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Evaluation of *CYP19* as a candidate gene for milk production traits in native cattle and buffalo populations of Southern India

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Abstract: Candidate gene approach facilitates searching causative polymorphisms that influence quantitative traits. Biosynthesis of estrogen catalyzed by aromatase, a product of the *CYP19* gene affect lactogenesis. The objective of the study was to determine the polymorphism in the promoter region of *CYP19* gene by PCR-RFLP and was undertaken on 502 bovine species belonging to Ongole (*Bos indicus*), Holstein Friesian crossbred, Jersey crossbred cattle, and Murrah buffaloes reared across Southern India. The frequency of the $CYP19^{A}$ allele was 0.90, 0.88, and 0.77 in Jersey crossbred, HF crossbred, and Ongole cattle, respectively. The *BB* genotypes could not be observed in Jersey crossbred cattle. Fixation of the *B* allele was evident in Murrah buffaloes. The Hardy–Weinberg equilibrium was verified and general linear model procedure was adopted to evaluate the effect of *CYP19*/*Pvu*II genotype on milk production traits. The *AA* genotypes (2111.53 ± 51.84 kg) in Jersey crossbred cattle, *AB* genotypes (2529.00 ± 238.08 kg) in Holstein Friesian crossbred and *BB* genotype (590.85 ± 92.29 kg) in Ongole cattle yielded more milk. The fat content of milk in *AB* genotypes was higher in all the cattle. The solid-not-fat and lactose content was more in the milk of *BB* genotypes and protein in *AB* genotypes of Ongole cattle. The fat, SNF, protein, and lactose content in Murrah buffalo milk was 7.26 ± 0.62%, 9.20 ± 0.19%, 3.44 ± 0.06%, 4.87 ± 0.11%, respectively. No significant ($p > 0.05$) effect of *CYP19*/*Pvu*II genotypes on milk production and composition was found in any of the genetic groups studied. The sequence analysis of *CYP19* P 1.1 revealed additional SNPs in all the cattle under study. A novel SNP at 82 nucleotides upstream of the *Pvu*II restriction site was observed in Murrah buffaloes.

Key words: *Bos indicus*, Ongole cattle, *Bubalus bubalis*, Murrah buffalo, *CYP19* polymorphism, milk performance

1. Introduction

Cattle husbandry is associated with the livelihood of farmers and the agricultural economy in India. The advent of improving the genetic potential of native cattle through artificial insemination and crossbreeding with exotic cattle gained momentum since 1950 and successfully made the country the world's largest milk producer. In this endeavor, the emphasis on the crossbreeding led to indiscriminate crossbreeding neglecting the native breeds. Protection, conservation, and promotion of indigenous breeds should be an essential part of cattle breeding policy of any nation, albeit aiming at the genetic improvement of native cattle. The impetus for the conservation and development of indigenous cattle of India derives from the ability to thrive on poor quality feed, high resilience to heat, and adaption in the tropics. These native zebu cattle were utilized across the globe to develop various genetic lines or crosses i.e. Brahman, Guzerat, Nelore Argentino and Sumba Ongole. Ongole cattle breed, popularly known as Nelore outside India is a dual-purpose indigenous breed that was regarded as a fair milker up to the early 1900s [1,2]. The advancement in farm technology and low yielding of the dual-purpose cattle had restricted these animals into the backyards of the progressive farmers who still maintain them with pride for traditional cattle shows. Developing a milk line through selection will help to restore the utility of these breeds and also secure the genetic diversity. Buffalo is another bovine species, the major source of milk in the subcontinent and its milk is preferred for taste and high fat content. India is the place of origin and domestication for Asiatic buffalo [3] having 57% of the world buffalo population [4] and much of their genetic potential is yet to be exploited.

The rapid improvement in traits of economic value in livestock species depend on identifying the underlying candidate genes [5] and exploiting their polymorphisms. *CYP19* gene encodes for the key enzymes of estrogen biosynthesis, aromatase cytochrome P450 [6]. Variation in *CYP19A1* gene causes pubertal failure [7] and regulation of transcription level of P450 aromatase enzyme is allied to a single nucleotide polymorphism (SNP) in the promoter region of the bovine *CYP19A1* gene affecting estradiol synthesis [8]. The lactogenic effect of prolactin is inhibited by estradiol in mammary tissue [9]. Imran et al. [10] illustrated the potential role of SNPs in the *CYP19A1* gene as markers for the selection of buffalos with better estrus behavior. The candidacy of *CYP19* for milk production traits arises from the fact that estrogen is involved in lactogenesis, influences mammary cells by increasing the number of prolactin and

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growth hormone receptors [11]. The physiological role of this gene directed the authors to hypothesize that *CYP19* influences milk production and hence, the present study was undertaken to determine the polymorphism in the *CYP19* gene and their association with milk production traits in Ongole, Holstein Friesian crossbred, Jersey crossbred cattle, and Murrah buffaloes in Southern India.

2. Materials and methods

2.1. Sampling of animals for blood collection

A total of 502 animals belonging to: Ongole cattle (n =135) maintained at Livestock Research Station, Lam, Guntur as a part of national policy on improvement of indigenous cattle and conservation of native genetic resources, Holstein Friesian (HF) crossbred (n = 114), Jersey crossbred (n = 96) cattle, and Murrah buffaloes (n = 157) reared in different organized farms (institutional and private) of Tamil Nadu, Andhra Pradesh and Kerala states in Southern India (Table 1, Figure 1) were sampled to collect whole blood samples for genomic DNA isolation [12]. The study areas come under tropical wet and, tropical wet-dry climatic zones [13]. A random sampling procedure was followed to collect blood from unrelated animals in the selected farms by verifying the pedigree records wherever available, and interviewing the farmers if in private farms. The crossbred cattle included in the study were from inter se mated population that were the progeny of nondescriptive cows crossed with either HF or Jersey bulls through artificial insemination as a part of breeding policy adopted in the country for increasing the milk production. The level of exotic inheritance in the crossbreds was maintained between 50% and 62.5%.

