Molecular markers: Genetic distance and mapping population concept of linkage maps

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Introduction

- ✓ Understanding biology and genetics at molecular level has become important for better understanding and manipulation of genome architecture.
- ✓ Identification of markers associated with desirable breeding traits are useful for marker assisted breeding and genomics and development of transgenics.
- ✓ It reduced breeding time and cycles required for crop improvement.
- ✓ Understanding the inheritance of molecular level of agriculture traits creates a new opportunity for plant breeding.

Markers

- ✓ Markers are the DNA sequence used for chromosome mapping for its specific site present in chromosome
- ✓ These are easily identifying characters of an individual.
- ✓ These are character whose pattern of inheritance can be followed at morphological, biochemical and molecular levels.

Morphological markers

- ✓ These are qualitative traits which can be detected visually.
- ✓ The have been found in nature due to mutagenesis.

Limitation

- ✓ It cause large effect on phenotype that they are undesirable in breeding programme.
- ✓ They mask the effect of linked genes and it makes them difficult to identify desirable linkage for selection.

- ✓ They are highly influence by environment and its gene interaction.

Biochemical markers

- ✓ These are the proteins produced by gene expression.
- ✓ Particularly isozymes are successfully used as biochemical markers.
- ✓ They are phenotypic markers which are affected by tissue, plant growth, etc.

Molecular markers

- ✓ It is a DNA sequence which is readily detected and whose inheritance can easily monitor.
- ✓ These are based on naturally occurring DNA polymorphism.
- ✓ It is the section of nucleotide sequence used for the identification of desired QTL or gene.
- ✓ Most widely used due to the abundance.
- ✓ Selectively neutral as they are non-coding sequence.
- ✓ Not affected by environmental factors.

Characteristics of molecular markers

- ✓ It must be polymorphic which measure the genetic diversity.
- ✓ Co-dominant inheritance which can detect different forms of markers.
- ✓ It should be evenly and frequently distributed in organism genome.

Application of molecular markers

- ✓ Phylogeny and plant evolution
- ✓ Diversity analysis of germplasm
- ✓ Genotyping of cultivars
- ✓ Barcoding sequence information from nuclear and organellar genome

Genetic Distance

- ✓ Genetic linkage occurs when particularly genetic loci for genes are inherently joined.
- ✓ Genetic loci on the same chromosome are physically connected and tend to stay together during meiosis, and are thus genetically linked called as autosomal linkage.
- ✓ Greater the distance between markers, the greater the chance of recombination occurring during meiosis
- ✓ Distance along a linkage map is measured in terms of the frequency of recombination between genetic markers
- ✓ Mapping functions are required to convert recombination fractions into **centiMorgans** (cM)
- ✓ When map distances are small (<10 cM), the map distance equals the recombination frequency
- ✓ However, this relationship does not apply for map distances that are greater than 10 cM

Linkage Mapping

i. Genetic linkage maps were originally built to map phenotypic mutants

ii. Modern linkage maps use molecular markers (predominantly, DNA markers).

iii. Different types of mapping populations are used.

iv. Mapping studies in diploid and allopolyploids use similar tools and techniques.

v. Linkage maps in autopolyploid necessitate different mapping strategies.

vi. Linkage maps are useful for:

- tagging markers along chromosomes

- identifying markers linked to genes and cloning genes

- identifying quantitative trait loci for traits of interest

- marker assisted selection

- comparative mapping and evolutionary studies

Mapping Population

- ✓ Mapping population is the population used for gene mapping.
- ✓ Commonly used mapping populations are obtained from controlled crosses.
- ✓ Selection of mapping population involves choosing of parents and determining a mating scheme.

Steps to construct the mapping population

- ✓ Select the parent plant
- ✓ Produce a mapping population
- ✓ Scoring in mapping population

i. Screening for polymorphism
ii. Scoring
iii. Linkage analysis

Select the parent plant

- ✓ For construction of map, the selected plant are genetically different or divergent to exhibit different polymorphism but not so much distant as the causes sterility.
- ✓ Wild species which shows different polymorphic can also be selected for cross.
- ✓ After selecting the surveyed plants, DNA should be isolated and digested with restriction enzyme and screened for polymorphism.

Production of a mapping population

- ✓ Requires a segregating plant population (i.e. a population derived from sexual reproduction).
- ✓ The parents selected will differ for one or more traits of interest.
- ✓ Population sizes: generally range from 50 to 250 individuals.
- ✓ For high-resolution mapping larger populations are required.
- ✓ If map is used for QTL studies then the mapping population must be phenotypically evaluated (i.e. trait data must be collected) before subsequent QTL mapping.
- ✓ In self-pollinated species, parents that are both highly homozygous (inbred).
- ✓ In cross pollinating species, the situation is more complicated being heterozygous.

