Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

KLY Tejaswini
Research Scholar, Department of Genetics and Plant Breeding, Agriculture College, Bapatla, Andhra Pradesh, India

PV Ramana Rao
Scientist, Department of Genetics and Plant Breeding, APRRI & RARS, Maruteru, Andhra Pradesh, India

Shashi Bhushan Kumar
Department of Soil Science & Agricultural Chemistry, BAU Ranchi, Jharkhand, India

(Special Issue- 5)
International Conference on
"Food Security through Agriculture & Allied Sciences"
(May 27-29, 2019)

Identification and utilization of qtls in crop improvement

KLY Tejaswini, PV Ramana Rao and Shashi Bhushan Kumar

Abstract
Most of the traits of interest in plant breeding (e.g., height, yield etc.) are quantitative with continuous (normal) distribution, multifactorial or complex traits. A quantitative trait is a measurable trait that depends on the cumulative action of many genes and their interaction with the environment.
A QTL is defined as "A region of the genome that is associated with an affect on a quantitative trait". A QTL can be a single gene, or it may be a cluster of linked genes that effect the trait. Among all the available markers, AFLP and SSR markers are most commonly used markers in the development of new linkage maps and QTL studies. Use of QTL linkage for identification of a particular trait was first performed by Payne (1918) [25], where he demonstrated that several of the loci that responded to selection for high scutellar bristle number in *Drosophila melanogaster* were closely linked to known markers on the first and third chromosomes. Thereafter several QTLs from different crops were identified which includes yield traits, pest and disease resistance traits, quality improvement traits etc., which can be used for improving those crops. Hence one can say that QTLs play a great role in crop improvement.
The process of constructing linkage maps and conducting QTL analysis is to identify genomic regions associated with traits which is known as QTL mapping (McCouch & Doerge, 1995). QTL mapping' is based on the segregation of genes and markers *via* chromosome recombination (called crossing-over) during meiosis (i.e. sexual reproduction), thus allowing their analysis in the progeny (Paterson, 1996). Although there have been numerous QTL mapping studies for a wide range of traits in diverse crop species, relatively few markers have actually been implemented in plant breeding programs. This is due to lack of adoption is that the markers used have not been reliable in predicting the desired phenotype. New developments and improvements in marker technology, the integration of functional genomics with QTL mapping, and the availability of more high-density maps would greatly affect the accuracy and efficiency of QTL Mapping. It is expected that the development of high resolution maps will also facilitate the isolation of actual genes (rather than markers) via 'mapbased cloning' (also 'positional cloning').

Keywords: QTL, Crop improvement, Polymorphic markers and cloning

Introduction
Plant breeding is an art as well as science that deal with traits of economic gain. Such traits require appropriate quantitative analysis and constant genetic improvement. For constant genetic improvement of the traits, continuous breeding efforts are necessary. The researchers should have a strong background of Mendelian genetics for application in plant breeding as most of the traits are carried from generation to generation by Mendelian inheritance. In many crops, significant improvement has been achieved by continuous breeding efforts. Most of the traits (e.g., plant height, yield etc.) are complex with continuous (normal) distribution. These traits showing continuous variation and are controlled by two or more separate genes and hence called Quantitative Traits (QT).
By definition, a quantitative trait is a measurable trait that depends on the cumulative action of many genes and their interaction with the environment and thereby producing continuous distribution of phenotypes (Sham *et al.,* 2002) [28]. These genes were termed as Polygenes by MATHER (1949) [20]. These genes segregate in Mendelian fashion in any given population, and in most cases their effects are approximately additive. (Kearsey and Pooni, 1996) [15]. These quantitative traits (QTs) are governed by many genes. Each gene produces its own effect whether small or big, negative or positive. All these genes together produce a

Correspondence
KLY Tejaswini
Research Scholar, Department of Genetics and Plant Breeding, Agriculture College, Bapatla, Andhra Pradesh, India

pronounced effect on the trait under study. These traits are usually quantified by measurement rather than counting.

The genetic improvement of QTs is a herculent task for the plant breeders because performance only partially reflects the genetic values of the individuals. Since the proposal of the multiple-factor hypothesis by Nillson-Ehle (1909) and East (1916), the genetic variation of a quantitative trait is due to collective effects of numerous genes, known as quantitative trait loci (QTLs) (Falconer and Mackay, 1996; Xu, 2010) [8, 35].

The term QTL was first coined by Gelderman (1975) [10]. A QTL is defined as "Agenome region that is associated with an effect on a quantitative trait". In other words, a quantitative trait locus/loci (QTL) is the location of individual locus or multiple loci in the genome that collectively affects a quantitative trait. Conceptually, a QTL can be a single gene, or it may be a cluster of linked genes that effect the trait.

Molecular Markers

Molecular markers *viz.,* RFLP, RAPD, AFLP, micro-satellites, ESTs, SNPs etc., are used for generation of linkage maps and identification of new QTLs. Until recent past, the most commonly used DNA markers in the QTL mapping were AFLP and microsatellites. In the recent times, Single nucleotide polymorphisms (SNPs) are gaining priority in QTL mapping studies. When compared to AFLP, microsatellites are preferred because of codominant in nature, distribution throughout the genome, highly variable and highly reproducible. While, SNPs are single nucleotide differences that distinguish two individuals and these are basedon sequencing studies. Expressed sequence tags (EST) are expressed sequences from cDNA libraries. EST markers include small insertion-deletions. EST mapping approach is used to both map QTL and studyits effects down to individual genes (Lexer *et al.,* 2004; Zhang *et al.,* 2004) [19, 38].

Markers – Qtl Linkage

The morphometric and molecular evaluation of a population are the key factors in identifying new QTLs and the disparities between molecular and morphometric evaluation would be narrowed if marker – QT linkage can be determined in an efficient manner. If a genetic marker is tightly linked to a trait, any alteration in the inheritance of marker reflects on the performance of the associated trait.

In one of the early studies of the century, Payne (1918) [25] demonstrated that loci that govern high scutellar bristle number in *Drosophila melanogaster* were closely linked to known markers on the first and third chromosomes.

