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DNA- Marker Assisted Selection

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Abstract

Marker assisted selection is a type of an indirect selection process in which we select a marker linked to the trait of interest rather than the trait itself. In broad sense it refers to type of selection which is based on quantitative trait loci. The basic pre-requisites of MAS are high throughput DNA extraction, linkage maps, association between molecular marker and traits of interest, high heritability of the gene of interest and markers. The various types of markers that are used in MAS includes morphological markers, cytological markers, biochemical markers and DNA based markers. The main advantages of MAS are rapid, cost effective, does not affected by environmental conditions and makes possible to select the trait of interest that is controlled by recessive alleles. It has been used in various fields of animal and plant breeding and it makes it possible to detect a gene of interest and transfer to next generation. It is an emerging technology which will help scientist in improvement of various varities of crops and species of animals.

Introduction

Marker assisted selection is defined as an indirect selection process where a trait of interest is selected based on a marker linked to a trait of interest, rather than on the trait itself. MAS refers to the selection which is based on quantitative trait loci. Marker assisted selection is also called as Marker assisted breeding or Marker aided selection. MAS is a combined product of conventional genetics and molecular biology. It is considered as one of the most efficient tool by the breeders for the selection of desirable traits in animals and plants. The marker may be morphological, biochemical or DNA/ RNA. Markers are closely related with the gene of interest due to the presence of genetic linkage on the chromosome. This concept will get more clear with the reference to the milk production in animals and disease resistance in plants. The marker that is specific for a particular trait is selected and the genetic region on the chromosome controlling the particular trait is identified. The words

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positive and negative selectable markers are crucial for molecular biology research. The positive selectable markers are those kind of markers that provide the host organism a selective advantage. However, negative selectable markers are chosen either to eradicate or inhibit the growth of host organism. Theoretically the marker assisted selection enhances the selection efficiency as it can be carried out on seeding material, shortening the time it takes to identify a genotype. Moreover there is more effect of environmental conditions in Marker assisted selection. Even if the trait of interest is controlled by recessive alleles. It can be easily detected.

Pre Requisites of MAS:

The basic pre-requisites of MAS are high throughput DNA extraction, linkage maps, association between molecular marker and traits of interest, high heritability of the gene of interest and markers. We require 100 to 1000 of plants or animals and their screening in most of the breeding programmes to detect desirable marker patterns. Moreover, there is demand of quick result and its implementation. Therefore, it is required high throughput DNA extraction and extraction system which can screened large number of DNA samples in quick succession of time. Markers that are selected to be used in MAS and detection of marker trait association is done on the basis of linkage maps. Once the association between the marker and trait is detected in the population, we will use dense marker maps to identify the markers that are closely present near the target site. The more knowledge related to marker and its trait we have the more easy it becomes to identify and understand the association between the marker and the trait.

Markers

With advances in the studies various types of markers have been developed. Now a days DNA based markers are widely used. The morphological markers were the first markers to be used in the indirect selection of trait of interest. The first report of association of genetic marker with a quantitative trait was observed in plants by Karl Sax in1923. In 1935 the linkage of flowering time with inherited gene for flower color was demonstrated by J. Rasmusson. Ideally a marker loci should have following characteristics such as it should be highly polymorphic, abundant, neutral and co dominant. Various type of markers include morphological markers, cytological markers, biochemical markers and DNA based markers.

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Types of Markers:

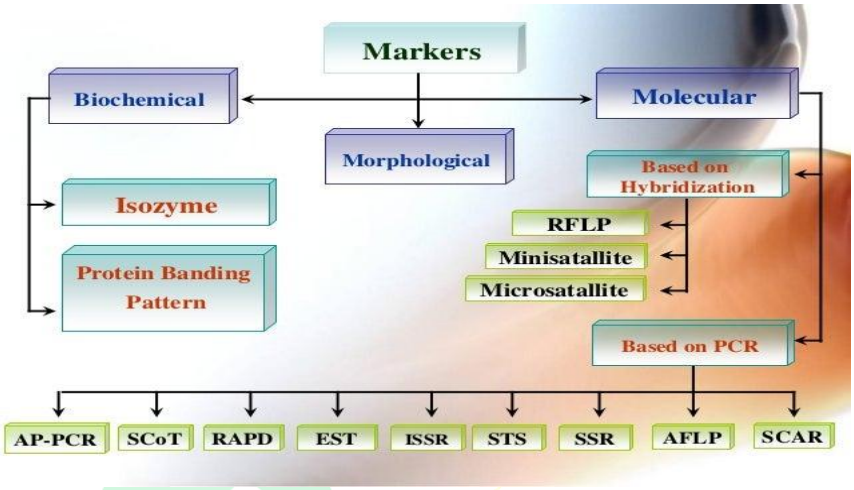

Fig.1 Types of Genetic Markers

Morphological markers were the first markers and are easily detected by naked eye. Examples are height, coloration, milk production etc. Proteins that are extracted or observed comes under the category of biochemical markers. Examples include isozymes and storage protein. Cytological markers can be identified microscopically and occurred in the form of chromosome bands. These chromosomal bands can be correlated with particular trait therefore, indicating that the locus responsible for our trait of interest is located near or within chromosomal band regions.

Microsatellites, Restricted Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Single Length Polymorphisms (SNPs). All comes under the category of DNA based markers.

Microsatellites

Microsatellites are also known as simple sequence repeats (SSRs), short tandem repeats (STRs) or variable number tandem repeat (VNTR) and are formed of 1-6 nucleotide repeats. Microsatellite can be defined as DNA motives which contain repetitive DNA. There are usually 5-50 times repetition. Within a host genome there are thousands of location where microsatellites can occur. The mutation rate is higher is higher in these regions as compared to other parts of the DNA. This leads to formation of high genetic diversity. These markers are highly polymorphic, species specific, co dominant and are present throughout the genome. Microsatellite markers can be di-nucleotide, tri-nucleotide, tetra-nucleotide and

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penta-nucleotide depending on the repeated number of nucleotides. The example of di-nucleotide microsatellite is TATATATATA sequence and example of tri-nucleotide include GTCGTCGTCGTCGTC sequence.

RFLP

RFLP can be detected and can be defined as the difference in the homologous DNA sequences. Restriction end nucleases is used for digestion of DNA sample and presence of fragments of different length can be detected. RFLP markers are highly locus specific and are able to detect both alleles in heterozygous. They are most commonly used in hereditary disease diagnostics, paternity test, genotyping and genome mapping. In simple terms RFLP is a difference in the size of DNA restriction fragments between individuals. RFLP is a co dominant marker and the advantage is that it is a non-PCR based approach (Based on Southern hybridization technique). So, there is no need of Primers however, we need restriction enzymes and large quantity of DNA to carry out this technique.

RAPD

RAPD is a type of PCR in which random amplification of DNA fragments is done. Advantage is that there is no requirement of the knowledge about DNA sequence. This is because the primers bind randomly to the parts of the DNA. With the help of random primers genetic diversity of an individual can be identified. It is a dominant marker which does not require the usage of restriction enzymes. There is intermediate reliability of RAPD as multiple, arbitrary primers are used which leads to questionable result. The requirement of DNA is less as compared to RFLP technique. A large number of primers ranging from bacteria to humans are commercially which can be subjected to RAPD technique.

AFLP

Selected DNA fragments that are obtained using the restriction enzymes are amplified and this leads to production of AFLP markers. Mainly two restriction enzymes are used for the digestion of high molecular DNA and one restriction enzyme is hexa-cutter and one is tetra-cutter. The primers used for the amplification of DNA fragments are labeled either by radioisotope or fluorescent dye. This helps in separation of amplified product on sequencing and can be easily visualized using autoradiography or laser based scanning. AFLP is a dominant marker which can be considered as the combination of RFLP and RAPD. There is

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no requirement of large amount of DNA however restriction enzymes. Two primers are must for successful implementation of AFLP.

SNPs

SNPs also known as snips and are most common type of genetic variation among individuals. They are differences in the DNA because of only one nucleotide. This usually arises due to addition or deletion of a nucleotide bases. The detection of these variations can lead to development of SNP markers. They are mainly used in genotyping of a last population for the detection of genetic disorders. These can be used for construction of genetic maps and provide knowledge regarding genotypes for genome wide association analysis. SNPs are highly polymorphic in nature. These markers have ability to reach higher density than any other type of marker. Now a days there is wide application of SNPs which includes identification of plants varieties, QTL analysis and construction of gene map.

