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Introduction to QTL mapping in plants

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Abstract: Understanding the genetic basis of complex, quantitative traits entails a combination of modern molecular genetic techniques and powerful statistical method. With the advancement in molecular marker analysis technology, it is now possible to analyze both the simply inherited and quantitative trait and identify individual genes controlling the traits of interest. Molecular markers could be used to tag Quantitative Trait Loci (QTL) to evaluate the contribution to the phenotypic aiming to accelerate the selection and genetic advances. The construction of detailed linkage maps with high levels of genome coverage for localized genes or QTLs those are associated with economically important trait like disease resistance in plants which helps in marker assisted selection, comparative mapping between different species, a framework for anchoring physical maps and the basis for map based cloning of genes. This review provides an introduction to molecular marker, mapping population and basic principles behind QTL map construction.

Key words: Quantitative trait locus, Linkage map, Molecular marker, Simple sequence repeats, Mapping population and Genetic distance

Introduction

Traditional methods of plant breeding have made a noteworthy input to crop improvement, but they have been slow and incompetent in targeting complex traits like yield, quality, drought and disease resistance. But, after the development of powerful biotechnological tools it is now possible to analyze both the simply inherited and quantitative traits and identify individual genes controlling the traits of interest. The development of genetics has been exponential with several milestones including determination of DNA as the genetic material, discovery of the double-helix structure of DNA, the development of electrophoretic assays of isozymes [1] and a wide range of molecular markers that reveals differences at the DNA level. Each of these milestones had led to a huge wave of development in genetics and plant breeding. Our understanding of organismal genetics now extends from phenotypes to molecular levels, which can lead to new or improved screening methods for selecting superior genotypes more efficiently in breeding strategies.

Quantitative characters have been a major area of study in genetics for over a century, as they are common features of natural variation in population of all eukaryotes, including crop plants. For many agriculturally important traits such as yield, quality and some forms of disease resistance, there were several genes segregating in a Mendelian fashion in any given population, and in most cases their effects were almost additive. These genes were termed 'polygenes' by Mather (1949). We now refer the polygenes by a more attractive abbreviation, 'QTL' (Quantitative Trait Loci), a term coined by Gelderman (1975). A QTL is defined as "a region of the genome that is associated with an effect on a quantitative trait". Conceptually, a QTL can be a single gene, or it may be a cluster of linked genes that affect the trait. The identification of QTLs based only on orthodox phenotypic evaluation is not possible. A major revolution in the characterization of quantitative traits that created opportunities to select for QTLs was initiated by the two major developments during the 1980s, First has been the discovery of extensive, yet easily visualized at the DNA level that could be used as markers and second has been the development of statistical packages that can help in analyzing

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variation in a quantitative trait in congruence with molecular marker data generated in a segregating population. One of the important application of molecular marker is the development of linkage mapping in any species it is also called genetic mapping. It refers to the determination of the relative positions of genes on a DNA molecule (chromosome) and of their distance between them. Genetic map indicates the position and relative genetic distances between markers along chromosomes. The first genetic map was published in 1911 by T. H. Morgan and his student, Alfred Sturtevant, who showed the locations of 6 sex-linked genes on a fruit fly chromosome. Linkage maps have been utilized for identifying chromosomal regions that contain genes controlling simple traits (controlled by a single gene) and quantitative traits using QTL analysis [2]. The process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with traits–is known as QTL mapping [3] [4].

Genetic resistance is the most cost-effective and environmentally appropriate approach to disease management in crop plants. Qualitative and quantitative disease resistance genes have been extensively studied in terms of genome location [5] [6]. The study of quantitative resistance genes by QTL tool allow for the systematic dissection of quantitative resistance in to estimates of locus number, location, effect, and interaction [7]. The use of cultivars resistant to multiple diseases is a necessity. The development of varieties with resistance to multiple diseases can be expected by information on the number, location and effect on the determinant genes.

