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Use of Molecular Markers in Parentage Testing, Genetic Diversity, Marker-Assisted Breeding and Disease Identification

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ABSTRACT

Conventional breeding programs have limitations while working with the quantitative traits and traits with low heritability. Furthermore, the identification of defective genes that can cause the development of a disease cannot be identified through conventional strategies until the development of the disease. The Discovery of molecular markers has made it easier for animal breeders and animal geneticists to enhance the productivity of animal breeding programs. Different kinds of molecular markers, RAPDs, SNPs, AFLPs, QTLs, and SSR are being used in animal breeding for gene mapping, phylogenetic studies, disease resistance studies, genetic conservation, and genetic diversity. Molecular markers also provide the advantage of working with low heritability and complex quantitative traits. QTL markers are being used for quantitative traits like milk production meat production because they are linked to quantitative trait genes. Marker-assisted breeding has helped the breeding programs to increase the efficiency of the breeding programs. Molecular markers like SNPs can be used to detect the mutation in genes at an early age. Microsatellites have been used at a very large scale for phylogenetic identification. In this review, we will discuss the importance and application of molecular markers in animal breeding and genetics.

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INTRODUCTION

Conventional breeding programs use mendelian inheritance for selective trait identification and application of Mendelian genetics for selective breeding (Qureshi et al., 2014). Likewise working with Mendelian genetics to improve the commercial traits of meat production, milk production, quality enhancement, and disease resistance mendelian genetics have limitations and cannot provide enough information to increase the production to compete with the demand of rapidly growing world population. Some wild breeds of animals that had low production in phenotypic traits were not used for breeding programs, because the conventional breeding focus on selection through phenotype, those breeds may have some

important genes that can play a vital role in diversity and survival are now gone. Similarly, due to excessive breeding of high production breeds, some local and native breeds are now extinct (Rout et al., 2008).

This excessive selection of animals for superior production and increased trait expression sometimes becomes the source of loss of some important genes in the animal population. Therefore, this has caused the loss of some local breeds, but these lost breeds can be a source of some important genes in the future trait development for humans (Notter 1999; Bruford et al., 2003; Toro et al., 2009). Similarly, a marker-assisted selection will also provide the opportunity to maintain the heritability of genes that are crucial for the survival of the genetic diversity of newly produced breeds. Furthermore, the use of molecular markers can help to trace some very important genes back to their original parent and can help to conserve the important animal. Similarly, molecular markers can help us screen the genome of an animal population and identify the important genes for production and survival, therefore this information can be helpful to conserve the animal that contains some important genes in their genomes.

Animal breeding programs can benefit from modern molecular techniques in different ways, for example, the use of molecular markers in selective breeding, parentage testing by molecular markers, and gene mapping. The use of molecular markers in animal breeding programs started in 1990. Olesen et al. (1999) and the breeding outcomes after this have shifted the focus of the scientific community from conventional breeding to selective breeding by marker-assisted selection. Also, in recent times the animal breeders are trying to increase the efficiency of breeding programs by combining both conventional and advanced techniques. Moreover, conventional breeding can take many years to improve a certain trait that has low heritable value. Conventional breeding while improving the traits that are commercially important overlook the importance of genetic diversity of the breeds. So, the newly developed animal may have high production but it will be susceptible to disease because of low genetic diversity (Singh et al., 2014). To decrease the susceptibility of animals along with higher production value marker-assisted selection can help to achieve greater goals. The new biotechnological techniques such as artificial insemination and embryo transfer and multiple ovulations have changed animal production drastically (Lindhe and Philipsson, 1998; Singh et al., 2010). Furthermore, the use of molecular biology for marker-assisted breeding has a positive impact on animal breeding, molecular markers can also be exploited to increase the disease resistance in animals for better productivity (Singh et al., 2012).

Molecular markers have a wide range of applications, they can be used to identify the foreign genes present in the animals. Molecular markers can also be used in transgene breeding to increase the production and disease resistance of animals (Erhardt and Weimann, 2007).