Sax (1923) [27] successfully demonstrated linkage between a genetic marker (a seed colour polymorphism due to a single gene) and a quantitative trait, seed weight, in *Phaseolus vulgaris.* He selected two parental lines, one of the which was homozygous for dominant pigment allele P and had an average seed weight of 48g. The second parent was homozygous for recessive allele p that lacks pigmentation and has an average seed weight of 21g. The F_2 of the cross is detailed below:

Table 1: Showing F2 of the cross between two selected parental lines

Marker genotype	PP	PP	PP
Seed weight genotype	SS	SS	SS
Seed weight (g)	30.7	28.3	26.4

If PPSS is assigned to parent 1 and PP SS to parent 2 based on pigmentation and seed weight, the genetic effects can be computed for the QT, seed weight. The mean of each marker genotype class can be estimated by multiplying the frequency of each genotype by its genotypic value and summing within the marker class. The F_2 mean was computed using the mendelian frequencies of ¼, ½, ¼ for the marker genotypes PP, Pp, pp. The differences between the PpSs genotype and average of the two homozygous equal to 28.3-1/2 (30.7+26.4) = -0.25g. The difference between the parents for seed weight was 27g and that in the F_2 for the same genotypes is 4.3g, the later is about 16% of the original parental differences, implying that 16% is the extent of association between the marker locus PP and the associated locus SS. In this experiment, it was assumed that the seed weight trait is also controlled by a single gene. Had it been only one major locus, what is now known as QTL or quantitative trait locus, and had there been complete linkage between PP and this QTL locus, the marker class difference given by SS-ss in the F_2 would have been equal to the parental difference. However, it rarely happens as the QTs are under the influence of several genes. But it is clear from the above simple experiment by Sax (1923) [27] that a marker that segregates in simple Mendelian pattern can be linked to QT and at the same time, the particular marker may not associate itself with all the loci (QTLs) that jointly express as QT. Hence, it is necessary for exploring many markers for establishing linkage with the QTL, so that all the markers together can explain the total variation in the QT.

In this study, a morphological marker was used but the same analogy is identical to that used of a molecular marker with a QT. The above analogy assumes to be simple to explain the association between a molecular marker and QTL. But in practice, there are many problems in optimization of statistical analysis and interpretation of the results. When Sax conducted his experiment, the influence of the environment in three different seasons on the expression of the seed weight in the parental, F_1 and F_2 generations was not considered. After understanding how a molecular marker is linked to a QT, this information can be extended from the reference population to other populations. Most of the earlier studies reported in the literature deals with markers with their locations in a linkage map linked to QTs based on already developed genetic material carrying the QTs.

Advancements in molecular marker technology combined with development of numerous analytical softwares, it is easy in the current scenario for combined analysis of genetic and phenotypic data which ultimately results in the identification of new QTLs/genes. These can be utilized for economic improvement for the benefit of the mankind especially in the field of agriculture. (Mackay 2001) [21]

Qtl Mapping

QTL mapping is the study of the alleles that are present in a locus and the phenotypes (physical forms or traits) produced by them. The process of constructing linkage maps and conducting QTL analysis is to identify genomic regions associated with traits which is known as QTL mapping.

Principles of QTL mapping

The basic principle involved in QTL mapping is studying the segregation pattern of genes and markers *via* chromosome recombination (crossing-over) during meiosis and subsequent analysis in their progeny (Paterson, 1996) [24]. Segregation of

mapping population into different genotypic classes based on selected marker and further application of correlative statistics to determine the significant difference of one genotype with another with respect to trait being measured.

Prerequisites of QTL mapping
1. The most important prerequisite for mapping studies is the availability of a good linkage map (Linkage map can be constructed using the marker data)
2. A segregating population obtained from parents that has contrasting differences for the trait(s) of interest and which allow for replication of each segregant.
3. Reliable phenotyping technique for assay of the trait(s) of interest
4. Reliable softwares for analyses

Objectives of QTL mapping
1. To identify the regions of the genome that influence the trait of interest
2. To analyze the effect of the QTL on the trait:
▪ What is the amount of variation caused by a specific region?
▪ What is the gene action associated with the QTL?
▪ Which allele is associated with the favorable effect?

Approaches for QTL mapping
1. Experimental crosses (Segregating Population)

Generation of a mapping population

▪ Backcrosses
▪ F_2/ F_3 population
▪ Recombinant inbred (RI) lines
▪ Double Haploids
▪ Near isogenic lines (NILs)

2. Association studies (Linkage disequilibrium, LD mapping)
▪ With candidate genes (direct approach)
▪ Localized association studies (chromosomal region)
▪ Whole-genome association studies

Briefly the QTL mapping studies involves
1. Making the crosses between divergent parents and generation of a suitable mapping population (F_2, F_3, RIL, BIL, DH etc.).
2. Genotyping of the population using a suitable molecular marker and generating marker data.
3. Generation of the linkage map using marker data (Genome size, genome coverage).
4. Phenotypic evaluation of the population in a single or different environments and collection of the data.
5. Mapping of QTLs
▪ Single marker analysis
▪ Interval mapping
▪ Multiple interval mapping
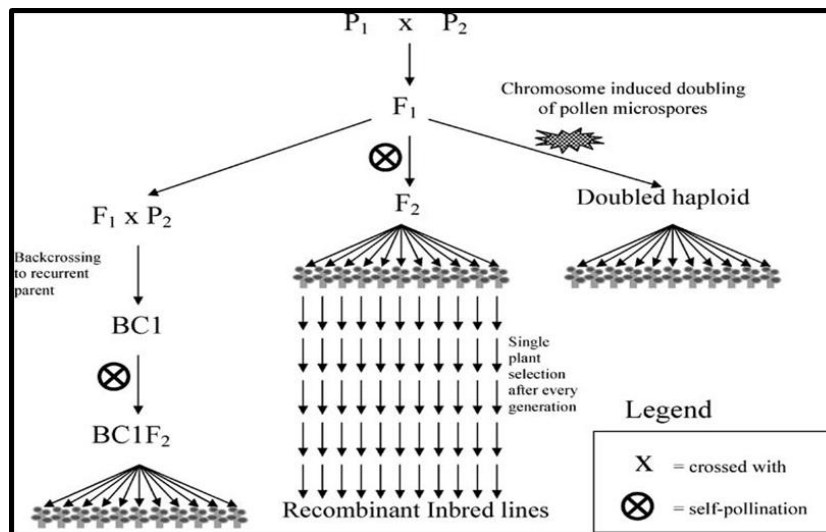▪ Composite interval mapping