The purpose of this paper is to review the basic concepts and principles behind genomic map construction, and to describe how major genes and quantitative trait loci (QTL) can be detected. It will be a useful reference for conventional plant breeders, physiologists, pathologists and other plant scientists.

1. QTL mapping

According to the Sutton's chromosomal theory of inheritance, genes (markers or loci) segregate via chromosome recombination during meiosis (i.e. sexual reproduction). Genetic mapping is based on this principle by which analysis of segregation of gene is made possible in the progeny [4]. According to the Mendel's second law of independent assortment segregation of alleles of one gene is independent of alleles of another gene as the chromosomes assort randomly into gametes during meiosis. The law of independent assortment always holds true for genes that are located on different chromosomes, but it does not always hold true for genes that are on the same chromosome. According to the Morgan, when two genes in a dihybrid cross were situated on the same chromosome, the proportion of parental gene combinations remains much higher than the non-parental type. Morgan attributed this due to the physical association or linkage of the two genes and coined the term linkage to describe this physical association of genes on a chromosome Thus, when two genes are close together on the same chromosome, they do not assort independently and are said to be linked. Genes that are closer together or tightly-linked will be transmitted together from parent to progeny more frequently than those genes located far apart.

The term recombination describe the generation of non-parental gene combinations. It is the process of exchange of genetic material between parents during meiosis process. At the beginning of meiosis, pair of homologues chromosome takes place followed by chiasma formation (crossing over) in which homologous chromosome pair intertwine and exchange sections of chromosome. Such process or set of processes is called recombination by which DNA molecules interact with one another to bring a rearrangement of the genetic information in an organism. During this recombination process formation of gametes takes place with new combination of genes that differ from either of the parents. This are called the recombinants and percentage of recombinants represents the recombination frequency. The observed recombination fraction between two loci is an estimate of one-half the number of chiasmata or crossover events between two loci because crossing over occurs at the four-strand stage and for single crossover events, only two of the four strands participate in the recombination. Two non-sister chromatids participate in the cross-over and the other two chromatids do not exchange chromosome segments. When two genes located on different chromosomes it assort

independently (unlinked) and have a recombination frequency of 50%, and if it is present on same chromosome (linked genes) than it have a recombination frequency that is less than 50%. The chance of a crossover producing recombination between genes is directly related to the distance between two genes. The lower the frequency of recombination between two markers, the closer they are situated on a chromosome and vice versa.

QTL mapping simply involves finding an association between a genetic marker and a phenotype that one can measure. For example, if all the tall plants among 250 individual pea plants of varying height have a particular allele of a genetic marker, then there is a very high probability that a QTL for plant tallness is associated with this marker in this population of plants. Therefore, the basic principle of determining whether a QTL is linked to a 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 individuals of one genotype differ significantly with the individuals of other genotype differ significantly with the individuals of other genotype with respect to the trait being measured.

2. Key steps for the QTL mapping

- Development of suitable mapping population from two parental strains with phenotypically contrasting trait (Highly disease resistance and susceptible variety).
- Selection of suitable molecular marker and development of saturated linkage map.
- Genotyping of mapping population.
- Use of appropriate statistical package to perform linkage analysis by using genotyping and phenotypic information.

3. Mapping population

The prime requirement for the construction of a linkage map is a segregating plant population (i.e. a population derived from sexual reproduction). The parents selected for the mapping population will differ for one or more traits of interest (e.g., highly disease resistant and highly disease susceptible); this is important to enhance the possibility of identifying a large set of polymorphic markers that are well distributed across the genome. Population sizes used in preliminary genetic mapping studies generally range from 50 to 250 individuals [8]. For the analysis of QTLs having small effects on the target trait, large number of individuals (~ 500) is required; however, a mapping population of a size of 200-300 individuals is sufficient for detection of QTLs with major effects.

