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Genetic Improvement of Tharparkar Cattle: A Review

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ABSTRACT

Tharparkar cattle are an important dual breed of desert which can be improved for sustainable milk production by using different molecular markers and through selective breeding. Although, genetic studies done on Tharparkar, are fewer, there is need of more attention on genetic studies and identification of useful markers and SNPs associated with production traits. This review article describes the production and reproduction performance of the breed and emphasizes more on genetic studies, breed characterization, marker assisted selection or identification of molecular markers, genomic assessment, *etc*. to improve the breed status. Molecular markers for heat tolerance, disease resistance, high fat and protein production, A2 milk composition identified in Tharparkar cattle positively encourage breeders to improve the breed for future use. It may help the government agencies to undertake systematic animal husbandry practices and breed improvement programmes so that high quality germ plasm may be provided to the farmers for breed improvement in rural area.

Key words: *Bos indicus*, Bos taurus, Genetic markers, Lactation, Polymorphism, Tharparkar.

Tharparkar cattle (*Bos indicus*) also known as White Sindhi is a dual purpose breed of Tharparkar district of Sindh province in Pakistan (Khan and Isani, 1994). It is lyre horned type of zebu cattle came into prominence during the First World War when some animals were taken to supply milk for the Near East army camps where their capacity for production under adverse feeding or poor quality forage and environmental conditions became apparent. Since then many breeding herds have come into existence in India and Pakistan. The breed is also called Thari, after the name of desert “Thar”. In India these are mainly found in Kutch (Gujarat), Jodhpur and Jaisalmer (Rajasthan). Milk yield of the breed under village condition is around 1660 kg, whereas under commercial farm condition the production is 2500 kg per lactation. Similar to other zebu cattle in India, Tharparkar has tick and parasite resistance, heat tolerance, ease of calving, longevity, reproducing for up to 20 years, drought resistant, bloat tolerant, good temperament and lean meat with even fat cover. Owing to indiscriminate breeding, cross breeding with exotic breeds, the population of pure bred animal has decreased during past decades. Hence there is an urgent need to conserve this breed which is only possible by using suitable tools for genetic improvement like development of breed markers and marker assisted selection to improve the productivity. Besides, the productivity of this breed may also be further improved by adequate feeding management and superior germplasm. Tharparkar cattle survive well, produce a good amount of milk, therefore, it is considered as a potential milch animal of the country (Gahlot, 1999; Chand, 2011). The review article emphasizes on genetic improvement of Tharparkar cattle, which is an important dual purpose breed of Thar desert.

Breed characteristics

Average animals of the Tharparkar breed are medium sized, strong with straight limbs and good feet, alert and sometimes wild and vicious. The usual colour of the cattle is white or grey. In males, the grey colour may be deepened, particularly on the fore and hind quarters. The head is of medium size, the forehead is broad and flat or slightly convex above eyes: the front of the horns and face are practically are on one plane. The eyes are full and bright. The eyelashes are black. The ears are somewhat long, broad and semi-pendulous and face forwards. Horns are set mostly curving gradually upwards and outwards. In the males the horns are thicker, shorter and straighter than in the females. The hump in males is moderately well developed, firm and placed in front of the withers. The dewlap is of medium size. The sheath in the male is of moderate length, and is semi-pendulous. The navel flap in the females is prominent. The size of cattle is variable. Shoulders are light and legs are comparatively short, but in good proportion to the body. The hooves are hard and black and are of moderate size. Thari cattle are very strong, resistant to several tropical diseases and can withstand harsh climatic and environmental conditions (Mason, 1996).

Production and Reproduction performance of Tharparkar

The productivity of dairy cows determined by its lifetime

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performance rather than on a single lactation performance. The Tharparkar breeding farms are located in Bihar, Haryana, Maharashtra, Rajasthan, Tamil Nadu and Uttar Pradesh (AH Report, 2016). These farms either belong to state Govt. or central Govt. The Performance traits of the Tharparkar breed mostly from data available from the organised breeding farms at different locations, like age at first calving (AFC), 305 days lactation yield (SLY), life time production (LTP), the number of milk days, milk yield per day, lactation length, dry period, service period, calving interval, repeatability, reproduction and growth have been reported by several workers.