Different types of molecular markers are being used in the field of animal breeding and animal biotechnology for multiple goal-achieving purposes. RAPD Random amplification of polymorphic DNA markers (RAPD) was the first molecular marker that was used by American scientists in 1990 (Deb and Chakraborty 2012). Since then scientists have developed different molecular markers that have a range of application in the plant as well as animal science, these markers include restriction fragment length polymorphism (RFLP), microsatellites, single nucleotide length polymorphism (SNPs), and simple sequence repeats (SSR) (Kiplagat et al., 2012). These molecular markers since their discovery have impacted animal and plant science in various ways, in this review we will look upon some significant role that has been played by molecular markers in animal breeding and animal production.

Molecular markers and parentage testing

Baron et al. (2002) reported a misidentification rate of 36% in 74 offspring from 9 alleged Gir cattle Bulls. They used microsatellite markers for the evaluation of progeny testing and found a significant difference in the DNA of alleged Bulls and offspring. Moreover, these tests did not include information about the genetic superiority of the Bulls. And their importance for the breeding programs. But the misidentification of the correct sire can have a negative effect on the breeding programs. Also, it has been suggested that before carrying out the breeding programs the paternity test can help correctly identify the superior sires with the required genetic information for the breeding program. Israel and Weller, (2000) reported that annually there is a 4.3% loss of genetic gain and the misidentification of animal for breeding cause a loss of 10% in dairy production. Fernandez et al. (2009) used 116 single nucleotide polymorphism (SNP) and 18 microsatellites (STR) to detect the polymorphism between 36 closely related Angus cattle and found that the information provided by SNPs were more helpful in distinguishing between the 36 closely related Angus cattle. Hence not only microsatellites but also the use of SNPs can help to detect the parent confusion among breeds and animals for successful breeding programs.

During the first decade of the 21st century, the use of microsatellites has been in high demand for parentage testing and tracing the polymorphism between the breeds of cattle, microsatellites have provided successful information for the breeding program. But in this decade scientists around the world have been comparing the use of single nucleotide polymorphism for parentage testing and microsatellites.

Özşensoy et al. (2014) reported the use of molecular markers for paternity testing of native Anatolian Turkish cattle breeds. They used microsatellites for paternity testing, in total they used 20 microsatellites loci in different Anatolian breeds of cattle. After amplification through a polymerase chain reaction, they used gel

electrophoresis to study the different fragment sizes of DNA. They identified 7 alleles that had PE value (probability of exclusion) greater than 0.9999 that showed they can be used to study the parentage analysis in the region.

These pedigree errors are a major reason for these losses in genetic gains and production values. The misidentification can arise from the mixing of records of animals on the farm, and during grazing mixing of animals can cause miscellaneous data, mixing of the labels, and sometimes mixing the artificial semen can cause mixing of genetic material between animals. Therefore, it is essential to establish the true parentage of the animals before establishing the breeding programs for expected gains (Sharma et al., 2015; Zhao et al., 2017). The people living in high altitudes depend upon Yak for their dairy needs, and mostly the feeding behavior of yak in these areas is grazing which can cause the mixing with other animals. The correct information about their genome is necessary for successful breeding programs. Pei et al. (2018) carried out research to identify the microsatellites present in the yak genome. They tested a total of 71 loci for microsatellites from which 35 loci generated excellent PCR, and from these 35 microsatellites, 17 microsatellites had high polymorphic value and can be used for differentiating the breeds among each other.

Selection of incorrect male animals for breeding will not only cause economical but also genetic losses, therefore correct identification of animals is necessary for the successful breeding program. Single nucleotide polymorphism can be used successfully to test the parentage in livestock. There are many examples of SNPs being used to determine the parentage in cattle breeding programs. Single nucleotide polymorphism is also being used successfully in marker-assisted breeding programs in cattle (Werner et al., 2004; Fisher et al., 2009). There are also examples of successful development of SNPs markers for parentage testing for international sheep breeds, several scientists have developed SNPs panel for testing the parentage successfully.

