

Original Research Article

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Assessment of Genetic Diversity in Gangatiri Cow by Employing RAPD Marker

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ABSTRACT

Random amplified polymorphic DNA- Polymerase chain reaction (RAPD-PCR) was employed to assess the genetic variability and phylogenetic relation among fifteen breed of Gangatiri animals Viz. fourteen animals (cow no.288, 348, 394, 365, 313, 364, 293, 319, 283, 208, 306, 322, 476, 478) are both sexes and one bull were selected randomly. The DNA samples were isolated from a total of fifteen animals from Gangatiri cows and three random primers were selected. The genetic distance was found higher between cow no. 288 and 476 ($D = 0.82697$) and lower ($D = 0.17647$) between cow no 478 and 476. The phylogenetic tree shows that cow no. 288 and 348 are closer, whereas cow no. 208 and 364 are distant to each other. The level of diversity as evident in these mutants can be harnessed in breeding for better varieties as the divergent genotypes are expected to result in high heterosis. The unique allele can be harnessed for genotype identity.

Keywords

RAPD, Genetic diversity, Fingerprints, Phylogenetic

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Introduction

Gangatiri is an indigenous dual purpose cattle breed of India It is originated in the region along the banks of Ganga River in eastern Uttar Pradesh and western part of Bihar state. The breeding tract of this breed includes mainly Ballia and Ghazipur districts of Uttar Pradesh, Rohtas and Shahabad districts of

Bihar. Population of Gangatiri cattle in Uttar Pradesh is 364.81 thousand out of this population, Mirzapur district is having highest population with 23.11 thousand cattle and 13.15 thousand Gangatiri cattle are found in Varanasi district as per the report of Uttar Pradesh State Biodiversity Board (2015). This is an important dual purpose breed of Northern part of India These cattle are well

adapted and medium input production system and produce 2.5 to 8.0 liter milk per day. Animal are rearing for milk production as well as ploughing, thrashing and power works. The lactation length is 150-250 days. Inter-calving period varies from 14-24 months. Gangatiri cattle are dual purpose indigenous cattle breed with good draught capacity. The average milk production of Gangatiri is 4.5 l/day and fat per cent varies from 4.1 to 5.2% (Anonymous 2006). Gangatiri is native of *duaba* area falling between Ganga and Ghaghara rivers. This is a medium size cattle breed reared mainly by resource poor farmers on zero or low input system for their livelihood (Singh *et al.*, 2007). A livelihood is socially sustainable when it can cope with and recover from stress and shocks and provide for future generations (Chambers and Conway, 1992).

Sustainable agriculture may be regarded as successful management of resources for agriculture to satisfy the changing human needs while maintaining or enhancing the quality of environment and conserving natural resources (FAO 1991). The breed significantly contributes to the liveliness of the people due to its good dual purpose. Polymorphic genetic markers are extremely useful for a number of application such as measurement of the amount of genetic diversity in species, discrimination between individual, strains or species, identification of markers linked to economically useful traits as well as analyses of animal kinship relationship, behavioral and population ecology. Random amplified polymorphic DNA- polymerase chain reaction technique has been used for estimation of the genetic variability among the breeds. RAPD markers are the randomly amplified target region of less functional part of the genome that does not strongly respond to selection on the phenotypic level. Such amplified region might accumulate more mutations, thereby offering a wider potential in assessing the intra/interbred genetic differentiation. The

RAPD –PCR has been used to estimate the genetic variability among livestock species. In the case of cattle, lots of researchers have been made to detect DNA polymorphisms between cattle breeds by RAPD analysis during last decades. Especially genetic marker for native cattle in several countries were investigated for the improvement of their native cattle, including Japanese black cattle, German native cattle, Zebu cattle, *Bos indicus* and *Bos Taurus*. Considering the above statement the present research work on “Assessment of genetic diversity of Gangatiri cow by employing random amplified polymorphic DNA (RAPD) markers” was undertaken to know the genetic diversity and relationship among Gangatiri cattle.

Materials and Methods

The experiment was carried out at the Micelles Life Science (Pvt.) Ltd., 4/462, Vibhav Khand, Gomti Nagar Lucknow, U.P. The details of materials and methods used are described as follows.

The total of fifteen animal of Gangatiri cows were selected from Department of Animal Husbandry and Dairying SHUATS. 5ml of blood was collected from each animal in a10 ml EDTA vacationer tube and kept in ice box during collection and then transported to the laboratory and stored at -20⁰C until the isolation of genomic DNA. The cattle identification numbers are given in table 1.

