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QTL Mapping: A Tool for Improvement in Crop plants

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Abstract

It is review paper and highlighting the importance of QTL Mapping in Crop plants. A QTL is defined as "a region of the genome or locus of gene that is associated with an effect on a quantitative trait". It is coined by Gelderman . Conceptually, a QTL can be a single gene, or it may be a group of linked genes that affect the trait. QTL mapping based on linkage and marker trait association can be effectively used for gene pyramiding, germplasm screening of diversified material for abiotic (salinity, cold, salt, drought) and biotic stresses (disease, pest) etc. The identification and location of specific genes mediating quantitative characters is having great importance in plant breeding. Proper development and understanding of the statistical background is essential for QTL mapping. A quantitative trait which is controlled by several genes, all the genes having small effects, additive in nature and is more affected by environment. Molecular markers are used to map QTL's. Mapping population includes F2, back crosses, recombinant inbred lines, and double haploids lines. Strong linkage disequilibrium at marker loci and allele of linked loci controlling the trait is essential feature of such type of population. QTL mapping is required Non-random mating populations. Objectives of QTL mapping is to offer direct mean to investigate the number of genes influencing the trait, to find out the location of the gene that affect traits of interest, to know the effect of genes on variation of the trait, to carry out study on linkage between genes of interest. The basic Principle is the co- segregation of marker locus and QTL together. Co-segregation is due to linkage between marker and QTL. Methods used for QTL mapping are single marker approach (SMA), simple interval mapping (SIM), composite interval mapping (CIM), multiple interval mapping(MIM). Various Factors affecting QTL mapping are number of genes controlling the target traits and their position, heritability of the traits, type and size of mapping population used in QTL mapping, type and number of markers in linkage maps, statistical method used.

Keywords: QTL mapping, molecular marker, population, methods, environment.

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[1]. It is based on measuring the mean difference between lines with contrasting marker alleles. This technique is preliminary step to find out 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[2].

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.

Efficient utilization of QTL mapping

QTL mapping is difficult to detect, localize and estimate the effective size of genes with a small effect. QTL mapping performs best for finding single genes with large effects. If repetition of QTL phenotyping experiment is low, QTL map will be unreliable. It requires a phenotypic screening system with high heritability.

Objectives of QTL mapping

To identify the region of the genome or gene that affects the quantitative trait of interest. To analyze the effect of the QTL on the trait. i. What amount of the variation for the target trait of interest is caused by a specific region?. ii. Which type of gene action is linked to QTL (Additive / Dominant Effect?). iii. What type of alleles are linked to favorable effect?

Basic prerequisites for successful QTL mapping

There should be focus on lines which are easy to observe in a good screen. Traits should be derived where difference between susceptible and resistant mapping populations from crosses between highly resistant and highly susceptible lines is there. Use highly reliable screening systems that are known to differentiate resistant from susceptible lines. Analysis should be based on the means of repeated screens rather than single trials. Ensure that repeatability of your screen is as high as possible (0.7 or higher).

Salient requirements of QTL mapping: A mapping population generated from phenotypically contrasting parents, saturated linkage map based on molecular markers, reliable phenotypic screening of mapping population and appropriate statistical package to analysis the genotypic information in combination with phenotypic information for QTL detection are the basic requirements of QTL mapping.

QTL mapping strategies

All marker-based mapping experiments have same basic strategy: i. First of all, we will choose parents which are different for a character. ii. Now, Screen the two parents for marker loci for polymorphism. iii. To create mapping populations like includes F_2 population, back crosses, recombinant inbred lines, and double haploids lines. iv. Phenotype screening. v. Contrast the mean of the MM and mm lines at every marker locus. If difference between mean of the MM and mm lines is more, there will be more chance of QTL detection. vi. To declare QTL where (MM-mm) is greatest. If difference between mean of the MM and mm lines is more, QTL effect will be more.

Principle of QTL mapping: The basic Principle is the co-segregation of marker locus and QTL generation after generation. Co segregation is due to linkage between marker and QTL to determine the linkage partition the mapping population into different genotypic classes based on progeny testing. There should be tight linkage between marker and target gene of interest so that they can co- segregate generation after generation. Situations where genes fail to segregate independently are said to display "linkage disequilibrium". QTL analysis, thus, depends on linkage disequilibrium. So, basic principle whether a QTL is linked to a marker.