International sheep genomic consortium (ISGC) reported the SNPs50k bead array in ovine, and there are examples of four sets of SNPs panels being used for New Zealand and Australian sheep parentage testing (Kijas et al., 2012). New Zealand's Ag research Centre reported SNPs to include 84 to 300 Autosomal panel of SNPs for parentage testing. The Australian research Centre CSIRO and Sheep CRC reported 382 SNPs for parentage testing and 88 SNPs were reported from the international sheep genomic consortium (Clark et al., 2014; Bell et al., 2013). Heaton et al. (2014) reported the SNPs panel for parentage testing can be very helpful in identifying the correct parentage in globally diverse sheep breeds. In their research, they also tried to identify a panel of SNPs that can be used for global sheep breeds parentage testing, and also, they developed the subset of these SNPs panel to be used for North American sheep. The research data of 74 breeds and 2915 sheep were provided by ISGC and they analyzed 47,693 autosomal SNPs for parentage testing of global sheep breeds. From these diverse number of SNPs, they selected 163 SNPs that had the

desirable characters for being used in parentage testing. These SNPs performed very well for parentage testing and they had a minimum allele frequency (MAF) value of more than 0.3 in average 48 breed groups. An SNP is considered highly polymorphic if its minimum allele frequency value is higher than 0.3. they concluded that this set of 163 SNPs can be very helpful and economical to apply to test the parentage of sheep breeds all over the world, and it can generate productive results for sheep breeding programs.

Marker-assisted breeding

The traditional breeding selection of animals is dependent upon phenotype selection and pedigree records, and sometimes mix approach is carried to select the animal for breeding (Henderson 1984). These traditional breeding techniques do not give appropriate information about the selection by phenotype and the interaction of these genes with the environment, and also these techniques are not useful among the traits that have low heritability and also that have late expression mechanism. The conventional breeding technologies are also helpless when the commercially important traits are sex-limited traits and the linkage information about these traits is limited. Therefore, it is necessary to depend upon such technologies that are helpful in these complex situations and can provide the information necessary for the breeding programs (Beuzen et al., 2000; Barillet 2007; Mirkena et al., 2010). Therefore, in recent times the use of molecular markers is getting popular for animal selection, and this approach is being called marker-assisted selection. The idea of marker-assisted breeding tells that the quantitative traits genes have specific sequences in their nucleotide sequence, and these are specific to different quantitative traits (Gianola et al., 2003). Therefore, these markers can be used for specific quantitative trait selection and exploited for improved breeding output.

The markers-assisted selection in breeding is most popular in cattle breeding programs, and according to recent research approaches single nucleotide polymorphic markers (SNP) are considered most suitable for cattle breeding (MeuWissen et al., 2001). While this approach is being used by the scientist for research purpose also it is being exploited by commercial agricultural farms for marker-assisted breeding for cattle. But because SNPs marker needs capital and is not very economical the small animal breeders are still dependent upon traditional breeding methods. Moniruzzaman et al. (2014) said that to carry out successful marker-assisted breeding, it is necessary to detect the quantitative trait loci, gene mapping, marker genotyping, and genetic evaluation of the animal, all of this information will lead to the successful selection of animal for breeding purposes. And because most of the commercial traits in animals as well as in plants are quantitative e.g. milk yield, meat production, and protein content the selection of animals for breeding by phenotype will not produce successful results. Therefore, the

use of QTL markers for the selection of animals for quantitative traits is a very useful technique to ensure productive results for the breeding programs.