2.2. DNA amplification and genotyping by PCR-RFLP

The promoter region is vital in controlling the transcription of a gene and a change in its sequence could alter the regulation. Hence, the genomic region comprising *CYP19* promoter P1.1 was considered to amplify using the primers (F: 5'-CTCTCGATGAGACAGGCTCC-3'; R: 5'-ACAATGCTGGGTTCTGGACT-3') as described by Jedrzejczak et al. [11]. Polymerase chain reaction (PCR) was performed on genomic DNA in a reaction volume of 15 µL comprising of PCR mastermix (2x Taq Master Mix Red, Ampliqon, Odense, Denmark) along with 0.5 pmol each of forward and reverse primer. The cycling conditions for amplification of *CYP19* were 94 °C/2 min (initial denaturation), followed by 30 cycles comprising denaturation (95 °C/15 s), primer annealing (55 ˚C/30 s), extension (70 ˚C/2 min) and a final extension (72 ˚C/5 min). The amplicon of each sample (10 µL) was subjected to restricted fragment length polymorphism (RFLP) analysis using 10 units of *Pvu*II for 4 h/37 °C to genotype each animal with respect to SNP at cleavage site. All the digested samples were electrophoresed in agarose gel at 80V for 40 min and visualized with Gel Doc XR+ to determine genotypes.

2.3. Milk samples and lactation data for association analysis

Out of the animals (502) sampled for determining the polymorphism, only the farm-bred cows that were having

Table 1. Details on the farm location and number samples collected.

Breed	Location of farm	No. of blood samples	No. of milk samples
Jersey crossbred	1. College of Agricultural Science, TNAU, Madurai, Tamil Nadu	16	11
	2. College of Agricultural Science, TNAU, Coimbatore, Tamil Nadu	19	16
	3. Post Graduate Research Institute in Animal Science, Katupakkam, Thiruvalluru, TANUVAS, Tamil Nadu	41	25
	4. Vishwas Dairy Farm, Madurai, Tamil Nadu	20	20
	Total	96	72
HF crossbred	1. College of Agricultural Science, TNAU, Madurai, Tamil Nadu	7	4
	2. College of Agricultural Science, TNAU, Coimbatore, Tamil Nadu	16	15
	3. Vishwas Dairy Farm, Madurai, Tamil Nadu	8	5
	4. District Livestock Farm, Ooty, Nilagiri, Tamil Nadu	28	9
	5. College of Veterinary Science, Pookote, Wyanad District, Kerala	30	17
	6. Ayroor farm, Padijarethera, Wyanad, Kerala	10	-
	7. Ganga Dairy, Meenangadi, Wayanad, Kerala	15	-
	Total	114	50
Ongole cattle	1. Livestock Research Station, Lam, S.V.V.U., Guntur District, Andhra Pradesh	135	62
	Total	135	62
Murrah buffaloes	1. Post Graduate Research Institute in Animal Science, Katupakkam, TANUVAS, Tamil Nadu	35	7
	2. Buffalo Research Station, Venkataramanna Gudem, S.V.V.U, West Godavari District, Andhra Pradesh	86	27
	3. Dhanalakshmi dairy farm, Nidamanur, Krishna District, Andhra Pradesh	36	4
	Total	157	38
GRAND TOTAL		502	222