Fig 1: Schematic representation showing procedure of obtaining BC populations, RILs, DHs

The basic requirement for construction of a linkage map and identification of new QTLs is a segregating plant population. The parents selected for generating the mapping population should have contrasting differences for one or more traits of interest. The size of the mapping population depends on the type of the population used and generally it varies from study to study and crop to crop. For a preliminary study, the size of the population can vary from 50 to 250 individuals however, larger populations are required for high-resolution mapping.
The generation of the mapping population also depends on the mode of reproduction. Generally in self-pollinating species, mapping populations are generated using highly homozygous parents (inbreds). In cross pollinating species which do not tolerate inbreeding, the generation of a suitable mapping population is little difficult. That too in polyploidy species it

is much more difficult. Mapping populations in cross pollinating species may be derived from a cross between a heterozygous parent and a haploid or homozygous parent (Wu *et al.*, 1992) [34]. For instance, in crops like white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) which are highly cross pollinated, F_1 generation mapping populations were successfully developed by pair crossing heterozygous parents that were distinctly different for important traits associated with plant persistence and seed yield (Barrett *et al.*, 2004; Forster *et al.*, 2000) [2, 9]. In each species, different segregating populations can be generated and utilized for mapping studies but each type of population has its own advantages and disadvantages (McCouch & Doerge, 1995; Paterson, 1996) [22, 24].

Types of mapping population
Quantitative trait locus (QTL) mapping requires parental lines that differ genetically for the trait of interest. The parental lines are crossed to generate F_1 individuals. These F_1s are selfed to generate F_2 or backcrossed to either of the parents to generate backcross progeny. Both of these (selfing or backcrossing) produce individuals that contain recombinant fractions of the genome of each parental line. The phenotype for each of these recombinant individuals or lines is assessed using a suitable experimental design. Also, the genotype of these individuals is assessed using markers that are polymorphic between the parental lines. Sometimes double haploids are generated which can also be used for mapping studies.

The choice of mapping population varies depending upon the objectives of experiment, resources available and time schedule. A segregating population produced from parents that have contrasting target traits can be utilized for mapping. The parent lines should be genetically divergent. The size of mapping population depends upon what type of mapping population is used and also on the genetics of the target trait. The different types of mapping populations used for QTL mapping are

- F_2 Population derived from selfing of F_1
- Back cross population derived by crossing of F_1 with either of the parents
- Recombinant inbred lines(RILs) derived by selfing of F_1 for 4-5 generations
- Double haploid lines (DHLs) derived by doubling of the chromosomes of haploids

F_2 population derived from selfing of the F_1 hybrids can be used as base population for mapping studies especially in self-pollinated species. The major advantage in utilizing F_2 population for mapping is that it requires very short time in generation.
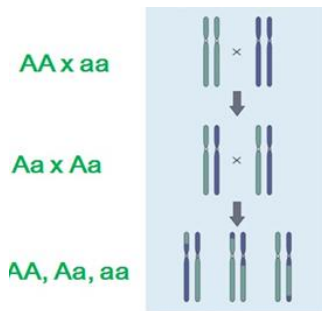

Fig 2: Population

Also, backcross (BC) populations, derived by crossing the F_1 hybrid to one of the parents can be utilized for basic mapping studies. The main advantage is that it is easy to construct and require only a short time to produce.
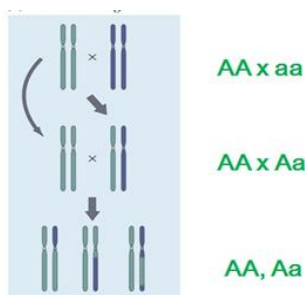

Fig 3: Single backcross (BC_1F_1)

Recombinant inbred lines (RILs) are generated by selfing of F_2 plants generation after generation for 4-5 times ($F_6 - F_7$). RILs consist of a series of homozygous lines, each containing a unique combination of chromosomal segments from the original parents. The length of time needed for producing RIL populations is the major disadvantage, because usually six to eight generations are required.
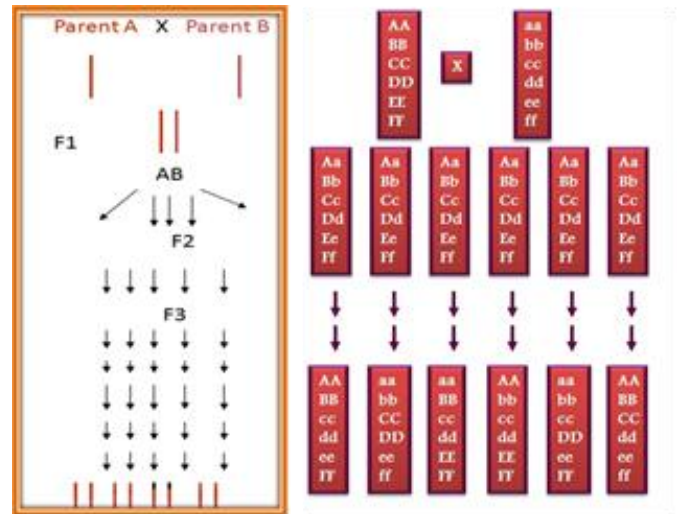

Fig 4: Recombinant Inbred Line (RIL)