Salisu et al. (2018) said that molecular markers are not only helpful for selection among quantitative traits but also can be exploited for those traits for whom the phenotypic measurements are not possible and also for those traits which have low heritability. Molecular markers such as single nucleotide polymorphism (SNP), restriction fragment length polymorphism (RFLP), and microsatellites (SSR) are being used in marker-assisted breeding programs because they are easy to use and can be amplified using polymerase chain reaction (PCR), but these markers can also be employed to see the polymorphism between the breeding population and checking the genetic diversity of a population. Marker-assisted breeding helps to identify the target genotypes by detecting the genetic marker present in that genotypes. For rapid genetic gain, the marker can be detected in the genotype of the parent and can be used to make the generation time short. The genetic markers are linked to quantitative traits and they are present in the nucleotide sequence of the QTL or a distance away from the actual gene. The QTL can be detected by detecting the genetic marker that is presently inclined to specific QTL because the specific genetic markers are present at a specific distance from the QTL, therefore they can be detected and the required trait genotype is easy to locate in a pool of genes. The selection of a mutant genotype and recessive gene can be made at a very early stage of the life of an organism using markers, and the phenotype of the organism can be predicted according to the marker identification. Moreover, the molecular markers can easily predict the sex-limited and low heritable traits, therefore paving way for easy and successful breeding programs and making the selected breeding easy. When the location of the marker is near to QTL, and a large number of markers present on one chromosome, high heterozygote frequency and the linkage disequilibrium than that marker generate the high results (Hiendleder et al., 2003).

Lahav et al. (2006) reported the marker-assisted selection in chicken-based upon the multi-trait economic index. They proposed a method that helps for selection that undergoes upon the number of traits. A total of 32 markers in this study were tested and out of these 32 tested markers, 5 markers give the most polymorphic values. And the results were used to choose to prepare and the breeding flock that after breeding gave the desirable results.

Bidinost et al. (2008) devised an experiment to identify the QTLs related to Merino sheep wool quality and sheep wool production, they selected chromosomes from eight different families, chromosome numbers 25, 11, 8, and 4 were selected to look QTLs related to wool production and wool quality. The body weight and greasy fleece weight were recorded at the adult shearing and hogget phase of life. The QTLs for yield were detected at chromosome number 25 which was the first time, the fleece weight and keratin type II gene were detected at chromosome number 4, and QTL

related to fiber diameter was detected on chromosome number 3. This study shows that the QTLs related to commercial importance can be detected at a very age using molecular markers. And these can be exploited to benefit at a very large scale in comparison to the conventional approach that does give precise information about the traits and their genes and their heredity patterns.

Molecular markers for disease identification

The use of a selection of animals through conventional breeding strategies for breeding programs is based upon selection by phenotype performance of the animal. For some reason, this selection by phenotype for quantitative traits like milk yield, body weight, and hair color can be done. But some complex traits are not measurable through phenotype measurements e.g. disease susceptibility and disease resistance, and because of this reason, we cannot depend upon the conventional breeding strategies for the selection of animals for these traits. Therefore, in these complex traits, the selection of animals for breeding through molecular markers is the most suitable choice (Dekkers 2004; Williams 2005). Because in the molecular markers selection criteria we can assure the presence of the gene that is responsible for the resistance against a certain disease is present in the desired animal or not, or we can check that either the animal has the gene that makes it susceptible for a particular infection is present or not. So here the molecular markers assisted selection can be more beneficial over the selection through phenotype.

One of the most important examples in mammals for disease-resistant is showed by sheep against the Scrapie. The prion gene PRNP present in specific genotypes is known to give the disease resistance to sheep against scrapie disease (Goldmann 2008). It was already reported various times that scrapie disease is a hereditary disease and is associated with a proteinaceous agent causing the disease, only a protein was involved in causing the scrapie. And if that protein was mutated then the resistance against the gene can be attained, and it was observed when the gene responsible for the production of protein was removed the mice became resistant (Prusiner 1982; Carlson et al., 1986; Hunter et al., 1989).