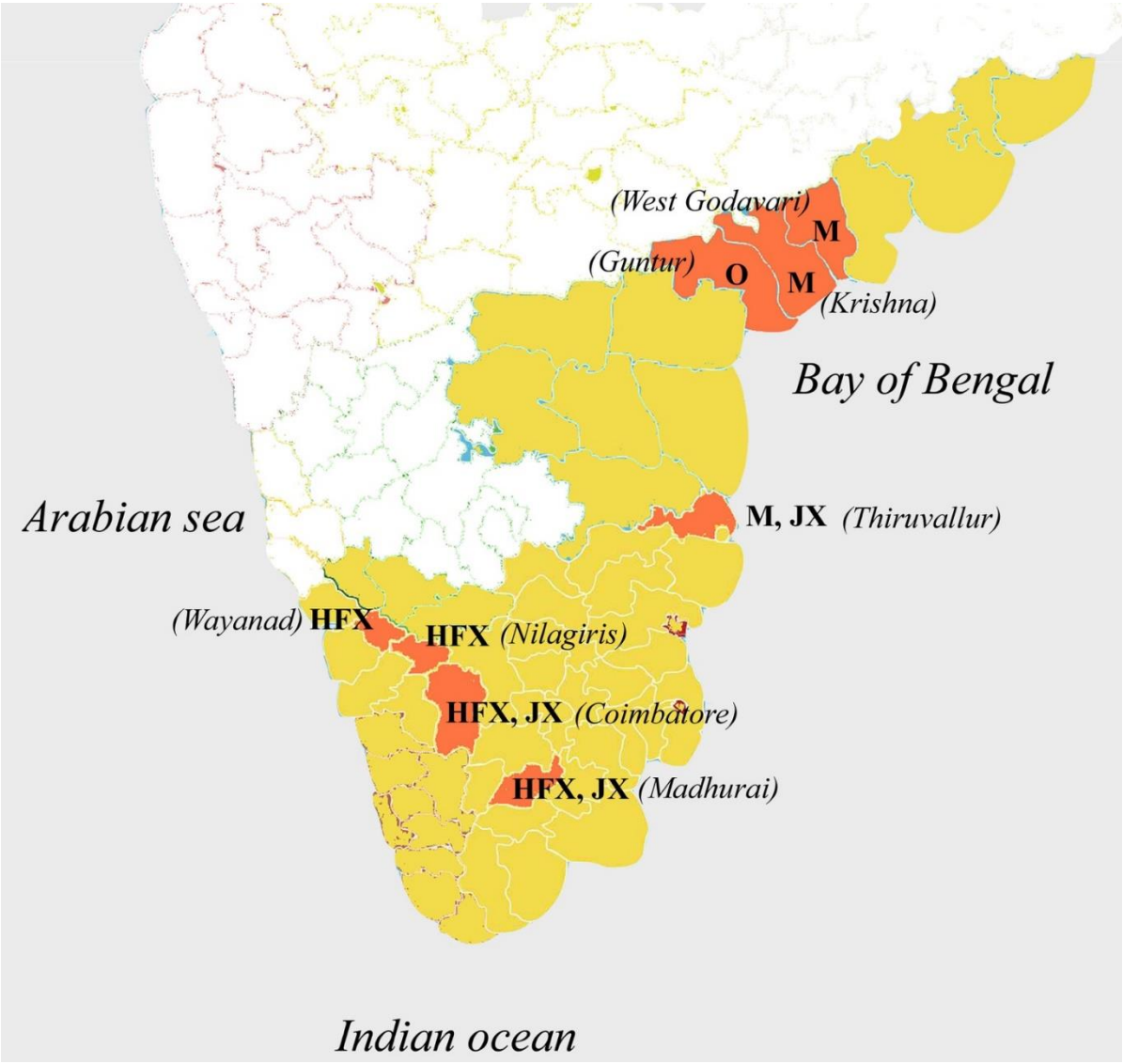

Figure 1. Cartographic depiction of location of farms sampled from various districts.

lactation records documented in farm inventory were included (Jersey crossbred = 145, HF crossbred = 141, Ongole = 154, Murrah = 107) for association study on lactation milk yield due to the reasons of reliability on the accuracy of records of animals purchased or procured from outside. To have uniformity across the farms and genetic groups, animals having records up to three lactations were only considered. Fat, protein, lactose and, solid-not-fat (SNF) contents of milk were determined (Lactoscan SL 30, MB Ver.60) in milk samples collected from 222 out of 502 animals that were lactating on the day of visit to the farm for blood sample collection. All the animals were under twice-daily milking regime and hand milked. The information on parity and stage of lactation was collected on the study group.

2.4. Statistical analysis

The estimation of allele frequencies, evaluation of equilibrium status and, population indices was performed using POPGENE 32 (Version 1.32) software [14].

The months were grouped for adjustment of the seasonal influences such that the within group differences were less than the between group (winter: January–February; summer: March–May; South-West monsoon: June–September; North-East monsoon: October–December). The stages of lactations were grouped corresponding to early (5 to 90 days), mid (91 to 180 days) and late (181 days and above) lactation. The influence of *CYP19* genotypes on lactation milk yield and milk composition traits was determined using the general linear model of SPSS Statistics 17 after adjusting for the effects of farm, season, and parity. The model adopted for lactation milk yield was $Y_{ijklm} = \mu + f_i + s_j + p_k + g_l + (g \times p)_{lk} + e_{ijklm}$, where Y_{ijklm} = value of observed lactation milk yield of an individual, μ = mean of the milk yield for the genetic group, f_i = fixed effect of farm, s_j = effect of season, p_k = effect of parity, g_l = effect of corresponding genotype, $(g \times p)_{lk}$ = genotype and interaction of lth genotype × kth parity, e_{ijklm} = random errors, assumed to be NID (O, σ^2_e).. Analysis on milk constituents was carried with, $Y_{ijkl} = \mu + f_i + p_j + s_k$

$+ g_l + (g \times p)_{lj} + e_{ijklm}$, where Y_{ijkl} = value of observed milk constituent of an individual, f_i = fixed effect of farm, p_j = effect of parity, s_k = effect of stage of lactation, g_l = effect of corresponding genotype, $(g \times p)_{lj}$ = interaction of lth genotype × jth parity, e_{ijklm} = random errors, assumed to be NID (O, σ^2_e). The dominance (D) and additive (A) genetic effects were estimated [15] for the genotypes, where D = AB − ½ (AA + BB) and A = ½ (AA – BB) and the ratio D/A indicates actual gene effects [16].

2.5. Sequence analysis

The PCR products corresponding to each genotype from the cattle genetic groups and Murrah buffalo from the present study were sequenced using Sanger's dideoxy chain termination method in both forward and reverse direction. The sequences were assembled using SeqBuilder program in DNASTAR Lasargene (DNASTAR, Madison, WI, USA) [17]. The assembled sequences were aligned along with corresponding sequences from other cattle and buffalo genetic groups obtained from the NCBI using Clustal W method in MegAlign software (DNASTAR Lasargene). A neighbor joining tree using default parameters was constructed in MEGAX software [18].

3. Results

3.1. *CYP19/Pvu*II polymorphism in cattle and buffaloes

Restriction fragment length polymorphism (RFLP) was employed for genotyping of the animals under each genetic group of cattle and Murrah buffaloes in the sample under study. The restriction fragments obtained for *CYP19*/*Pvu*II polymorphism (Figure 2) were *AA* (405 bp), *AB* (405, 327 and 78 bp), and *BB* (327 and 78 bp). The PCR-RFLP analysis revealed all the three genotypes in HF crossbred and Ongole cattle, whereas in Jersey crossbred only *AA* and *AB* were identified (Figure 2a). All the Murrah buffalo cows of the study set exhibited the DNA fragment of 327 and 78 bp representing *BB* genotype.

3.1.1. Population genetic index

The observed genotype and allele frequencies along with their expected genotypic frequencies in the examined groups were presented in Table 2. The *AA* genotype frequencies across the cattle genetic groups ranged between 0.60 and 0.79. The frequency of *A* allele was 0.90, 0.88, and 0.77 in Jersey crossbred, HF crossbred, and Ongole cattle, respectively. The analysis on *CYP19* loci revealed that the present populations were consistent with Hardy–Weinberg equilibrium ($p > 0.05$). The studied herds of Murrah buffaloes were monomorphic for *B* allele.