Selection of mapping population: Selection of populations is critical to successful linkage mapping and it could vary based upon the main objectives of the experiments, whether the molecular markers to be used for genotyping are dominant or co-dominant, timeframe as well as available resources. Several different populations like second filial generation (F2), backcross (BC), recombinant inbred lines (RILs), double haploids (DHs), and near isogenic lines (NILs) may be utilized for mapping within a given plant species, with each population type possessing advantages and disadvantages [4]. In self-pollinating species, mapping populations originate from parents that are both highly homozygous (inbred). In cross pollinating (outcrossing) species, the situation is more complicated since most of these species do not tolerate inbreeding.

Types of mapping population: F2 populations, derived from selfingF1 hybrids (derived by crossing two parents),and backcross (BC) populations, derived by crossing the F1 hybrid to one of the parents, are the simplest types of mapping populations developed for self-pollinating species. Inbreeding from individual F2 plants allows the construction of recombinant inbred lines (RIL) after the 7-8 population, which consists of a series of homozygous lines, each containing a unique combination of chromosomal segments from the original parents. If backcross selection is repeated at least for six generations, more than 99% of the genome from BC6 and above will be derived from recurrent parent [9]. Selfing of selected individuals from BC7F1 will produce BC7F2 lines that are homozygous for the target gene, which is said to be nearly isogenic with the recipient parent (NILs) [10]. Doubled haploid (DH) populations maybe produced by regenerating plants by the induction of chromosome doubling from pollen grains, however, the production of DH populations is only possible in species that are

amenable to tissue culture (e.g. cereal species such as rice, barley and wheat).

Advantages and disadvantages of mapping population: The primary advantage of F2 population is the ability to measure the effects of additive and dominance gene actions at specific loci; other advantages are that they are easy to construct and require only a short time to produce. As RILs are essentially homozygous, only additive gene action can be measured. The length of time needed for producing RIL and NIL populations is the major disadvantage, because usually six to eight generations are required. The major advantages of RIL and DH populations are that they produce homozygous or 'true-breeding' lines that can be multiplied and reproduced without genetic change occurring. F2 and BC populations are considered to be temporary populations because they are highly heterozygous and cannot be propagated indefinitely through seeds. RILs, NILs and DHs are permanent populations because they are homozygous or 'true-breeding' lines that can be multiplied and reproduced without genetic change occurring. Seeds from RILs, NILs and DHs can be transferred between different laboratories for linkage mapping to ensure that all collaborators examine identical material [11].

Mapping population in relation with dominant or co-dominant marker: Co-dominant marker as compared to dominant marker reveals more genetic information in F2 population. Dominant markers supply as much information as Co-dominant markers in RIL, NILs and DHs [12] because all loci are homozygous, or nearly so. BC populations can be useful for mapping dominant markers if all loci in the recurrent parent are homozygous, and the donor and recurrent parent have contrasting polymorphic marker alleles [13].

4. Molecular marker

Genetic marker: There are three major types of genetic markers: (1) morphological markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA [14]. The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant. DNA markers are the most widely used type of marker predominantly due to their abundance. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant.

Types, advantages and disadvantages of molecular marker: The first large scale efforts to produce genetic maps were performed mainly using RFLP markers, the best known genetic markers at the time [15] [16]. Different types of molecular markers used in plant sciences today are Restriction fragment length polymorphisms (RFLPs), microsatellites or simple sequence repeats (SSRs), expressed sequence tags (ESTs), cleaved amplified polymorphic sequence (CAPS), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeat (ISSR), Diversity arrays technology (DArT) and single nucleotide polymorphism (SNP). Each of this marker system has advantages and disadvantages (**Table 1**).

Classification of molecular marker: Molecular marker can be classified by number of different ways. DNA markers may be broadly divided into three classes based on the method of their detection: (1) hybridization-based; (2) polymerase chain reaction (PCR)-based and (3) DNA sequence-based.DNA markers can be categorized in to two groups based on ability to distinguish between individuals of the same or different species: (1) Polymorphic and (2) Monomorphic marker. Polymorphic markers can able to discriminate between the genotypes however monomorphic cannot. Polymorphic markers again classified in to two groups based on whether markers can discriminate between homozygote and heterozygote :(1) Co-dominant marker and (2) Dominant marker. Co-dominant marker can able to distinguish homozygotic and heterozygotic condition and however dominant marker cannot (**Table 1**).

