

# Exploring Genetic Variability and Breed Differentiation in Indigenous Cattle Using High-Density SNP (777K) Genotyping

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## ABSTRACT

In this study, we investigated the informativeness of the bovine high-density SNP genotyping assay in Pakistani indigenous cattle breeds, which include Achi, Bhagnari, Cholistani, Dajal, Dhanni, Kankrej, Lohani, Red Sindhi, Sahiwal, and Tharparker. Our goal was to evaluate minor allele frequency (MAF), genetic diversity, and the extent of linkage disequilibrium (LD) in these breeds. Additionally, we examined the performance of common SNP found on both the bovine high-density SNP and 54K SNP chips. We found that only 44% to 56% of the SNP were informative for further analysis in these breeds. The overall MAF ranged from 0.23 to 0.18 across these breeds. The mean Reynolds's genetic distance between these breeds varied from 0.191 to 0.574. We observed the onset of LD decay at an inter-marker distance of 140 kb. Our study revealed that the high-density SNP chip offers an informative genotyping platform for mapping quantitative trait loci (QTL) in cattle. The array data indicates a significant and untapped resource that can contribute to the evaluation of complex economic traits and the establishment of marker-assisted selection programs in the cattle industry.

## Article Information

Received 14 April 2024

Revised 05 February 2026

Accepted 13 February 2026

Available online 10 September 2026 (early access)

## Authors' Contribution

HM, AM and IA conceived and designed the study. HM and MFS supervised the research work, analyzed the data, and revised the manuscript. ZI and GB contributed to data interpretation and manuscript preparation. HM and WAK wrote the original draft of the manuscript. All authors read and approved the final version of the manuscript.

## Key words

Minor allele frequency, SNP polymorphism, Linkage disequilibrium, Cattle, Diversity

## INTRODUCTION

High-density single nucleotide polymorphism (SNP) genotyping has emerged as a preferred method for studying genetic variants due to its extensive coverage of the genome and the stability of SNP markers (Dadi *et al.*, 2012). The bovine high-density SNP BeadChip, a widely utilized genotyping array, provides information on 777,962 SNP markers and has been validated in various

cattle breeds, including both indicine and taurine breeds (Matukumalli *et al.*, 2009). These high-density SNP chips, such as the HD and 54K arrays, have proven invaluable for assessing cattle diversity and relationships (Decker *et al.*, 2014; Makina *et al.*, 2014), investigating linkage disequilibrium patterns (Mokry *et al.*, 2014; Makina *et al.*, 2015), conducting genome-wide association studies (Iso-Touru *et al.*, 2016), and identifying selection signatures (Zhao *et al.*, 2015; Stella *et al.*, 2010).

However, the effective application of high-density SNP genotyping in indigenous cattle breeds hinges on several crucial factors. These include the level of polymorphism exhibited by SNP markers, the distribution of minor allele frequency (MAF) within the population (Matukumalli *et al.*, 2009), and the extent of linkage disequilibrium (LD) among neighboring markers. Understanding these factors is essential for successful selection and breeding strategies tailored to the specific needs of indigenous cattle populations (Makina *et al.*, 2015).

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0030-9923/2026/0001-0001 \$ 9.00/0



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In this context, the present study aimed to assess the MAF, extent of LD, and genetic diversity within Pakistani cattle breeds. The bovine high-density SNP genotyping assay will serve as a powerful tool in deciphering the genetic landscape of indigenous cattle populations, including Achi, Bhagnari, Cholistani, Dajal, Dhanni, Kankrej, Lohani, Red Sindhi, Sahiwal, and Tharparker breeds. By evaluating the performance of these high-density SNP chips in these specific breeds, we can shed light on the suitability of this genotyping platform for QTL mapping, identification of complex economic traits, and the development of marker-assisted selection programs within the Pakistani cattle industry.

This study holds significant promise for advancing our understanding of the genetic architecture and diversity of Pakistani indigenous cattle breeds, enabling more targeted and efficient breeding programs to improve desired traits and overall productivity in these valuable livestock populations.

## MATERIALS AND METHODS

### *Animal sampling and genotyping*

Samples of Achi, Bhagnari, Cholistani, Dajal, Dhanni, Kankrej, Lohani, Red Sindhi, Sahiwal, and Tharparker cattle were collected from various government livestock stations ([Supplementary Table I](#)). A 10 mL jugular blood sample was collected from each animal using a Falcon tube containing EDTA as an anticoagulant. Genomic DNA was extracted from the blood samples using organic methods as described in a previous study by [Mustafa \*et al.\* \(2017\)](#). The quality of the extracted genomic DNA was assessed using gel electrophoresis to ensure integrity, and the quantity was measured using a Nanodrop Spectrophotometer (Nanodrop ND-1000). Genotyping was performed at BFG, USDA, USA, using the Bovine high-density SNP BeadChip, which contains 777,962 SNP. The genotyping process followed the standard manufacturer's protocol (Illumina, Inc., San Diego, CA, USA). Genotyping data from a 54K SNP chip were retrieved from [Decker \*et al.\* \(2014\)](#).

### *Quality control (QC)*

To ensure data quality, SNP located on the sex chromosomes (X and Y), mitochondrial DNA (mtDNA), and unmapped SNP were excluded from the dataset, focusing only on autosomes for further analysis. Samples containing more than 10% missing genotypes were excluded from the analysis. SNP that had a MAF of  $\leq 0.05$ , a call rate  $\leq 95\%$ , and exhibited departure from Hardy-Weinberg equilibrium (HWE) ( $p \leq 0.001$ ) were eliminated from further analysis. Quality control procedures were

performed using PLINK software ([Purcell \*et al.\*, 2007](#)). Similar quality control measures were applied to both the bovine high-density SNP and 54K SNP BeadChip for common SNP.

### *MAF*

MAF was computed for each breed across all 29 autosomes using the default settings in PLINK version 1.9 ([Purcell \*et al.\*, 2007](#)). Differences in MAF among breeds were assessed using the SPSS package, with a significance level set at  $p < 0.001$ . SNP were classified into four MAF categories: MAF = 0 (monomorphic),  $0 < \text{MAF} < 0.05$  (rare),  $0.05 \leq \text{MAF} < 0.1$  (intermediate), and  $0.1 \leq \text{MAF} \leq 0.5$  (common). The proportions of SNP for each category were determined for both the 777K and 54K chips in all the analyzed breeds.

### *LD level*

LD was assessed in the selected breeds by computing the correlation coefficient ( $r^2$ ) for allele frequencies at pairs of loci using PLINK ([Purcell \*et al.\*, 2007](#)). Pairwise linkage disequilibrium ( $r^2$ ) was estimated between adjacent SNP across all 29 autosomes for each breed, utilizing both the Bovine HD SNP BeadChip and the 54K SNP BeadChip. To examine LD decay, the genome was divided into 10 bins based on inter-marker interval (20 kb). The average linkage disequilibrium ( $r^2$ ) was calculated and plotted against each bin.

### *Diversity analysis*

Diversity was measured using a set of LD-pruned markers, where SNP in strong LD with an  $r^2$  value greater than 0.5 were excluded from the analysis. The Reynolds's genetic distance between breeds and the average heterozygosity for each breed across the SNP were calculated using ARLEQUIN software ([Excoffier \*et al.\*, 2005](#)). The Reynolds's genetic distance provides a measure of genetic differentiation between breeds based on allele frequencies, while average heterozygosity reflects the level of genetic variation within each breed.

