

**Quantitative Trait Locus (QTL) Mapping****Mangave S. S. and Patil N. S.**

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**SUMMARY**

Comparison of linkage analysis and association mapping for QTL detection revealed that linkage mapping is more useful for genome-wide scan for QTLs, while association mapping gives more precise location of an individual QTL. Therefore, linkage analysis may be preferred for preliminary location of QTLs and then use association mapping for more precise location. Association mapping is prone to the identification of false positives, especially if the experimental design is not rigorously controlled. For example, population structure has long been known to induce many false positives and accounting for population structure has become one of the main issues when implementing association mapping in plants. Also, with increasing numbers of genetic markers used, the problem becomes separating true from false positive and this highlights the need for independent validation of identified association. The examples of association mapping studies performed in three most important crops' germplasm largely demonstrate the flourish of crop genomics era with the utilization of powerful LD-based association mapping tool. Currently, a number of such studies are in progress for various other crops in many laboratories worldwide. The near-future completion of genome sequencing projects 2 Quantitative Trait Loci Mapping in Plants: Concepts and Approaches 53 of crop species, powered with more cost-effective sequencing technologies, will certainly create a basis for application of whole-genome-association studies. This will provide with more powerful association mapping tool(s) for crop breeding and genomics programs in tagging true functional associations conditioning genetic diversities, and consequently, its effective utilization.

**INTRODUCTION**

QTL mapping is process of locating genes with effects on quantitative traits using molecular markers. There are two types of traits, one type is quantitative type and another type is qualitative type. Here, quantitative type show continuous variation and qualitative type show discontinuous variation. Qualitative type is generally governed by few genes or single genes and fall into a few distinct phenotypic classes called as discrete classes. These classes can predict the genotypes of the individuals. Molecular markers are ideal to study QTL's and to map QTL's, which can be effectively used in MAS. It can be defined as the marker-facilitated genetic dissection of variation of complex phenotypes through appropriate experimental design and statistical analyses of segregating materials (Angaji SA, 2009). It is based on measuring the mean difference between lines with contrasting marker alleles. This technique is preliminary step to findout target desirable genes for marker-aided backcrossing. So far, only successful with disease resistance and stress tolerance genes having very large effects. QTL mapping is basic research activity requiring careful planning of crosses and high-precision phenotyping. A major breakthrough in the characterization of quantitative traits that created opportunities to select for QTLs was initiated by the development of DNA (or molecular) markers in the 1980s.

**Principle of QTL Analysis**

Identifying a QTL or a gene within a plant genome is like finding the proverbial needle in a haystack. However, QTL analysis can be used to divide the haystack in manageable piles and systematically search them. In simple terms, QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers. Markers are used to partition the mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Tanksley1993; Young1996 ). A significant difference between phenotypic means of the groups (either 2 or 3), depending on the marker system and type of population, indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait.

**Why QTL Mapping**

QTL mapping is used to offer direct mean to investigate the number of genes influencing the trait, to find out the location of the gene and to know the effect of dosage of these genes on variation of the trait. Genetic

mapping is the first step to map based cloning. It is used for DNA based marker assisted selection (MAS) and carrying out study on linkage between genes of interest.

### Mapping Populations Used in QTL Mapping

Various types of mapping population may be produced from the heterozygous F1 hybrids:

- Double haploid lines (DHLs): Plants are regenerated from pollen (which is haploid) of the F1 plants and treated to restore diploid condition in which every locus is homozygous. Since the pollen population has been generated by meiosis, the DHLs represent a direct sample of the segregating gametes.
- Backcross (BC) population: The F1 plants are backcrossed to one of the parents.
- F2 population: F1 plants are selfed.
- F2:3/F2:4 lines:  $F_2^3/4$  plants tracing back to the same F2 plant, also called F2 families.
- 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.

### Construction of Genetic/Linkage Maps

A linkage map may be thought of as a 'road map' of the chromosomes derived from two different parents. Linkage maps indicate the position and relative genetic distances between markers along chromosomes. Construction of a linkage map, using genotyping data generated on any of the above mentioned mapping populations, is an important step before initiating any QTL analysis. In a segregating mapping population, there is a mixture of parental and recombinant genotypes. The frequency of recombinant genotypes is used to calculate recombination fractions, 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, (Sehgal et al). 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). Two commonly used mapping functions that convert recombination frequency into centimorgan (cM) distance are the Kosambi mapping function, which assumes that recombination events influence the occurrence of adjacent recombination events, and the Haldane mapping function, which assumes no interference between crossover events. Linkage between markers 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 (Risch 1992). LOD values of  $>3$  are typically used to construct linkage maps. LOD values may be lowered in order to detect linkage over a greater distance or to place additional markers within maps constructed at higher LOD values (Collard et al. 2005). Linked markers are grouped together into linkage groups, which represent chromosomal segments or entire chromosomes.

