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Quantitative Trait Locus Mapping: Some Biological and Statistical Considerations

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Abstract

Many important traits in plant and animal populations such as yield, quality, and resistance/tolerance to biotic and abiotic stresses are controlled by many genes with small effects and are known as quantitative traits (also 'polygenic,' 'multifactorial' or 'complex' traits). The regions within genomes that contain genes associated with variation of quantitative traits are known as quantitative trait loci (QTL). A key development in the field of complex trait analysis was the establishment of large collections of molecular/genetic markers, which could be used to construct detailed genetic maps of both experimental and domesticated species. These maps provided the foundation for the modern-day QUANTITATIVE TRAIT LOCUS (QTL) mapping methodologies. The identification of QTLs can help to understand how many genomic regions significantly contribute to the trait variation in a population and how much variation is due to additive, dominant or epistatic effects of QTLs. Although the basic principle of QTL mapping has been established in Sax's work on beans, the identification of QTLs based only on conventional phenotypic evaluation is possible. A major breakthrough in the characterization of quantitative traits was initiated by the development of RFLP markers which created opportunities to select QTLs. After that generally, biparental populations are used to map QTLs, in which the marker genotype and trait phenotype data are analyzed to detect the association between the two. The advent of molecular marker technology and the development of detailed linkage maps in various organisms made it possible to dissect QTs into discrete genetic factors. This review focuses the discussion on the biological considerations and statistical methods used for mapping QTLs.

Keywords: QTL mapping, RILs, SIM, molecular markers

1. Introduction

Much of the natural variation observed in the crops, domestic animals, and other populations is due to much more minor genetic changes in many genes. With laid down of the basic theoretical foundations of quantitative genetics by R.A. Fisher and the establishment of Quantitative Genetics, the focus was to partition the overall variation of quantitative traits into genetic and environmental ones. With the development and advancement of polymorphic markers in many species, one of the interesting and applied areas of researches in genetics, plant, and animal breeding is to partition genetic variation to individual quantitative trait loci (QTL) in the genome as well as interaction among them (Zeng et al., 1999; Doerge, 2002). A QTL is a region of the genome that is associated with an effect on a quantitative trait. Conceptually, a QTL can be a single gene, or it may be a cluster of linked genes that affect the trait. The aims behind QTL analysis are to:

- ✓ detect the genomic regions affecting the trait: where are the QTLs
- ✓ determine how much of the variation for the trait is caused by a specific region
- ✓ identify the gene action associated with the QTL additive/dominant effects
- ✓ identify the allele associated with the favorable effect
- ✓ determine whether there is a 'hot-spot on particular chromosomes for particular traits or is there a relatively random distribution
- ✓ assign breeding values to lines or families based on their genotypes at one or more QTLs.

The general steps in QTL mapping using bi-parental populations include (1) selection of parental lines that differ for traits of interest and generating segregating population, (2) selection of molecular markers such as SSR, and SNP for screening the two parental lines, (4) genotyping and phenotyping of the mapping population, and (5) detection of QTL using a suitable statistical method (Xu et al., 2017).

Mapping population

Detection of QTLs needs segregating populations which can be natural populations or populations developed from a cross between two lines. In practice, the most commonly used experimental designs for locating QTL start with two parental inbred lines, $P_1$and P_2, differing both in trait values and in the marker (M, N, ...) variants or alleles (M_1, M_2, N_1, N_2, ...) they carry. The F_1 individuals obtained from the cross of two homozygote lines are heterozygous at all markers and QTL regions. From the

F_1 population, crosses are made to generate F_2, backcrosses (BC), doubled haploids (DH), recombinant inbred lines (RIL), and near-isogenic lines (NIL) populations (figure 1) (Singh & Singh, 2015a; Xu et al., 2017).

