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Assessment of genetic diversity and genetic characterization of Nili Ravi buffalo breed utilizing microsatellite markers

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Abstract

Background: Livestock contribution to Pakistan's GDP is 11.2% and it engages 8 million of rural families for their livelihood. Estimated population of buffalo is 40.00 million heads in Pakistan mostly consisting of low genetic worth population resulting in low productivity and pressure on natural resources. Assessment of genetic diversity and genetic characterization of indigenous livestock resources is an essential step towards conservation and to gauge effects of various breed improvement efforts on population genetics.

Methods: In present study genetic diversity of Nili Ravi buffalo population was assessed. A total of 196 unrelated Nili Ravi buffaloes from Punjab, Pakistan were sampled in this study. Genomic DNA was extracted and subjected to amplification using FAO recommended 12 Short Tandem Repeats (STRs) microsatellite markers. Among 12 microsatellite loci, 11 were successfully amplified (TGLA227, BM2113, ETH10, SPS115, TGLA126, TGLA122, INRA23, BM1818, ETH3, ETH225 and BM1824) whereas microsatellite locus TGLA53 was not amplified. Amplicons were resolved by genetic analyzer instrument and gene mapper software. Allele count, frequencies, gene diversity, heterozygosity, polymorphic information content (PIC) and linkage disequilibrium values were calculated by using Microsatellite toolkit v3 and Power Marker version 3.25.

Results: A total of 96 alleles were detected in 196 samples with average of 8.73 alleles per locus and range of 5 alleles (ETH 3) to 18 alleles (ETH 225) per locus. Gene diversity ranged from 0.198 (BM1824) to 0.841 (ETH225), observed heterozygosity values ranged from 0.081 (ETH10) to 0.831 (BM2113) and PIC values ranged from 0.191 (BM1824) to 0.825 (ETH225).

Conclusion: This study which will serve as a baseline to understand genetic dynamics of Nili Ravi buffalo breed. Highly polymorphic nature of STR markers will help in understanding effects of various breed improvements efforts on genetic diversity of Nili Ravi breed in future studies.

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Introduction

Hearing impairment (HI) is a heterogeneous infirmity The livestock genetic resources are integral part of agricultural ecosystem. They contribute to nutritional requirements of population by providing meat and milk. Other products and by-products from livestock resources such as hide, fiber, manure and fuel provide raw material for other industries. This contribution constitutes significant share of GDP of under develop and developing countries. Livestock sector accounts for 11.2% of total GDP of Pakistan. As most of livestock in Pakistan is raised in rural areas in small holder setups, it engages about 8 million families of rural community providing more than 35-40% of their income [1].

The water buffalo (*Bubalus bubalis*) population in Pakistan is estimated at 40.00 Million with estimated milk production of 36.18 Million tons [1]. Buffalo population in Pakistan consists of five breeds' i.e. Nili, Ravi, Nili Ravi, Azakheli and Kundi. Nili and Ravi are morphologically distinct breeds but due to intensive interbreeding among population, it is becoming increasingly difficult to find purebred Nili or Ravi specimen in field. Breed characterization based on morphological features is insufficient to understand breed's genetic dynamics, improvement and conservation. Hence molecular studies of these breeds are warranted to underscore genetic variations and to gauge effect of various breed improvement efforts in buffalo breeds of Pakistan [2]. Selection of high producing animals based on performance of their progeny and disseminating their germplasm through artificial insemination (AI) is being carried out successfully in Punjab toimprove genetic potential of native buffalo breeds. On the other hand such efforts create pressure on genetic diversity of population due to availability and utilization of few high genetic worth males in AI programs resulting in low genetic diversity in population. Food and Agricultural Organization (FAO) in collaboration with International Society of Animal Geneticists (ISAG) addressed this issue by publishing guidelines on Measurement of Domestic Animal Diversity (MoDAD) [3].