## RESULTS

### *Descriptive statistics of SNP*

After passing the quality control criteria, a total of 345,432 (44%), 404,223 (52%), 410,059 (53%), 437,183 (56%), 411,843 (53%), 426,137 (55%), 409,186 (53%), 433,812 (53%), 344,889 (44%), and 404,331 (53%) SNP were observed out of 777,962 SNP in Achi, Bhagnari, Cholistani, Dajal, Dhanni, Kankrej, Lohani, Red Sindhi, Sahiwal, and Tharparker breeds, respectively ([Table I](#)). For the 54K BeadChip, the number of informative SNP

in these breeds was 17, 182 (32%), 19,598 (36%), 19,771 (37%), 19,671 (36%), 19,961 (37%), 19,781 (37%), 19,216 (36%), 19,430 (36%), 17,165 (32%), and 19,597 (36%). After applying the filtering criteria, the retained SNP covered a total genomic region of 2510.4 Mb across all

autosomes. Among the autosomes, BTA1 had the longest length of 158.49 Mb, while BTA25 had the shortest length of 42.9 Mb. BTA1 also exhibited the highest number of filtered SNP, whereas BTA26 had the lowest number (Table II).

**Table I. SNP filter after quality control (QC) from the bovine high density (777K) SNP and 54K BeadChips in Pakistani indigenous breeds.**

| Breed      | N  | SNP Call Rate ( $\leq 95\%$ ) | MAF ( $\leq 0.05$ ) | HWE ( $p < 0.001$ ) | Filtered SNP     |
|------------|----|-------------------------------|---------------------|---------------------|------------------|
| Achi       | 18 | 121 095 (4 865) *             | 30 9966 (28 425)    | 236 (07)            | 345 432 (17 182) |
| Bhagnari   | 14 | 37 722 (3 422)                | 30 3975 (27 189)    | 235 (24)            | 404 223 (19 598) |
| Cholistani | 13 | 29 198 (3 491)                | 30 3690 (27 078)    | 304 (25)            | 410 059 (19 771) |
| Dhanni     | 10 | 24 893 (3 488)                | 30 2812 (27 116)    | 233 (27)            | 437 183 (19 671) |
| Dajal      | 10 | 28 876 (3 475)                | 30 3317 (27 551)    | 239 (24)            | 411 843 (19 961) |
| Kankraj    | 12 | 29 831 (3 446)                | 30 2894 (27 801)    | 241 (23)            | 426 137 (19 781) |
| Red Sindhi | 13 | 29 973 (3 802)                | 30 3932 (27 541)    | 237 (22)            | 409 186 (19 216) |
| Lohani     | 19 | 29 741 (3 881)                | 30 4471 (27 844)    | 242 (25)            | 433 812 (19 430) |
| Sahiwal    | 14 | 121 011 (4 863)               | 30 9933 (28 426)    | 236 (07)            | 344 889 (17 165) |
| Tharparkar | 13 | 37 744 (3 422)                | 30 3881 (27 188)    | 236 (26)            | 404 331 (19 597) |

mt; mitochondrial, MAF; minor allele frequency, HWE; Hardy-Weinberg equilibrium; {Unmapped, sex chromosomes, mt SNP number for HD Chip (42 699) and for 54K chip (2 131)}. \*Parentheses values indicate 54K chip SNP.

**Table II. Chromosome-wise filtered SNP numbers in indigenous Pakistani cattle breeds for both the high density (777k) SNP and 54K SNP BeadChips.**

| BTA | length (Mb) | Achi           | Bhagnari      | Cholistani    | Dajal         | Dhanni        | Kankraj       | Lohani        | Red Sindhi    | Sahiwal       | Tharparkar    |
|-----|-------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 1   | 158.49      | 22 811 (1163)* | 22 811 (1312) | 29 887 (1163) | 28 897 (1163) | 29 887 (1321) | 25 811 (1242) | 26 417 (1301) | 29 887 (1132) | 22 811 (1163) | 26 676 (1255) |
| 2   | 136.7       | 19 443 (956)   | 19 443 (944)  | 24 629 (936)  | 27 925 (936)  | 24 629 (942)  | 24 509 (1001) | 22 355 (1036) | 24 629 (1007) | 19 443 (936)  | 22 629 (1006) |
| 3   | 121.5       | 17 509 (901)   | 22 811 (987)  | 22 509 (877)  | 25 880 (877)  | 22 509 (998)  | 23 905 (978)  | 20 150 (938)  | 22 509 (981)  | 17 509 (877)  | 20 010 (927)  |
| 4   | 120.5       | 16 671 (862)   | 24 509 (871)  | 20 905 (874)  | 22 978 (874)  | 20 905 (982)  | 22 170 (874)  | 19 911 (921)  | 20 905 (981)  | 16 671 (874)  | 18 905 (962)  |
| 5   | 121.2       | 16 232 (722)   | 23 905 (820)  | 19 170 (724)  | 22 966 (724)  | 19 170 (887)  | 21 485 (724)  | 19 966 (819)  | 19 170 (891)  | 16 232 (724)  | 19 170 (886)  |
| 6   | 119.3       | 19 380 (1022)  | 22 170 (1039) | 22 485 (1047) | 22 510 (1047) | 22 485 (1033) | 21 071 (1063) | 22 110 (1179) | 22 485 (1091) | 19 380 (1047) | 22 485 (1144) |
| 7   | 112.2       | 16 432 (756)   | 21 485 (761)  | 21 671 (796)  | 22 485 (796)  | 21 671 (841)  | 20 452 (987)  | 21 554 (906)  | 21 671 (897)  | 16 432 (796)  | 19 671 (1023) |
| 8   | 113.4       | 17 296 (732)   | 21 071 (769)  | 20 452 (796)  | 21 671 (796)  | 20 452 (752)  | 20 318 (881)  | 20 004 (887)  | 20 452 (877)  | 17 296 (796)  | 20 452 (872)  |
| 9   | 106.5       | 16 818 (801)   | 20 452 (903)  | 20 318 (805)  | 20 452 (805)  | 20 318 (993)  | 20 214 (807)  | 19 368 (840)  | 20 318 (798)  | 16 818 (805)  | 19 001 (869)  |
| 10  | 104.4       | 13 914 (747)   | 20 318 (791)  | 19 914 (762)  | 20 318 (762)  | 19 914 (886)  | 19 198 (803)  | 15 772 (796)  | 19 914 (788)  | 13 914 (762)  | 16 022 (803)  |

Table continues on next page.....