Oner et al. (2011) said that transmissible spongiform encephalopathy TSE or Scrapie has been existing in Europe for 250 years in goats and sheep. The scrapie is a degenerative neural prion disease that has been fatal for sheep and goats. And the susceptibility to disease shows polymorphism on the PrP gene, and this polymorphism has been detected on different codons in sheep. These codons on the PrP gene in sheep 171, 154, and 136 shows polymorphism against scrapie disease. And this polymorphism can be studied and exploited to infer disease resistance in sheep against scrapie. Alsayed et al. (2019) also experimented to check the

polymorphism of the PRNP gene for prion disease, two Palestinian breeds Assaf and Awassi were selected for the study, and a total of 38 animals were tested. To check the susceptibility against the disease they selected valine, arginine, glutamine (VRQ), and alanine, arginine, glutamine (ARQ) at codons 136, 154, and 171. In the study another genotype was found that showed the polymorphism at two different codons V121 and L23Hof PRNP gene locus. Yaman et al. (2015) conducted a similar experiment with Merino breeds of sheep from Turkey, to check the polymorphism of the PRNP gene. The experiment was able to identify 5 genotypes (ARR/ARR, ARR/ARQ, ARQ/ARQ, ARR/VRQ, and ARQ/VRQ) and three alleles (ARR, ARQ, and VRQ) related to scrapie susceptibility.

Goldmann (2008) reported that chronic wastage disease (CWD) in sheep is very lethal for sheep health and also, bovine spongiform encephalopathy (BSE) is not only damaging the animal health but is injurious for humans health. Both diseases CWD and BSE are believed to have a genetic basis and are causing a lot of damage to the economy on a global scale. Scrapie is a prion disease that is caused by protein coded by the PRP gene which makes it susceptible to the disease. The Antriodactyles order is also believed to have a genetic polymorphism that makes it susceptible to prions to cause these diseases. And this genetic polymorphism can be exploited through genetic markers to identify the PRP gene and use some biotechnological approaches to make the gene silent, once the gene is silent the protein will no longer be synthesized and that will make the sheep resistant to the disease.

Pena et al. (2015) reported that the aspergillus fumigatus that is causing the aspergillosis in humans and animals, is single species based on morphological and chemical similarities. But after they applied the molecular markers on different strains of aspergillus fumigatus, they found these strains were genetically distinct from each other. They used two approaches, a PCR based restriction fragment length polymorphism RFLP and randomly amplified polymorphic DNA RAPDs, they collected the aspergillus fumigatus sensu lato and aspergillus fumigatus sensu stricto samples from Brazil and Argentina. Both the PCR-RFLP and RAPD showed the same band patterns after analysis. Moreover, they showed a genetic similarity of 78% regardless of their geographical isolation and a genetic similarity coefficient of 0.6 to 1, which shows they contain genetic variability at the intraspecific level.

Genetic diversity and molecular markers

Natural and artificial selection animal have changed the genetics of animals in different ways, both selections contributed to evolution to develop the new genetic basis in animals that can help them to survive in a challenging environment. These changes brought about by the artificial selection were to develop the new breeds of domestic animals so they can give higher production of milk and meat (Blott et al., 1999; Rosenberg et al., 2001). Öner et al. (2019) investigated the genetic diversity of

cattle breeds in turkey, five native Turkish cattle breeds were investigated using 22 microsatellites referred by FAO. In this study, it was able to identify a significant genetic diversity in the local cattle breeds. Furthermore, in Turkey, the conservation of genetic diversity in sheep breeds was started in early 2000, but still, the field needs a rigorous effort from the scientific community. Karsli et al. (2020) published their investigation regarding the use of 21 microsatellites to check the genetic diversity in four native Turkish sheep breeds Güney Karaman, Kangal, Norduz, and Karakas. The experiment showed that Norduz and Güney Karaman sheep breeds have become genetically distinct from the Akkaraman breeds because they faced different environmental conditional over the years. Kirikci et al. (2020) reported the use of 9 microsatellites to check the genetic diversity of native most populous breeds in Black See Karayaka and concluded that this breed has subpopulations unique to some regions that needed a thorough study for conservational programs.