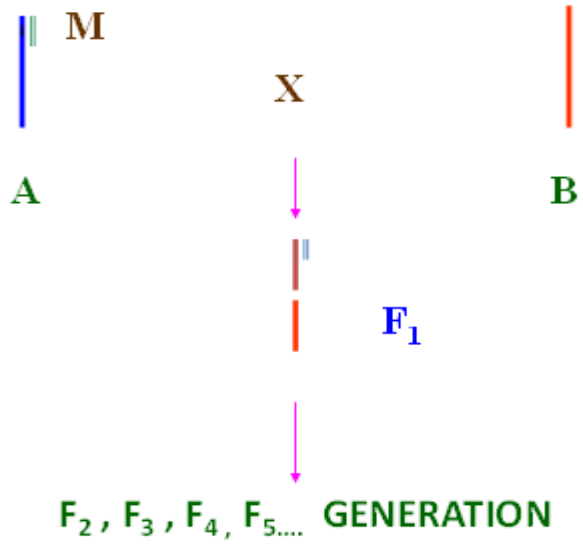

Figure-1
Factors effecting power of QTL mapping

QTL's are statistically worked out from data generated in an experiment. The following factors affect the power of QTL mapping:

Number of genes controlling the target traits and their position: Position of gene on chromosome affects the success of QTL mapping. If genes will remain close to concerned genetic marker, there will be more chance of detection of target traits or target genes. It is based on banding pattern of markers used. If genes will remain away from concerned genetic marker, there will be more chance of crossing over. It affect banding pattern of markers used. In this condition, it will be difficult to determine position of target genes.

Heteritability of the genes segregating in a mapping population: Generally characters governed by oligogenes or single genes are having high heteritability than governed by polygene.

Type of mapping population used in QTL mapping: Non random mating population is required for QTL mapping. It is result of mutation, natural selection, random drift etc.

Size of mapping population used in QTL mapping: In large sample size, QTL with small effects can not be observed but QTL with large effects can be observed. In small sample size also, QTL with small effects can not be observed but QTL with major effects can be observed.

Type and number of markers in linkage maps: If there is more number of markers used, amount of precision of estimation of both QTL position and effect will be more. Here, co-dominant marker shows three types of genetic difference while dominant marker shows two types of genetic difference. so, co-dominant marker provide more information than dominant marker regarding recombination with in marker intervals.

Phenotyping of mapping population and sample size: The target quantitative traits are measured as precisely as possible and limited amounts of missing data can be tolerated. The power to resolve the QTL location is confined first by sample size and then by genetic marker coverage of the genome. Generally, the number of individuals in a sample might appear to be large but missing data or skewed allele frequencies in the population cause the effective sample size to diminish, thus sacrificing the statistical power. Sometimes, it is must to sacrifice population size in favour of data quality and this trade off means that only major QTL (with relatively large effect) can be detected. QTL Data is typically pooled over locations and replications to obtain a single quantitative trait for the line. It is also preferred to measure the target trait(s) in experiments conducted in multiple (and appropriate) locations to have a better understanding of the QTL x environment interaction, if any.

QTLs and the signature of selection

Orr (1998) developed a sign test that compares the number of plus alleles present in the high condition of a trait with a model of neutrality assuming either equal or differential allelic effects. Consequently, QTL data can provide evidence for the presence of directional selection, when one can demonstrate a polarity to allelic substitution. This approach has been used in such divergent organisms as sunflowers and Lake Malawi cichlids to help quantify the dominant selective agents responsible for the diversification of the respective organisms.

Detection and locating of QTL: The figure1 describe the construction and use of a near-isogenic line (NIL) for identification of high probability for QTLs. Initially, a donor and recurrent parent are crossed and subsequent repeated back crosses to recurrent parent lead to a reduction of the donor genome contribution. With marker assisted selection (MAS), a panel of NILs that tile the genome can be constructed. The resulting panel members can be tested for a range of phenotypic traits for the detection and locating of QTL candidates.