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

Sr. No.	Co-dominant /Dominant	Advantages	Disadvantages	References
1.	**Restriction Fragment length polymorphism (RFLP)**			
	Co-dominant	• Highly reproducible, • Transferable across the population • Robust and reliable • Locus specific	• High quality and quantity of DNA required • Radiolabeled probes required • Time consuming, laborious and expensive • Limited polymorphism • Not amenable for automation	[17] [18]
2.	**Microsatellite or Simple sequence repeats (SSRs)**			
	Co-dominant	• Highly reproducible • Transferable across the population • Robust and reliable • Locus specific • Amenable for automation and technically simple	• High development cost • Primer development is highly time consuming and laborious • Usually polyacrylamide electrophoresis required which is again laborious and time consuming	[19] [20]
3	**Express sequence tag (EST-SSR)**			
	Co-dominant	• Highly reproducible, robust and reliable • High degree of sequence conservation • Transportable across the pedigree and species • Enable a transfer of linkage information between species	• Marker development is limited to species for which sequencing database already exist	[21]
4	**Amplified fragment Length Polymorphism (AFLP)**			
	Dominant	• Highly reproducible • Highly polymorphic • Used for any organisms without sequence information • Provide good genome coverage	• High quality and quantity of DNA required • Complicated methodology	[22]
5	**Randomly amplified polymorphic DNA (RAPD)**			
	Dominant	• Quick and simple • Inexpensive • Small quantity of DNA required	• Non-reproducibility • Generally not transferable	[23] [24]
6	**Inter simple sequence repeats (ISSRs)**			
	Dominant	• Highly polymorphic • Simple	• Non-reproducibility • Generally not transferable	[25] [26]

5. *Genotyping of mapping population by polymorphic molecular marker:*

It is critical that sufficient polymorphism exists between parents in order to construct a linkage map. So, the next step in the construction of a linkage map is to identify polymorphic molecular markers that reveal differences between parents. The choice of DNA markers used for mapping may depend on the availability of characterized markers or the appropriateness of particular markers for a particular species. Those, molecular marker give more polymorphism are generally selected for the desired trait. In general, cross pollinating species possess higher levels of DNA polymorphism compared to inbreeding species; mapping in inbreeding species generally requires the selection of parents that are distantly related. In many cases, parents that provide adequate polymorphism are selected on the basis of the level of genetic diversity between parents [27]. Along with the polymorphism, markers which give high density linkage map are more suitable for QTL generation. A methodology for constructing dense genetic linkage maps has been reported [28].

First, polymorphic markers have to identify and test in parents, those gives good polymorphism between selected parents are generally selected. Secondly, they must be screen in entire population from F1 plants to all F2 plants individual. Each of the selected molecular markers has to test with each and every individuals of F2 population to study the segregation of marker in the progeny. This is called the genotyping of the population. At the same time, phenotypic variation pattern also have to measure from the F2 population. The significant deviation of segregation ratio of molecular marker (genotypic) and phenotypic trait can be analyzed by using suitable biostatistics method or software by comparing it with the expected segregation ratio. The expected segregation ratios for Co-dominant and dominant Markers in F2 population are

1:2:1(AA:Aa:aa) and 3:1 respectively. Generally, markers will segregate in a Mendelian fashion although distorted segregation ratios may be encountered [29].

6. *Linkage analysis of markers*

Linkage map finally prepare by using computer programs by coding data for each molecular marker on each individual of a population. Numerous computer packages available for linkage analysis like JoinMap, MAPMAKER/EXP, GMENDEL, LINKAGE, Map Manager QTX [30]. Among them JoinMap is widely used.

