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Conceptual Paper

Tools of Marker Assisted Selection for Livestocks

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In the field condition there has been huge loss of production observed due to increased susceptibility to diseases, decreased growth and suboptimal production level Apart from these there are indirect loss in revenue in terms of cost of treatment of diseases and culling of animals. Further there is no new introduction of antibiotics in recent past has led to increased drug resistance within farm animals. Specialized breeding practices can enhance resistance/tolerance enhance animal health and have beneficial effect on the prevalence of the infection. If breeding is done for tolerance, which is nothing but net impact on the performance, at any given level of infection, it would not necessarily affect the pathogen. But if the breeding is done for resistance it would reduce the disease transmission possibly by imposing selection pressure upon the pathogen. Hence, it would be disadvantageous to breed animals tolerant to a disease when the aim is to reduce the transmission of infection. Therefore, selection to create resistance is needed in the field livestock. Disease resistance to disease is multifactorial and polygenic in nature and absolute resistance is difficult to be achieved. But the selection for genetic resistance along with other disease control measures can be effective to reduce the parallel challenges in livestock health management. Therefore, genes controlling the expression of an affected phenotype can be identified and then its frequency can be reduced through selection and the frequency of the desirable phenotype can be increased in the population. This can be achieved through Marker assisted selection.

Markers are any entity that can distinguish one target from the lot. Markers are mainly categorized as [a] morphological/phenotypic markers, [b] biochemical markers and [C] genetic markers. Phenotypic markers includes height, weight, disease resistance. Biochemical markers are changes in the structures and configuration of proteins, isoenzymes etc, which can be reflected on the fingerprint gel. The most important one are genetic markers which evaluates individuals based on variation in DNA sequences. For instance, RAPD, RFLP, AFLP, SSR, SNPs etc.

Technically, marker assisted selection is an indirect method of selection process where a trait of interest is selected not based on trait itself but on a marker linked to it. So, the purpose is to combine all genetic information of markers and the QTL (quantitative trait loci) with the phenotypic information to improve genetic evaluation and selection in animal breeding program.

Genetic markers

These markers are generated though mutations in genetic code. An ideal genetic marker is desired to be polymorphic, is in linkage-disequilibrium with the gene of interest, should be stably inherited, reproducible, easily detectable, and not affected by environment. The following markers are listed below-

Microsatellites

These are simple sequences repeats present uniformly throughout the DNA sequence. These are 2- 6 bp length repeats.eg. (CA) n, (CAG)n. FAO (UN) has preferably recommended microsatellite markers to study genetic diversity. There are different recommendations for different species because of their evolutionary differences. For instance, 25 markers in 25-50 animals have been recommended in livestock species.

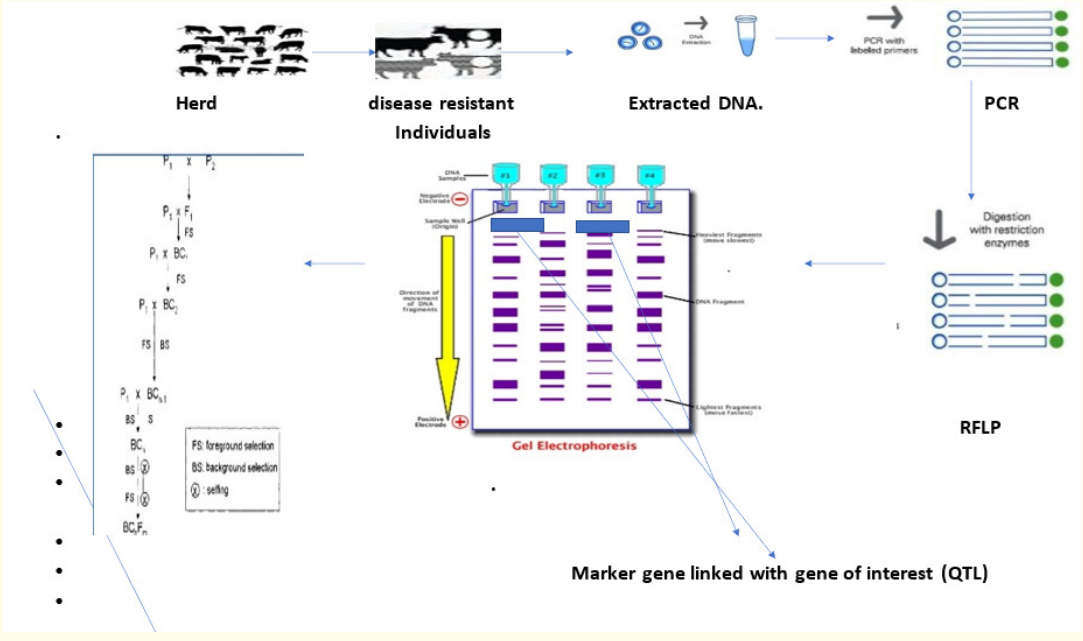

Figure 1: Flow of marker assisted selection.