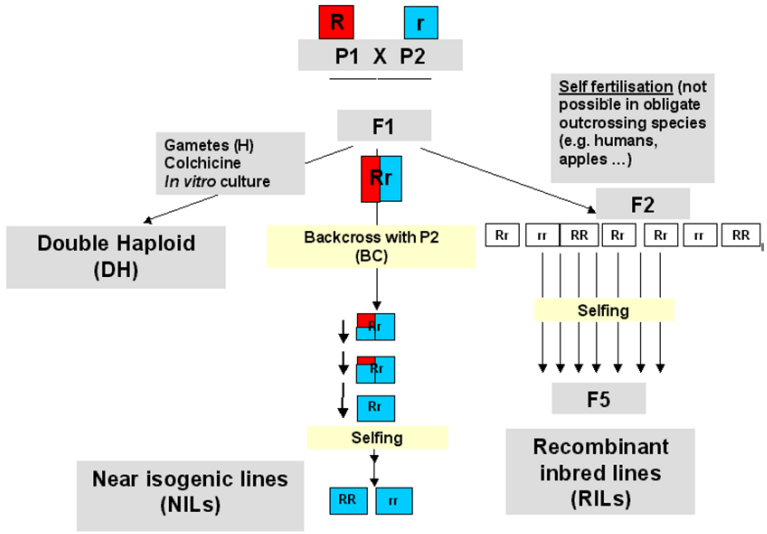


Figure 1. A schematic representation of the various bi-parental mapping populations.

F2: A F_2 population is generated by selfing or sib-mating of the F_1 individuals from a cross between the two homozygote parents (figure 1). F_2 population consisted of all parental allele combinations and each individual is expected to have a unique combination of linkage blocks from the two parental lines. In the process of F_2 population generation, only a single meiotic cycle, in other words, only one round of recombination can occur between any two loci. Therefore, the estimates of linkage between pairs of loci based on F_2 populations are not more accurate and the estimated genetic distances between two loci are likely to be greater than those detected by the population undergoing several rounds of recombination. F_2 populations are the best suited for preliminary linkage analysis and QTL mapping. The expected ratios for dominant and codominant markers in an F_2 population are 3:1 and 1:2:1, respectively. The development of F_2 populations requires the minimum times compare to the other segregating populations and it takes only two generations. The presence of all possible genotypes at a locus in F_2 populations provides the estimates of additive, dominance, and epistatic components of the

genetic variance. Each individual in the population captures the recombination events from both male and female parental gametes, which makes it ideal for identifying heterosis QTLs.

Backcrosses (BC): BC populations are generated by crossing F_1 plants with either of the two parental lines (figure 1). Due to the absence of one of the two possible homozygote genotypes at each locus in BC, genetic analysis using dominant markers can be performed only when the marker allele is absent in recurrent parent, but this is not the case in codominant markers. Backcross exhibits 1:1 ratio for dominant and codominant markers. The individuals in BC populations undergone only one cycle of recombination. Therefore, in BC populations like F_2 populations, recombination is not fixed and cannot be evaluated in replicated trials, which makes them unsuitable for QTL mapping. Compared to an F_2 population, a backcross population is less informative for linkage mapping because recombination among markers occurs in only one set of gametes (*i.e.*, the F_1) (Lander et al., 1987).

Doubled haploid (DH)*:* DH plants are usually produced by anthers/pollen culture of F_1 plants followed by chromosome doubling of haploid plants using colchicine (figure 1). The DH lines are completely homozygous at all the loci and recombination in DH lines is fixed, therefore they can be multiplied and maintained indefinitely and can be evaluated in replicated trials. The expected Mendelian ratio for each locus in a DH population irrespective of the dominant or codominant nature of the genes is 1:1. DHs. DH populations, like RILs, are perpetual as they can be multiplied and maintained indefinitely and can be evaluated in replicated trials.

Recombinant inbred lines (RILs)*:* RILs are a set of homozygous lines produced by continuous inbreeding/selfing of individual F_2 plants *via* the single seed descent (SSD) method (figure 1). The RIL population generated by adequate generations of selfing consists of homozygotes lines with different recombination from parental genomes. The expected ratio of the two homozygotes lines at each locus in the population is 1:1. Due to several cycles of recombination, RILs enable the detection of markers located much closer to the target gene than is possible with F_2, DH, and BC populations. Since RILs are homozygous, like DHs they can be propagated indefinitely without any further change in their genotype and recombination structure; this makes RILs essentially a perpetual or permanent mapping population (Burr et al., 1988).

Near-isogenic lines (NILs): NILs are developed by continuous backcrossing to recurrent parent followed by a single generation of selfing. They are homozygous lines that are identical in genotype, except for a single gene/locus or a variable length of the genomic regions flanking the target locus. They may also differ for some random genomic segments located elsewhere in the genome. Like DHs and RILs, NILs are homozygous and perpetual mapping resources, but they are usually used for fine mapping of a specific gene/genomic region and are not common populations for the construction of linkage map and QTL mapping (Muehlbauer et al., 1988).

