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# QTL Mapping - Methods and Applications

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Many agriculturally valuable traits such as yield, seed size and disease traits show a continuous variation and are governed by polygenes (Mather, 1949). This continuous variation of metric traits is due to simultaneous segregation of many genes with cumulative effect (Yule, 1906) and also due to superimposition of variation arising from environmental or non-genetic causes. In nature, most genetic variation in readily observable traits is polygenic (Tanksley, 1993). Biometrical or quantitative genetics deals with inheritance of quantitative traits and analysis of these traits is generally carried out from means, variance and covariance. Nilson Ehle (1908) presented experimental evidence to support the hypothesis of Yule (1906) with the inheritance of seed colour in wheat as multiple factor hypothesis or polygenic inheritance. Johannsen (1909) demonstrated that both heritable and non-heritable factors contributed to variation in seed weight of beans. East (1916) presented conclusive evidence of polygenic inheritance and cumulative effects in the inheritance of corolla length in *Nicotiana longiflora*. Fisher (1918) divided the genetic variance into three components: additive, dominance and interaction or epistatic components. Hyaman and Mather partitioned the interaction component again into additive × additive, additive × dominance and dominance × dominance. Many cases the linkage between major genes and polygenes have been reported, notably first report of the association of seed size with seed coat pigmentation in beans by Sax (1923). Later Thoday (1961) put porth the idea of mapping the polygenes using the association of single gene markers.

The regions within genomes that contains genes associated with a particular quantitative trait are called ad Quantitative trait loci (coined by Gelderman, 1975). An important milestone in the study of quantitative traits is the availability of molecular markers and advanced molecular techniques for QTL and mapping. QTL mapping principally depends on linkage disequilibrium *ie* co-segregation of marker locus and QTL. Hence the basic principle of determining whether a QTL is linked to marker is to partition the mapping population into different genotypic classes based on genotypes at the marker locus, and the apply correlative statistics to determine whether the individual of one genotypes differ significantly with the individuals of other genotype with respect to the trait being measured.

Molecular markers are fragments of DNA, found at specific locations of genome preferably in non-coding regions. First used DNA markers are Restriction fragment length polymorphism (RFLP) is based on restriction site changes in the target DNA (Table 1) and subsequent hybridization with probe DNA. Later PCR based markers, *eg.* RAPD and microsatellites has been developed. RFLP markers are robust and reliable but limited by reproduction problems as well as time consuming, laborious expensive procedures. SSR markers have wide applicability in QTL mapping because of their genomic abundance, hyper-variability, multi-allelic nature and amenable to automation. SNPs include single base differences between homologous DNA fragments plus small insertions and deletions (InDels) (Solis *et al.*, 2008). SNPs are bi-allelic in nature and useful for quantifying the linkage disequilibrium. They are highly amenable to automation and potentially, can be used to create very high density genetic maps for association mapping (Wang *et al*.1999).

The construction of linkage map and QTL analysis essentially requires a segregating population derived from cross between two contrasting parents for target trait and mapping population must be evaluated phenotypically before subsequent QTL mapping. The type of mapping population to be developed mainly depends on the objectives of the experiment, timeframe and resource availability for undertaking the QTL analysis. Most widely used mapping population are $F_2$, $F_2$ derived $F_3$ ($F_{2:3}$), backcross (BC), recombinant inbred lines (RILs), near-isogenic lines (NILs) and double haploids (DHs).

A $F_2$ population is developed by selfing (or intermating for cross pollinated species) among $F_1$ individuals. These populations are suitable population for preliminary mapping and takes less time and effort for development. Backcross populations are developed by crossing the $F_1$ with one of the two parents used in the initial cross. The major limitation of $F_2$ or backcross populations is that the populations are not eternal. RILs are developed by single seed descent method (SSD) from a $F_2$ population, a rapid generation advancement method leads to development of series of homozygous or near homozygous lines with a unique combination of chromosomal segments contributed from original parents. RILs can be evaluated in multi-location replicated trails (Table 2).