Near isogenic lines (NIL) are generated by repeated backcrossing of the F_1 plant with either of the parents. Likewise, backcross inbred lines (BILs) are generated repeated backcrossing (2-4 times) of the F_1 plants to the recurrent parent followed by selfing. Also, the major disadvantage in using NILs or BILs is that it requires more time and effort in generation.
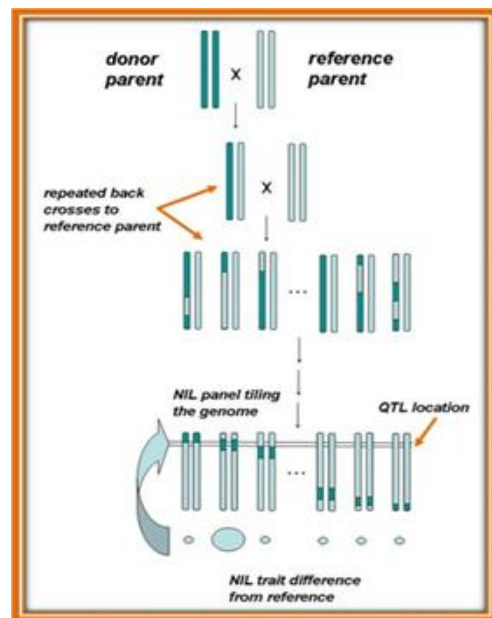

Fig 5: Near Isogenic Line (NIL) Backcross Inbred Line (BIL

Doubled haploid (DH) populations are produced through tissue culture of pollen grains and by doubling of the chromosomes in haploids by colchicine treatment. However, the production of DH populations is only viable in species that respond well to tissue culture (e.g. cereal species such as rice, barley and wheat).
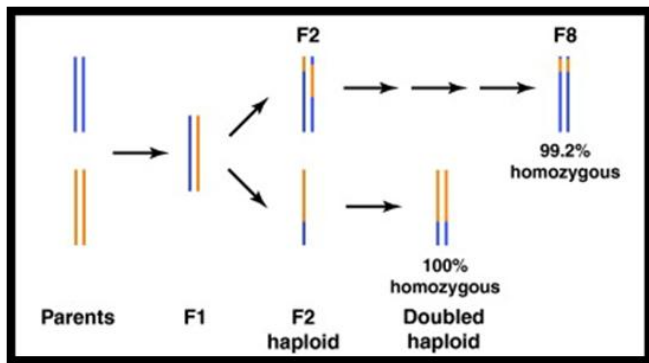
Fig 6: Double Haploid (DH)

The major advantages of using RIL and DH populations are that they are homozygous. Hence, both RIL and DH populations are very useful for QTL mapping when compared to other populations. Furthermore, seed from individual RIL or DH lines may be shared with others for further linkage analysis and the addition of markers to existing maps, ensuring that all collaborators examine identical material (Paterson, 1996; Young, 1994) [24, 36].

Identification of Polymorphic markers & Genotyping
The second step in the construction of a linkage map is to identify polymorphic markers between the contrasting parents. It is imperative to study the polymorphism between the parents using selected markers. In other words, DNA markers that reveal differences between parents are to be determined. The choice of DNA markers utilized in the mapping studies majorly depend on the availability of characterized markers or the appropriateness of particular markers for a particular species. It is very crucial to have sufficient polymorphism between parents in order to construct a linkage map (Young, 1994) [36]. When compared to self-pollinated crops, cross pollinating species possess higher levels of DNA polymorphism. In most of the cases, parents that are genetically divergent are selected to have sufficient polymorphism. (Joshi & Nguyen, 1993; Anderson *et al.*, 1993; Yu & Nguyen, 1994 and Collard *et al.*, 2003) [14, 1, 37, 4]
In some polyploid species such as sugarcane, identifying polymorphic markers is more complicated (Ripol *et al.*, 1999) [26]. For mapping of polyploidy species, using diploid relatives of polyploid species is of great use. However, diploid relatives may not exist for all polyploid species (Wu *et al.*, 1992, Ripol *et al.*, 1999) [26].
After identification of the polymorphic markers between the parents, these markers are utilized to evaluate entire mapping population including the parents (and F_1 hybrid, if possible). This is referred to as "Genotyping" of the mapping population.

Generating the linkage map
The genotyping of the segregation population is followed by studying the segregation pattern of these markers and to generate the linkage map. Linkage maps indicate the position and relative genetic distances between markers along chromosomes. Linkage maps plays a crucial role in identifying chromosomal segments containing genes and QTLs associated with traits of interest. For instance, in a segregating population, there is a combination of parental and recombinant genotypes. Recombination fractions and genetic distance between markers are estimated based on the frequency of recombinant genotypes. The position of markers,

their relative order and distances between markers can be determined by analyzing the segregation of the markers. If the recombination frequency between two markers is less it indicates that they are closely present on the same chromosome. While, markers having recombination frequency of 50% or more are designated as 'unlinked' and these are located either far on the same chromosome or on different chromosomes. Mapping functions are used to convert recombination fractions into map units called centi-Morgans (cM). In genetic mapping, the studied markers are placed in order indicating the relative genetic distances between them. To detect reliable QTLs, it is desirable to have a saturated marker map. In a saturated map, the markers are present from one end to the other end of the chromosome with very less distance so that recombination events rarely occur between them. In practice, it is considered to be less than 10 recombinations per 100 meiosis, or a map distance of less than 10cM.

The generated genotypic data is then analyzed using several statistical software tools (Mapmarker or Joinmap or Mapdisto or ICIM etc). For construction of a linkage map. The segregation pattern for each of the markers is analyzed. The segregation patterns are in accordance with the type of mapping population used. Each software has a specific format for coding data for each polymorphic marker on each individual of a mapping population. There is a provision for missing data also in most of the softwares. Linkage analysis can be performed manually for a few markers but it not feasible to manually analyze large number of markers. Further determination of linkages and construction of maps is not possible by manual analysis.

Further linkage analysis is conducted to generate the linkage map. Linkage between markers is usually calculated using different algorithms viz., LOD score, recombination frequency, marker distance, group number etc. The basic algorithm is odds ratios (i.e. the ratio of linkage versus no linkage). This is expressed in a logarithmic ratio and as logarithm of odds (LOD) value or LOD score.