The χ^2 differences between genotype and allele frequencies of Jersey crossbred and HF crossbred were not significant, whereas the differences in genotype (df = 2, χ^2 value = 12.05 and 9.213) and allele (df = 1, χ^2 value = 11.51 and 8.96) frequencies between Ongole and crossbred cattle were significant ($p < 0.001$). The observed and expected heterozygosity at the loci in all the populations did not differ significantly. The polymorphic information content (PIC) was 16% to 29% and the F_{IS} estimates were negative for all genetic groups ranging from 0.05 to 0.37. The same parameters could not be estimated in Murrah buffaloes because the *B* allele was fixed in the population resulting in a single genotype.

3.2. Association of polymorphism with milk production traits

3.2.1. Milk yield

All the cattle populations included in the study were found to be polymorphic for *CYP19*/*Pvu*II, and in HF crossbred cows only one animal was with *BB* genotype (Table 2) and hence, the animal was not included in the association analysis. The least squares mean for various factors were presented in Table 3. The *AA* genotypes of Jersey crossbred cows yielded higher milk yield (2111.53 ± 51.84 kg, n = 112) than *AB* genotypes (2077.52 ± 100.08 kg, n = 33) whereas, in HF crossbred *AB* (2529.00 ± 238.08 kg, n = 18) genotypes recorded high milk yield compared to *AA* genotypes (2317.54 ± 92.44, n = 123). In Ongole cattle, *BB* genotype yielded more milk (590.85 ± 92.29 kg, n = 8) than the *AA* (544.78 ± 27.15 kg, n= 91) and *AB* (466.73 ± 35.51 kg, n= 55) genotypes. The difference in least squares mean of lactation milk yields between *CYP19* genotypes in different genetic groups of cattle after adjusting for the effects of farm, season, and parity were statistically nonsignificant ($p > 0.05$). Among the other factors studied, location of farm only had significant effect ($p < 0.05$) on lactation milk yield. The least squares mean of lactation milk yield in Murrah buffaloes was 1917.45 ± 59.20 kg. **3.2.2. Milk composition**

The least squares mean for the milk constituents are presented in Table 4. The mean values for all the milk constituent traits differed significantly ($p < 0.01$) between the farms. The parity and stage of lactation had nonsignificant ($p > 0.05$) effect on the studied traits. The fat content of milk in *AB* genotypes was higher than *AA* genotypes in both Jersey crossbred (5.15 ± 0.86%) and HF crossbreds (4.71 ± 0.71%). The SNF, protein, and lactose contents were recorded more in *AA* genotypes of Jersey crossbred but in HF crossbred group it was more in *AB* genotypes. In Ongole cattle the *AB* genotypes showed higher fat (4.09 ± 0.30%) and protein (3.53 ± 0.26%) content. SNF content was high in *BB* genotype and was 9.43 ± 0.35%. All the differences in milk constituents between the genotypes were observed to be nonsignificant ($p > 0.05$). The fat, SNF, protein, and lactose content in Murrah buffalo milk was 7.26 ± 0.62%, 9.20 ± 0.19%, 3.44 ± 0.06%, 4.87 ± 0.11%, respectively.

3.3. Sequence analysis

The PCR amplicons were subjected to Sanger sequencing and submitted to GenBank (Crossbred cattle: KT596709.1 to KT596713.1; Murrah buffalo: KT596714.1 and KT596715.1; Ongole: KT596716.1 to KT596719.1). The alignment of sequence with the reference (Z69241.2) revealed the reported A>G transition (rs208717235) in all the cattle genetic groups under study (Figure 3). In the present study, the SNPs corresponding to the position 2 (G>T) and 34 (G>C) in sequences of zebu cattle and