Minisatellites

These are 10-60bp long repeats can go upto 100 nucleotides long. These repeats are present only at the telomere regions. Minisatellites cannot be easily amplified by PCR, and it is computationally not so compatible. And since they are present on telomere region they are known as VNTRs (Variable number tandem repeats).

SNPs (Single Nucleotide Polymorphism)

Change of a single nucleotide at a position leading to polymorphism. At any particular location 4 SNPs are possible but normally 2 can be seen. SNPs repeat in the genome of livestock at the rate of 100-300 bp. These are better controlled and analyzed using simple software, and are ubiquitous, stably inherited.

RFLP (Restriction Fragment Length Polymorphism)

These are hybridization-based markers wherein DNA is isolated, amplicon is generated then digested using restriction enzymes. The electrophoresis is done to separate fragments and hybridization is done (southern blot/radiolabelled probe). The results were recorded using autoradiography. RFLP is a co-dominant, highly reproducible marker, and can be used in genetic mapping. But, its demerits include, it is time taking, hazardous to health and need a polymorphic probe.

RAPD (Random amplified polymorphic DNA)

It is a dominant marker, and it is the least polymorphic marker under consideration. PCR products are run on gel electrophoresis, blotting is not required, minute amount of DNA is needed and there is no requirement of prior knowledge of DNA. These markers are the least heritable.

AFLP (Amplified fragment length polymorphism)

It includes both RFLP and PCR. DNA is undergone Restriction digestion, adapters of 17-21 nucleotides sequence long are used for ligation at two ends, which should include a restriction site. The amplification is done using specific primers. In this method, the sequence of DNA need not to be known, only the sequence of the adapter should be known. It is more reproducible than RAPD. But it requires very good quality DNA.

SSCP (Single strand conformational polymorphism)

In this method, there are conformational differences in single stranded nucleotide sequence of identical length which is induced by differences in the sequences under certain experimental conditions. The property allows sequences to be distinguished by the means of gel electrophoresis which separates fragments according to their different conformation.

Applications

- MAS has been implemented in so many case studies. One important one was BLAD (Bovine leucocyte adhesion disease) in HF calves, which is an autosomal recessive disease. Here, bulls and cows heterozygous for the traits are healthy, due to which a recessive allele can spread undetected for many generations. Here, MAS was a reliable tool to identify the heterozygous carrier. It was found that the deleterious allele for BLAD had two mutations in the CD18 gene. In that second mutation, the nucleotide (G) replaces adenine(A) so the amino acid glycine is produced instead of aspartic acid. When these PCR products are treated with the restriction enzyme Taq 1, the enzyme recognizes TCGA sequences and cuts between T and C nucleotides. Thus, each strand generates DNA fragments consistent with the presence or absence of the restriction site TCGA on the strand. In normal homozygous individual due to the presence of normal TCGA restriction site, after RE digestion with Taq1, two segments one of 32bp and other of 26 bp was got on agarose gel. In BLAD carrier, due to the absence of restriction site on the second strand, three segments i.e 32bp, 36bp and complete 58bp were reflected on agarose gel. And in BLAD affected one, due to absence of restriction site on both the strand there was no digestion, and a complete undigested strand of 58bp was recovered on the gel. Hence, just by observing the bands on the agarose gel BLAD carrier and BLAD affected individuals can be detected and culled from the herd. Thus, genetic diseases can be detected using MAS and the healthier individuals can be selected.
- High-Throughput Livestock Genomes Genotyping and sequencing has revealed a single nucleotide polymorphism which is being deployed in massively parallel fashion on DNA microarrays. These microarrays are enabling genome-wide association studies to identify genotype- phenotype correlation for both simple and more complex traits.
- The International Society of Animal Genetics is supporting transition from microsatellite to SNPs, have released a list of 200 SNPs to be used in Bovine parentage verification (100 SNPs as core panel, 100 SNPs as additional panel). The 200 SNPs are the part of the BovineLDcontent(>8000SNPs) widely used as based content SNP chip for genomic selection worldwide.