The production performance and reproduction depend on genetic and environmental (non-genetic) factors. Patel *et al*., (2012) studied the effect of season of calving on milk production in Tharparkar cows and observed that cows calved during winter and rainy seasons had 13 and 22.6% higher milk yield respectively than those calved in summer and autumn seasons. The milk fat was observed higher during winter and autumn season (5.25 and 5.24%) than the summer and rainy season (4.30 and 4.64%) in Tharparkar cows, whereas the SNF % was almost similar in all seasons (Patel *et al*., 1994). Choudhary *et al*., (2019) also estimated herd life (3080.55±84.34 days), productive life (1903.17±77.17 days), life time milk yield (9414.55±406.5 Kg) and milk yield/day of lactating life (5.32±0.30 Kg) based on data consisting of 284 lactation records of Tharparkar herd over a period of 11 years. In the study, they also found that sire significantly affected the herd life, number of days in milk and parity. Similar observations were also studied by Mukherjee *et al.* (1999); Kumar, (1999). Chaudhary *et al*. (2019) estimated heritability of life time performance traits which were moderate to high heritable. They estimated heritability of herd life (0.80±0.24), productive life (0.73±0.53) and life time milk yield (0.50±0.49) respectively. Dry period in Tharparkar cows ranged from 100.93 days to 105.61±19 and 105.03±2.09 days (Patel *et al*., 2000; Gahlot, 1999; Mishra *et al*., 2017). Similarly, the lactation length was estimated to be 288.68±3.14 days (Chand, 2011), 279.19±3.27 days (Kishore, 2016) and 307.30±1.08 days (Gahlot, 1999).

Average lactation yield was estimated to be more or less 2000 Kg (Gahlot, 1999; Chand, 2011). Patel *et al*., (2000) estimated average per day milk as 4.65±0.13 whereas Chand (2011) and Kishore (2016) estimated 7.44±0.06 Kg and 7.45±0.09 respectively, indicating a scope of genetic improvement in productivity. Lifetime performance traits like herd life and productive life of Tharparkar cattle were also estimated as 2657.2 days to 3540.57±29.74 days and 1460 to 1867.34±96.82 days, respectively (Gahlot, 1999). Productive performance of Tharpakar cattle at various herds was also compared by Chaudhary *et al*. (2018). Average age of first calving in Tharparkar cattle as 1876.17±40.66 and 1769.07±29.80 days were estimated by Chand (2011) and Mishra *et al*. (2018). Average calving interval reported as 399.97±2.44 days (Mishra *et al*., 2017) was less than 455.60±8.50 to 528.02±13.74 days reported earlier by Rahumathula *et al*. (1994) indicating scope of genetic improvement in the breed. Service period was also estimated as 132.29±9.37 to 252.14±13.49 days, 152.04±4.58 days, 122.04±4.26 days and 117.53±2.39 days by Gahlot *et al*. (2002), Chand (2011), Kishore (2012) and Mishra *et al*. (2017), respectively indicating improvement in average service period. Patel *et al*. (2001) revealed that Tharparkar cows conceived within 2 months after calving exhibited lower Dry period (47 days), Calving interval (323 days) and Lactation length (276 days). However, higher Lactation milk yield (1601 kg) was observed in animals those conceived between 4 to 5 months after calving.

Necessity of genetic testing in breeding bulls

Genetic studies or genetic testing need be conducted on breeding bulls or bull calves stationed at frozen semen production units and bull rearing centres as these bulls/bull calves are extensively used for artificial insemination (AI) in India so that genetic defects in any of these bulls should not spread to a large number of populations (Patel *et al*., 2006; 2007; Patel and Patel, 2014). Tharparkar bulls are fewer or not available in frozen semen production units in India therefore, genetic studies or testing is also very rare but it is extremely important to have genetic testing of the bulls done prior to its use for breed improvement.

The association between abnormal chromosome constitutions and disorders of sexual development in domestic animals has been recorded since the beginnings of conventional cytogenetic analysis. Alterations in chromosome number and structure have been found associated with direct effects on fertility and reproductive outcome in cattle. A few Tharparkar bulls were karyotyped (Kumar, 2010; Ahmed *et al*., 2020). Genetic disorders, especially autosomal recessive, are major concern worldwide as the carriers or heterozygous in cattle population look normal and therefore, often used for breeding through artificial insemination (AI) based on their genetic merits. Most of genetic diseases occur in Holstein and other European breeds, therefore, screening of such breeds is very common worldwide. In India, it is mandatory to screen genetic disease in breeding bulls used for AI programmes. Therefore, a very few Tharparkar cattle are screened for Genetic disease (Kotikalapudi *et al.,* 2017) and found normal for BLAD whereas, two HF x Tharparkar crossbreds were found carrier and one affected for BLAD (Yathish *et al*., 2010) indicating that the mutant gene transmitted from Holstein.