| BTA   | length (Mb) | Achi               | Bhagnari           | Cholistani         | Dajal              | Dhanni             | Kankraj            | Lohani             | Red Sindhi         | Sahiwal            | Tharparkar         |
|-------|-------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 11    | 107.1       | 13 151<br>(871)    | 20 214<br>(867)    | 18 151<br>(807)    | 20 914<br>(807)    | 18 151<br>(798)    | 15 151<br>(789)    | 16 720<br>(855)    | 18 151<br>(914)    | 14 151<br>(807)    | 17 571<br>(836)    |
| 12    | 91.3        | 12 872<br>(541)    | 19 198<br>(898)    | 16 872<br>(874)    | 19 198<br>(567)    | 16 872<br>(587)    | 12 872<br>(698)    | 15 418<br>(642)    | 14 984<br>(671)    | 12 872<br>(567)    | 14 584<br>(707)    |
| 13    | 84.1        | 11 361<br>(572)    | 11 361<br>(631)    | 15 361<br>(976)    | 18 989<br>(596)    | 14 361<br>(596)    | 14 361<br>(667)    | 12 990<br>(637)    | 13 502<br>(781)    | 11 361<br>(596)    | 13 502<br>(611)    |
| 14    | 83.7        | 12 109<br>(681)    | 13 109<br>(673)    | 14 109<br>(650)    | 17 361<br>(711)    | 13 109<br>(650)    | 13 109<br>(891)    | 17 345<br>(723)    | 15 313<br>(771)    | 11 109<br>(650)    | 15 313<br>(879)    |
| 15    | 85.3        | 11 295<br>(601)    | 11 295<br>(647)    | 13 295<br>(861)    | 14 109<br>(852)    | 12 295<br>(849)    | 15 295<br>(761)    | 14 079<br>(657)    | 15 374<br>(701)    | 10 295<br>(605)    | 11 334<br>(561)    |
| 16    | 81.9        | 10 778<br>(429)    | 11 778<br>(754)    | 11 978<br>(762)    | 12 778<br>(607)    | 11 978<br>(753)    | 13 978<br>(791)    | 14 476<br>(573)    | 14 391<br>(720)    | 10 978<br>(507)    | 12 341<br>(672)    |
| 17    | 75.3        | 10 871<br>(402)*   | 10 971<br>(661)    | 10 971<br>(590)    | 10 871<br>(954)    | 10 971<br>(779)    | 12 971<br>(891)    | 12 073<br>(444)    | 13 155<br>(611)    | 10 871<br>(560)    | 13 155<br>(558)    |
| 18    | 66.8        | 9 190<br>(514)     | 9 171<br>(566)     | 9 190<br>(678)     | 9 190<br>(771)     | 10 190<br>(587)    | 10 990<br>(788)    | 9 601<br>(507)     | 12 127<br>(622)    | 9 290<br>(478)     | 11 127<br>(476)    |
| 19    | 63.7        | 7 786<br>(301)     | 7 736<br>(432)     | 7 786<br>(523)     | 7 786<br>(613)     | 10 786<br>(691)    | 9 986<br>(613)     | 9 544<br>(501)     | 9 786<br>(561)     | 7 786<br>(423)     | 10 097<br>(475)    |
| 20    | 71.7        | 10 473<br>(691)    | 10 473<br>(656)    | 10 473<br>(560)    | 10 483<br>(560)    | 10 473<br>(568)    | 10 473<br>(560)    | 9 355<br>(409)     | 10 573<br>(560)    | 10 473<br>(563)    | 10 319<br>(643)    |
| 21    | 71.5        | 9 945<br>(511)     | 9 945<br>(531)     | 9 945<br>(583)     | 9 995<br>(789)     | 9 945<br>(551)     | 11 945<br>(463)    | 11 360<br>(539)    | 10 941<br>(463)    | 9 968<br>(463)     | 12 279<br>(433)    |
| 22    | 61.4        | 8 671<br>(415)     | 8 871<br>(741)     | 8 871<br>(615)     | 8 871<br>(725)     | 8 871<br>(595)     | 10 671<br>(565)    | 97 83<br>(529)     | 10 871<br>(715)    | 8 891<br>(515)     | 10 293<br>(537)    |
| 23    | 52.4        | 7 651<br>(672)     | 7 651<br>(681)     | 7 651<br>(634)     | 7 651<br>(634)     | 7 681<br>(678)     | 9 551<br>(634)     | 9 265<br>(518)     | 7 751<br>(634)     | 7 687<br>(634)     | 8 582<br>(751)     |
| 24    | 62.2        | 6 681<br>(471)     | 6 681<br>(451)     | 6 681<br>(545)     | 6 681<br>(783)     | 6 681<br>(395)     | 9 381<br>(345)     | 6 919<br>(363)     | 6 621<br>(345)     | 6 681<br>(345)     | 7 090<br>(349)     |
| 25    | 42.91       | 5 720<br>(243)     | 5 722<br>(198)     | 5 720<br>(462)     | 5 742<br>(262)     | 5 790<br>(379)     | 8 820<br>(362)     | 4 388<br>(220)     | 9 740<br>(162)     | 5 738<br>(162)     | 4 582<br>(198)     |
| 26    | 51.8        | 3 400<br>(174)     | 3 400<br>(365)     | 3 400<br>(243)     | 3 400<br>(156)     | 3 890<br>(178)     | 7 400<br>(152)     | 8 168<br>(376)     | 7 590<br>(191)     | 3 480<br>(156)     | 6 875<br>(333)     |
| 27    | 45.5        | 6 084<br>(144)     | 6 084<br>(282)     | 6 084<br>(291)     | 6 034<br>(214)     | 6 284<br>(267)     | 7 064<br>(211)     | 7 127<br>(378)     | 7 184<br>(125)     | 6 098<br>(114)     | 7 151<br>(294)     |
| 28    | 46.3        | 5 565<br>(135)     | 5 865<br>(240)     | 5 866<br>(151)     | 5 575<br>(174)     | 5 852<br>(193)     | 6 663<br>(124)     | 6 949<br>(367)     | 6 865<br>(324)     | 5 575<br>(124)     | 7 037<br>(276)     |
| 29    | 51.3        | 5 323<br>(136)     | 5 723<br>(122)     | 5 715<br>(186)     | 5 473<br>(116)     | 5 723<br>(232)     | 6 323<br>(116)     | 6 019<br>(355)     | 6 953<br>(116)     | 5 079<br>(116)     | 6 078<br>(261)     |
| Total | 2510.4      | 34 5432<br>(17166) | 40 4223<br>(19593) | 41 0059<br>(19771) | 43 7183<br>(19671) | 41 1843<br>(19961) | 42 6137<br>(19781) | 40 9186<br>(19216) | 43 3812<br>(19430) | 34 4889<br>(17165) | 40 4331<br>(19597) |

\*Parentheses values indicate 54K chip SNP.

#### SNP polymorphism

Figures 1 and 2 depict the approximate proportions of SNP in these breeds. Approximately 33% of the bovine high-density SNP were monomorphic (0), while 46% of the 54K BeadChip SNP were monomorphic in these breeds. The proportions of minor allele frequencies for the bovine high-density SNP BeadChip ranged from

11.5% in Dhanni, Dajal, and Kankrej to 10.8% in Achi, and from 12.8% in Tharparkar to 12.1% in Sahiwal. For the 54K BeadChip, the proportion of common SNP ranged from 56.2% in Dhanni to 44.3% in Sahiwal cattle. The proportion of common SNP on the 54K BeadChip varied across the breeds, ranging from 32% in Achi and Sahiwal cattle to 37% in Cholistani, Dhanni, and Kankrej cattle.

