Strategies for Genetic Mapping of Agronomic Traits

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Genetic mapping- Genetic mapping is based on the use of genetic techniques to construct maps showing the position of the genes and other sequence features of the genome.

The main steps involved in genetic mapping are-

1. Selection of target trait.
2. Identification of parents differing in the trait of interest.
3. Development of appropriate mapping population.
4. Screening the population for the target trait.
5. Parental polymorphism survey using molecular markers.
6. Co-segregation analysis.
7. Construction of linkage map.
8. Marker validation in alternate genotype/population.
9. Utilisation of marker in breeding programmes.

1. Selection of target trait

- Traits can be qualitatively or quantitatively inherited. Qualitatively inherited traits are controlled by one or few genes –have major effect on particular trait and follows Mendelian segregation- not influenced by Genetic and environment background. Eg-BLB resistance in rice
- Quantitatively inherited traits are controlled by many genes/loci. Each locus has small effect on a trait and cumulative effect of alleles at all loci controlling the trait determines the trait expression. They are influenced by environment and genetic background. Difficult to map. Eg- yield, quality and drought tolerance.

2. Identification of parents differing in the trait of interest

- For success of genetic mapping, one needs at least two parents differing for the alternative forms of the trait of the interest. For an examples two parental lines one having gene for resistance and the other having gene for susceptibility.

3. Development of appropriate population segregating for the trait of interest

- In case of quantitatively inherited traits, the early generation segregating populations like F_2, F_3, BCF_1 can be used. Geneticists also prefer to use advanced generation materials like F_6, F_7, RIL, NILS and DH.
- For mapping quantitatively inherited traits advanced generation materials like RIL, NILS and DH are more appropriate.
- Breeders also use a strategy called AB-QTL strategy wherein through backcrossing the population is developed and simultaneously phenotype to identify co-segregating markers.

4. Screening the population for the target trait (Phenotyping)

- Adequate care should be taken while phenotyping since success of mapping efforts depends mainly on precise phenotyping. The method of phenotyping differs significantly between qualitatively and quantitatively inherited traits. For qualitatively inherited traits pest and disease resistance phenotyping involves exposing the individuals of the population to a particular biotype/pathotype of pest/disease and scoring the plants for resistance/susceptibility after a particular time interval.
- For quantitative traits, the process of phenotyping involves analysis of individual component characters that contribute towards that the overall expression of the target trait. For an example when mapping for QTLs for yield, it is necessary to phenotype individual components of yield.
- Performing the experiment in replicated multilocation trails helps to avoid the uncertainties induced by the environment.

5 & 6. Parental polymorphism survey using molecular markers

- After developing the population and phenotyping the next step is to identify markers that co-segregate with trait of interest. This requires analysing the polymorphism among the parental lines with molecular markers.
- If mapping is done with co-dominant markers like SSRs, it is necessary to scan the parental lines with a set of uniformly spaced SSR markers (12-16 per chromosome) and identify at least 6-8 polymorphic markers per chromosome. If dominant markers like RAPD and ISSRs are selected for the study, then parental polymorphism survey should done with as much markers as possible.Once a set of markers polymorphic between the parental lines has been identified, the next step is to carry out co-segregation analysis for these markers. A simple strategy called 'bulked-segregant analysis' can be used to quickly identify markers, which co-segregate with trait of interest

Bulk Segregant Analysis

- For example, A set of resistant and susceptible F_2 lines (usually 10-15 lines in each case) are bulked separately and analysed with parental polymorphic markers, if a fragment (here after markers) present in the resistant donor, absent in susceptible recipient, present in the resistant bulk and absent in the susceptible bulk, then the marker is most probably associated with resistance.
- The marker is then analysed individually in all the lines of the resistant and susceptible bulks. If the marker is present in a majority (>70%) of individuals constituting resistant bulk and absent in a majority of individuals constituting the susceptible bulk, then it can be assumed that the marker is linked to the resistance.
- Based on the extent of resistant individuals showing amplification for resistant linked marker linkage distance is determined. Check the presence of the marker in the resistant or susceptible individuals of the population.Once the marker is confirmed to clearly co-segregate with trait phenotype in majority of individuals of the population, then the next step is to identify its chromosomal location. Thus, if an SSR marker is identified to tightly co-segregate with trait phenotype in a population, then tentative chromosomal location of the gene controlling the trait can be easily identified and more SSR markers in the vicinity of the co-segregating markers can be used for locating the exact position of the gene.

7. Construction of linkage map

- Linkage maps are basically, a kind of road maps of chromosomes drawn based on segregation pattern of markers. They indicate the position and relative genetic distances between markers along the chromosomes. A clear co-segregation data is required to draw linkage map.Based on co-segregation patterns, percentage recombination is calculated for each pair of markers in terms of cM. Statistical software's like Map maker, Map manager, join map, cartographer and linkage can be used for construction of linkage map.The co-segregation data has to be fed to the computer in excel format and the software automatically, constructs linkage map after calculating ' LOD' score for each pair of markers.
- For constructing linkage maps for quantitative traits, usually marker intervals showing association with trait phenotype are identified and based on the extent to which these intervals are showing association, linkage distances are calculated using the software 'MAPmaker-QTL after taking into consideration of LOD scores.

8. Marker validation in alternate genotypes/ populations

- Once a marker or sets of markers are identified to be tightly linked to a particular gene, the next step is to validate the markers and their linkage distances in alternate populations.
- Alternate populations can be developed by selecting another donor line possessing the same resistance gene and crossing it with a susceptible parent. Before deploying markers in practical breeding, it is always ideal to validate the same in 1-2 alternate populations.

9. Utilization of markers in breeding programmes

- Once closely linked markers (<2 cm) & flanking markers (<5 cm on either side of the gene) are identified and validated in alternate populations, they are ready for use in MAB. Flanking markers have distinct advantage compared to single marker since selection based on flanking markers will eliminate false positive.
- When a marker or set of markers are to be deployed in practical breeding, it is necessary to verify whether they are polymorphic between the parental lines used in the particular breeding programme. If the markers are polymorphic, then they can be reliably deployed to track the introgression of the gene in the breeding programmes.

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