Extraction of genomic DNA from blood samples

DNA was extracted from blood by MLS blood genomic DNA kit protocol. The present of DNA in the extracted sample was confirmed by running the sample in a 1% agarose gel at 80V for 48 minutes. The quality of DNA was measured by spectrophotometer at 260nm wavelength.

PCR reaction

Primer selection

In this study three universal RAPD primers were selected. List of the primers along with their sequences are shown in table 2.

PCR amplification

PCR reaction were performed on each DNA sample in a 25 μ l reaction mix containing gDNA 1.0 μ l, RAPD primer (100ng/ μ l) 2.0 μ l, dNTPs (10mM) 1.0 μ l, TaqPol Assay buffer (10X) 2.5 μ l, Taq DNA Polymerase (3U/ μ l) 0.5 μ l and water 18 μ l. DNA amplification was performed by thermal cycle. The amplification program included an initial denaturation step of 94 $^{\circ}$ C for 5 minutes followed by 40 cycles of 94 $^{\circ}$ C denaturation 1 minute, 54 $^{\circ}$ C annealing 1 min, and 72 $^{\circ}$ C extension for 2 minutes. The final extension was performed at 72 $^{\circ}$ C for 15 minutes and held at 8 $^{\circ}$ C.

Gel electrophoresis of PCR amplified product

Prepare 1X TBE by diluting appropriate amount of 10X TBE buffer. Weigh 0.5 g of agarose and add to 50 ml of 1X TBE. This given 1% agarose gel. Boil till agarose dissolved completely and a clear solution results. Add ethidium bromide to molten agarose to a final concentration of 0.5 μ g/ml (from a stock of 10 mg/ ml in water), when temperature is 50 $^{\circ}$ C. Meanwhile place the combs of electrophoresis set such that it was approximately 2cm away from the cathode. Pour the agarose solution in the central part of the tank (the thickness of the gel should be 0.5 – 0.9 cm). Keep the gel undisturbed at room temperature for the agarose to solidify. Pour 1X TBE buffer into the gel tank till the buffer level stands at 0.5 – 0.8 cm above the gel surface. Gently lift the combs ensuring that the wells remain intact. Connect the power cord to

the electrophoretic power supply according to the convention, red - anode and black – cathode. Mix 5 μ l of gel loading buffer with 15 μ l of the sample and load the wells. Set the voltage to 50V and switch on the power supply. Switch off the power supply when the tracking (Bromophenol blue) from the well reaches $\frac{3}{4}$ of the gel. Observe the bands under UV transilluminator for checking the DNA bands and photographed using a digital camera (Fig. 1).

Data analysis

Only distinct and prominent band were scored for estimation of various parameters. The presence and absence of band was recorded as “1” and “0” respectively. The binary coded characters (1, 0) were used for genetic analysis. Statistical analyses were carried out for estimation of genetic distance, identity and phylogenetic relationship among 15 breed of Gangatiri cow. The relationship among breeds of Gangatiri cow was analyzed by generating dendrogram using Nei genetic distances with UPGMA (Un- weighed pair group method using arithmetic average) analysis through PHYLIP software.

Results and Discussion

The DNA fragment to be enlarged is determined by selecting primers. Primers are short, artificial; DNA strand often nor more than 50 and usually only 17 to 22 base pair long that are complimentary to the beginning or the end of the DNA section to be enlarged. They are extremely rich in the genomes of eukaryotes, polymorphic and more often than not co-dominant and manageable among dissimilar mapping populations. The variation in band profile was observed within the breed however most of the bands were not variable between individuals f the same breed. The majority of RAPD primer gave distinctly reproducible bands in all the breeds some of

the primer produced highly polymorphic patterns, but other produced less polymorphic products. The band profile was found similar to the study of Appa Rao *et al.*, who reported that the variations may exist between individual of same species.

The genetic distances between breeds were calculated using Nei equation through POPGENE software (Table 3). In the present study an individual primer produced more variable results as compared to results

obtained by all primers hence all primer data were pooled for estimation of genetic parameters. Neighbor-joining method is proposed for reconstructing phylogenetic trees from evolutionary distance data. The principle of this method is to find pairs of operational taxonomic units (OTUs [= neighbors]) that minimize the total branch length at each stage of clustering of OTUs starting with a star like tree. Cow no. 476 and 348 has maximum distance value 0.82697 and 478 and 476 has minimum distance value 0.17647.