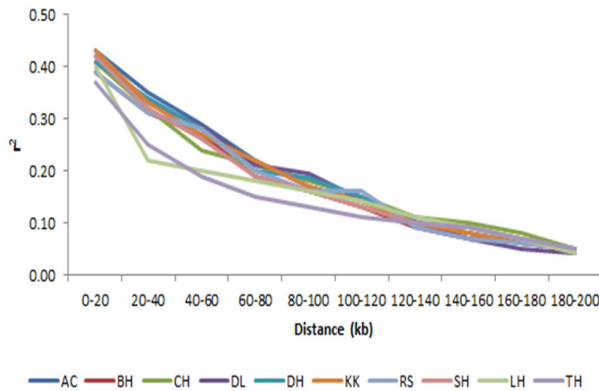


Fig. 1. LD ( $r^2$ ) decay in indigenous Pakistani breeds.

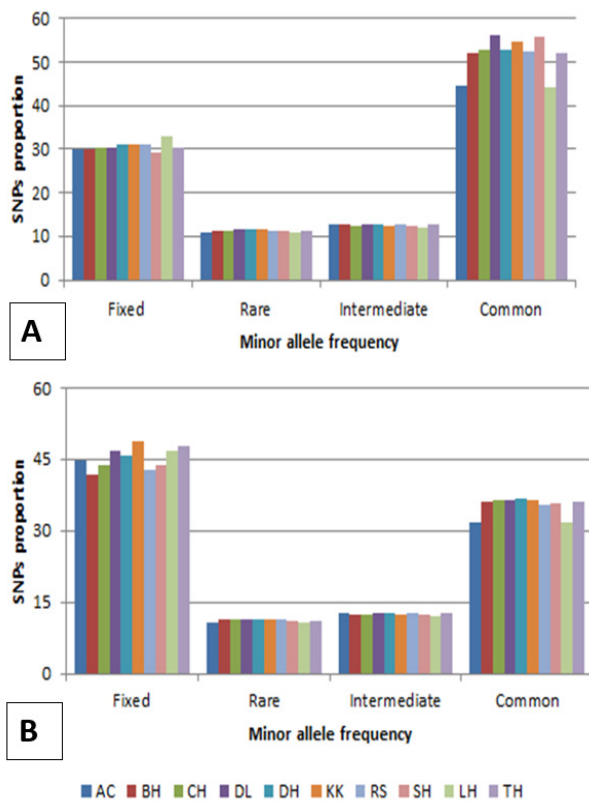


Fig. 2. SNPs proportion under different minor allele frequencies for Bovine HD SNPs (A), and the 54K SNPs (B) BeadChip in indigenous breeds.

#### MAF

Table IV presents the average MAF for each chromosome in the examined breeds. The Bovine high-density SNP BeadChip showed an overall mean MAF ranging from 0.23 to 0.18. In contrast, the overall mean MAF for the 54K SNP BeadChip was relatively lower.

Notably, the Tharparker and Kankrej breeds exhibited significantly lower overall mean MAF (0.01) for both BeadChips, indicating a higher abundance of polymorphic markers in these two breeds.

#### LD level

The whole-genome LD analysis was performed for the first time in these breeds, with an overall mean  $r^2$  value of 0.33 and 0.35 in Lohani cattle using both BeadChips (Table III).

#### Genetic analysis

Genetic diversity was assessed by calculating the expected (HE) and observed (HO) heterozygosity using a dataset consisting of 138,181 and 21,532 SNP from the high-density (HD) and 54K BeadChips, respectively, as presented in Table V. Prior to analyzing genetic diversity, SNP with high LD ( $r^2 > 0.5$ ) were removed. The values of HO and HE were found to be highly similar, indicating a random sampling of these breeds. Among the breeds, Tharparker demonstrated relatively higher diversity with an HO value of 0.733, compared to Lohani (HO= 0.722) and other breeds. Table VI illustrates the Reynold's genetic distance in these breeds. The genetic distance across breeds ranged from 0.191 (between Achi and Tharparker) to 0.574 (between Dajal and Bhagnari) based on the Bovine high-density SNP BeadChip. On the 54K BeadChip, the genetic distance varied from 0.037 (between Red Sindhi and Achi) to 0.394 (between Dajal and Bhagnari).

## DISCUSSION

The Illumina Bovine Chips are a very informative source of SNP for different applications in the cattle industry. The most important application of these chips in cattle might be genome-wide association studies and genomic selection for the improvement of milk and meat traits. The discoveries of thousands of SNP have made it possible to compute appropriate and accurate genomic breeding values for breed improvement (Meuwissen *et al.*, 2001; VanRaden *et al.*, 2009). Nowadays, BeadChips are routinely used in the cattle industry in different countries for selection and breed improvement programs (Hayes *et al.*, 2009).

In this study, we observed 345,432 (44%), 404,223 (52%), 410,059 (53%), 437,183 (56%), 411,843 (53%), 426,137 (55%), 409,186 (53%), 433,812 (53%), 344,889 (44%), 404,331 (53%) SNP of the bovine high-density SNP numbers in Achi, Bhagnari, Cholistani, Dajal, Dhanni, Kankrej, Lohani, Red Sindhi, Sahiwal, and Tharparker, respectively. The informative SNP number of the 54K BeadChip in these breeds was 17,182 (32%), 19,598 (36%),

**Table III. Chromosome-wise mean inter-marker distance (kb) in indigenous Pakistani cattle breeds for the bovine high density (777K) and 54K SNP chips.**