Molecular characterisation for breed purity and relationship with other breeds

Molecular characterization was carried out by using Random Amplification Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) method with a group of 42 Tharparkar cattle along with 30 animals of Rathi breed (Sharma *et al*., 2004). Twenty three random primers were screened out of which 15 primers yielded satisfactory amplifications and were usedfor further analysis by Sharma group and they found

average numbers of polymorphic fragments per primer 7.07±0.86 in Rathi and 6.80±0.61 in Tharparkar cattle respectively. The percentage of polymorphic bands in these two cattle breeds were 86 and 87%, respectively. Within breed genetic similarities for pooled over primers in the animals of Rathi and Tharparkar breeds were 0.577±0.30 and 0.531±0.02, respectively, on the basis of band frequency (BF) and 0.645±0.04 and 0.534±0.04, respectively, on the basis of band sharing (BS). Averages of between breed genetic similarities for pooled over primers were 0.97 and 0.92 according to BF and BS, respectively, which reflect a higher degree of genetic similarity between Rathi and Tharparkar cattle breeds. High estimates of between breed genetic similarities for pooled over primers indicated that either Rathi is having decent from Tharparkar or both the cattle breeds are having common descent. The low value of Index of genetic distances between these two cattle breeds may be due to the fact that Rathi and Tharparkar cattle breeds are the native of Thar Desert in Northwest India. Similarly, a panel of 19 microsatellite markers was developed for breed characterization in a herd of 44 Tharparkar and Red Sindhi breeds of cattle in Pakistan, which was 100% successful study (Azam *et al*., 2012). Pattern of allelic frequencies of most of the microsatellite markers were clearly distinct between two breeds. As a result of the present study a reliable, efficient and very informative panel of microsatellite markers was successfully developed which was capable to interpret individual identity, forensic cases and breed characterization in cattle.

Marker assisted selection- An important tool for genetic improvement

A genetic marker is a sequence of a DNA located on a particular chromosome and usually associated with a particular gene or trait. These markers are useful for a specific trait as a tool for selection in animals (Gizaw *et al*., 2007). Markers could be chromosomal changes (karyotypes); translocations, deletions, inversions, *etc*. Similarly, markers could also be defined as biochemical, such as the blood type and isozymes which represent biochemical traits that could be analyzed by protein electrophoresis. The most advanced of these markers are molecular markers which are based on the nucleotide sequence mutations within the individual's genome; they are the most reliable markers available (Yang *et al*., 2013). Till now, many types of molecular markers have been identified and utilized to detect the variation among individual and population. Markers can be identified and assessed by various molecular analyses; Restriction fragment length polymorphism (RFLP), Single-strand conformation polymorphism (SSCP), Simple sequence repeats (SSR)/microsatellites and Single Nucleotide Polymorphism (SNP). Deb *et al.* (2014) detected an SNP using RT-PCR in a bovine CACNA2D1 gene located at G519663A locus on chromosome 4. They revealed that GG genotype is associated with the lowest somatic cell score (SCS) and the transcript abundance of CACNA2D1 mRNA and was favorable for the mastitis resistance. More markers were also found to be associated with mastitis in dairy cattle (Yuan *et al*., 2012; Asaf *et al.,* 2015; Wang *et al.,* 2007). Similarly, a marker for Johne's disease (JD), Foot and mouth disease (FMD), Tuberculosis (TB), were also identified (Ruiz *et al*., 2010; Pinedo *et al.,* 2009; Singh *et al.,* 2014; Maryam *et al.,* 2012) in various breeds of cattle.

Polymorphism in bovine lymphocyte antigen (BOLA-DRB3.2) Gene in Tharparkar Cattle was studied (Bhushan *et al.,* 2007). Bovine lymphocyte antigens are suitable molecular and immunological markers associated with disease resistance. Tharparkar breed showed very limited polymorphism with respect to the reported patterns by various workers (Gelhaus *et al*., 1995; Aravindakshan *et al.,* 1999).

Rachagani and Gupta (2008) estimated gene frequency of kappa casein, which is considered to be marker for milk yield and protein, in Sahiwal and Tharparkar breeds. The frequencies of alleles A and B in the Sahiwal and Tharparkar breeds were estimated as 0.873 and 0.127 and 0.857 and 0.143, respectively. Genotype BB of the kappa-casein gene is a marker which had more influence on the monthly milk yield, 305-days milk yield, monthly solids-not-fat (SNF) yield, and monthly protein yield in cattle.