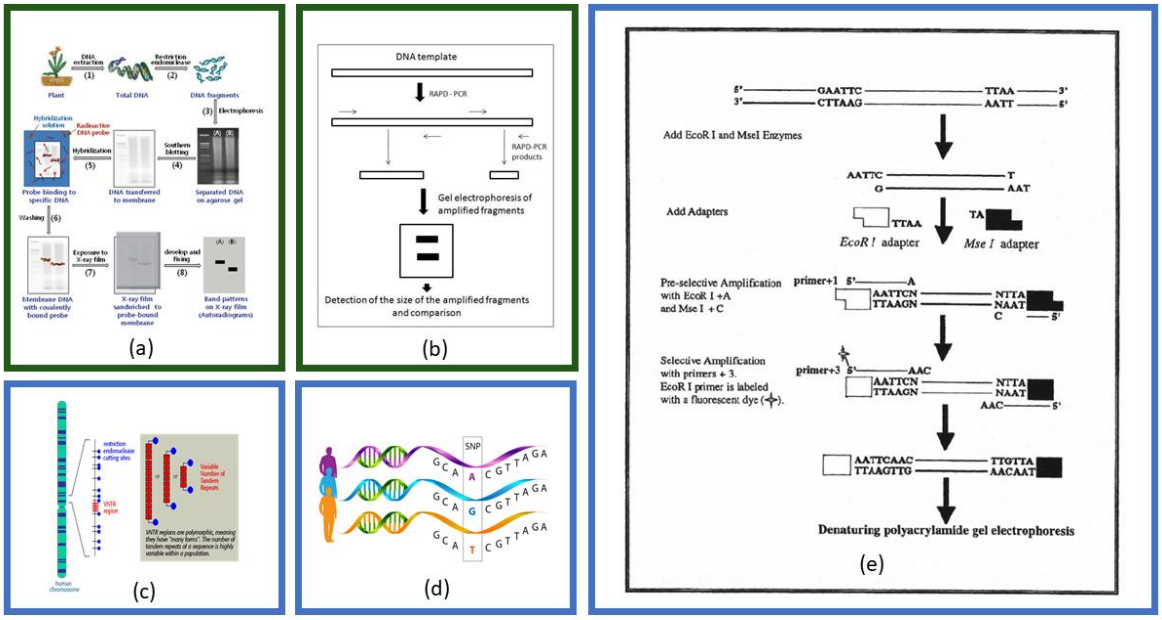

Fig.2 An Overview of functioning of various DNA Markers

(a) RFLP; (b) RAPD; (c) Microsatellite (VNTR); (d) SNPs; (e) AFLP

Advantages of MAS

Genetically superior species can be identified easily and quickly when a marker is linked with it. As most of the traits shown by animal expresses at later stage of life, these traits can be studied during early age of life. In the traditional breeding programme, we study number of

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consecutive generation for a trait of interest. It takes upto years and using MAS this time can be saved. Moreover, markers give us the information of trait of interest that is how it works. This is beneficial in selecting future generations. Other advantages include their use in marker assisted breeding, genetic diversity assessment and development of new variety. Environmental conditions has no effect on marker assisted selection moreover, a resistance of an animal can be determined using this method. As some diseases occur during a particular season therefore, MAS and detect the disease irrespective of the season. Phenotypically detection of a trait is impossible if it is controlled by recessive allele but MAS makes it possible and can detect recessive alleles controlling a trait of interest. MAS is cheap and cost effective as multiple markers can be used on the same DNA. When there are multiple genes controlling the same trait it becomes difficult to select them phenotypically. However, MAS makes this possible.

Limitations of MAS

As compared to conventional method it is expensive and requires specialized trained person to carry out the procedure. So, there are chances of getting a false positive reaction if recombination between marker and gene occurs. This usually happens in the case of DNA markers. There are chances that marker developed for one population may not function in the other population. There are chances of over estimation of a gene especially when two or more genes are present closely to each other.

Applications Of MAS

There are various applications of MAS in plant as well as animal breeding. It is applicable in formation of gene pyramid for diseases. Quality traits and production traits of plants and animals such as milk production, crop yield, crop height can be improved with help of MAS. The trait of interest can be easily detected and MAS makes it possible to transfer the desirable traits from one species to another. MAS can be applied in the gene introgression in which wild type gene (trait of interest) can be transferred into normal population. MAS can be applied in the field of genetic improvements, both in plants and animals.

Conclusion

There has been remarkable improvement in the field of animal as well as plant breeding. Moreover, traditionally used breeding programmes have made it possible and to take this field to next level there is a requirement of new emerging technologies. MAS is one

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of the emerging technologies that had great impact in the field of genetics moreover, its incorporation with commonly used breeding programmes had made improvement in plants and animals related to their various traits. Improvement of these traits will help the breeders. The only drawback MAS faces is high cost of operation. Studies have provide information that novel technologies in the field of marker development will potentially reduced the cost of MAS. This will make MAS tool widely effective in plants and animal breeding.

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