Breed characterization exploiting Short Tandem Repeats (STRs) markers is recommended method by FAO. Briefly, STRs are tandem repeats of 2 to 7 nucleotide innon coding regions of genome. STRs are excellent source of measuring quantitative genetic variation in population owing to their polymorphism. STRs are non-coding regions hence they are not prone to selection pressure. Moreover, they are randomly distributed in genome thus providing reliable genetic dynamics of population. Using STRs panels in genetic characterization is also helpful in interpretation and utilization of data at global scale as well as its comparison with other genetically characterized breeds. Using similar panel of STRs markers in future will also show clearer picture of genetic trends in that breed. Therefore, the present study was designed to genetically characterized and to assess its genetic diversity in Nili Ravi buffalo population of Punjab province of Pakistan utilizing STRs markers [4].

Methods

A total of 196 blood samples were taken from genetically unrelated animals across Punjab. Samples were taken from adult female animals present at Livestock experiment station (LES) Bhunikey district Kasur, LES Rakh Ghulaman district Bhakkar, LES Khushab, LES ChakKatora district Hasilpur, LES Haroonabad district Bahawalnagar and from field area in Punjab. All sampled animals were phenotypically true to Nili Ravi breed. Venous blood samples of 5 to 10 ml were taken from each animal in vials containing EDTA as anticoagulant. Samples were stored at -20 °C prior to DNA extraction. DNA was extracted using Favor Prep ® DNA isolation kit (cat No. FABGK 001-1) following kit manual. DNA was quantified by Nano Drop One^{c} (Thermo Scientific®) spectrophotometer and diluted appropriately for PCR reaction. Samples were amplified in thermal cycler (Bio Rad® C-1000 Touch) by using Bovine Genotyping Panel 1.2 (Thermo Scientific® cat No. F904S). Samples were initially denatured at 98 °C for 1 min following by 30 cycle of amplification. Thermal profile for amplification was: 98 °C for 20 sec for denaturation, 60 °C for 75 sec for annealing and 72 °C for 30 sec for extension. 5 minutes at 72 °C were provided for final extension. Every batch included standard DNA specimen provided with Bovine genotyping 1.2panel for accurate genotyping and allele calling. Samples were genotyped by using Genetic Analyzer 3130 (Applied Biosytems®) with GeneScan™ 500 LIZ® as size standard. GeneMapper® Software version 5 was used for analysis of genotyping data and allele calling. Microsatellite loci TGLA227, BM2113, ETH10, SPS115, TGLA126, TGLA122, INRA23, BM1818, ETH3, ETH225 and BM1824 were amplified successfully, whereas Microsatellite locus TGLA53 was not amplified. Resulting data were analyzed for number and frequency of alleles, expected heterozygosity and polymorphism information contents (PIC), Linkage disequilibrium (LD) Matrix by using Microsatellite toolkit v3 and Power Marker version 3.25 tool.

Results

A total of 96 alleles were detected in 196 samples with average of 8.73 alleles per locus and range of 5 alleles (ETH 3) to 18 alleles (ETH 225) per locus. Details of locus wise number of alleles are shown in table 1. Overall population statistics calculated by using Microsatellite toolkit v3 are described in table 2. Gene diversity, Heterozygosity and Polymorphic information content values were calculated by Power Marker version 3.25 tool [5]. Heterozygosity values indicate genetic variability and population bottlenecks. Gene diversity or expected Heterozygosity is probability of finding heterozygous alleles in a random sample from population under Hardy-Weinberg Equilibrium[6].It ranged from 0.198 (BM1824) to 0.841 (ETH225). Observed Heterozygosity values were highest0.831 for locus BM2113 and lowest 0.081 for locus ETH10. Polymorphic information content (PIC) values ranged from 0.191 (BM1824) to 0.825 (ETH225). The Expected and Observed heterozygosity as well as PIC values for each locus are shown in Table 3.

Locus	TGLA227	BM2113	ETH10	SPS115	TGLA126	TGLA122	INRA23	BM1818	ETH3	ETH225	BM1824
No. of Alleles Detected	6	9	7	9	9	11	6	8	5	18	8
Chromosome	18	2	5	15	20	21	3	23	19	9	1

Table 1: Locus wise allele numbers.

Sample size	Loci typed	Unbiased Hz	Unbiased Hz SD	Obs Hz	Obs Hz SD	No Alleles	No Alleles SD
196	11	0.7245	0.0257	0.9483	0.0072	8.73	3.35

Table 2: Population statistics.