Markers are assigned to linkage groups using the odds ratios (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 [31]. LOD values of >3 are typically used to construct linkage maps. A LOD value of 3 between two markers indicates that linkage is 1000 times more likely (i.e. 1000:1) than no linkage (null hypothesis). Higher critical LOD values will result in more number of fragmented linkage groups, each with smaller number of markers while small LOD values will tend to create few linkage groups with large number of markers per group. Two markers are placed in distinct linkage groups if they are not linked to any member of the other group. Linkage groups represent chromosomal segments or entire chromosomes.

A difficulty associated with obtaining an equal number of linkage groups and chromosomes is that the polymorphic markers detected are not necessarily evenly distributed over the chromosome, but clustered in some regions and absent in others [4]. In addition to the non-random distribution of markers, the frequency of recombination is not equal along chromosomes. The accuracy of measuring the genetic distance and determining marker order is directly related to the number of individuals studied in the mapping population.

7. *Determination of genetic (MAP) distance*

The mapping procedure is basically a process of building a map by adding loci one by one, starting from the most informative pair of loci (loci pair with most linkage information). At each step, a marker is added to the map on the basis of its total linkage information with the markers that were placed earlier on the map. For each added locus the best position is searched and a goodness-of-fit measure is calculated.

Distance along a linkage map is measured in terms of the frequency of recombination between genetic markers. If the distance between genetic markers is greater than the chance of recombination occulting during the meiosis is also greater. Mapping functions are required to convert recombination fractions into centiMorgans (cM) because recombination frequency and the frequency of crossing-over are not linearly related. Two commonly used mapping functions 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.

8. *Statistical methods to detect QTLs*

Several statistical methods used for the detecting QTL, while minimizing the occurrence of false positive (declaring an association between a marker and QTL when in fact one does not exist). Widely used methods for detecting QTLs are (i) single marker analysis, (ii) simple interval mapping, (iii) composite interval mapping and (iv) Multiple Interval Mapping. (i) Single marker approach is the simplest method sometimes referred to as the single factor analysis of variance (SF-ANOVA). Linear regression, ANOVA and t-test statistical methods mainly have been used for the single marker analysis. (ii) Simple interval mapping evaluates the target association between the trait values and the genotype of a hypothetical QTL (target QTL) at multiple analysis points between pair of adjacent marker loci (the target interval). (iii) Composite internal mapping developed by Jansen and Stam (1994) combine interval mapping for a single QTL in a given interval with multiple regression analysis on marker associated with other QTL.(iv) Multiple intervals mapping (MIM) is the extension of interval mapping to multiple QTLs, just as multiple regression extends analysis of variance. MIM allows one to infer the location of QTLs to positions between markers, make proper allowance for missing genotypes data and can allow interactions between QTLs [32].

Discussion

The genes that control quantitative disease resistance in plants have long been too difficult to identify or characterize precisely. This overwhelming task resolved after the advent of Quantitative Trait Loci (QTL) mapping. With molecular marker and QTL mapping, complex form of disease resistance and their underlying genes are now far more accessible. Genetic map based on molecular markers are available for several economically important plants, like Arabidopsis, maize, rice, wheat, barley, tomato, potato, sunflower, pea, bean, rye, millet, cotton, soybean, sorghum, cowpea, tobacco, turnip rape, cauliflower, alfalfa, carrot, sugarcane, sugar beet, coffee and grape.

Despite lack of precise information about the molecular nature of the QTL, introgression of QTLs into elite lines or germplasm and marker assisted selection for QTLs in breeding could be undertaken in some crop plants such as maize, tomato and rice with reasonable success. QTLs conferring resistance to downy mildews of maize was mapped and validated at IARI [33] [34]. It also transferred two major QTLs for downy mildew resistance into CM 139 an elite but downy mildew susceptible inbred line.

There are still some important caveats regarding QTL analysis. Only the QTLs of largest effect and those closest to a marker locus, will show statistically reliable association. Particularly important is fine mapping or high resolution mapping of the QTL, if the QTL information is to be effectively applied in field. We believe that recent developments and improvements in marker techniques, the integration of functional genomics, better theoretical models and high throughput strategies are expected to enable greater power and precision in detection of QTL and utility of QTL information for crop improvement in future.

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