QTL MAPPING

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For most of the period up to 1980, the study of quantitative traits has involved statistical techniques based on means, variances and covariances of relatives. These studied provided a conceptual base for partitioning the total phenotypic variance into genetic and environmental variances, and further analysing the genetic variance in terms of additive, dominance and epistatic effects. From this information, it became feasible to estimate the heritability of the trait and predict the response of the trait to selection. It was also possible to estimate the minimum number of genes that controlled the trait of interest. However, little was known about what these genes were, where they are located, and how they controlled the trait(s), apart from the fact that for any given trait, there were several such genes segregating in a Mendelian fashion in any given population, and in most cases their effects were approximately additive (Kearsey and Pooni, 1996). These genes were termed 'polygenes' by Mather (1949). Sax's (1923) experiment with beans demonstrated that the effect of an individual locus affecting a quantitative trait could be isolated though a series of crosses resulting in randomization of the genetic background with respect to all genes not linked to the genetic markers under observation. Even though all of the markers used by Sax were morphological seed markers with complete dominance, he was able to show a significant effect on seed weight associated with some of his markers.

It was realized that most of the commercially important traits in crop plants are quantitative in nature. Each of these quantitative traits is controlled by many genes which were termed as polygenes by Mather (1949). With the aid of molecular markers and appropriate statistical tools one can identify chromosome loci each carrying one or more genes controlling quantitative or complex trait. Each such identified locus is described as quantitative trait loci (QTL). A QTL is defined as "a region of the genome that is associated with an effect of a quantitative trait." So a QTL can be a single gene, or it may be a cluster of linked genes that affect the traits. QTL mapping studies have reported in most of the crop

plants for diverse traits like yield, quality disease and insect pest resistance, abiotic stress tolerance and environmental adaptation.

Principles of QTL Mapping

Identifying a gene or QTL with in a plant genome is like finding the needle in a hay stack. QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers. The markers are used to partition the mapping population in to different genotypic classes based on genotypes at the marker locus, and apply the correlative statistics to determine whether the individual of one genotype differ significantly with the individuals of other genotype with respect to the trait under study. A significant difference between phenotypic means of the two / more groups depending on the marker system and type of population indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the trait. A significant P value obtained for the differences between the marker and QTL is due to recombination. The closer a marker is from a QTL, the lower the chance of recombination occurring between marker and QTL. Therefore, the QTL and marker will be usually be inherited together in the progeny, and the mean of the group with the tightly-linked marker will be significantly different ($P < 0.05$) to the mean of the group without the marker. When a marker is loosely-linked or unlinked to a QTL, there is independent segregation of the marker and QTL. In this situation, there will be no significant difference between means of the genotype groups based on the presence or absence of the loosely linked marker. Unlinked markers located far apart or on different chromosomes to the QTL are randomly inherited with the QTL; therefore, no significant differences between means of the genotype groups will be detected.

Steps in QTL Mapping

The various steps in the identification and characterization of quantitative trait loci (QTL) for use in marker assisted selection are presented in figure 2. The process of QTL mapping involves the four major steps, which were discussed below under following subheadings.

Developing of mapping population: A suitable mapping population generated from phenotypically contrasting parents is prerequisite for QTL mapping (E.g.: highly resistant and susceptible lines). The parental lines used in development of mapping population should be genetically diverse, which enhance the possibility of identifying a large set of polymorphic

markers that are well distributed across the genome. Several different populations may be utilized for mapping within given plants species. With each population type possessing advantages and disadvantages. The mapping population could vary based on the objective of study, the time frame line and resources available for undertaking QTL mapping. The ability to defect QTL in F2 or F2 derived populations and RILs are relatively higher than other mapping population. The F2:3 families have the advantage that it is possible to measure the effects of additive and dominant gene actions at specific loci. The RILs are essentially homozygous and only additive gene action can be measured, the advantage with RILs is that the experiments can be performed at several locations in multiple years.The size of the mapping population for QTL analysis depends on several factors viz., type of mapping population used for QTL analysis, genetic nature of the target trait, objective of the study, and resources available for handling a sizable mapping population in terms of phenotyping and genotyping. From the practical point of view the purpose of QTL mapping is to detect the QTL, with major effects, and it is possible only when large number of individuals say 500 or more being used for QTL analysis. So in general size of the mapping population is around 200-300 individuals.

Generating saturated linkage map: Mapping means placing the markers in order, indicating the relative genetic distance between them and assaying them to their linkage groups on the basis of recombination values from all pair wise combination between the markers. Linkage map indicates the position and relative genetic distance between markers along chromosomes. We can analyse the segregation patterns for each of the markers by screening the mapping population using polymorphic molecular markers, which is referred as genotyping. A vanity of molecular markers viz., RFLPs, RAPD, SSRs, AFLP, and SNPs etc have been used to identify individual QTLs and to find out effects and position of these QTLs.

Phenotyping of mapping population: The target quantitative traits have to be measures as precisely as possible. Strictly speaking there should not be any missing date, but limited amounts of missing data can be tolerated. The missing data in the population causes the effective in the sample size and intern affect the power of QTL mapping. The data is pooled over location and replication to obtain a single quantitative value for the line. It is also necessary to measure the target traits in experiments conducted in multiple location to have better understanding of the QTL x Environment interaction.

QTL detection using statistical tools: The basic purpose of QTL mapping is to detect QTL, while minimizing the occurrence of false positive (Type I Error) i.e. declaring an association between a marker and QTL when in fact it does not exists). The tests for QTL or trait association are often performed by the following approaches:

A) Single marker approach, B) Simple interval mapping, C) Composite interval mapping, D) Multiple interval mapping.

QTL Application

The introgression of QTLs into elite lines / germplasm, and maker-aided selection (MAS) for QTLs in crop improvement has to be undertaken in some of the crop like Maize (Li et al., 2008), Tomato (Stevens et al., 2007) and Wheat (Naz et al., 2008). The plant breeders may need not to know the precise location of QTL as the QTL has large effect and can be introgressed using marker assisted back crossing (MABB). In Maize the QTLs with major effects which conferring resistance to downy of mildews has been identified and transferred into CM139 elite but downy mildew- susceptible inbred line (George et al., 2003; Nair et al., 2005). QTLs so identified for diverse traits in different crops have been met in crop improvement especially to enhance the yield and to develop disease resistance elite lines.

Utility and Prospects

QTL mapping plays significant roles to identify genetic regions responsible to important phenotype variation. One of the common strategies of QTL mapping uses a large number of RILs, which are established for at least several generations of inbreeding (typically up to F_6 or F_7). QTL Information Despite lack of precise information about the molecular nature of the QTL, introgression of QTLs into elite lines or germplasm, and marker-assisted selection (MAS) for QTLs inbreeding could be undertaken in some crop plants such as maize, tomato and rice, with reasonable success. Plant breeders may not need to know the precise locations of the QTL, so long as the QTL has large effect, and can be introgressed using marker-assisted backcrossing. The methods available will enable them to pick such useful QTL, which could well have been missed by conventional phenotypic selection. Also, another important advantage of the markers is in the reduction of linkage drag during the introgression of QTL by backcrossing. At IARI, we have mapped and validated QTLs conferring resistance to downy mildews of maize (George et al., 2003; Nair et al., 2005) and

have recently transferred two major QTLs for downy mildew resistance into CM139,an elite but downy mildew-susceptible inbred line.

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