Over the years, bi-parental mapping populations have been used to map QTLs for various traits in crop plants such as barley (Hussain et al., 2016; Barati et al., 2017; Du et al., 2019; Capo-Chichi et al., 2021) wheat (Badakhshan et al., 2008; Azadi et al., 2015; Ehdaie et al., 2016; Guo et al., 2020; Wang et al., 2021), rice (Li et al., 2016; Amoah et al., 2020; Li et al., 2020).

Principle of QTL mapping

Quantitative trait loci (QTL) analysis is a methodology that combines DNA marker and phenotypic trait data to locate and characterize genes that influence quantitative traits. The individuals in the mapping population are partitioned into different groups based on their marker genotypes to determine whether significant differences exist between groups with respect to the trait being measured. A significant difference between phenotypic means of the groups (either 2 or 3) indicates that the marker locus is used to partition the mapping population is linked to a QTL controlling the trait (Tanksley, 1993; Young, 1996; Collard et al., 2005).

For genotyping of the population, DNA markers are first used to 'screen' (or evaluate) the parents of a mapping population for polymorphisms, detectable differences in marker patterns. After polymorphic markers are identified, they are used to evaluate each line or individual of the mapping population. Each line is scored for having the marker pattern corresponding to one or the other parent. The number of polymorphic markers needed for a QTL study will depend on the genome size of the species, the average spacing between markers, and the objectives of the study. In the next step, polymorphic markers will be used for the construction of a linkage/genetic map consisting of linkage groups in which relative positions and distances (cM) of loci are determined. In the saturated linkage map, the number of linkage groups is equal to the number of haplotype chromosomes in the species. Before linkage analysis, each marker locus is generally analyzed for evidence of segregation distortion, the deviation of observed segregation ratios from the ratios expected with Mendelian inheritance.

Statistical methods to map QTLs

Three commonly used methods for detecting QTLs in the bi-parental populations are single-marker analysis, simple interval mapping, and composite interval mapping (Liu, 1998; Tanksley, 1993).

Single-marker analysis: It also called 'single-point analysis' is the simplest method that examines the association of single marker variants with the trait variation at a

time. To calculate the strength of the association between genotype and phenotype, the mapping population is split into two or three groups, according to their marker genotypes, then the mean trait value of these two or three classes is compared. If the difference is significant, then this provides initial evidence for the location of a QTL in the neighborhood of the marker (Young, 1996). In the simplest case, linear equations can be developed to describe the relationship between a trait and each molecular marker using the following form: Y = μ + f(marker) + e, where, Y is the trait value, μ is the population mean, f(marker) is a function of the molecular marker, and e is an error. The linear model can be assessed by using t-tests, analysis of variance (ANOVA), and linear regression.

The t-test is the simplest method to test for trait men difference between two marker groups. For example, let $\hat{\mu}_{MM}$ and $\hat{\mu}_{Mm}$ be the observed trait means of individuals with marker genotypes *MM* and *Mm* for a marker in a backcross population, the *t* statistics for testing significance between $\hat{\mu}_{MM}$ and $\hat{\mu}_{Mm}$ is:

$$t = \frac{\hat{\mu}_{MM} - \hat{\mu}_{Mm}}{\sqrt{s^2\left(\frac{1}{n_{MM}} + \frac{1}{n_{Mm}}\right)}}$$

Where s^2 is the pooled sampling variance, and n_{MM} and n_{Mm} are corresponding sample size in each marker class. Significant *t* statistics show the presence of putative QTL in the vicinity of the tested marker locus. After locating the QTL, it is possible to estimate the effect of detected QTL on trait variance using the following formula: $\epsilon(\hat{\mu}_{MM} - \hat{\mu}_{Mm)=(1-2r)a}$, where ϵ denote the expectation, *a* is the effect of identified QTL and *r* is the recombination frequency between marker locus and QTL. It should be considered that using the backcross population it is not possible to separate QTL additive and dominance effects and *a* indicates the QTL genetic effect. In the case of DH and RIL populations, $\epsilon(\hat{\mu}_{MM} - \hat{\mu}_{mm)/2=(1-2r)a}$, where *a* is an estimate of the QTL additive effect.

