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Molecular Techniques in Animal Biotechnology

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Abstract
Animal husbandry is seen as an essential part of agriculture, which is crucial for the prosperity of a country. Selecting and sustaining unique characteristics that influence economic value is the intended aim of genetic research. Various molecular techniques based on PCR are AFLP (Amlified Fragment Length Polymorphism), RAPD-PCR (Randomly Amplified Polymorphic DNA), PCR-RFLP (PCR-Restriction Fragment Leght Polymorphism) and Microsatellites. Molecular techniques; It is widely used in fields such as biology, agriculture, medicine, veterinary, forestry and fisheries. With molecular techniques, genetic variation in populations, genetic (linkage) maps, mapping of quantitative traits (QTL), marker-assisted selection (MAS) studies, phylogenetic analyses, parent and gender determination, and genetic diagnosis of some diseases are performed. This review examines recent molecular biology closely. Molecular biology and the livestock sector together have revolutionized science of agriculture.
Key words: *Molecular techniques, AFLP, PCR-RFLP, RAPD-PCR, microsatellites*

Introduction

Polymorphism is the occurrence of two or more alternative structures due to different alleles in a population. Polymorphism can be seen at the level of the whole individual, as differences at the level of proteins (protein polymorphism) or DNA (DNA polymorphism).

Protein polymorphism studies, which have been widely used since the 1960s, have been replaced by molecular techniques with the development of recombinant DNA technology and the discovery of PCR (polymerase chain reaction). Faster and more accurate results are obtained with molecular techniques. In recent years, various molecular techniques (AFLP, RAPD, RFLP, Microsatellites) have been developed.

Molecular techniques; It is widely used in fields such as biology, agriculture, medicine, veterinary, forestry and fisheries. With molecular techniques, determination of genetic structure in populations, marker-assisted selection (MAS) studies, genetic maps, phylogenetic analyses, parent and gender determination, diagnosis of some diseases, etc. is being done.

Here, the introduction of molecular techniques (AFLP, RAPD, RFLP, Microsatellites) and genetic polymorphism, genetic mapping and QTL (Quantitative Trait Loci) mapping studies performed with these techniques in cattle, sheep and goats are discussed.

Molecular Techniques

AFLP

(Amlified Fragment Length Polymorphism)

Vos et al. (1995), In the AFLP technique developed by genomic DNA is divided into smaller fragments with cutting enzymes. Short primers are designed and these fragments (adapters) are pre-amplified by PCR. Then, long primer combinations are designed from these pre-amplification products and amplified again by PCR. The resulting PCR products are evaluated by visualizing them on the gel.

AFLP markers have been used to determine genetic polymorphism in cattle (Ajmone-Marsan et al., 1997), goats (Ajmone-Marsan et al., 2001), and to characterize and screen clinical mastitis in Canadian Holstein cattle (Sharma et al., 2006).

RAPD-PCR

(Randomly Amplified Polymorphic DNA-PCR)

RAPD-PCR (random amplified polymorphic DNA) is a genetic analysis method in which short oligonucleotides (10 bp) are designed and randomly amplified DNA fragments are obtained by polymerase chain reaction (Williams et al., 1990). Polymorphism is screened on the basis that the binding site of a primer is present in one individual and absent in the other individual (dominant-recessive). Genetic variation can be determined quickly and easily with large numbers of DNA fragments produced

RAPD markers have been used to determine genetic polymorphism in sheep (Chuswa et al., 1996) and cattle (Parejo et al., 2002). Sahin et al. (2005) determined genetic polymorphism in hair goats from the Antalya region by the RAPD-PCR method. They calculated the genetic distance in Antalya region goats as 0.3536 and the average heterozygosity rate as 0.3691.

PCR-RFLP

(PCR-Restriction Fragment Leght Polymorphism)

While RFLP analysis was previously performed using radioactively labeled probes (RFLPs), today it is performed based on PCR (PCR-RFLP). The relevant DNA molecule amplified by PCR is cut using cutting enzymes. Cutting enzymes recognize known nucleotide sequences of DNA and cut them specifically at these points. Then, the cut DNA is separated on agarose gel. Depending on the presence or absence of cutting enzyme recognition sites, allelic variants are visualized in agarose gel and genetic differences can be detected (Özköse et al., 2002).