LOD score

$$Odds\ ratio = \frac{Probability\ of\ the\ data\ occurring\ with\ a\ QTL}{Probability\ of\ the\ data\ occurring\ with\ no\ QTL}$$

LOD values of >3 are used to construct a precise linkage map but sometimes researchers construct the linkage map with LOD values >2. When LOD value is given as 3, it indicates that linkage if 1000 times likely than no linkage. To detect greater level of linkage, sometimes the LOD value is lowered. Also different algorithms were used for grouping of the markers before the linkage analysis.
The software will group all the linked markers into linkage groups. These linkage groups can either by part of the chromosomes or complete chromosomes. The distance between the markers on each linkage group is measured in terms of recombination frequency. In other words, the distance between the markers is measured in terms of centiMorgans (cM). When the distance between the markers on alinkage group are small (<10 cM), the map distance equals the recombination frequency. One simple rule to choose the best algorithms for construction of linkage map is that the best method should give the shortest linkage map.
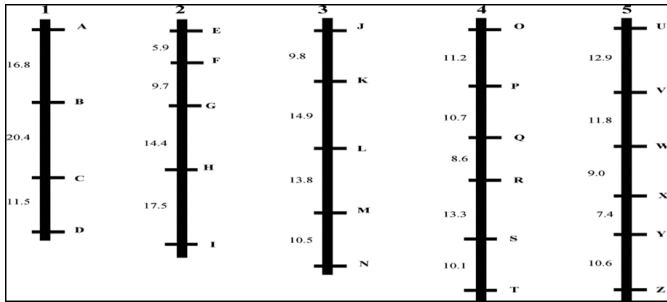
Fig 7: Example showing Map distance between markers in linkage map

Phenotyping of mapping population

Phenotyping involves the precise measurement of the target quantitative trait with limited amounts of missing data. Size of the sample and genetic marker coverage are the key factors in resolving the QTL location for a particular trait. Sometimes, size of the population might be compromised for increasing the precision of data and in such instances generally major QTLs are detected. The pooled data (including locations and replications) should be utilized for the construction of linkage map. For a better understanding of the QTL and environment, it is always preferable to measure the target traits in multiple environments.

Methods to detect QTLs

Widely-used methods for detecting QTLs are
1. Single-marker analysis (SMA)
2. Simple interval mapping (SIM)
3. Composite interval mapping (CIM)
4. Multiple interval mapping (MIM)

Single Marker Analysis (SMA)

Single marker analysis (SMA), also called as single factor analysis (SF-ANOVA) or single point analysis is a good start for novice researchers in QTL mapping but is equally good for practical data analysis. In earliest studies of QTL mapping, SMA is mostly used. (Edwards *et al.,* 1987; Weller *et al.,* 1988) [7, 32] The analysis and interpretation of the data in SMA is simple when compared to other methods. It can be computed using basic softwares. The gene orders and complete linkage map are not necessary in SMA. F-test for the difference between marker genotype classes is highly significant at locus.
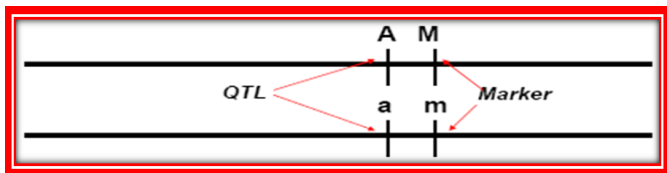

Fig 8: Single Marker Analysis (SMA)

Limitations of Single Marker Analysis

SMA is very simple in compuataion but this approach has many limitations
1. As the distance between the marker and QTL increases, the likelihood of QTL detection decreases significantly
2. In this method, itcannot be determined whether the markers are associated with one or more QTLs.
3. The effects of QTL are likely to be under estimated because they are confounded with recombinations frequencies.

Simple Interval Mapping (SIM)

This method was proposed by Lander and Botstein (1989) [17]. SIM evaluates the association between the target trait values and the genotype of a hypothetical QTL at multiple analysis points between pair of adjacent marker loci (target interval).Interval mapping (IM) is considered as a second level of QTL mapping. QTL mapping by this method requires prior construction of a marker genetic map.
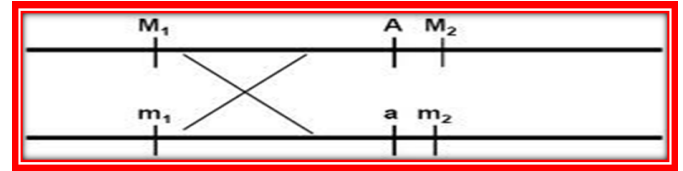

Fig 9: Simple In treval Mapping (SIM)

The interval mapping/ SIM approach is based on the joint frequencies of a pair of adjacent markers and a putative QTL flanked by the two markers. The most widely used approach in QTL mapping is interval mapping. By using tightly linked markers for analysis, it is possible to compensate for recombination between markers and the QTL, there by increasing the probability of statistically detecting the QT, and providing an unbiased estimate of QTL effect.

However, when multiple QTLs are segregating in a cross (which is usually the case), SIM fails to take into account genetic variances caused by other QTLs. In such a case, SIM suffers essentially from the same short comings of single marker analysis.

Composite Interval Mapping (CIM)

Composite interval mapping is a combination of interval mapping and linear regression of the markers. It takes into account other genetic markers in addition to an adjacent pair of linked markers for interval mapping. This method was developed by Jansen and Stam (1994) [13]. CIM evaluates the possibility of a target QTL at multiple analysis points across each intermarker interval. It considers a marker interval plus a few other well-chosen single markers in each analysis, so that *n-1* tests for interval QTL associations are performed on a chromosome with *n* markers.

The advantages of CIM are as follows
1. Mapping of multiple QTLs can be accomplished by the search in one dimension.
2. By using linked markers as cofactors, the precision of the mapping is increased and even QTLs outside the region are not affected.
3. By eliminating much of the genetic variance by other QTL, the residual variance is reduced which thereby increases the power of QTL detection.