In the F_2 population, individuals are classified into three genotypes groups based on each codominance marker locus, therefore ANOVA will be powerful than *t*-test for the significant test of the phenotypic means of genotypic groups. The analysis gives an F statistic and provides a quick and simple method to detect which markers are associated with a QTL. Since F_2 population consisted of all three possible genotypes in marker locus, therefore additive and dominance effects of QTL can be estimated as: $\hat{\mu}_{MM} - \hat{\mu}_{mm})/2 = a(1-2r)$ and $\hat{\mu}_{Mm} - (\hat{\mu}_{MM} + \hat{\mu}_{mm})/2 = d(1-2r)^2$, where let $\hat{\mu}_{MM}$, $\hat{\mu}_{mm}$ and $\hat{\mu}_{Mm}$ be the observed trait means of individuals with marker genotypes *MM*, *mm* and *Mm* for a marker in an F_2 population

Linear regression is the most commonly used statistical method for detection association between a marker locus and traits variation because the marker coefficient of determination (R^2) explains how much of the phenotypic variation is associated with the QTL linked to the marker. The marker and trait association is tested using the linear model of $y_i = \beta_0 + \beta_{x_i} + e_i$, where y_i is the trait value of the i^{th} individual in a population, β_0 is the mean (intercept), β is coefficient of regression showing the association, x_i is a dummy variable related to the i^{th} individual marker genotype taking a value of 1 and 0 for *MM*, and *Mm* marker genotypes in a BC population, respectively and e_i is a random residual variable for the i^{th} individual. The marker with significant regression coefficient is the one that is linked to the QTL.

In genetic terms, this method relies on the linear relationship between the size differences in the marker classes phenotypic means and the recombination frequency between the QTL and the individual marker, which is expressed as $1/2D_i = a_i(1 - 2r_i)$, where D_i and r_i are the difference between the phenotypic means of i^{th} markers classes and the recombination frequency between the QTL and the i^{th} marker, respectively (Kearsey & Hyne, 1994). At the true position of the QTL, this is linear regression of $½D_i$ on $(1- 2r_i)$ with sloped which passes through the origin.

Single marker analysis is simple and does not need a linkage map, and QTL mapping was initially carried out by looking for an association between genotypes at individual markers and phenotypic variation of target traits. There are three problems with this approach (Lander & Botstein 1989):

i) the analysis cannot determine whether a significant marker effect is due to one or QTLs.

ii) even in the cases of a single QTL, it cannot determine the significant marker effect is due to closely linked QTL with small effect or distantly linked with large effect.

iii) the method cannot estimate the likely positions of the QTLs, and the QTL effect is confounded with the QTL distance from marker, i.e., recombination frequencies

Simple interval mapping (SIM): To overcome some problems of single-marker analysis, Lander and Botstein (1989) proposed simple interval mapping based on the maximum likelihood method which makes use of linkage maps and analyses intervals between a pair of adjacent linked markers along chromosomes simultaneously, instead of analyzing single markers. In SIM, the interval between two adjacent markers is tested for the presence of a putative QTL by performing a likelihood ratio test (LRT).

To perform the test, the Likelihood is calculated for a given set of parameters (particularly QTL effect and QTL position) given the observed data on phenotypes and marker genotypes. The estimates for the parameters are those where the likelihood is highest. Finally, measure of the strength of evidence for the presence of a QTL at give interval, *e.g.,* λ can be tested with a likelihood ratio test using likelihood ratio (LR); $LR = ln\frac{\Pr(y|QTL\ at\ \lambda, \mu_{MM\lambda}, \mu_{Mm\lambda}, \sigma_\lambda}{(y|no\ QTL,\ \mu,\ \delta)}$ or logarithm of odd (LOD), $LOD = log_{10}\frac{\Pr(y|QTL\ at\ \lambda, \mu_{MM\lambda}, \mu_{Mm\lambda}, \sigma_\lambda}{(y|no\ QTL,\ \mu,\ \delta)}$, where $\mu_{MM\lambda}$, $\mu_{Mm\lambda}$ and σ_λ are the maximum likelihood estimates, assuming a single QTL at position λ. There is a one-to-one correspondence between LR and LOD, and LR can be translated into LOD as $LOD = 0.217LR$. LOD is the most commonly used test statistics in SIM and LOD =3 means that the top model (presence of QTL) is 1000 times more likely than the bottom model (absence of QTL). This test is performed at any two adjacent marker intervals in each linkage group. If the likelihood ratio test statistic at a given markers interval exceeds a predefined critical threshold or equal to the critical threshold, a QTL is estimated at the position of the maximum test statistic at that interval. Depend on the size of genome, density of markers in linkage map and type and size of population, the threshold at %5 significant level over a whole genome is generally between 2 and 3.5 on LOD score (Zeng, 1994). Alternatively, using QTL mapping software the relevant threshold for a given data set can be determined from the data by using permutation or bootstrap test. The QTL likelihood curve of the LOD is derived by plotting the LOD score against marker position in the genome. The LOD curve achieves the critical threshold or above it indicates the presence of a QTL at this position (figure 2).