In cattle, κ-casein gene (Damiani, et al., 1990), calpastatin gene (Bishop et al., 1993), somatotropin gene (Zhang et al., 1993), myoglobin gene (Agaba and Kemp, 1994), growth hormone PCR-RFLP technique was used to determine genetic polymorphism in the gene (Unainian et al., 1994) and mtDNA of European, African and Indian cattle populations (Loftus et al., 1994).

In sheep, genetic polymorphisms in the prolactin gene (Vincent and Rothschild, 1997), calpastatin gene (Palmer et al., 1998), and β-lactoglobulin gene (Feligini et al., 1998; Elmacı et al., 2006) were determined using the PCR-RFLP technique.

In goats, β-casein gene (Papalardo et al., 1996), αS2-casein gene (Cosenza et al., 1998), κ-casein gene (Yahyaoui et al., 2001), β-lactoglobulin gene (Pena et al., 2000).) in mtDNA (Palma et al., 1997), PCR-RFLP technique was used to determine genetic polymorphism.

Microsatellites

Microsatellites (STR: short tandem repeat) are short tandemly repeated sequences and are polymorphous loci found in the genome. These repeated base sequences are 2-6 base pairs long and are short DNA segments that are repeated sequentially in any of the mono, di, or tetra nucleotide permutations within the genome (Bishop et al., 1994). The length of microsatellite DNA segments is generally 100-250 bp. Microsatellite markers are widely used in genetic mapping and QTL mapping, in the investigation of genetic diversity or genetic relationships in populations based on the nuclear genome.

Microsatellite markers have been used to determine genetic polymorphism in European cattle breeds (Cañón et al., 2001), Asian cattle breeds (Kim et al., 2002), and African cattle breeds (Ibeagha et al., 2004).

Altınalan (2005) determined the genetic characterization of Turkey's native cattle breeds (Native Black, Gray Breed, Southern Yellow-Red Kilis, and Eastern Anatolian Red) with microsatellite markers (26 pieces). In the phylogenetic analysis of the study in which Black Red cattle were used as control material, it was reported that Anatolian native breeds formed a unique cluster together.

Soysal et al. (2005) investigated the genetic relationships of Turkish domestic (İvesi, Akkaraman, Kıvırcık) and hybrid sheep breeds (Konya Merino, and Türkgeldi) with microsatellite markers (OarFCB20, OarFCB304, MAF65). In grouping; It was stated that three different clusters were formed: 1) İvesi, 2) Akkraman-Konya Merino, 3) Kıvırcık-Türkgeldi.

In 57 sheep breeds from Europe and the Middle East from 15 countries (Peter et al., 2006), in Turkey Akkaraman, Morkaraman and Tuj sheep breeds (Uzun et al., 2006), in 45 goat breeds from 15 European and Middle Eastern countries (Cañón et al., 2006) determination of genetic polymorphism was made with microsatellite markers.

Genetic (linkage) maps were created with microsatellite markers in cattle (Bishop et al., 1994; Ihara et al., 2004), goats (Vaiman et al., 1996) and sheep (Crawford et al., 1995).

QTL (quantitative trait loci) for milk production traits in sheep (Diez-Tascón et al., 2001), growth and carcass traits in cattle (Casas et al., 2004), milk production, health and reproductive traits (Ashwell et al., 2002) Mapping was done with microsatellite markers.

Conclusion

Today, Türkiye is among the countries rich in biodiversity, that is, endemic or local plant and animal species in the world. In particular, it is necessary to determine the genetic characterization of our domestic animal genetic resources with molecular techniques, QTL mapping of economic yield characteristics and molecular marker assisted breeding (MAS: Marker Assisted Selection) studies should be carried out with these results. However, with the application of MAS in the future, it will be possible to perform identification at the molecular level in the selection, evaluation and certification of breeding animals.

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