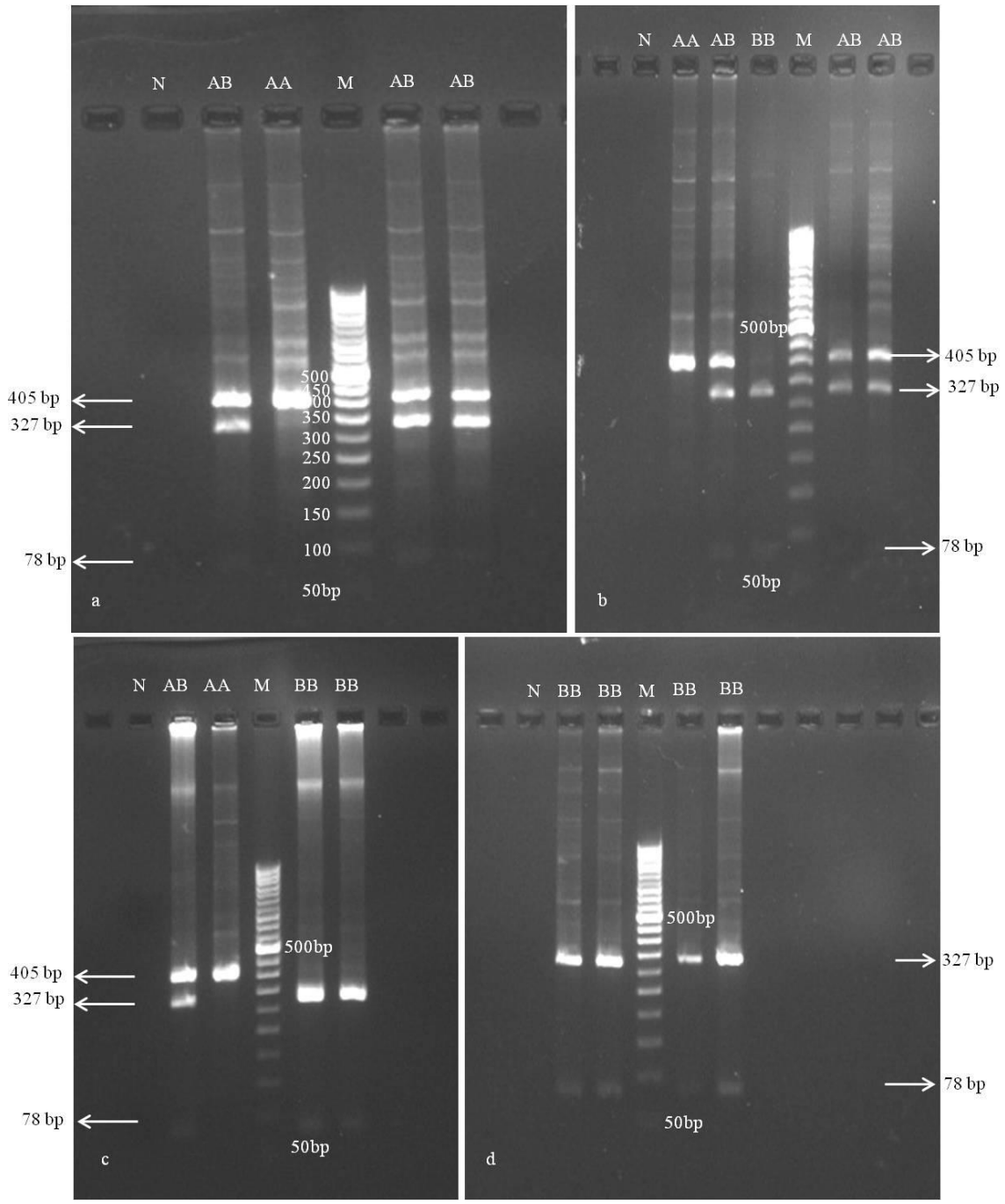

Figure 2. *CYP19*/*Pvu*II pattern of PCR product of 405 bp fragment from different genetic groups. a) Jersey crossbred, b) HF crossbred, c) Ongole cattle, d) Murrah buffaloes *AA* (405 bp). *AB* (405, 327, and 78 bp) and *BB* (327 and 78 bp);
M: 50 bp DNA marker, NC: negative control.

crossbreds were novel observations. The phylogenetic analysis on promoter region of *CYP19* gene of studied populations performed along with the corresponding sequences available in GenBank was presented in Figure 4.

4. Discussion

4.1. Polymorphism in different genetic groups

The polymorphic bands derived on digestion of the P 1.1 region of *CYP19*/*Pvu*II from different bovine genetic groups were suggestive of variation in all the cattle genetic groups, but not in Murrah buffaloes. The A>G transition at 1044 nt is the recognition site for *Pvu*II enzyme and was reported earlier [19].

The absence of *BB* genotypes in present Jersey crossbreds was in accordance with earlier observations in Jersey population [20]. The $CYP19^{A}$ frequency in the studied herds of Jersey crossbred, HF crossbred and Ongole cattle were 0.90, 0.88, and 0.77, respectively. The $CYP19^{A}$ frequency in HF crossbred cattle is in accordance with the report on Polish Holstein Friesian cattle [21]. Higher frequencies of $CYP19^{A}$ allele in HF cattle [22,23] and fixation [11] and near to fixation [20] in Jersey cattle were

Table 2. Distribution of genotypes and allele frequencies at *CYP19/Pvu*II loci in cattle and buffaloes.

Breed/group	Number of animals (n)	Observed genotypic frequency			Allele frequency		Expected genotype frequency			χ^2 value	p value
		AA	*AB*	*BB*	*A*	*B*	*AA*	*AB*	*BB*		
Jersey crossbred	96	0.79 (76)	0.21 (20)	0	0.90	0.10	0.80 (77)	0.19 (18)	0.01 (1)	1.29^{ns}	0.25
HF crossbred	114	0.76 (87)	0.23 (26)	0.01 (1)	0.88	0.12	0.77 (87.67)	0.22 (24.67)	0.01 (1.66)	0.39^{ns}	0.53
Ongole	135	0.60 (80)	0.36 (49)	0.04 (6)	0.77	0.23	0.60 (80.8)	0.35 (47.4)	0.05 (6.8)	0.19^{ns}	0.66
Murrah	157	0	0	1 (157)	0	1	-	-	-	-	-

Figures in parentheses are the number of animals, ns: not significant ($p > 0.05$), degrees of freedom = 1.

Table 3. Least squares mean (± S.E) of lactation milk yield (kg) for *CYP19* genotypes in different genetic groups of cattle.

Main effect/subclass	Jersey crossbred		HF crossbred		Ongole	
	n	Milk yield	n	Milk yield	n	Milk yield
Overall mean		2094.52 ± 57.61		2423.27 ± 125.69		534.12 ± 33.82
Farm		*		*		
1	30	1692.30^{a} ± 105.97	14	1670.26^{a} ± 239.39	1	534.12 ± 33.82
2	44	2749.05^{c} ± 75.10	22	2996.25^{b} ± 193.78	-	
3	19	2101.05^{b} ± 125.11	17	2675.97^{b} ± 220.82	-	
4	52	1835.70^{a} ± 78.49	74	1982.58^{a} ± 149.72	-	
5	-		14	2791.31^{b} ± 213.38	-	
Parity						
1	65	1934.90 ± 75.76	70	2102.03 ± 135.61	60	556.91 ± 64.93
2	49	2094.22 ± 85.75	43	2453.76 ± 184.92	52	539.99 ± 55.27
3	31	2254.47 ± 109.98	28	2714.03 ± 284.20	42	505.45 ± 56.36
Season						
Winter (Jan–Feb)	32	2084.54 ± 101.84	24	2518.10 ± 190.94	28	535.04 ± 53.34
Summer (Mar–May)	25	2053.75 ± 105.97	41	2648.70 ± 170.69	46	521.62 ± 47.14
South-West monsoon (June–Sep)	56	2090.84 ± 76.73	41	2211.65 ± 154.71	44	583.74 ± 50.20
North-East monsoon (Oct–Dec)	32	2148.98 ± 93.45	35	2314.64 ± 166.88	36	496.08 ± 50.16
CYP19 genotype						
AA	112	2111.53 ± 51.84	123	2317.54 ± 92.44	91	544.78 ± 27.15
AB	33	2077.52 ± 100.08	18	2529.00 ± 238.08	55	466.73 ± 35.51
BB	-	-	-	-	8	590.85 ± 92.29

n = number of observations; * =Significant ($p < 0.05$), means with at least one common superscript within classes do not differ significantly ($p > 0.05$).