Some computer software uses multiple regression of phenotypic data on marker genotypic data to perform SIM as described by Haley and Knott (1992). The results of SIM based on regression analysis are very similar to those obtained with the maximum likelihood approach, except in the presence of a large proportion of missing marker data. However, computationally the multiple regression approach is faster than the maximum likelihood method and also more robust if the assumption of a Gaussian distribution of residuals is violated. The F-values obtained in the regression analyses are converted into LOD scores by using the transformations (Haley & Knott, 1992).

Simple interval mapping is statistically more powerful than single-marker analysis and provides a LOD score curve that allows localization of the QTL onto the linkage map. In addition, the estimate of QTL effect is more reliable and is not confounded with the QTL distance from the marker. Finally, the missing marker genotype data are taking into account, which enhances reliability of the findings. However, SIM has some limitations. The method assumes a single QTL in the interval of two

adjacent markers. The QTL effect can be biased when more than one QTL present at the market interval and if two QTLs locate close to each other, it will detect a single "ghost" QTL. Implementation of SIM requires more computation time than single-marker analysis (Singh & Singh, 2015b).

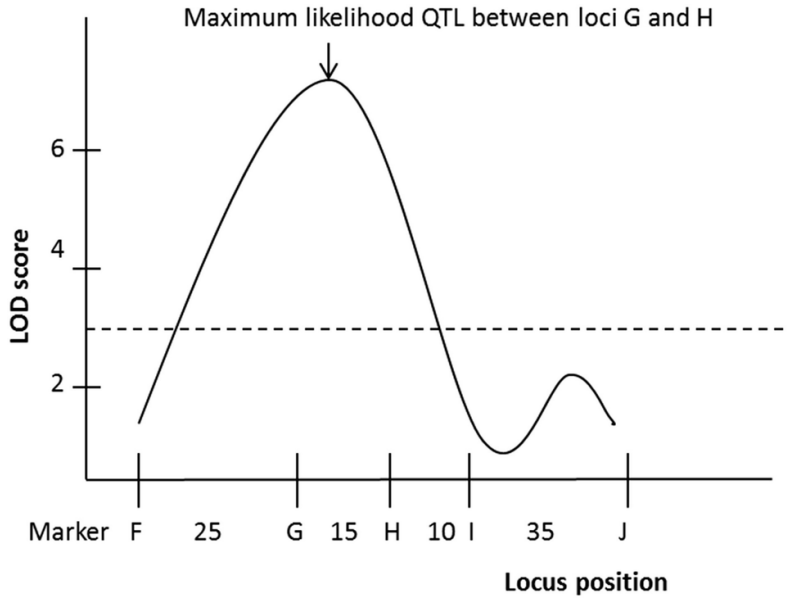


Figure 2. The LOD curve indicating that the most likely QTL position (peak of the curve). Adapted from Boopathi (2020).