**Table 1: Advantages and disadvantages of most commonly-used DNA markers for QTL analysis**

| **Molecular marker** | **Restriction fragment length polymorphism (RFLP)** | **Random amplified polymorphic DNA (RAPD)** | **Simple sequence Repeats (SSRs) or microsatellites** | **Amplified fragment length polymorphism (AFLP)** | **Single nucleotide polymorphism (SNPs)** | **Diversity Arrays Technology (DArT) markers** |
|---|---|---|---|---|---|---|
| Codominant or Dominant | Codominant | Dominant | Codominant | Dominant | Codominant | Dominant |
| Advantages | Robust, Reliable, Transferable across populations | Rapid, simple, inexpensive, Multiple loci from a single primer possible, less DNA required, no prior knowledge of DNA sequence is required | Simple, Robust and reliable, Transferable between populations | Multiple loci, High levels of polymorphism | Reliable, small PCR products, genome wide distribution, May possibly multiplex hundreds or thousands on one chip, Sample processing may be completely automated, No stutter products | Generic, hybridization-based and cost-effective fingerprinting method, does not require DNA sequence information, scored with high accuracy, high-throughput marker system |
| Disadvantages | Time-consuming, laborious, expensive, more DNA required | Generally not transferable, Low reproducibility | require polyacrylamide electrophoresis | Complicated methodology, Large DNA required, skill required | Less alleles, Mixture interpretation is more difficult | less polymorphism information per locus |

NILs can be generated by backcrossing the $F_1$ plants to the recurrent parents and selecting the trait of interest in each generation. NILs developed through backcrossing are similar to recurrent parent except for the gene of interest. Linkage drag is a potential problem in constructing NILs and require many generations for development. Doubled haploids (DH) are generated by chromosome doubling form pollen grains of $F_1$s and hence they are products of one meiotic cycle, and hence comparable to $F_2$ in terms of recombination information. DHs are permanent mapping population and hence can be replicated and evaluated over locations and years and maintained without any genetic change. The size of mapping population depends on type of mapping population, genetic nature of the target traits, objectives of the experiment, resources available for handling a sizable population.

## Quantitative trait loci (QTL)

In QTL mapping, association between phenotypic values and presence/absence of alleles of markers, that have been mapped onto a linkage map is analyzed and the size of the allelic effect of the detected QTL can be estimated.

Basic steps in QTL analysis are as follows

1. Make cross and generate mapping population
2. Identify markers that are polymorphic between the parents
3. Generate marker data
4. Generate linkage maps of molecular markers
5. Collect phenotypic measurements of QTL trait
6. Map QTLs (Association of QTL with marker).

## Methods of determining association of QTL and trait

1. Single marker analysis
2. Interval mapping
3. Composite interval mapping
4. Multiple interval mapping

## Mapping in polyploid genomes

Polyploid origin can be divided into autopolyploids, which are derived from a whole genome duplication event of the same ancestral chromosome set, and allopolyploids, which are derived from a hybridization event of alternate parental genomes followed by genome duplication (Pignatta *eta al*, 2010). In an allopolyploid species, such as wheat, there are specific meiotic pairings among the homologous chromosomes and construction of linkage is similar to diploids except for multiple genomes. The construction of linkage maps in polyploids is more challenging than in diploids due to the complexities of gene segregation and recombination during meiosis *viz*., multiple allele segregation, double reduction and mixed bivalent and quadrivalent pairing among homologous chromosomes. A commonly used approach to construct molecular genetic maps in polyploids is based on single dose fragments (SDF) (Wu *et al*., 1992). The construction of a linkage map in autotetraploid species, using either codominant or dominant markers scored on two parents and their full-sib progeny. The steps in construction are: the dose of each DNA fragment(electrophoretic band) is determined initially, pairs of loci are tested for linkage according to their dosage and configuration, distance between linked loci calculated and orderd along the chromosomes and finally the chromosomes are collected into homologous groups (Ripol *et al*.1999).

New sequencing and genotyping technologies have enabled researchers to generate high density SNP genotype data for mapping populations. Linkage analysis and QTL mapping using SNP dosage data in a tetraploid potato mapping population by Hacket *et al*. (2013) followed the below procedure. In polyploid species, SNP data usually contain a new type of information, the allele dosage, which is not used by present methodologies for linkage analysis and QTL mapping in diploids. They extended existing methodology to use dosage data on SNPs in an autotetraploid mapping population. The SNP dosages are inferred from allele intensity ratios using normal mixture models. The steps of the linkage analysis (testing for distorted segregation, clustering SNPs, calculation of recombination fractions and LOD scores, ordering of SNPs and inference of parental phase) are extended to use the dosage information. For QTL analysis, the probability of each possible offspring genotype is inferred at a grid of locations along the chromosome from the ordered parental genotypes and phases and the offspring dosages. A normal mixture model is then used to relate trait values to the offspring genotypes and to identify the most likely locations for QTLs. The allele intensity ratios are mapped as quantitative traits to check that their position and phase agrees with that of the corresponding SNP. This analysis confirms most SNP positions, and eliminates some problem SNPs to give high-density maps for each chromosome. This methodology can be applied to construct high-density linkage maps in any autotetraploid species, and could also be extended to higher autopolyploids.