also reported in few earlier studies. On perusal of literature, studies on *CYP19* polymorphism in *Bos indicus* breeds could not be found except for the report on Rathi cows [24] in which the frequencies were 0.65, 0.32 and 0.03 for *AA*, *AB*, and *BB* genotypes, respectively and the $CYP19^{A}$ allele was more frequent (0.81). The frequency of $CYP19^{A}$ allele in Iranian native cattle which are related to zebu cattle ranged from 0.89 to 0.98 [25]. The $CYP19^{A}$ allele frequency in Ongole cows was found to be much lesser than reported in Rathi and Iranian cows.

The differences in genotypic and allelic frequencies at *CYP19* locus of Ongole cattle with both the crossbred cattle were significant ($p < 0.001$). Similar observation of significant difference was reported between Iranian native cattle and taurine cattle [21]. The genetic diversity analysis demonstrated Ongole cattle as having the more heterozygosity (0.36) and the high polymorphism information content (0.292) indicative of good heterozygous state, and hence Ongole cattle could be considered as a reliable source of genetic variability for *CYP19*. The negative F_{IS} values that were indicative of heterozygote excess were not significant. The variation in the allele frequency with other genetic groups reported could be due to the differences in the utility of breeds (dairy/dual/draught) and crossbreeding of indigenous cattle with exotic *Bos taurus*.

Comparable to the present observation on Murrah buffaloes the fixation of *B* allele was also evident in Egyptian river buffaloes [26].

4.2. Association of polymorphism with milk production traits

The *AA* genotypes of Jersey crossbred cows (2111.53 ± 51.84 kg), *AB* genotypes (2529.00 ± 238.08 kg) in HF crossbred, and *BB* genotype in Ongole cattle (590.85 ±

Table 4. Least squares mean (± S.E) of milk constituents for *CYP19* genotypes in different genetic groups of cattle.

	Jersey crossbred					HF crossbred					Ongole				
Main effect/subclass	n	Fat %	SNF %	Protein %	Lactose %	n	Fat %	SNF %	Protein %	Lactose %	n	Fat %	SNF %	Protein %	Lactose %
Overall mean		4.68 ± 0.45	8.91 ± 0.15	3.26 ± 0.56	4.91 ± 0.08		4.24 ± 0.26	9.22 ± 0.21	3.35 ± 0.07	5.06 ± 0.11		3.60 ± 0.23	9.33 ± 0.15	3.48 ± 0.10	5.12 ± 0.08
Farm		**	*	*	**		*	**	**	**					
1	11	2.97^{a} ± 0.90	8.14^{a} ± 0.31	2.98^{a} ± 0.11	4.45^{a} ± 0.17	4	2.54^{a} ± 0.87	8.38^{a} ± 0.63	3.07^{a} ± 0.23	4.60^{a} ± 0.34	62	3.60 ± 0.23	9.33 ± 0.15	3.48 ± 0.10	5.12 ± 0.08
2	20	5.74^{b} ± 0.65	8.88^{b} ± 0.22	3.28^{b} ± 0.08	4.85^{b} ± 0.12	5	4.70^{b} ± 0.79	10.19^{b} ± 0.57	3.71^{b} ± 0.21	5.56^{b} ± 0.31					
3	16	4.96^{b} ± 0.89	9.59^{c} ± 0.30	3.53^{c} ± 0.11	5.43^{c} ± 0.17	15	4.57^{b} ± 0.54	9.82^{b} ± 0.39	3.59^{b} ± 0.15	5.38^{b} ± 0.21					
4	25	4.04^{b} ± 0.62	9.02^{b} ± 0.21	3.30^{b} ± 0.08	4.93^{b} ± 0.12	16	4.75^{b} ± 0.63	10.35^{b} ± 0.46	3.77^{b} ± 0.16	5.65^{b} ± 0.25					
5		-	-	-	-	9	4.85^{b} ± 0.58	8.40^{a} ± 0.42	3.00^{a} ± 0.15	4.51^{a} ± 0.23					
Genotype															
AA	58	4.29 ± 0.42	8.99 ± 0.14	3.30 ± 0.05	4.96 ± .08	40	3.96 ± 0.30	9.21 ± 0.22	3.35 ± 0.08	5.02 ± 0.12	34	3.52 ± 0.25	9.32 ± 0.12	3.42 ± 0.08	5.11 ± 0.06
AB	14	5.15 ± 0.86	8.79 ± 0.29	3.22 ± 0.11	4.86 ± 0.16	9	4.71 ± 0.71	9.71 ± 0.51	3.54 ± 0.19	5.30 ± 0.28	22	4.09 ± 0.30	9.27 ± 0.38	3.53 ± 0.26	5.08 ± 0.21
BB		-	-	-	-		-	-	-	-	6	2.94 ± 0.36	9.43 ± 0.35	3.49 ±0.27	5.17 ± 0.19

n = number of observations, SNF = solid-not-fat, HF = Holstein Friesian, * = significant ($p < 0.05$), ** = significant ($p < 0.01$).