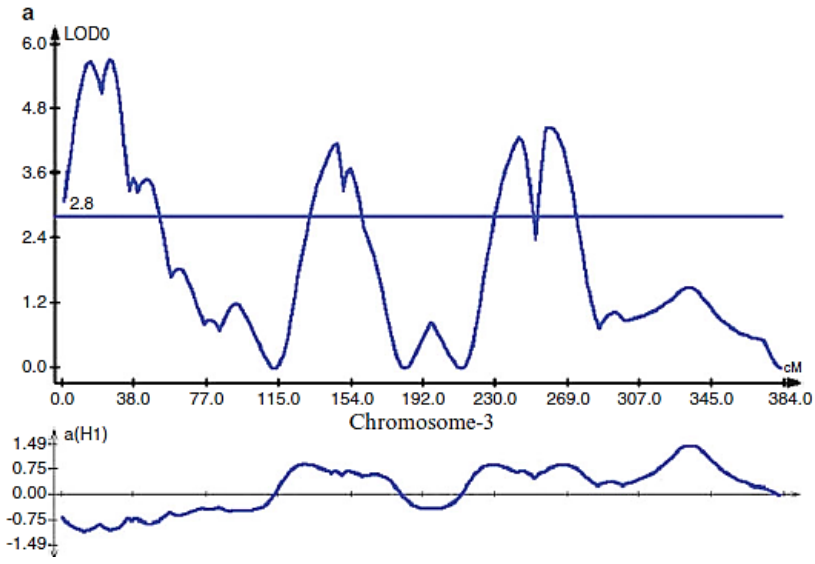
Composite interval mapping (CIM)

Simple interval method uses genome-wide scanning for detecting the position of a QTL throughout the genome. But this method can lead to biased estimates of QTL positions and effects when multiple QTLs occur on the interval of adjacent markers because it makes use of a single-marker interval between adjacent markers at a time and has no test has been performed to eliminate the effect of other QTLs outside the interval. Therefore, if a real QTL is located near a marker interval with no QTL, interval mapping may still detect a "ghost" QTL due to the linkage between the real QTL and the interval being tested (We et al., 2007). To overcome this problem, two almost identical methods namely "composite interval mapping" and "multiple-QTL model" or "marker-QTL- marker" (MQM) were proposed by Zeng (1994) and Jansen and Stam (1994), respectively. CIM combines interval mapping for a single

QTL in a given interval with multiple regression analysis on markers associated with other QTLs to control the effects of QTLs present in other marker intervals of the same or other chromosomes.

As CIM is an extension of SIM and uses some selected markers as cofactors (covariates) in the model to control for the genetic variation in other possibly linked and unlinked QTL based on the following model, $y_i = \mu + Z_i B + \sum_{r=1}^{m} X_{ir}\beta_r + e_i$, where yi is the ith individual phenotypic trait value; m μ is the overall mean; B is a column vector for the effects of a putative QTL, which depends on the type of mapping population; Zi is a row vector of predictor variables corresponding to the effects of the putative QTL; Xir is a row vector of predictor variables corresponding to the rth cofactor marker; βr is a column vector with the coefficient of the rth cofactor marker; and ei is the random error (Silva et al., 2012).

The main advantages of CIM are (1) By the search in one-dimension, multiple QTLs can be mapped, (2) By using linked markers as cofactors, the test is not affected by QTL outside the region, thereby the precision of QTL mapping is increased, (3) By eliminating much of genetic variance by other QTL, the residual variance is reduced and, consequently, the power of QTL detection is improved (Zeng, 1994). Due to these reasons, the CIM is more accurate and powerful than SIM in detecting QTLs (figure 3). However, CIM algorithm has some limitations such as (1) The different methods of cofactor selection, e.g., unlinked marker control, all marker control, and stepwise regression may produce different and sometimes contradictory results, and (2) In the presence of epistasis, CIM is inefficient because the method is unable to detect interacting QTLs.



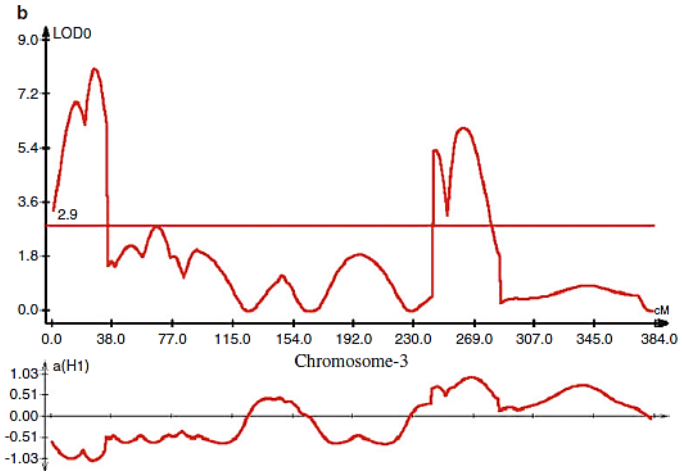


Figure 3. LOD score graphs obtained by (a) simple interval mapping (SIM) and (b) composite interval mapping (CIM) for 1,000-grain weight in rice (Adopted from Singh and Singh, 2015)

2. Bootstrap Method in QTL Mapping

One important problem in QTL mapping is large confidence intervals (CI) associated with QTL locations in segregation populations (Kearsey 1998a). The reliability depends on the heritability of individual QTL. Simulations have shown that the 95% CI of QTLs in an F_2 population of 300 individuals is more than 30 cM while it is difficult to reduce the CI to much less than 10 cM even for a very highly heritable QTL; more markers beyond a density of one every 15 Cm do not help much.