Table.1 Cattle’s identification numbers and sex

Code	Cow Name
1	288
2	348
3	394
4	365
5	313
6	364
7	293
8	319
9	283
10	208
11	306
12	322
13	476
14	478
15	Bull

Table.2 List and sequences of primer used in the experiment

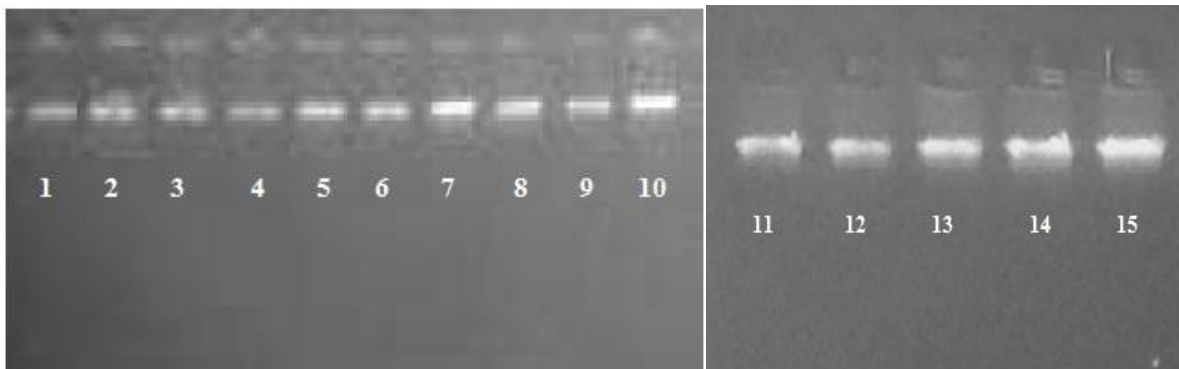
S. N.	Primer	GC %
Primer 1	5’ – CCCHGCAMCTGMTCGCACHC – 3’	60%
Primer 2	5’ – AGGHCTCGATAHCMGVY – 3’	41.18
Primer 3	5’ – MTGTAMGCTCCTGGGGATTCHC – 3’	50%

Table.3 Estimation of pair wise genetic distance between experimental Gangatiri cattle using Nei's equation