Beta-lactoglobulin is one of two major whey proteins, found in the milk of animals including cattle. These whey proteins with kappa casein play a crucial role in the coagulation and curdling of milk. This role in coagulation is also important for cheese production. β-lactoglobulin is also found in a number of genetic variants of which A and B are predominant. The variants differ by two amino acid substitutions in the polypeptide chains and two single nucleotide substitutions in the β-lactoglobulin. Variant A has Aspartic acid (GAT) and Valine (GTG) at 64 and 118 codon, whereas variant B has Glycine (GGT) and Alanine (GCG). Milk produced by β-lactoglobulin AA-genotype has been found to contain more lactoglobulin, less casein and less fat than that obtained from BB cows (Walawski *et al*., 1994). Rachagani *et al.* (2006) revealed in their studies that the allele B of β-Lactoglobulin occurred at a higher frequency than the allele A in both Sahiwal and Tharparkar breeds. They estimated gene frequency of A and B alleles as 0.17 and 0.83 and 0.39 and 0.61 in Sahiwal and Tharparkar breeds respectively indicating good quality of milk.

Bovine milk constitutes about 85% water, 15% milk sugar lactose, protein, fat, and minerals. In milk there are two major protein groups known as caseins and whey proteins. Caseins account for 80% of bovine milk protein (Niki *et al*., 1994), whereas both major whey proteins constitute about 14% (Roginski, 2003). Beta-casein is 30% of the total protein content in cow's milk. Bovine milk contains 4 caseins: alpha s1 (CSN1S1, 39-46% of total caseins), alpha s2 (CSN1S2, 8-11%), beta (CSN2, 25-35%) and kappa (CSN3, 8-15%) (Roginski, 2003). There is also gamma-casein that is a product of the degradation of beta-casein (Ostersen *et al.,* 1997). There are 13 genetic variants of beta-

casein: A1, A2, A3, A4, B, C, D, E, F, H1, H2, I, G. The most common forms of beta-casein in dairy cattle breeds are A1 and A2, while B is less common and A3 and C are rare (Farrell *et al*., 2004). Presence of proline (CCT) and histidine (CAT) amino acid in the peptide chain at position 67 of the beta-casein may give rise to two variants A2 and A1 beta-casein respectively (Roginski, 2003). The cause for concern with milk containing A1 beta-casein is that histidine at the 67th amino acid position allows a digestive enzyme to cut out a 7 amino acid segment of the protein immediately adjacent to that histidine. However, proline is present in that location in A2 beta-casein, that same segment is either not separated at all or the separation occurs at a very low rate. The 7 amino acid segment that is separated from A1 beta casein is known as beta-casomorphin-7, often abbreviated as BCM-7 (Kostyra *et al*., 2004), which adversely affect health of human. Studies have revealed that A1 allele is more frequent in exotic cattle (A1 milk) while Indian native dairy cows have only A2 allele and hence are a source for safe milk (Mishra and joshi 2009). Indian milk breeds, including Tharparkar produce 100% A2 milk.

When a cell experiences environmental stress, it stops or at least slows down most of its original functions, such as transport processes, DNA, RNA and protein synthesis. However, there are proteins which preferentially expressed under these restrictive conditions. HSP70 is one of these proteins. 70-kDa heat shock proteins (Hsp70s) assist a wide range of folding processes, including the folding and assembly of newly synthesized proteins, refolding of misfolded and aggregated proteins, membrane translocation of organellar and secretory proteins and control of the activity of regulatory proteins (Ryan and Pfanner, 2002; Pratt and Toft, 2003). Thus, polymorphism in HSP70 is a major importance in thermo-tolerance development in cattle. Bhat *et al*., (2016) studied the effect of heat shock protein 70 (HSP70) polymorphism on thermotolerance in Tharparkar cattle. They revealed, on sequencing, one single-nucleotide polymorphism with G > T substitution at a position that led to a change of amino acid aspartate to tyrosine in allele A. Thus, AA-genotype is superior was found to be most thermos-tolerant genotype with the highest heat tolerance coefficient (HTC).