Types of mapping population

- ✓ F2 Population
- ✓ F2 derived F3 population
- ✓ Backcross Mapping population
- ✓ Recombinant Inbred Lines (RILs)
- ✓ Near Isogenic Lines (NILs)
- ✓ Double haploids (DHs)

F2 population

- ✓ It is developed by selfing among F1 individuals.

Merits:

- ✓ It requires less time for development and can be developed with minimum efforts

Demerits:

- ✓ Quantitative traits cannot be precisely mapped using F2 population.
- ✓ It is not a long term population.

F2 derived F3 population

- ✓ It is obtained by selfing the F2 individuals for a single generation.

Merits:

- ✓ It is suitable for specific situations like mapping QTLs and recessive genes
- ✓ It is used for reconstitution of respective F2 plants.

Demerit:

- ✓ It is not long term population.

Backcross Population.

- ✓ It is developed by crossing the F1 with one of two parents used in the initial cross.
- ✓ Backcross with recessive parent is usually used in genetic analysis.

Merits:

- ✓ It requires less time to be developed.
- ✓ It is used for marker assisted backcross breeding.

Demerits:

- ✓ This population is not eternal.

Near Isogenic Lines.

- ✓ NILs are developed by repeated selfing or backcrossing the F1 individuals to the recurrent parents

Merits:

- ✓ They are immortal mapping population.
- ✓ They are suitable for tagging the trait and quite useful in functional genomics.

Demerits:

- ✓ It requires many generations for development.
- ✓ It is not useful for linkage mapping.
- ✓ Linkage drag is a potential problem in constructing NILs.

Recombinant Inbred Lines.

- ✓ They are developed by single seed selections from individual plants of an F2 population.

- ✓ They are produced by continuous selfing or sib mating the progeny of individual members of an F2 population until complete homozygosity is achieved.

Merits:

- ✓ RILs can be propagated indefinitely without further segregation.
- ✓ They are useful in identifying tightly linked markers.

Demerits:

- ✓ It requires many generation to develop RILs and developing RILs are relatively difficult in crops with high inbreeding depression.

Doubled Haploids

- ✓ Doubled Haploids are developed by chromosome doubling of anther culture derived haploid plants from F1.
- ✓ They are product of one meiotic cycle.

Merits:

- ✓ They can be replicated and evaluated over locations and years and maintained without any genotypic change.
- ✓ They can be useful for both qualitative and quantitative characters.
- ✓ It can be used for instant production of homozygous lines.

Demerits:

- ✓ Suitable culturing methods / haploid production methods are not available for number of crops.
- ✓ Anther culture induced variability.

Scoring in mapping population

- ✓ Once the mapping population is obtained DNA is isolated from each individual plant in population.
- ✓ It is important the chromosome of each plant is mapping.
- ✓ It should contains a unique array of parental chromosome segment.

Steps for scoring technique

Screening for polymorphism

- ✓ Probes are selected from library and tested against the parent to determined which restriction enzyme will detect the polymorphism between the parents.
- ✓ It required no. of enzyme depending upon the variation.

Scoring

- ✓ When a suitable enzyme detected it can be scored in mapping population.
- ✓ To accomplish this a series of agarose gel must prepared and filters are prepared from them.
- ✓ These filter consist of one restriction enzyme and each filter set contain one digested DNA from each individual population.

Linkage analyses

- ✓ Data obtained from linkage scoring at mapping population used to construct linkage map.
- ✓ It is base on the degree to which probes tend to consegregate.

Specialised mapping

- ✓ Bulk Segregant Analysis (BSA)
- ✓ Combining markers and population
- ✓ Characterization of mapping population
- ✓ Segregation distortion in linkage mapping

Bulk Segregant Analysis

- ✓ Indirect selection by using qualitative traits.

- ✓ It is useful in analysis of conventional markers.
- ✓ It is base on the principle of segregant population.
- ✓ F2 population is divided in two pools of contrasting individuals.
- ✓ It helps in selecting the recessive alleles and dominant alleles.

Combining markers and population

- ✓ The segregation ratio is jointly determined by the nature of marker and types of mapping populations.
- ✓ Markers such as AFLP, RAPD are often scored as dominant markers whereas markers like RFLP, Microsatellite shows a co- dominant

Characterization of mapping population

- ✓ The molecular genotype of any individual is independent of environment, it is not influenced by GxE interaction
- ✓ However trait phenotype could be influenced by the environment, particularly in case of quantitative characters.
- ✓ Therefore, it is important to precisely estimate the traits value by evaluating genotypes in multilocation testing

Segregation distortion of markers

- ✓ Significant deviation from expected segregation ratio in a given marker population combination is referred to as segregation distortion.
- ✓ Several reasons for segregation distortion:

i. Gamete/ zygote lethality

ii. Meiotic drive

iii. Sampling and selection during population development

References (if any)

1. **Molecular Markers in Plants- Robert J. Henry**
2. **Molecular markers in plant genetics and biotechnology- Dominique Vienne**

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