrawat S. Gene pyramiding: An overview. Int. J. Curr. Res. Biosci. Plant Bio. 2016; 3:22-28.
13. Melchinger AE, Utz HF, Schon CC. Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low

power of QTL detection and large bias in estimates of QTL effects. Genetics. 1998; 149:383-403.
14. Miedaner T, Zhao Y, Gowda M, Longin CFH, Korzun, V. Genetic architecture of resistance to *Septoria tritici* blotch in European wheat. BMC Genomics. 2013; 14:858.
15. Morris M, Dreher K, Ribaut JM, Khairallah M. Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker assisted selection. Mol. Breed. 2003; 11:235-247. (doi:10.1023/ A:1022872604743)
16. Parlevliet JE. Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. Euphytica, 1978; 27:369-379.
17. Price AH. Believe it or not, QTLs are accurate! Trends Plant Sci. 2006; 11:213-216. (doi: 10.1016/j.tplants.2006.03.006)
18. Ragimekula N, Varadarajula NN, Mallapuram SP, Gangimeni G, Reddy RK. Marker assisted selection in disease resistance breeding. J Plant Breed Genet. 2013; 1:90-109.
19. Ribaut JM, Hoisington D. Marker-assisted selection: new tools and strategies. Trends Plant Sci. 1998; 3:236-239. (doi:10.1016/S1360-1385(98)01240-0)
20. Sanchez AC, Brar DS, Huang N, Li Z, Khush GS. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. Crop Sci. 2000; 40:792-797.
21. Sehgal D, Singh R, Rajpal VR. Quantitative trait loci mapping in plants: Concepts and approaches. Mol Breeding for Sustainable Crop Improvement, Sustainable Development and Biodiversity, Springer International Publishing Switzerland, 2016.
22. Shan X, Blake TK, Talbert LE. Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. Theor. Appl. Genet. 1999; 98:1072-1078. (doi:10.1007/s001220051169).
23. Sharp PJ. Validation of molecular markers for wheat breeding. Aust. J Agric. Res. 2001; 52:1357-1366. (doi:10. 1071/AR01052)
24. Suresh S, Malathi D. Gene pyramiding for biotic stress tolerance in crop plants. Weekly Sci. Res. J. 2013; 1:2321-7871.
25. Tanksley S. Mapping polygenes. Ann. Rev. Genet. 1993; 27:205-233. (doi:10.1146/annurev.ge.27.120193.001225)
26. Vincelli P. Genetic engineering and sustainable crop disease management: Opportunities for case-by-case decision-making. Sustain. 2016; 8:495.
27. Weising K, Nybom H, Wolff K, Kahl G. DNA Fingerprinting in plants principles, methods, and applications second edition. CRC Press Taylor and Francis Group, 2005, 277-291.
28. Wieczorek A. Use of biotechnology in agriculture-Benefits and risks. College of Tropical Agriculture and Human Resources (CTAHR), University of Hawaii, USA, 2003.
29. Yadav S, Anuradha G, Kumar RR, Vemireddy LR, Sudhakar R. Identification of QTLs and possible candidate genes conferring sheath blight resistance in rice (*Oryza sativa* L.). Springer Plus, 2015; 4:175.
30. Zwart RS, Thompson JP, Godwin ID. Genetic analysis of resistance to root-lesion nematode (*Pratylenchus thornei*) in wheat. Plant Breed. 2004; 123:209-212. (doi:10.1111/ j.1439-0523.2004.00986.x)