Several approaches have been explored to overcome this problem. A statistical method called ***'bootstrapping'*** is used to overcome this problem in QTL mapping. Bootstrap is a resampling method, which provides a very robust procedure for constructing CI for QTL position (Walling et al., 1998). It involves resampling points from one's data, with replacement, to create a series of bootstrap samples of the same size as the original data. Suppose the original data set consists of n individuals. A bootstrap sample is generated by drawing n values, with replacement, from the original data set. Such a sample will have some of the original values present multiple times and others not present at all. A series of N such samples are generated and an estimate (map position in this case) is computed for each, generating a distribution of estimates (the empirical distribution). The variation among the resulting estimates is taken to indicate the size of the error involved in estimating from original data. The resulting 95% bootstrap confidence interval has its lower

value the estimate corresponding to the 2.5% cumulative frequency point of the empirical bootstrap distribution, while the upper value is corresponding to the upper 97.5% 0f the bootstrap distribution. Simulation studies showed that this approach usually yields CI very close to the correct length when at least 200 bootstrap samples are used (Lynch & Walsh, 1998).

Software for QTL mapping

A number of freely available and commercial software packages are used for mapping QTLs in experimental populations. Hereby, some most commonly used and freely available software will be introduced.

WinQTL Cartographer is the most commonly used software for mapping QTL mapping which is available free at http://statgen.ncsu.edu/qtlcart/WQTLCart.htm (Wang et al., 2012). Data imports and exports can be performed in a variety of formats and empirical threshold LOD scores are calculated by permutation, and confidence intervals for QTL positions are estimated by the bootstrap method. Various types of QTL mapping methods including single-marker analysis, SIM, CIM, MIM with epistasis, Bayesian interval mapping, multiple trait analysis, and multiple trait MIM analysis are implemented in the software.

QTL IciMapping is freely available public software capable of building high-density linkage maps and mapping quantitative trait loci (QTL) in biparental populations (Meng et al., 2015). Multiple functionalities for linkage analysis are available in the software including segregation distortion analysis (SDL), construction of linkage maps in biparental populations (MAP), construction of consensus map from multiple linkage maps with common markers (CMP). The QTL mapping options such as mapping of additive, dominant, and digenic epistasis genes (BIP), mapping of additive and digenic epistasis genes with chromosome segment substitution lines (CSL), and QTL-by-environment interaction analysis (MET) are also implemented in *QTL IciMapping*. It can use marker and phenotypic data files in form of plain text or MS Excel formats. The outputs of software contain the summary of the completed linkage maps, Mendelian ratio test of individual markers, estimates of recombination frequencies, LOD scores, genetic distances, results at all scanning positions, identified QTL, permutation tests, and detection powers for up to six mapping methods.

R/qtl is an extensible, interactive environment for mapping QTLs in experimental populations derived from inbred lines crosses. It has been designed as an add-on program to the statistical language/software R, which is freely available from http://www.r-project.org. The single-QTL genome scans and two-dimensional, two-QTL genome scans, under a normal model, with the possible inclusion of covariates,

by the EM algorithm, Haley–Knott regression, and multiple imputation are various functions for QTL mapping in *R/qtl*. 2001). Further, non-parametric interval mapping for performing single-QTL genome scans as well as binary trait mapping are also implemented in R/qtl package (Broman et al., 2003).

Conclusions

QTL mapping studies have a long and rich history and have played important roles in plant and animal breeding as well as in gene cloning and characterization; however, there is still a great deal of work to be done. Through development of appropriate segregation populations and selection of effective statistical and molecular biological tools are essential for practical implementation of QTL mapping in applied sciences. Therefore, understanding the biological and statistical basis of methods applied for QTL mapping will enable breeders to determine the ideal genes and genotypes from QTL studies and utilizes them in breeding program.

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