| BTA | Achi             | Bhagnari         | Cholistani       | Dajal            | Dhanni           | Kankraj          | Lohani           | Red Sindhi       | Sahiwal          | Tharparkar       |
|-----|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 1   | 6.94<br>(135.77) | 6.14<br>(135.77) | 6.94<br>(135.77) | 6.34<br>(135.77) | 6.94<br>(135.77) | 6.94<br>(135.77) | 5.99<br>(125.87) | 6.94<br>(135.77) | 6.94<br>(135.77) | 5.93<br>(121.41) |
| 2   | 7.02<br>(145.65) | 7.02<br>(132.56) | 7.02<br>(145.65) | 7.02<br>(145.65) | 7.02<br>(145.65) | 7.02<br>(145.65) | 6.12<br>(135.96) | 7.02<br>(145.65) | 7.02<br>(145.65) | 6.04<br>(132.04) |
| 3   | 6.93<br>(137.63) | 6.03<br>(137.63) | 6.23<br>(137.63) | 6.93<br>(137.63) | 6.93<br>(137.63) | 6.93<br>(137.63) | 6.02<br>(130.45) | 6.93<br>(137.63) | 6.93<br>(137.63) | 6.06<br>(128.91) |
| 4   | 7.23<br>(137.63) | 7.13<br>(137.63) | 7.23<br>(137.63) | 7.23<br>(137.63) | 7.23<br>(137.63) | 7.23<br>(137.63) | 7.06<br>(124.86) | 7.23<br>(137.63) | 7.23<br>(137.63) | 6.38<br>(130.65) |
| 5   | 7.46<br>(166.74) | 7.21<br>(166.74) | 7.16<br>(166.74) | 7.46<br>(166.74) | 7.46<br>(166.74) | 7.46<br>(166.74) | 6.07<br>(136.81) | 7.46<br>(166.74) | 7.46<br>(166.74) | 6.31<br>(146.61) |
| 6   | 6.16<br>(113.17) | 6.16<br>(113.17) | 6.16<br>(113.17) | 5.16<br>(113.17) | 6.16<br>(113.17) | 6.16<br>(113.17) | 5.40<br>(103.89) | 6.16<br>(113.17) | 6.16<br>(113.17) | 5.31<br>(100.74) |
| 7   | 6.85<br>(141.20) | 6.05<br>(141.20) | 6.85<br>(141.20) | 6.85<br>(141.20) | 6.85<br>(112.15) | 6.85<br>(141.20) | 5.22<br>(109.95) | 6.85<br>(141.20) | 6.85<br>(141.20) | 5.72<br>(123.77) |
| 8   | 6.54<br>(141.72) | 6.14<br>(112.12) | 6.54<br>(141.72) | 6.54<br>(141.72) | 6.54<br>(141.72) | 6.54<br>(141.72) | 5.65<br>(124.50) | 6.54<br>(141.72) | 6.54<br>(141.72) | 5.53<br>(127.17) |
| 9   | 6.28<br>(130.21) | 6.08<br>(130.21) | 6.18<br>(130.21) | 6.28<br>(130.21) | 6.28<br>(130.21) | 6.28<br>(130.21) | 5.46<br>(120.61) | 6.28<br>(130.21) | 6.28<br>(130.21) | 5.56<br>(124.91) |
| 10  | 7.49<br>(135.16) | 7.49<br>(135.16) | 7.29<br>(135.16) | 7.49<br>(135.16) | 7.49<br>(135.16) | 7.49<br>(135.16) | 6.61<br>(128.25) | 7.49<br>(135.16) | 7.49<br>(135.16) | 6.51<br>(129.38) |
| 11  | 7.08<br>(132.67) | 7.08<br>(132.67) | 7.08<br>(132.67) | 7.08<br>(132.67) | 7.08<br>(131.11) | 6.08<br>(132.67) | 6.41<br>(128.31) | 7.08<br>(132.67) | 7.08<br>(132.67) | 6.10<br>(125.36) |
| 12  | 7.41<br>(160.42) | 7.01<br>(160.42) | 7.41<br>(160.42) | 6.41<br>(160.42) | 7.41<br>(160.42) | 7.41<br>(160.42) | 5.90<br>(128.61) | 7.41<br>(160.42) | 7.41<br>(160.42) | 6.25<br>(141.65) |
| 13  | 6.43<br>(140.29) | 6.13<br>(140.29) | 6.13<br>(140.29) | 6.43<br>(140.29) | 6.43<br>(140.29) | 6.43<br>(140.29) | 5.90<br>(137.23) | 6.43<br>(140.29) | 6.43<br>(140.29) | 6.24<br>(131.82) |
| 14  | 7.54<br>(128.09) | 7.04<br>(128.09) | 4.14<br>(128.09) | 7.24<br>(128.09) | 7.54<br>(110.14) | 7.54<br>(128.09) | 6.48<br>(94.68)  | 7.54<br>(128.09) | 7.54<br>(128.09) | 5.44<br>(115.17) |
| 15  | 6.81<br>(139.56) | 6.11<br>(139.56) | 5.21<br>(139.56) | 6.21<br>(139.56) | 6.81<br>(139.56) | 4.81<br>(139.56) | 6.48<br>(110.91) | 6.81<br>(139.56) | 6.81<br>(139.06) | 6.39<br>(128.49) |
| 16  | 6.15<br>(133.34) | 6.75<br>(111.13) | 6.85<br>(133.34) | 6.35<br>(112.12) | 6.85<br>(133.34) | 5.85<br>(133.34) | 4.80<br>(120.42) | 6.85<br>(133.34) | 6.85<br>(145.11) | 5.69<br>(120.24) |
| 17  | 7.17<br>(126.11) | 5.17<br>(101.23) | 7.17<br>(120.10) | 5.17<br>(126.11) | 7.17<br>(126.11) | 7.17<br>(126.11) | 5.65<br>(113.85) | 7.17<br>(126.11) | 7.17<br>(126.11) | 5.39<br>(115.98) |
| 18  | 8.12<br>(136.90) | 7.12<br>(101.13) | 4.12<br>(136.90) | 6.20<br>(136.90) | 8.20<br>(141.11) | 8.20<br>(136.90) | 5.64<br>(137.47) | 8.20<br>(136.90) | 8.20<br>(136.19) | 5.69<br>(129.05) |
| 19  | 6.17<br>(128.22) | 6.20<br>(131.10) | 6.27<br>(122.81) | 5.37<br>(153.45) | 6.87<br>(153.45) | 4.87<br>(153.45) | 5.75<br>(133.38) | 6.87<br>(112.14) | 6.87<br>(153.45) | 5.67<br>(126.44) |
| 20  | 7.20<br>(127.39) | 7.20<br>(121.34) | 7.20<br>(131.81) | 6.20<br>(127.39) | 7.20<br>(127.39) | 7.20<br>(140.00) | 6.21<br>(111.27) | 7.20<br>(127.39) | 7.20<br>(112.14) | 5.92<br>(117.12) |
| 21  | 6.19<br>(147.32) | 6.00<br>(122.67) | 4.19<br>(147.32) | 6.19<br>(147.32) | 6.19<br>(147.32) | 6.90<br>(147.32) | 6.70<br>(132.68) | 6.90<br>(147.32) | 6.90<br>(100.01) | 6.38<br>(127.18) |
| 22  | 6.36<br>(118.87) | 6.16<br>(118.87) | 4.86<br>(118.87) | 6.86<br>(103.01) | 6.26<br>(110.12) | 6.86<br>(101.14) | 6.30<br>(113.30) | 6.86<br>(118.87) | 6.86<br>(118.07) | 5.84<br>(110.66) |

*Table continues on next page.....*

| BTA   | Achi              | Bhagnari          | Cholistani        | Dajal             | Dhanni            | Kankraj           | Lohani            | Red Sindhi       | Sahiwal           | Tharparkar        |
|-------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|-------------------|
| 23    | 7.48<br>(234.73)  | 7.18<br>(234.73)  | 7.48<br>(234.73)  | 7.18<br>(112.17)  | 7.18<br>(221.15)  | 7.48<br>(103.18)  | 6.28<br>(183.44)  | 7.48<br>(134.14) | 7.48<br>(234.03)  | 5.85<br>(207.23)  |
| 24    | 6.11<br>(123.90)  | 6.71<br>(123.90)  | 6.21<br>(122.77)  | 6.21<br>(131.01)  | 6.21<br>(123.90)  | 6.50<br>(123.90)  | 5.12<br>(122.84)  | 6.40<br>(123.90) | 7.11<br>(123.10)  | 5.95<br>(117.55)  |
| 25    | 7.46<br>(314.41)  | 7.46<br>(121.01)  | 7.16<br>(281.66)  | 7.46<br>(401.12)  | 7.46<br>(314.41)  | 5.46<br>(314.41)  | 6.20<br>(254.04)  | 7.46<br>(314.41) | 7.46<br>(314.01)  | 6.11<br>(232.44)  |
| 26    | 14.96<br>(132.10) | 10.23<br>(266.54) | 12.11<br>(286.01) | 12.33<br>(286.01) | 12.44<br>(114.10) | 11.24<br>(114.01) | 11.71<br>(137.14) | 7.77<br>(286.01) | 14.13<br>(112.11) | 6.04<br>(120.67)  |
| 27    | 8.90<br>(289.92)  | 6.19<br>(407.89)  | 4.90<br>(401.18)  | 5.39<br>(401.18)  | 5.90<br>(401.18)  | 6.90<br>(401.18)  | 5.59<br>(157.36)  | 8.90<br>(210.17) | 8.90<br>(407.01)  | 11.23<br>(119.34) |
| 28    | 7.15<br>(407.61)  | 7.15<br>(407.61)  | 5.50<br>(201.11)  | 7.35<br>(221.16)  | 5.50<br>(403.11)  | 7.50<br>(407.61)  | 6.43<br>(179.86)  | 7.50<br>(407.61) | 7.50<br>(404.21)  | 6.49<br>(136.24)  |
| 29    | 8.19<br>(406.21)  | 7.19<br>(404.45)  | 8.19<br>(406.21)  | 6.29<br>(406.21)  | 6.90<br>(406.21)  | 7.90<br>(406.21)  | 7.36<br>(177.35)  | 8.90<br>(399.01) | 8.90<br>(406.11)  | 7.28<br>(134.32)  |
| $\mu$ | 7.30<br>(169.41)  | 6.74<br>(166.10)  | 6.54<br>(170.03)  | 6.73<br>(170.73)  | 7.05<br>(171.04)  | 6.94<br>(168.78)  | 6.22<br>(135.01)  | 7.19<br>(167.70) | 7.44<br>(171.62)  | 6.18<br>(131.81)  |