This artificial selection leads to the development of several breeding programs that started a new dimension in animal production, the goals were commercially oriented which lead to the development of several breeds that gave superior production of milk meat and other traits.

Demir and Balcioğlu (2019) studied the genetic diversity of three Turkish breeds Anatolian Black and Eastern Anatolian Red and Turkish Grey Steppe and Holstein Friesian cattle breeds. They used 20 microsatellites in total and 120 animals from 4 different breeds and selected 204 different alleles. After studying the heterozygosity values of both Holstein breed and Turkish breeds he concluded that there was enough genetical distance between the Turkish and Holstein breeds that they can be distinguished as different breeds.

But in recent years the focus has shifted towards knowing the genetic basis of these newly developed breeds, moreover, the goal of most of the studies is to evaluate the genetics of global domestic animals to check the variation among them. Because the domestic animal that is being farmed locally are also bred varieties and it is suitable to know their genetic basis to check for variation in their genes. The most appropriate approach to check the genetic variation among different breeds of animals is through molecular markers (Bjornstad and Roed 2002). Microsatellites have proved to be very useful in developing the variation in the genetics of farm animals like cattle and sheep. And they are being used at a large scale to check the genetic diversity of domestic animals in global breeds.

Totally 120 individuals of 4 breeds were genotyped using 20 microsatellite markers and 204 different alleles, of which 31 were private alleles, were detected.

Berthouly et al. (2008) carried research to check the genetic diversity in Asian and French breeds of chicken. They compared their results to the global AVIANDIV

project, they used 22 microsatellites and compared them to those 14 loci of AVIANDIV. The AVIANDIV findings can be very helpful to the research of checking the diversity of local chicken breeds. After their research, they found that Huatung and Coucou de Rennes French breeds contributed a lot to the global chicken diversity, after comparing their result to14 loci of AVIANDIV they concluded that the French Maran breed contributed the most to the genetic diversity globally.

Tefiel et al. (2020) carried out research to compare the relationship between Turkish and Algerian sheep breeds. They use 18 microsatellites recommended by Fao and were able to report that the Turkish and Algerian goat breeds are very much different breeds, and this research Showed that goat breeds are genetically separate from each other.

Maintaining the genetic diversity of animals is very crucial for domestic animals because the loss of breeds means the loss of civilization and the loss of some very important genes. Moreover, the breeding for a commercial trait may cause the loss of some underperforming breeds that may have some very important genes that contribute to survival in many situations (Baumung et al., 2004). Peter et al. (2007) reported the genetic diversity study in 57 sheep varieties of Europe in different European countries. The research was carried through microsatellite molecular markers. microsatellites have become a global tool for analyzing the global diversity of farm animals. Baumung et al. (2004) reported the research carried out in 93 different countries, microsatellites were used to analyze the genetic diversity of ruminants in these countries in 87 different research programs. therefore, we can say that conserving genetic diversity is very crucial for human survival as well as research programs, and molecular markers have too much to offer in this perspective.

CONCLUSION

Since their discovery, molecular markers have changed the world of genetics and breeding in plants and animals. The idea of polymorphic regions on DNA has helped scientists to draw new ideas that have revolutionized the breeding of animals. Molecular markers have been important for parentage testing, disease identification, marker-assisted breeding, and many other important fields. Molecular markers are of different types, randomly amplified polymorphic DNA (RAPD), microsatellites, single nucleotide polymorphism (SNP), and Quantitative trait loci. Microsatellites have helped in distinguishing the parentage of animals, and microsatellites have been used on a very large scale for marker-assisted breeding, quantitative trait loci are being used for selecting traits that are economically and commercially important. But in recent years SNPs have been important for animal breeding programs and the mutation tracing for disease diagnosis at an early age. The application of molecular

markers does not stop here, molecular markers can be exploited at a very large scale for genetic diversity and conservation of important animal breeds. Molecular markers have so much to offer for animal genetic and breeding science.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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