92.29 kg) yielded more milk yield compared to cows with other genotypes within the breed. All the differences were statistically nonsignificant ($p > 0.05$) as also reported by Jędrzejczak et al. [23] in HF crossbreds and Kowalewska-Luczak et al. [20] in Jersey cattle. Significant ($p < 0.05$) high milk yield by *AA* genotype was reported in Black-and-White cows [22]. In Simmental cattle [27], which are also dual-purpose, akin to Ongole, *BB* genotypes produced more milk ($p < 0.05$).

The mean values for all the milk constituent traits between the farms differed significantly ($p > 0.01$) indicating the influence of management and or nutritional aspects on milk composition traits. The fat content of milk in *AB* genotypes was higher in all the cattle genetic groups studied. The SNF, protein, and lactose contents were recorded more in *AA* genotypes of Jersey crossbred, but in HF crossbred cattle *AB* genotypes recorded higher values for protein and fat content. In Ongole cattle the *AB* genotypes showed higher protein (3.53 ± 0.26%), whereas *BB* genotype recorded higher SNF and lactose percent. All the differences were observed to be nonsignificant ($p > 0.05$) for the differences in milk constituents in the studied genetic groups. Higher fat and protein contents in milk from *AB* genotypes of Jersey [20] and *AA* genotypes in HF [28] cattle were reported by other researchers. The variations with the reported influences of the *CYP19*/*Pvu*II genotypes on milk traits might be due to the differences in the utility and nature of breeds and the factors incorporated in the model for analysis. The fat, SNF, protein, and lactose content in Murrah buffalo milk was 7.26 ± 0.62%, 9.20 ± 0.19%, 3.44 ± 0.06%, 4.87 ± 0.11%, respectively.

The analysis on gene effects revealed that the highest lactation milk yield, protein and lactose content in Ongole cattle were associated with *BB* genotype cows, and the corresponding D/A estimates 4.4, 1.9, and 2.0 revealed overdominant effects on the traits [16]. In the *AB* genotypes for fat (D/A = 2.9) and SNF (D/A = –0.04) the overdominant and additive effects were evident, respectively.

4.3. Comparative sequence analysis

A previously reported SNP (rs526245293) resulting in A>T in the promoter region of bovine *CYP19* was observed only in HF crossbred cattle in the present study. Few of the SNPs which were earlier reported in the dbSNP repository (rs447719822, rs522510850, rs444719967, rs208680202, rs449136722, rs461641261, rs481655852) were not observed in any of the genetic groups. An additional G>T and G>C was observed upstream of rs208717235 (A>G) in all the cattle.

The SNPs observed in Murrah buffaloes were consistent with the sequence of Egypt river buffalo (MF490279.1) submitted by Aboelenin et al. [26]. A novel SNP found at nucleotide position 2 (G>T) was not previously reported and is novel for Murrah buffalo.

Species-wise divergence was evident on subjecting the *CYP19* promoter sequences for phylogenetic analysis (Figure 4). The homology among Murrah buffaloes of Indian origin and Egyptian buffalo resulted in a single clad and *Bos* genus clustered differently indicating that *Bubalus bubalis* have a distant phylogenetic relationship with cattle. Except for the Ongole cattle with *AA* genotype all the cattle were clustering out from taurine group. Interestingly, *BB* genotypes of cattle branched out of *AA* and *AB* genotypes.