\*Parentheses values indicate 54K chip SNP.

**Table IV. Chromosome wise average minor allele frequency (MAF) distribution in indigenous Pakistani cattle.**

| BTA | Achi             | Bhagnari          | Cholistani       | Dajal            | Dhanni           | Kankraj          | Lohani           | Red Sindhi       | Sahiwal          | Tharparkar       |
|-----|------------------|-------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 1   | 0.247<br>(0.183) | 0.223<br>(0.223)* | 0.222<br>(0.222) | 0.200<br>(0.200) | 0.247<br>(0.191) | 0.247<br>(0.112) | 0.241<br>(0.112) | 0.231<br>(0.231) | 0.247<br>(0.247) | 0.212<br>(0.112) |
| 2   | 0.248<br>(0.193) | 0.221<br>(0.221)  | 0.221<br>(0.221) | 0.201<br>(0.201) | 0.248<br>(0.183) | 0.248<br>(0.164) | 0.220<br>(0.164) | 0.222<br>(0.222) | 0.248<br>(0.248) | 0.101<br>(0.164) |
| 3   | 0.250<br>(0.191) | 0.215<br>(0.215)  | 0.204<br>(0.204) | 0.204<br>(0.204) | 0.250<br>(0.193) | 0.250<br>(0.166) | 0.213<br>(0.166) | 0.221<br>(0.221) | 0.250<br>(0.112) | 0.200<br>(0.166) |
| 4   | 0.246<br>(0.199) | 0.246<br>(0.246)  | 0.246<br>(0.246) | 0.201<br>(0.201) | 0.200<br>(0.191) | 0.223<br>(0.245) | 0.215<br>(0.245) | 0.204<br>(0.112) | 0.223<br>(0.164) | 0.121<br>(0.245) |
| 5   | 0.253<br>(0.194) | 0.211<br>(0.180)  | 0.253<br>(0.253) | 0.221<br>(0.221) | 0.212<br>(0.199) | 0.221<br>(0.189) | 0.211<br>(0.189) | 0.246<br>(0.164) | 0.221<br>(0.166) | 0.202<br>(0.189) |
| 6   | 0.261<br>(0.199) | 0.201<br>(0.110)  | 0.261<br>(0.261) | 0.218<br>(0.218) | 0.201<br>(0.194) | 0.215<br>(0.122) | 0.211<br>(0.122) | 0.253<br>(0.166) | 0.215<br>(0.245) | 0.201<br>(0.122) |
| 7   | 0.252<br>(0.252) | 0.200<br>(0.180)  | 0.252<br>(0.131) | 0.221<br>(0.221) | 0.201<br>(0.199) | 0.246<br>(0.140) | 0.201<br>(0.140) | 0.261<br>(0.245) | 0.246<br>(0.189) | 0.180<br>(0.140) |
| 8   | 0.258<br>(0.164) | 0.201<br>(0.121)  | 0.258<br>(0.258) | 0.213<br>(0.213) | 0.200<br>(0.112) | 0.211<br>(0.181) | 0.200<br>(0.181) | 0.252<br>(0.189) | 0.211<br>(0.122) | 0.110<br>(0.181) |
| 9   | 0.255<br>(0.166) | 0.255<br>(0.110)  | 0.255<br>(0.161) | 0.206<br>(0.206) | 0.202<br>(0.164) | 0.201<br>(0.211) | 0.201<br>(0.211) | 0.258<br>(0.122) | 0.201<br>(0.140) | 0.180<br>(0.211) |
| 10  | 0.245<br>(0.245) | 0.245<br>(0.242)  | 0.245<br>(0.156) | 0.216<br>(0.216) | 0.210<br>(0.166) | 0.200<br>(0.170) | 0.255<br>(0.170) | 0.255<br>(0.140) | 0.200<br>(0.181) | 0.121<br>(0.170) |
| 11  | 0.248<br>(0.189) | 0.200<br>(0.244)  | 0.248<br>(0.125) | 0.200<br>(0.200) | 0.201<br>(0.245) | 0.201<br>(0.176) | 0.245<br>(0.176) | 0.245<br>(0.181) | 0.201<br>(0.211) | 0.110<br>(0.176) |
| 12  | 0.243<br>(0.243) | 0.201<br>(0.212)  | 0.202<br>(0.200) | 0.201<br>(0.131) | 0.200<br>(0.189) | 0.255<br>(0.184) | 0.200<br>(0.184) | 0.248<br>(0.211) | 0.255<br>(0.170) | 0.242<br>(0.184) |
| 13  | 0.252<br>(0.140) | 0.201<br>(0.101)  | 0.201<br>(0.101) | 0.201<br>(0.131) | 0.201<br>(0.122) | 0.125<br>(0.193) | 0.201<br>(0.193) | 0.202<br>(0.170) | 0.245<br>(0.176) | 0.244<br>(0.193) |
| 14  | 0.257<br>(0.181) | 0.212<br>(0.200)  | 0.214<br>(0.189) | 0.212<br>(0.121) | 0.202<br>(0.140) | 0.200<br>(0.180) | 0.201<br>(0.180) | 0.201<br>(0.176) | 0.200<br>(0.184) | 0.212<br>(0.180) |

Table continues on next page.....