When compared to SIM, CIM is more powerful tool hence it is being used frequently in recent studies.

Multiple Interval Mapping (MIM)

MIM method uses multiple marker intervals simultaneously to fit multiple putative QTL in the mapping studies. With the MIM approach, the precision and power of QTL mapping could be improved. Also, the estimation of epistasis between different QTLs and heritability of quantitative traits can be performed using MIM.

Classification of Qtls
Major and Minor QTLs
The newly identified QTLs may be classified as 'major' or 'minor' depending on the phenotypic variation explained by a QTL (based on the R^2value):
- Major QTLs will account for a relatively large amount of R^2 (e.g. >10%)
- Minor QTLs will usually account for < 10% R^2.

Generally, QTLs with higher R^2 value will be stable and express across the environments whereas QTLs with less phenotypic variance are highly influenced by the environment and are not stable.

Suggestive, Significant and non-significant QTLs
QTLs can also be classified as (1) Suggestive, (2) Significant and (3) Highly significant.

Significant and highly-significant QTLs were given significance levels of 5 and 0.1%, respectively. Suggestive QTL is one that would occur once at random in a QTL mapping study.

Factors affecting the detection of QTLs
1. The number of genes controlling the target trait and their genome positions is the major factor in detection of QTLs.
2. The genetic interactions between the genes influence the QTL detection.
3. The heritability of the trait also influence the QTL detection.
4. The type and size of mapping population are also the key factors in QTL mapping.
5. The density and coverage of markers in the construction of the linkage map has a strong influence.
6. Statistical methodology employed and significance levels used for QTL mapping.

Merits of QTL mapping
1. Identification of novel genes governing the target traits.
2. When earlier approaches like using mutants fail to detect genes with phenotypic functions, QTL mapping can help.
3. Identification of new functional alleles with known function.
4. Natural variation studies provide insight into the origins of plant evolution

Limitations of QTL mapping
1. It mainly identifies loci with large effects
2. The number of QTLs detected, their position and effects are subjected to statistical error
3. In most studies, small additive effects / epistatic loci are not detected and may require further analyses

Problems and possible solutions in QTL mapping
First and most importantly, QTL locations obtained from segregating populations have very large confidence intervals (CIs). These CIs should be less than 5 cMbut often they are often more than 30 cM (Van Ooijen, 1992, Darvasi *et al.*, 1993, Hyne *et al.*, 1995) [30, 5, 12]. It is now well established that having more markers beyond about one every 10-20 cM

does not reduce the CI and that the only way to reduce it is to increase the population size considerably (Hyne *et al.*, 1995) [12]. The reason for these large CIs is simply the lack of recombination at meiosis (Boenhnke, 1994; Kearsey and Pooni, 1996; Guo and Lange, 2000) [15, 11]. Most chromosomes survive meiosis with either one cross over or none at all and thus one needs to sample a very large number of meiosis to have enough cross over's to map the QTL with accuracy. The smaller the heritability of the trait, the larger the population required.

Further recombination can be achieved by randomly mating the populations for two or more generations and this is particularly beneficial for closely linked genes. However, both very large populations and several generations of are seldom practical options in a breeding programme, particularly when there is large amount of genetically uniform material (inbred or F_1). Back crosses and F_2s allow very large populations and hence are efficient, but the spaced plant trials needed to assess them are not very useful for traits of agricultural importance, such as yield, because the conditions are not typical.

A second problem of concern is presence of multiple QTL on a chromosome. It is difficult to distinguish two QTL that are less than 20 cM apart, even with QTL of moderate heritability and hence two or more QTL within this interval may be misinterpreted as one (Leberton *et al.*, 1998) [18]. This can result in a large QTL being located between the two true QTL if they are linked in coupling and possibly no QTL being identified if they are linked in repulsion. Either way, one is missed both in the location and in the size of the QTL effect.

A third problem is a statistical one. QTL location by whatever method involves scanning each chromosome for the likely position of the QTL. This inevitably implies that a large number of possible positions are tested and those likelihood of containing a QTL exceeds are accepted. To avoid too many false positives, the test probability level is adjusted downwards to allow for the multiple tests. This is the concomitant result of increasing the probability of false negatives.

The only real solution to those problems of QTL location in populations is to repeat the experiments using a completely different sample of genotypes derived from the same population. One should then test whether there are QTL in this second population located at the positions identified in the first. This is an approach that the human geneticists realized some time ago and all good human genetics experiments now have an initial identification sample followed by a final testing or confirmation sample.

Utilization of QTLs in different crops
In several crops like rice, wheat, maize, barley, cotton and other vegetable and fruit crops, analysis of advanced backsross population/RILs etc. led to identification of new QTLs for many traits including yield, quality, resistance to biotic and abiotic stresses (Baohuawang and PengW. chee, 2010). These QTLs were incorporated in the breeding programmes for the genetic improvement of the particular crop.