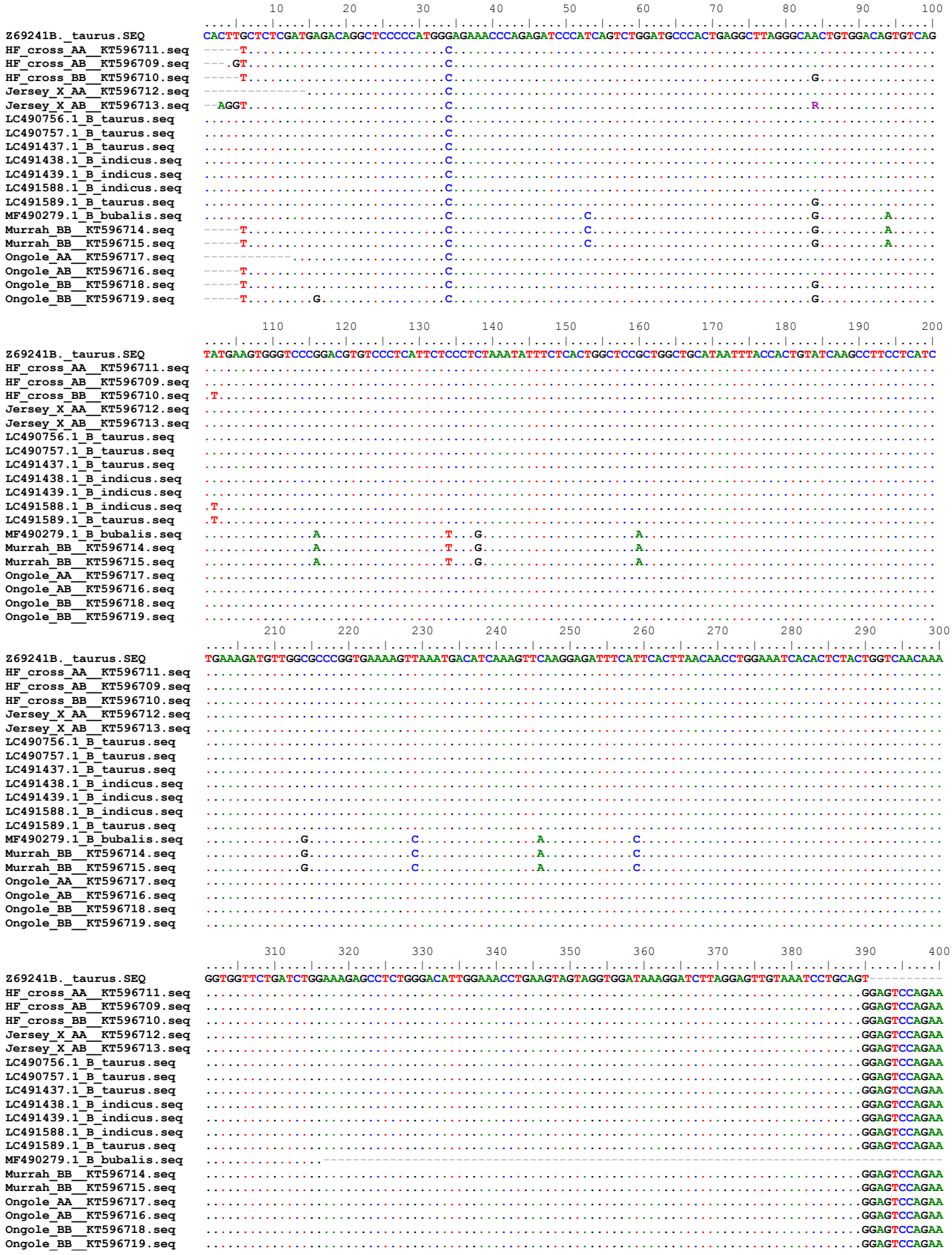

Figure 3. Multiple sequence alignment showing variations in *CYP19* promoter region in various cattle genetic groups and Murrah buffalo. Cleavage site for *Pvu*II: Nucleotide position 84 (rs208717235, A>G).

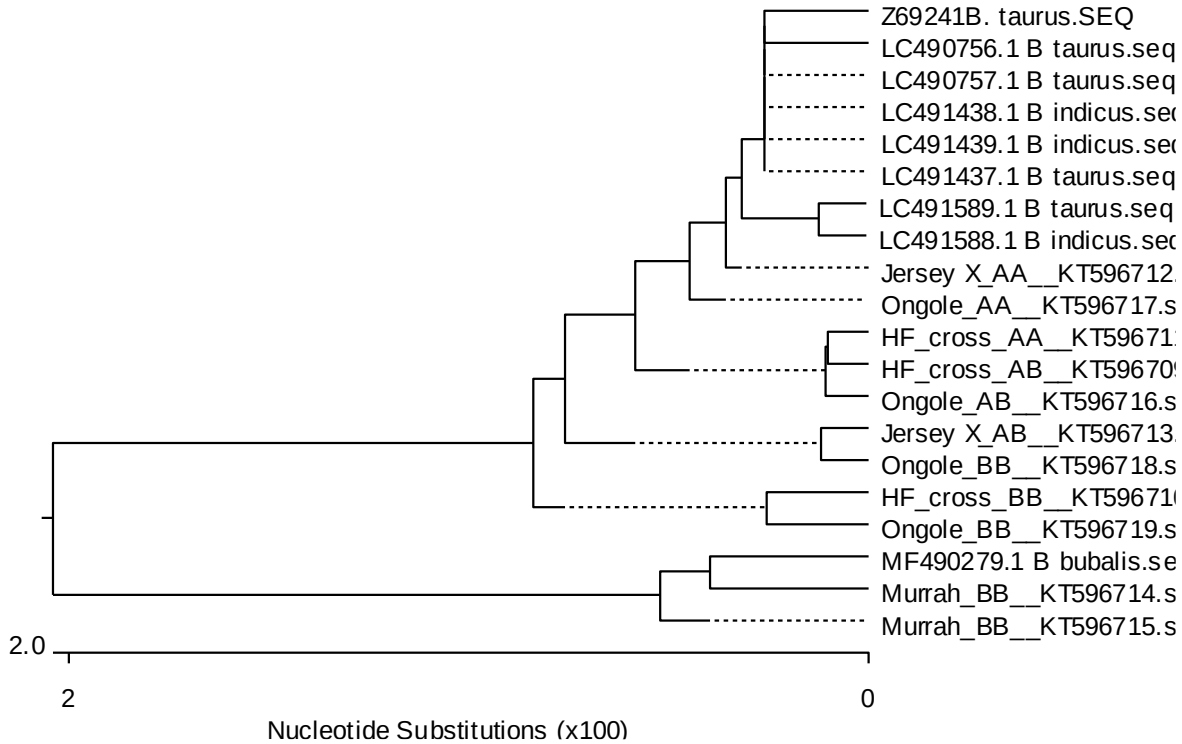

Figure 4. The phylogentic analysis on promoter region of CYP19 gene depicting *Bos indicus* cattle and Murrah buffalo genotypes segregating into a different cluster.

5. Conclusion

The present study indicated polymorphism in the P1.1 region of the *CYP19* gene is nonsignificantly associated with milk production in Southern Indian cattle breeds. Few of the earlier studies have shown a significant influence of *CYP19* gene variation on milk production traits [22,27]. Interestingly no specific allele or genotype is associated with an increase in milk production traits across bovine genetic groups. The *AA* genotype or heterozygotes are advantageous in crossbred cattle. Ongole is a dual-purpose animal with moderate milk production but the *BB* genotype is associated with highest milk production. The Murrah, being one of the best milk producing buffalo, is used to upgrade less productive buffaloes [29,30] having *B* allele fixed. Detection of additional polymorphisms in the promoter region would help in predicting whether the locus is directly influencing the milk production or it is a linked polymorphism that is responsible for the association.

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Ethical statement

This study did not involve any experimentation with animals except drawing ~ 3 mL blood from jugular vein aseptically by the veterinarian hence approval of Institutional Animal Ethics Committee/CPCSEA was exempted by the competent authority (No. 389/PG/AGB/VCRI/2011). Applicable guidelines were followed for the care and use of animal after the written informed consent from the owners of the animals.

Conflict of interest

The authors declare that they do not have any conflict of interest.

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