Development of introgression lines- For development of introgression lines to define and map QTLs for crop improvement, Schauer *et al*. have mapped the metabolic and fruit-quality QTLs in tomato introgression lines previously developed through multiple rounds of self- and back-crossing (to the cultivated parent) between an elite cultivar, *Solanum lycopersicum* var. Roma, and a wild tomato plant, *Solanum pennellii*, to generate 76 independent introgression lines of tomato plants harboring chromosome segments from the wild relative4. Selection of specific, homozygous, single, overlapping chromosome introgressions in this population both simplifies QTL localization and defines linked DNA markers for use in crop improvement.

Statistical Methods for QTL Mapping (B.M. Prasanna, IARI, PUSA, New Delhi-110 012): Tests for QTL/trait association are often performed by the following methods:

Table1
Advantages and disadvantages of most commonly-used DNA markers for QTL analysis, Collard *etal*

Molecular (C) marker	Restriction fragment length polymorphism (**RFLP**)	Random amplified polymorphic DNA (**RAPD**)	Simple sequence **repeats(SSRs)∗** or‘microsatellites’	Amplified fragment length polymorphism (**AFLP**)
Codominant(C) or Dominant (D)	**Codominant**	**Dominant**	**Codominant**	**Dominant**
Advantages	Robust, Reliable, Transferable across, populations	Rapid, simple , Inexpensive, Multiple loci from a single primer possible, less DNA required	simple, Robust and reliable, Transferable between, populations	Multiple loci, High levels of polymorphism produced
Disadvantages	Time-consuming, laborious, expensive, more DNA required, Less polymorphism,	Generally not transferable, Less reproducibility	Time-consuming, laborious, Usually require polyacrylamide electrophoresis	Complicated methodology, Large DNA required

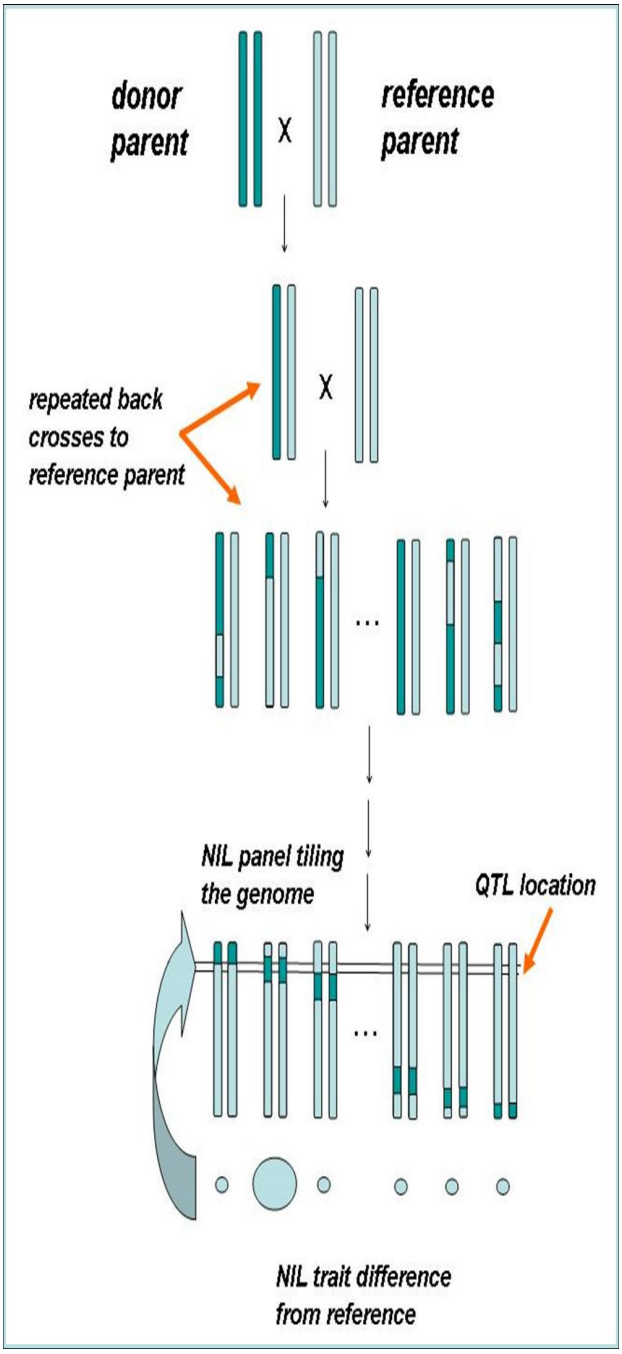

Figure-2
Detection and locating of QTL, Comparative Evolutionary Genomics of cotton