Distance Matrix Table RAPD

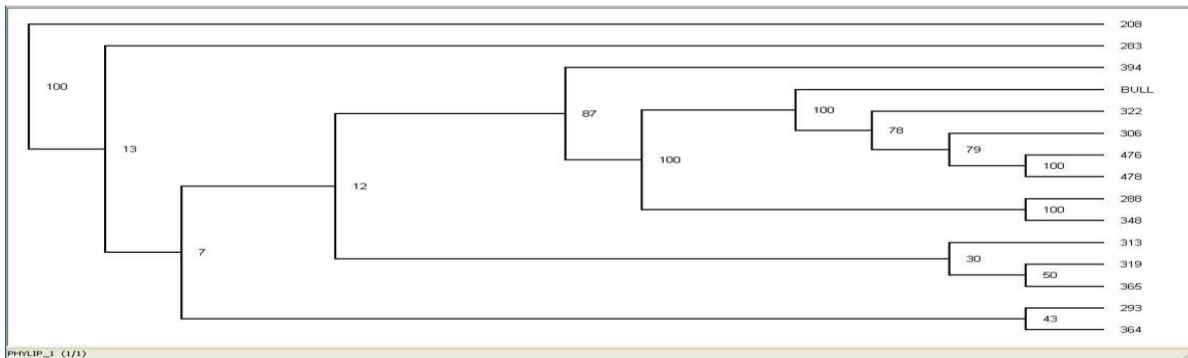
	288	348	394	365	313	364	293	319	283	208	306	322	476	478	BULL
288		0.21865	0.62724	0.64851	0.62007	0.61724	0.61433	0.61806	0.62963	0.61775	0.74661	0.76056	0.82045	0.81955	0.73483
348	0.21865		0.62909	0.60784	0.63636	0.63636	0.60208	0.64437	0.63148	0.60900	0.74194	0.75120	0.82697	0.82609	0.73913
394	0.62724	0.62909		0.42857	0.40741	0.42126	0.39689	0.41270	0.38586	0.33852	0.74054	0.80226	0.79939	0.81040	0.74263
365	0.64851	0.60784	0.42857		0.34809	0.33333	0.29381	0.27379	0.34387	0.23048	0.72703	0.77534	0.78235	0.78107	0.74479
313	0.62007	0.63636	0.40741	0.34809		0.31890	0.38132	0.29762	0.34545	0.31907	0.71351	0.74011	0.75684	0.77982	0.72118
364	0.61724	0.63636	0.42126	0.33333	0.31890		0.28731	0.28731	0.34981	0.35010	0.28358	0.75000	0.78723	0.80057	0.82235
293	0.61433	0.60208	0.39689	0.28381	0.38132	0.28731		0.34962	0.30784	0.26199	0.74874	0.75916	0.79272	0.80282	0.74065
319	0.61806	0.64437	0.41270	0.27379	0.29762	0.34981	0.34962		0.42300	0.32707	0.73711	0.77957	0.79827	0.79130	0.72890
283	0.62963	0.63148	0.38586	0.34387	0.34545	0.35010	0.30784	0.42300		0.26960	0.73615	0.77410	0.77515	0.79762	0.73298
208	0.61775	0.60900	0.33852	0.23048	0.31907	0.28358	0.26199	0.32707	0.26960		0.72864	0.77487	0.77031	0.79155	0.74065
306	0.74661	0.74194	0.74054	0.72703	0.71351	0.75000	0.74874	0.73711	0.73615	0.72864		0.32773	0.24883	0.30806	0.31518
322	0.76056	0.75120	0.80226	0.77534	0.74011	0.78723	0.75916	0.77957	0.77410	0.77487	0.32773		0.37056	0.35385	0.36100
476	0.82045	0.82697	0.79939	0.78235	0.75684	0.80057	0.79272	0.79827	0.77515	0.77031	0.24883	0.37056		0.17647	0.36111
478	0.81955	0.82609	0.81040	0.78107	0.77982	0.82235	0.80282	0.79130	0.79762	0.79155	0.30806	0.35385	0.17647		0.39252
BULL	0.73483	0.73913	0.74263	0.74479	0.72118	0.76709	0.74065	0.72890	0.73298	0.74065	0.31518	0.36100	0.36111	0.39252	

Fig.1 Genomic DNA extracted from 15 blood samples loaded on 1% agarose gel lanes



1 to 10 – gDNA of Samples 1 to 10 and Lanes 11 to 15 – gDNA of Samples 11 to 15.

Fig.2 Phylogenetic relationship among breed of Gangatiri based on RAPD pooled data from three primer using UPGMA analysis through neighbor procedure of phylip version 3.5 (POPEGENE software)



The phylogenetic relationship among these breeds was revealed through the UPMA dendrogram base on Nei's genetic distance through POPGENE software (Fig. 2). Visualization of breed relationship using NJ tree obtained from Nei's genetic distance shows clustering of breeds in conformity to the geographic location of populations. The result show that cow no. 476 and 478 are closely related similarly cow no. 288 and 348 are also closely related to each other. Nearest distance as per tree from bull is cow no. 322 and largest distance is 208.

Genetic diversity is of great significance for breeding program. Germplasm characterization is an important component of programmes on effective and efficient management, utilization of animal genetics resources. RAPD markers are randomly distributed throughout the genome, but most reason of the genome is not expressed at the phenotypic level. As a result marker like RAPD may accurately assay the degree of genetic change between two genomes but they may not necessarily reflect the divergence in term of changes in traits of livestock improvement. The differences of genetic variations in the present study found in fifteen experimental cattle samples were high and therefore, there is a scope for future improvement of local cattle. More research needs to be done on the native cattle and the choice of cattle for conservation must take into account any available information on productivity traits of economic value.

This study reveals the phylogenetic relationship among these mutants and their parents. The exotic line was different from the rest with the three molecular markers, indicating the difference in their pedigree and geographical origin. Cow no. 476 and 348 has maximum distance value 0.82697 and 478 and 476 has minimum distance value 0.17647. The result obtained here will be

useful for breeders who are willing to explore the possibility of pyramiding genes that are responsible for rose like flower mutations, reduced petal mutation and others studied. The level of diversity as evident in these mutants can be harnessed in breeding for better varieties as the divergent genotypes are expected to result in high heterosis. The unique allele can be harnessed for genotype identity. From the clustering pattern and genetic relationship obtained using RAPD markers, breeders can identify the diverse from different cluster and employ them in their future breeding programmes for the selection of parents to generate mapping populations.

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