The details of their review are as follows

Table 2: Details of identified QTLS in different crops

Crop	[a]Population(s)	Traits/Genes evaluated	Authors
Tomato	BC$_2$ and BC$_3$	A battery of horticultural traits	Tanksley et al. (1996)
	BC$_3$ and BC$_4$	A battery of horticultural traits	Fulton et al. (1997)
	BC$_2$ and BC$_3$	19 agronomic traits	Bernacchi et al. (1998a)
	NILs derived from AB populations	A battery of important agronomic traits	Bernacchi et al. (1998b)
	BC$_2$ and BC$_3$	30 horticultural traits	Fulton et al. (2000)
	BC$_2$ and BC$_3$	Biochemical properties	Fulton et al. (2002)
	RIL,BC$_3$S$_1$, BC$_3$S$_3$,QTL-NILs	Six quality traits	Chaïb et al. (2006)
	BC$_2$, IL and RIL	Ascorbic acid content	Stevens et al. (2007)
Rice	BC$_2$ testcross population	12 agronomically important quantitative traits	Xiao et al. (1998)
	BC$_4$F$_2$, BC$_4$F$_3$ and NILs	Heading date	Yamamoto et al. (2000)
	BC$_2$F$_1$ and BC$_2$F$_2$	Yield, yield components and morphological traits	Thomson et al. (2003)
	BC$_2$F$_2$	Yield and yield component traits	Septiningsih et al. (2003a)
	BC$_2$F$_2$	14 seed quality traits	Septiningsih et al. (2003b)
	BC$_3$F$_3$ and BC$_3$F$_4$	Blast resistance	Wu et al. (2004)
	BC$_3$F$_1$ hybrid families	Grain quality and grain morphology	Li et al. (2004)
	RILs and BC$_3$F$_3$	Rice blast disease	Liu et al. (2004)
	BC$_2$ testcross progeny	13 agronomic traits pertaining to yield and its components	Marri et al. (2005)
	F$_{2:3}$ developed from BC$_5$F$_6$ line	Agronomic traits	Yoon et al. (2006)
	BC$_3$F$_4$ NILs	Yield-related traits	Xie et al. (2008)
	A set of 12 BC$_2$F$_6$ progenies	Yield and yield components	Cheema et al. (2008)
	A BC$_3$F$_3$ line named BC116	Rice blast disease	Manosalva et al. (2009)
Barley	BC$_2$F$_2$	Yield, yield compotent and quality	Pillen et al. (2003)
	BC$_3$F$_3$	Grain yield and its components	Li et al. (2006)
	BC$_2$F$_{6:8}$	Resistance to seedling disease and adult plant spot blotch	Yun et al. (2006)
	BC$_2$F$_8$	Morphological and agronomical traits	Gyenis et al. (2007)
	BC$_2$DH populations and candidate ILs	The major photoperiod response gene *Ppd*-H1	von Korff et al. (2004)
	BC$_2$DH population	Powdery mildew, leaf rust resistance and scald resistance	von Korff et al. (2005)
	BC$_2$DH population S42	9 agronomic traits	von Korff et al. (2006)
	BC$_2$DH population S42	7 malting parameters	von Korff et al. (2008)
	ILs derived from the AB population S42	Resistance to powdery mildew and leaf rust	Schmalenbach et al. (2008)
	ILs derived from the AB population S42	7 Agronomic traits	Schmalenbach et al. (2009)
Wheat	BC$_2$F$_2$ and BC$_2$F$_3$	Yield and yield components	Huang et al. (2003)
	BC$_2$F$_1$	Seven agronomic traits	Huang et al. (2004)
	BC$_2$F$_{2:4}$	12 Agronomic traits	Narasimhamoorthy et al. (2006)
	BC$_2$F$_3$	Quality traits	Kunert et al. (2007)
	BC$_2$F$_3$	Leaf rust resistance	Naz et al. (2008)
Maize	BC$_2$ testcrosses	Grain yield, grain moisture and plant height	Ho et al. (2002)
	BC$_2$F$_2$ and NILs	Grain yield components	Li et al. (2007)
	BC$_2$S$_1$, F$_{2:3}$ and NILs	9 plant traits	Li et al. (2008)
	BC$_2$F$_1$	Root aerenchyma formation	Mano and Omori (2008)
Cotton	BC$_3$F$_2$	Fiber elongation	Chee et al. (2005a)
	BC$_3$F$_2$	Fiber length	Chee et al. (2005b)
	BC$_3$F$_2$	Fiber fineness and micronaire	Draye et al. (2005)

[a]Notes: NIL, near-isogenic line; IL, introgression line; BC$_2$DH, advanced backcross (BC$_2$) doubled haploid population; RIL, recombinant inbred line; AB populations, advanced backcross populations.

Applications
1. Improve the efficiency for breeding traits with low heritability or that can be measured in one sex (Soller and Beckmann, 1988; Lande and Thompson, 1990) [29, 16].
2. Transgenic technology can even be applied to quantitative traits.
3. In medicine, the improved methods of prevention is possible when alleles causing predisposition to multifactorial diseases are identified.
4. Quantitative genetic theory will be made more realistic when the number and properties of the genes are known, and thus helps us in better understanding of the evolution.

Conclusions
- Although there have been numerous QTL mapping studies for a wide range of traits in many crop species but the major flaw is in usuage of these QTLs in plant breeding programs.
- The main reason for this lack of adoption is that the markers used have not been reliable in predicting the desired phenotype.
- New developments and improvements in marker technologyie, combining the QTL mapping studies with functional genomics is the need of the hour. Also, the availability of more high-density maps would greatly affect the accuracy and efficiency of QTL Mapping.
- New types of markers and high-throughput marker techniques should play an important role in the construction of genetic maps. Also, these techniques should not be too expensive.
- Due to the abundance of single nucleotide polymorphisms (SNPs) and development and use of high-throughput SNP detection systems, in the near future SNP markers play a major role in mapping studies.
- The latest trends are to combine QTL mapping with methods in functional genomics, developed for the study of gene expression.
- These techniques include expressed sequence tag (EST) and microarray analysis, which can be utilized to develop markers from genes themselves.
- Development of high resolution maps will help in the isolation of actual genes via 'map based cloning'.

Future Prospects
The future aspects of study for QTL mapping studies is
- Constant improvement of molecular platforms ie., development of new types of cost effective markers.
- New types of genetic materials which can help in identification of minor but stable QTLs
- Advances in Bioinformatics