Pattern Recognition Receptors (PRRs) are proteins capable of recognizing molecules frequently found in pathogens (the so-called Pathogen-Associated Molecular Patterns-PAMPs), or molecules released by damaged cells (the Damage-Associated Molecular Patterns-DAMPs). Common classes of PAMPs include lipopolysaccharide (cell wall of gram negative bacteria), peptidoglycan (cell wall of gram positive bacteria), polypeptide (*e.g.* flagellin) and nucleic acids (dsRNA in viruses). Similarly the common class of DAMPs is endogenous danger molecules that are released from damaged or dying cells and activate the innate immune system by interacting with PRRs. DAMPs contribute to the host defence system. PRRs are divided into two main groups, including membrane-bound PRRs known as Toll-like receptors (TLRs) and cytoplasmic PRRs (NOD-like-receptors [NLRs] and RIGI-like-receptors [RLRs]) (O'Donovan *et al*., 2020). Several previous studies, in both human and animals, have shown that sequence variations in TLR genes influences immune responsiveness, inadequate activation of the innate immune system, weakened recognition of pathogen's PAMP, hereafter interfere with innate-immune activation. The genetic variations in TLR2 gene may enhance the risk of infectious diseases in human and domestic animals. Iqbal *et al*., (2020) studied polymorphic patterns in the complete coding sequences of Toll-like receptor2 (TLR2) gene in the Tharparkar cattle breed originating in the Tharparkar district in Sindh province in Pakistan. They found that the genetic variations of TLR2 gene provided important genetic insight into disease resistance in Tharparkar cattle.

Genetic diversity and genomic assessment

Knowledge about genetic diversity is very essential for the management and sustainable utilization of livestock genetic resources. In view of this a recent genome-wide assessment of genetic diversity, linkage disequilibrium and haplotype block structure in Tharparkar cattle breed of India was studied by Saravanan *et al*. (2020) wherein they presented a comprehensive genome-wide analysis of genetic diversity, Region of Homozygosity (ROH), inbreeding, linkage disequilibrium, effective population size and haplotype block structure in Tharparkar cattle of India. For their studies, 24 Tharparkar cattle were selected and genotyped with the Illumina BovineSNP50 arrays. After quality control, 22,825 biallelic SNPs were retained, which were in Hardy–Weinberg equilibrium (HWE), Minor allele frequency (MAF) > 0.05 and the genotyping rate >90%. The overall mean observed and expected heterozygosity were 0.339±0.156 and 0.325±0.129, respectively. The average minor allele frequency was observed as 0.234 with a standard deviation of ± 0.131. They identified a total of 1832 Region of homozygosity (ROH) segments and also observed the highest autosomal coverage of 13.87% on chromosome 23.

Till now, genetic improvements of zebu /*Bos indicus* cattle breeds are less progressive as compared to Exotic / *Bos taurus* breeds due to insufficient molecular genetic information. However, due to advent of Next Generation Sequencing (NGS), cattle genomic studies are now well established. Available data of SNPs based on *Bos Taurus* (Iqbal *et al*., 2019). They also did a comparative analysis in various *Bos indicus* breeds of Pakistan using coding region SNPs, which revealed a close relationship between the best milking indigenous breeds; Red Sindhi and Sahiwal. However, Bhagnari and Tharparkar being popular for their adaptation to dry and extremely hot climates were found to share the highest number of SNPs. Besides, existing bovine SNP array chips have always predicted a significant number of monomorphic SNPs in case of zebu cattle breed. The density of SNPs present across the genome of *Bos indicus* was comparatively higher (1 SNP in every 285 bp) than that

of *Bos taurus* (1 SNP in every 714 bp) (Gibbs *et al.* 2009; Nayee *et al*., 2018). Identification of genome-wide SNPs and microsatellites and high-quality SNPs to milk production, fertility, carcass, adaptability and immune response of economically important traits was carried out in Tharparkar Breed (Devadasan *et al*., 2020). They summarised a total of 146,011 SNPs identified with respect to *Bos Taurus* reference genome, which are indicus specifc, out of which 10,519 SNPs were found to be novel. Similarly, a total of 87,047 SNPs was identified with respect to *Bos indicus* reference genome. After final annotation of SNPs identified with respect to *Bos indicus* reference genome, 2871 SNPs were found to be associated in 383 candidate genes having to do with milk production, fertility, carcass, immune response and adaptability traits. Following that, 2571 microsatellites were identified. The information mined from the data might be of importance for the future breed improvement programs, conservation efforts and for enhancing the SNPs density of the existing bovine SNP chips.

CONCLUSION

Survivability of the animal often depends on its ability to cope with or adapt to the existing environmental conditions. So to sustain livestock production in an environment challenged by climate change, the animals must be genetically suitable and have the ability to survive in diversified environments. Conventionally, identification of animals with superior genetic traits that were economically beneficial was the fundamental reason for identifying biomarkers in animals. Thus selection of favourable molecular markers or biomarkers for economical traits by which Tharparkar cattle can be improved are of paramount importance. Furthermore, the improved breeding, feeding and management practices are also important so that the genetic potential of Tharparkar cattle can be exploited for improvement of the breed. It is required to investigate more genetic markers to improve the productivity of the Tharparkar cattle.

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