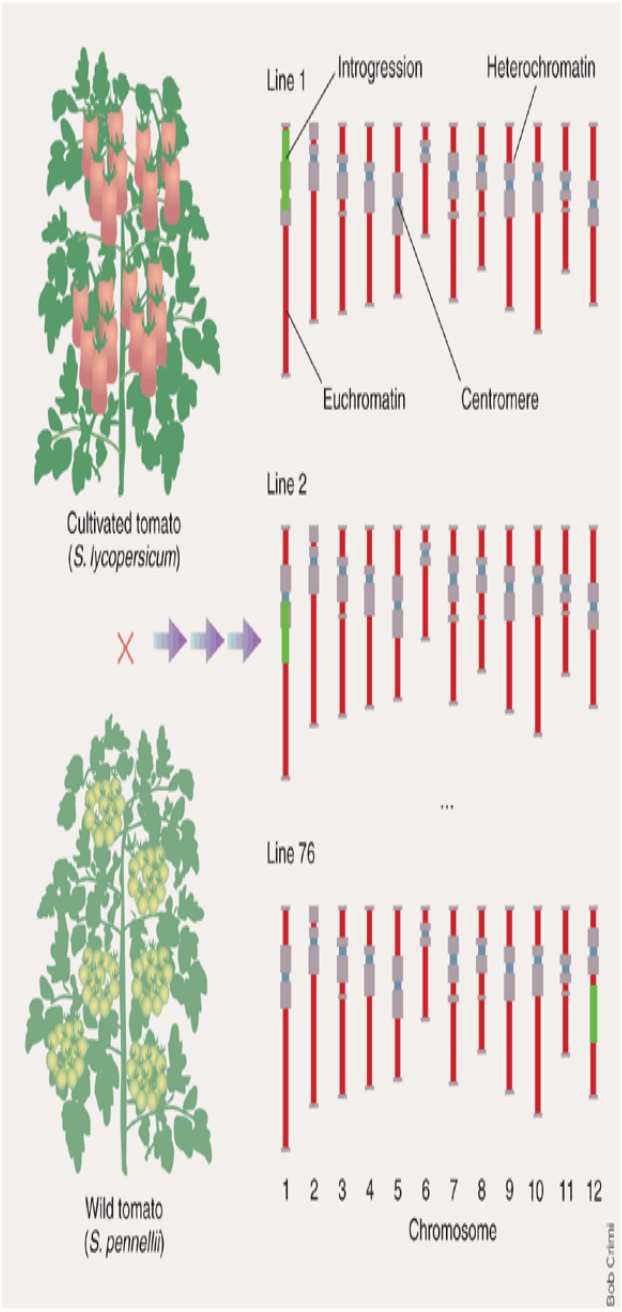

Figure-3
Development of introgression lines for QTL mapping in crop improvement[7]

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

Simple Interval Mapping (SIM)

SIM was first proposed by Lander and Botstein[8] 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[9] 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: i. mapping of multiple QTLs can be carried out by the search in one dimension; ii. by using linked markers as covariates, the test is not affected by QTL out of region, thereby increasing the precision of QTL mapping; and iii. 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.

Conclusion

QTL maps based on linkage studies and marker trait association can be effectively utilized for gene pyramiding , germplasm screening of diversified material for abiotic (salinity, cold, salt, drought) and biotic stresses (disease, pest) etc. The identification and location of specific genes mediating quantitative characters is having great importance in plant breeding. QTL analysis is helpful in assessing possible number of loci, their distribution in the genome, equality of effects and manner of their action. DNA markers are very useful for information about number and position of QTLs because they are highly polymorphic, abundant and co-dominant in nature. High resolution linkage maps based on various molecular markers are required for preparation of for QTL analysis. Proper development and understanding of the statistical background is essential for QTL mapping. The technique of Marker-assisted selection and QTL mapping should be adopted at large scale for all major crops.

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