References

1. Anderson J, Churchill G, Autrique J, Tanksley S, Sorrells M. Optimizing parental selection for genetic linkage maps. Genome. 1993; 36:181-186.
2. Barrett B, Griffiths A, Schreiber M, Ellison N, Mercer C, Bouton J *et al.* A microsatellite map of white clover. Theoretical Applied Genetics. 2004; 109:596-608.
3. Boehnke M. Limits of resolution of linkage studies: Implications for the positional cloning of human disease genes. American Journal of Human Genetics. 1994; 55:379-390.
4. Collard BCY, Pang ECK, Taylor PWJ. Selection of wild *Cicer* accessions for the generation of mapping populations segregating for resistance to ascochyta blight. Euphytica. 2003; 130:1-9.
5. Darvasi A, Weintreb A, Minke V, Weller S, Soller M. Detection marker QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics. 1993; 134:943-951.
6. East EM. Studies on size inheritance in Nicotiana. Genetics. 1916; 1:164-176.
7. Edwards MD, Stuber CW, Wendel JFC. Molecular marker facilitated investigations of quantitative trait loci in maize. Genetics. 1987; 116:113-125.
8. Falconer DS, Mackay TFC. Introduction to Quantitative Genetics, 4th edition, Harlow UK, Longman Group, Prentice Hall, London, 1996, pp. 464.
9. Forster JW, Jones ES, Kolliker R, Drayton MC, Dumsday JL, Dupal MP *et al.* Development and implementation of molecular markers for crop improvement. In: 'Molecular Breeding of Forage Crops.' In: G. Spangenberg (ed.), Proceedings of the 2nd International Symposium, Lorne and Hamilton, Victoria Australia, November 19–24. Kluwer Academic Publishers, London, 2000, pp. 101-133.
10. Geldermann H. Investigafions on inheritance of quantitative characters in animals by gene markers. I. Methods. Theoretical Applied Genetics. 1975; 46:319-330.
11. Guo SW, Lange K. Genetic mapping of complex traits: promises, problems and prospects. Theoeretical Population Biology. 2000; 57:1-11.
12. Hyne V, Kearsey MJ, Pike DJ, Snape JW. QTL analysis: unreliability and bias in estimation procedures. Molecular Breeding. 1995; 1:273:282.
13. Jansen RC, Stam P. High resolution of quantitative traits into *via* interval mapping. Genetics. 1994; 136:1447-1455.
14. Joshi C, Nguyen H. RAPD (random amplified polymorphic DNA) analysis based intervarietal genetic relationships among hexaploid wheats. Plant Sciences. 1993; 93:95-103.
15. Kearsey MJ, Pooni HS. The genetic analysis of quantitative traits, Chapman and Hall, London, 1996.
16. Lande R, Thompson R. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics. 1990; 124:743-756.
17. Lander ES, Botstein D. Mapping mendelian factors underlying quantitative traits using RFLP linkage map. Genetics. 1989; 121:185-199.
18. Leberton CM, Visscher PM, Haley CS, Semikhodskii A, Quarrie KA. A non-parametric bootstrap method for testing close linkage Vspleotrophy of coincident quantitative trait loci. Genetics. 1998; 150:931-943.
19. Lexer C, Lai Z, Rieseberg LH. Candidate gene polymorphisms associated with salt tolerance in wild sunflower hybrids: implications for the origin of Helianthus paradoxus, a diploid hybrid species. New Phytologist. 2004; 161:225-233.
20. Mather K. Biometrical genetics, 1st edition. London: Methuen, 1949.
21. Mackay TFC. Quantitative trait loci in Drosophila. Nature Reviews Genetics. 2001; 2:11-20.
22. Mc Couch SR, Deorge RW. QTL mapping in rice, Trends in Genetics. 1995; 11:482-487.
23. Nilsson-Ehle H. Kreuzungs untersuchungen an Haferund Weizen. –Acudemic Disserfarion, Lund, 1909, pp. 122.
24. Paterson AH. Making genetic maps. In: A.H. Paterson (Ed.). Genome Mapping in Plants. R. G. Landes Company, San Diego, California; Academic Press, Austin, Texas, 1996, pp. 23-39.
25. Payne F. The effect of artificial selection on bristle number on *Drosophila ampelophila* and its interpretation. Proceedings of National Academy of Sciences. 1918; 4:55-58.
26. Ripol MI, Churchill GA, Da Silva JAG, Sorrells M. Statistical aspects of genetic mapping in autopolyploids. Genetics. 1999; 235:31-41.
27. Sax K. The association of size differences with seed coat pattern and pigmentation in Phaseolus vulgaris. Genetics. 1923; 8:552-560.
28. Sham PC, Purcell S, Chemy SS, Abecasis GR. Powerful regression based quantitative trait linkage analysis in general pedigrees. Am J Hum Genet, 71,2002. 238-253
29. Soller M, Beckmann JS. Genomic genetics and the utilization for breeding purposes of genetic variation between populations. Proceedings of the 2nd international conference on Quantitative Genetics. Sinauer, Sunderland, Mass, USA, 1988, pp. 161-188.
30. Van Ooijen JW. Accuracy of mapping quantitative trait loci in autogamous species. Theoretical Applied Genetics. 1992; 84:803-811.
31. Wang B, Peng Chee W. Application of advanced backcross qualitative trait locus (QTL) analysis in crop improvement. Journal of Plant Breeding and Crop Science. 2010; 2(8):221-232.
32. Weller JI, Song JZ, Heyen DW, Lewin HA, Ron M. A new approach to the problem of multiple comparisons in the genetic dissection of complex traits. Genetics. 1998; 150:1699-1706.
33. Weller JI, Wyler A. Power of different sampling strategies to detect quantitative trait loci variance effects. Theoretical Applied Genetics. 1992; 83:582-588.
34. Wu KK, Burnquist W, Sorrells ME, Tew TW. The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theoretical Applied Genetics. 1992; 83:294-300.
35. Xu S. An expectation-maximization algorithm for the Lasso estimation of quantitative trait locus effects. Heredity. 2010; 105:483-494.
36. Young ND. Constructing a plant genetic linkage map with DNA markers: DNA Based Markers in Plants. The Netherlands. Kluwer Academic Publishers, 1994, pp. 39-57.
37. Yu LX, Nguyen HT. Genefic variation detected with RAPD markers among upland and lowland rice culfivars (*Oryza sativa* L.). Theoretical Applied Genetics. 1994; 87:668-672.

38. Zhang WK, Wang YJ, Luo GZ, Zhang JS, He CY, Wu XL *et al.* QTL mapping of ten agronomic traits on the soybean (*Glycine max* L. Merr.) genetic map and their association with EST markers. Theoretical Applied Genetics. 2004; 108:1131-1139.