| BTA | Achi             | Bhagnari         | Cholistani       | Dajal            | Dhanni           | Kankraj          | Lohani           | Red Sindhi       | Sahiwal          | Tharparkar       |
|-----|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 15  | 0.211<br>(0.211) | 0.200<br>(0.121) | 0.215<br>(0.188) | 0.256<br>(0.111) | 0.200<br>(0.181) | 0.101<br>(0.210) | 0.212<br>(0.210) | 0.214<br>(0.184) | 0.201<br>(0.193) | 0.101<br>(0.210) |
| 16  | 0.212<br>(0.170) | 0.212<br>(0.202) | 0.211<br>(0.178) | 0.234<br>(0.166) | 0.204<br>(0.211) | 0.201<br>(0.191) | 0.200<br>(0.191) | 0.215<br>(0.193) | 0.201<br>(0.180) | 0.200<br>(0.191) |
| 17  | 0.211<br>(0.176) | 0.201<br>(0.201) | 0.203<br>(0.189) | 0.227<br>(0.154) | 0.202<br>(0.170) | 0.212<br>(0.110) | 0.202<br>(0.110) | 0.211<br>(0.180) | 0.212<br>(0.210) | 0.121<br>(0.110) |
| 18  | 0.203<br>(0.184) | 0.201<br>(0.280) | 0.212<br>(0.101) | 0.256<br>(0.143) | 0.201<br>(0.176) | 0.200<br>(0.171) | 0.201<br>(0.171) | 0.203<br>(0.210) | 0.200<br>(0.191) | 0.202<br>(0.171) |
| 19  | 0.212<br>(0.193) | 0.200<br>(0.110) | 0.200<br>(0.200) | 0.258<br>(0.121) | 0.203<br>(0.184) | 0.212<br>(0.162) | 0.201<br>(0.162) | 0.212<br>(0.191) | 0.212<br>(0.110) | 0.201<br>(0.162) |
| 20  | 0.212<br>(0.180) | 0.202<br>(0.180) | 0.200<br>(0.176) | 0.223<br>(0.154) | 0.202<br>(0.193) | 0.101<br>(0.121) | 0.200<br>(0.121) | 0.200<br>(0.110) | 0.201<br>(0.171) | 0.280<br>(0.121) |
| 21  | 0.210<br>(0.210) | 0.210<br>(0.121) | 0.201<br>(0.182) | 0.210<br>(0.155) | 0.212<br>(0.180) | 0.200<br>(0.122) | 0.202<br>(0.122) | 0.200<br>(0.171) | 0.201<br>(0.162) | 0.110<br>(0.122) |
| 22  | 0.211<br>(0.191) | 0.201<br>(0.110) | 0.204<br>(0.181) | 0.244<br>(0.171) | 0.200<br>(0.210) | 0.121<br>(0.121) | 0.210<br>(0.121) | 0.201<br>(0.162) | 0.244<br>(0.121) | 0.180<br>(0.121) |
| 23  | 0.211<br>(0.193) | 0.200<br>(0.234) | 0.201<br>(0.177) | 0.234<br>(0.156) | 0.201<br>(0.191) | 0.202<br>(0.202) | 0.201<br>(0.202) | 0.204<br>(0.121) | 0.251<br>(0.122) | 0.121<br>(0.202) |
| 24  | 0.210<br>(0.195) | 0.201<br>(0.222) | 0.221<br>(0.122) | 0.245<br>(0.167) | 0.203<br>(0.110) | 0.201<br>(0.201) | 0.200<br>(0.201) | 0.201<br>(0.122) | 0.211<br>(0.121) | 0.110<br>(0.201) |
| 25  | 0.211<br>(0.180) | 0.202<br>(0.181) | 0.218<br>(0.175) | 0.222<br>(0.122) | 0.201<br>(0.171) | 0.180<br>(0.180) | 0.201<br>(0.180) | 0.221<br>(0.121) | 0.212<br>(0.212) | 0.234<br>(0.180) |
| 26  | 0.210<br>(0.210) | 0.200<br>(0.177) | 0.221<br>(0.182) | 0.228<br>(0.171) | 0.200<br>(0.162) | 0.110<br>(0.110) | 0.200<br>(0.200) | 0.218<br>(0.218) | 0.219<br>(0.219) | 0.222<br>(0.121) |
| 27  | 0.210<br>(0.193) | 0.204<br>(0.182) | 0.213<br>(0.165) | 0.244<br>(0.156) | 0.201<br>(0.121) | 0.180<br>(0.180) | 0.214<br>(0.214) | 0.249<br>(0.249) | 0.201<br>(0.201) | 0.240<br>(0.121) |
| 28  | 0.216<br>(0.183) | 0.202<br>(0.191) | 0.206<br>(0.144) | 0.236<br>(0.152) | 0.203<br>(0.122) | 0.121<br>(0.121) | 0.200<br>(0.111) | 0.236<br>(0.236) | 0.200<br>(0.200) | 0.239<br>(0.239) |
| 29  | 0.212<br>(0.212) | 0.201<br>(0.188) | 0.216<br>(0.121) | 0.236<br>(0.144) | 0.236<br>(0.121) | 0.110<br>(0.110) | 0.201<br>(0.112) | 0.236<br>(0.236) | 0.212<br>(0.212) | 0.230<br>(0.230) |
| μ   | 0.230<br>(0.194) | 0.209<br>(0.183) | 0.222<br>(0.180) | 0.223<br>(0.170) | 0.208<br>(0.172) | 0.189<br>(0.164) | 0.209<br>(0.168) | 0.225<br>(0.181) | 0.219<br>(0.179) | 0.180<br>(0.170) |

\*Parentheses values indicate 54K chip SNP.

**Table V. Mean LD ( $r^2$ ) and heterozygosity ( $H_o$  and  $H_e$ ) in indigenous Pakistani breeds.**

| Breed      | Linkage dis-equilibrium (LD) |      | Observed heterozygosity ( $H_o$ ) |       | Expected heterozygosity ( $H_e$ ) |       |
|------------|------------------------------|------|-----------------------------------|-------|-----------------------------------|-------|
|            | 777K                         | 54K  | 777K                              | 54K   | 777K                              | 54K   |
| Achi       | 0.33                         | 0.23 | 0.691                             | 0.571 | 0.675                             | 0.535 |
| Bhagnari   | 0.33                         | 0.23 | 0.695                             | 0.545 | 0.692                             | 0.522 |
| Cholistani | 0.33                         | 0.23 | 0.704                             | 0.613 | 0.691                             | 0.561 |
| Dajal      | 0.33                         | 0.23 | 0.64                              | 0.56  | 0.675                             | 0.545 |
| Dhanni     | 0.33                         | 0.23 | 0.709                             | 0.634 | 0.692                             | 0.532 |
| Kankrej    | 0.33                         | 0.23 | 0.69                              | 0.567 | 0.672                             | 0.582 |
| Lohani     | 0.35                         | 0.23 | 0.722                             | 0.601 | 0.691                             | 0.531 |
| Red Sindhi | 0.33                         | 0.23 | 0.693                             | 0.567 | 0.691                             | 0.581 |
| Sahiwal    | 0.33                         | 0.23 | 0.703                             | 0.61  | 0.683                             | 0.593 |
| Tharparker | 0.33                         | 0.23 | 0.733                             | 0.632 | 0.683                             | 0.591 |

19,771 (37%), 19,671 (36%), 19,961 (37%), 19,781 (37%), 19,216 (36%), 19,430 (36%), 17,165 (32%), 19,597 (36%) after quality control filtration. The SNP numbers in this study are in close agreement with the previously reported SNP numbers (446,986 to 547,615) after filtration in different indicine breeds, namely Nellore, Sahiwal, Red Sindhi, and Gir cattle, using the bovine high-density SNP BeadChip (Espigolan *et al.*, 2013; Porto-Neto *et al.*, 2013; Makina *et al.*, 2014; Mustafa *et al.*, 2014, 2017). However, fewer numbers of filtered SNP (166,742 to 410,059) were also reported in Iranian and Indian cattle breeds (Karimi *et al.*, 2017; Dash *et al.*, 2017).