**Table 2: Most commonly used mapping population for linkage and QTL mapping**

| Mapping Population | $F_2$ Population | $F_2$ derived $F_3$ ($F_2$:$F_3$) | Backcross Inbred Lines (BIL) | Doubled haploids (DH) | Recombinant Inbred Lines (RIL) | Near Isogenic Lines (NILs)s | Chromosomal Segmental Substitution Lines |
|---|---|---|---|---|---|---|---|
| Generation | Selfing or sib mating of the $F_1$ individuals generated by crossing the selected parents | Selfing the $F_2$ individuals for a single generation | Crossing the $F_1$ with either of the parents | Chromosome doubling of anther culture derived haploid plants from $F_1$ | Single seed descent method of generation advancement | Generated either by repeated selfing after backcross or backcrossing the $F_1$ plants to the recurrent parents. | Series of plants with chromosome segments of the donor parent in the recurrent parental chromosome background. |
| Merits | Requires less time and efforts for development, Best population for preliminary mapping | Mapping quantitative traits and mapping recessive genes, | Useful for marker-assisted backcross breeding | Instant production of homozygous lines, saves time, permanent mapping population for both qualitative and quantitative traits | Can be propagated indefinitely without further segregation | Immortal mapping population, suitable population for tagging the trait and functional genomics studies | does not require linkage map construction for QTL identification Used for mapping, cloning QTL genes and breeding, Immortal lines |
| Demerits | One cycle of meiosis, Cannot be evaluated in replicated trials over locations and years, | Not immortal | The recombination information in case of backcrosses is based on only one parent (the $F_1$),<br>Not immortal. | Recombination from the male side alone is accounted, | Requires many seasons / generations, difficult in developing in crops with high inbreeding depression | require many generations for development, useful for molecular tagging of the gene concerned, but not for linkage mapping | Undesirable traits linked to the target genes |

## Applications of QTL mapping

QTL mapping provides the information about the putative locations and QTL effects influencing a quantitative trait. Several QTLs have been mapped and identified yield, disease resistance, drought tolerance, grain quality traits in crop plants are presented Table 4 and 5. Novel QTLs can be introgrssed into elite germplasm lines and marker assisted selection could be undertaken in crop plants. It is possible to reduce linkage drag during the introgression of QTL by backcross programme.

Quantitative trait locus (QTL) mapping is a highly effective approach for studying genetically complex forms of plant disease resistance. With QTL mapping, the roles of specific resistance loci can be described, race-specificity of partial resistance genes can be assessed and interactions between resistance genes, plant development and the environment can be analysed (Young, 1996). DNA markers tightly linked to quantitative resistance loci (QRLs) controlling QDR can be used for marker-assisted selection (MAS) to incorporate these valuable traits. Quantitative disease resistance (QDR) confers a reduction, rather than lack, of disease and has diverse biological and molecular bases as revealed by cloning of QRLs and identification of the candidate gene(s) underlying QRLs (Dina St and Clair, 2010).

The advanced backcross method (AB-QTL) has been used for the simultaneous identification and transfer of favorable quantitative trait locus (QTL) alleles from unadapted material to elite lines (Tanksley and Nelson 1996). By this approach, QTLs having positive effects on key agronomic traits (e.g., fruit weight and soluble-solids content) were discovered in the wild species *Lycopersicon pimpinellifolium*, *Lycopersicon pennellii* and *Lycopersicon hirsutum*, and transferred to cultivated tomato (*Lycopersicon esculentum*) ( Eshed and Zamir 1994; Tanksley et al. 1996). Development of new high-throughput markers with high density maps and combining QTL mapping with functional genomics will facilitate map based cloning and further cloning of QTLs.map-based

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