### Detection of QTLs

Four widely used methods for detecting QTLs are single-marker analysis, interval mapping by maximum likelihood, interval mapping by regression and composite interval mapping.

### Types of QTL Mapping:- Single Marker Approach

The single marker approach is also known as single factor analysis of variance or single point analysis. It is widely used method for quick scanning of whole genome to determine best QTLs. It is used for each marker locus which is free from other loci. Generally, this technique is unable to determine QTL position. F-test is used for determination of significant differences between various genotypes groups.

Some major limitations of this approach:

- The method cannot determine whether the markers are associated with one or more QTLs.
- Chance of QTL detection decreases with distance between marker and QTL.
- Effects of QTL is underestimated of confounding with recombination frequencies.
- Its accuracy is less compare to other methods.

### Simple Interval Mapping (SIM)

SIM was first proposed by Lander and Botstein and it is based on linkage map. It can be called as two marker approach. Here, QTL is determined in interval generated between two markers at various points. It gives more accurate results compare to single marker approach but less than CIM and MIM technique. In this technique, likelihood ratio test is used to determine every QTL position in interval created by both markers. SIM is mostly preferred as it can be easily performed through statistical packages such as MAPMAKER/QTL. Lander and Botstein, 1989 developed formulae for significance levels appropriate for interval mapping when the genome size, number of chromosomes, number of marker intervals, and the overall false positive rate desired are given. However, when various QTLs are segregating in a cross, SIM will not take into consideration genetic variance due to other. In such a case, SIM is having same limitation as in single marker analysis.

### Composite Interval Mapping (CIM)

CIM and MQM techniques are developed by Jansen and Stam (1994). It is used to minimize effects of various linked QTLs. It is based on one QTL and other markers used as covariates. This technique gives more precise results and used to exclude bias due to another QTLs (non-target QTLs) linked to target QTL. It used to fit the parameters for a single QTL in one interval. The partial regression coefficient is used to determine genetic variance due to non-target QTLs. It considers a marker interval and a few other selected single markers in each QTL analysis, so that n-1 tests for interval-QTL associations are conducted on a chromosome with n markers.

The merits of CIM are as follows:

- Mapping of multiple QTLs can be carried out by the search in one dimension.
- By using linked markers as covariates, the test is not affected by QTL out of region, thereby increasing the precision of QTL mapping.
- By eliminating as much as the genetic variance produced by other QTL, the residual variance is reduced, thereby the efficiency of determination of QTL is increased. CIM is more efficient than SIM, but not widely used in QTL mapping as in SIM.

### Multitrait Interval Mapping (MIM)

It is recent method of QTL Mapping. Multiple Interval Mapping (MIM) is the extension of interval mapping to multiple QTLs, just as multiple regression extends analysis of variance. It is used to map multiple QTLs. This method is potential tool for detection of QTL X QTL interaction.

### QTL Mapping Software's

There are over 100 genetic analysis software packages (linkage analysis and QTL mapping). Here, we list some features of the most commonly used software packages.

#### MapMaker/QTL (<ftp://genome.wi.mit.edu/pub/mapmaker3/>)

A user-friendly, freely distributed software program runs on almost all platforms. It analyzes F2 or backcross data using standard interval mapping.

#### MQTL

MQTL is a computer program for CIM in multiple environments. It can also perform SIM. Currently, MQTL is restricted to the analysis of data from homozygous progeny (double haploids, or RILs). Progeny types with more than two marker classes (e.g., F2) are not handled.

#### PLABQTL (<http://www.uni-hohenheim.de/~ipspwww/soft.html>)

PLABQTL is a freely distributed computer program for CIM and SIM of QTL. Its main purpose is to localize and characterize QTL in mapping populations derived from a biparental cross by selfing or production of double haploids. Currently, this program is the easiest software for composite interval mapping.

#### QTL Cartographer (<http://statgen.mcsu.edu/qtlcart/cartographer.html>)

QTL Cartographer is a QTL software written for either UNIX, Macintosh, or Windows. It performs single-marker regression, interval mapping, and composite interval mapping. It permits analysis from F2 or backcross populations. It displays map positions of QTLs using the GNUPLOT software.

**MapQTL** (<http://www.cpro.dlo.nl/cbw/>)

MapQTL is a licensed software program. It performs Kruskal–Wallis test (single-marker analysis), CIM, and multiple interval mapping on almost all kinds of mapping populations.

### **Qgene**

Qgene is a QTL mapping and marker-aided breeding package written for Macintosh. It has a user-friendly graphical interface and produces graphical outputs. QTL mapping is conducted by either single-marker regression or interval regression.

### **SAS**

SAS is a general statistical analysis software. It can detect QTL by identifying associations between marker genotype and quantitative trait phenotype by single-marker analysis approach such as ANOVA, t-test, GLM, or REG. summarizes QTL mapping studies in three most important staple crops, viz. wheat, maize, and rice, for various traits using different marker systems, analysis procedure, and software programs.

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