The average intermarker distance for the bovine high-density BeadChip varied from 6.21 to 7.72 kb in Lohani and Cholistani breeds, respectively. Similarly, for the 54K SNP BeadChip, the average intermarker distance ranged from 6.18 kb in Tharparker cattle to 7.44 kb in Sahiwal cattle.

**Table VI. Reynold's genetic distance based on the bovine HD SNP (below the diagonal) and 54K SNP (above the diagonal) chips among breeds.**

| Breed      | Achi  | Bhagnari | Cholistani | Dajal | Dhanni | Kankrej | Lohani | Red Sindhi | Sahiwal | Tharparker |
|------------|-------|----------|------------|-------|--------|---------|--------|------------|---------|------------|
| Achi       |       | 0.292    | 0.085      | 0.161 | 0.161  | 0.068   | 0.083  | 0.037      | 0.082   | 0.084      |
| Bhagnari   | 0.471 |          | 0.342      | 0.394 | 0.316  | 0.342   | 0.308  | 0.321      | 0.314   | 0.303      |
| Cholistani | 0.157 | 0.524    |            | 0.180 | 0.141  | 0.123   | 0.107  | 0.078      | 0.174   | 0.141      |
| Dajal      | 0.216 | 0.574    | 0.282      |       | 0.152  | 0.214   | 0.113  | 0.132      | 0.192   | 0.205      |
| Dhanni     | 0.137 | 0.513    | 0.208      | 0.244 |        | 0.106   | 0.083  | 0.084      | 0.087   | 0.112      |
| Kankrej    | 0.163 | 0.531    | 0.231      | 0.301 | 0.266  |         | 0.104  | 0.101      | 0.192   | 0.182      |
| Lohani     | 0.143 | 0.509    | 0.206      | 0.27  | 0.195  | 0.222   |        | 0.066      | 0.084   | 0.131      |
| Red Sindhi | 0.127 | 0.506    | 0.186      | 0.265 | 0.192  | 0.217   | 0.187  |            | 0.089   | 0.121      |
| Sahiwal    | 0.137 | 0.509    | 0.205      | 0.27  | 0.196  | 0.222   | 0.192  | 0.189      |         | 0.115      |
| Tharparker | 0.191 | 0.539    | 0.254      | 0.315 | 0.243  | 0.267   | 0.24   | 0.236      | 0.240   |            |

Furthermore, for the 54K SNP BeadChip, the average intermarker distance ranged from 131.81 kb in Tharparker cattle to 171.62 kb in Cholistani cattle. Dash *et al.* (2017) reported similar intermarker distance means for Sahiwal, Tharparker, and Gir breeds. In contrast, Espigolan *et al.* (2013) reported a lower intermarker distance mean of 4.9 kb in Nellore cattle, while Karimi *et al.* (2015) estimated a higher mean intermarker distance of 15.4 kb in Iranian cattle using the bovine high-density SNP BeadChip.

MAF represents the frequency of the less common allele at a particular locus within a population. It serves as a crucial indicator of genetic diversity within the population. In our research, we computed the minor allele frequency for the SNP present in Pakistani indigenous cattle breeds. The MAF exhibited variations across various cattle breeds. For instance, in the Achi breed, the MAF ranged from 0.008 to 0.5, with an average of 0.156. In the Bhagnari breed, the MAF ranged from 0.01 to 0.496, with an average of 0.172. Similarly, in the Cholistani breed, the MAF ranged from 0.002 to 0.497, with an average of 0.159. The Dajal breed displayed a MAF ranging from 0.008 to 0.498, with an average of 0.157. The Dhanni breed exhibited a MAF ranging from 0.006 to 0.5, with an average of 0.158. In the Kankrej breed, the minor allele frequency ranged from 0.01 to 0.496, with an average of 0.174. In the Lohani breed, the MAF varied from 0.006 to 0.5, with an average of 0.162. The Red Sindhi breed exhibited a MAF ranging from 0.008 to 0.5, with an average of 0.16. In the Sahiwal breed, the MAF ranged from 0.01 to 0.5, with an average of 0.169. In the Tharparker breed, the MAF exhibited a range of 0.008 to 0.496, with an average of 0.162. These observations emphasize the presence of genetic diversity within the Pakistani indigenous cattle breeds.

It is important to note that the MAF values reported here are specific to the samples analyzed in our study and may vary in different populations or across different

studies. Additionally, further research and analysis are needed to fully understand the genetic polymorphism and diversity in Pakistani indigenous cattle breeds using larger sample sizes and more comprehensive genomic analyses.

## CONCLUSION

In conclusion, our study provides insights into the SNP polymorphism in Pakistani indigenous cattle breeds, including information on the number of SNP, intermarker distances, and MAF. This knowledge contributes to our understanding of the genetic diversity and potential for genetic improvement in these cattle breeds, which can have implications for breeding programs and conservation efforts.

## DECLARATIONS

### Acknowledgement

This project received partial funding from the Higher Education Commission of Pakistan (HEC-NRPU-16844) and NCLBGandG, PMAS-Arid Agriculture University, Murree Road, Shamsabad, Rawalpindi. We extend our gratitude to the United States Department of Agriculture's Agricultural Research Service (USDA-ARS) for their invaluable technical support. We also appreciate the cooperation of the livestock experimentation stations in Pakistan for providing the necessary blood samples.

### Funding

This research was partially funded by the Higher Education Commission (HEC) of Pakistan under the National Research Program for Universities (NRPU-16844) and supported by the National Center for Livestock Breeding, Genetics and Genomics (NCLBG&G), PMAS-Arid Agriculture University Rawalpindi.

*IRB approval*

IRB approval was obtained from the Institutional Ethical Review Committee vide Notification DAS/ 258.

*Ethical statement*

All animal handling and sampling procedures were conducted in accordance with the ethical guidelines approved by the Institutional Animal Care and Use Committee (IACUC) / Ethical Review Committee of UVAS, Lahore, Pakistan.

*Supplementary material*

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20240414091942>

*Generative AI and AI assisted technology statement*

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

*Statement of conflict of interest*

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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## Supplementary Material

# Exploring Genetic Variability and Breed Differentiation in Indigenous Cattle Using High-Density SNP (777K) Genotyping

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**Supplementary Table I. Sampling details (Mustafa *et al.*, 2017).**

| Population | N  | Purpose       | Province           |
|------------|----|---------------|--------------------|
| Achi       | 18 | Milk and meat | Khyber Pakhtunkhwa |
| Bhagnari   | 14 | Work          | Balochistan        |
| Cholistani | 13 | Milk and meat | Punjab             |
| Dhanni     | 10 | Work and milk | Punjab             |
| Dajal      | 10 | Work and meat | Punjab             |
| Kankraj    | 12 | Work and meat | Sindh              |
| Lohanni    | 19 | Work and milk | Balochistan        |
| Red Sindhi | 13 | Milk          | Sindh              |
| Sahiwal    | 14 | Milk          | Punjab             |
| Tharparkar | 13 | Work and milk | Sindh              |

N, number of sampled animals. Milk, Meat, and Work indicate the reported production/use purpose of each population. Province indicates the province listed for each population in the sampling information.

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0030-9923/2026/0001-0001 \$ 9.00/0



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