Locus	Expected Heterozygosity	Observed Heterozygosity	PIC values
TGLA227	0.6960	0.5561	0.6530
BM2113	0.7749	0.8316	0.7422
ETH10	0.2931	0.0816	0.2777
SPS115	0.7512	0.5357	0.7139
TGLA126	0.7980	0.4745	0.7709
TGLA122	0.7687	0.3520	0.7390
INRA23	0.6369	0.2194	0.5873
BM1818	0.7502	0.3827	0.7236
ETH3	0.4133	0.4847	0.3519
ETH225	0.8411	0.4898	0.8254
BM1824	0.1983	0.1582	0.1912

Table 3: Expected heterozygosity, observed heterozygosity and PIC values of Nili Ravi buffalo.

Locus												
TGLA227	Fragment Length (BP)	69	71	73	75	77	79					
	Count	4	73	16	89	181	16					
	Frequency	0.0102	0.1862	0.0408	0.2270	0.4617	0.0408					
	SD	0.0062	0.0201	0.0110	0.0229	0.0272	0.0122					
BM2113	Fragment Length (BP)	121	123	125	127	129	131	135	137	139		
	Count	25	18	70	110	15	2	1	126	23		
	Frequency	0.06377551	0.045918	0.178571	0.280612	0.038265	0.005102	0.002551	0.321429	0.058673		
	SD	0.0119139	0.010314	0.017857	0.021384	0.009494	0.003589	0.002545	0.022385	0.013083		
ETH10	Fragment Length (BP)	204	210	218	222	232	236	238				
	Count	30	1	1	1	2	28	2				
	Frequency	0.07653061	0.002551	0.002551	0.002551	0.005102	0.071429	0.005102				
	SD	0.01641561	0.002545	0.002545	0.002545	0.005089	0.015726	0.005089				
SPS115	Fragment Length (BP)	244	248	250	256	258	260	268	270	272		
	Count	1	1	115	141	32	3	19	19	3		
	Frequency	0.00255102	0.002551	0.293367	0.359694	0.081633	0.007653	0.048469	0.048469	0.007653		
	SD	0.0025445	0.002545	0.025704	0.025709	0.014152	0.004385	0.011166	0.011166	0.004385		
TGLA126	Fragment Length (BP)	118	122	124	126	128	130	132	134	138		
	Count	1	20	18	64	85	21	2	2	125		
	Frequency	0.00255102	0.05102	0.045918	0.163265	0.216837	0.053571	0.005102	0.005102	0.318878		
	SD	0.0025445	0.011397	0.010927	0.019941	0.02312	0.014146	0.005089	0.005089	0.028553		
TGLA122	Fragment Length (BP)	129	131	133	135	137	139	141	147	197	199	201
	Count	1	1	2	5	72	54	9	2	66	150	12
	Frequency	0.00255102	0.002551	0.005102	0.012755	0.183673	0.137755	0.022959	0.005102	0.168367	0.382653	0.030612
	SD	0.0025445	0.002545	0.005089	0.005631	0.023595	0.020586	0.0083	0.005089	0.018356	0.03434	0.011764
INRA23	Fragment Length (BP)	193	195	197	199	207	209					
	Count	100	207	43	8	1	1					
	Frequency	0.25510204	0.528061	0.109694	0.020408	0.002551	0.002551					
	SD	0.02963794	0.032707	0.01722	0.007067	0.002545	0.002545					
BM1818	Fragment Length (BP)	255	257	267	269	273	275	277	279			
	Count	8	32	62	169	25	20	3	12			
	Frequency	0.02040816	0.081633	0.158163	0.431122	0.063776	0.05102	0.007653	0.030612			
	SD	0.00706659	0.014605	0.022296	0.031993	0.01296	0.011954	0.004385	0.008562			
ETH3	Fragment Length (BP)	105	107	115	117	125						
	Count	94	2	2	9	285						
	Frequency	0.23979592	0.005102	0.005102	0.022959	0.727041						
	SD	0.01820334	0.003589	0.005089	0.007475	0.021127						
ETH225	Fragment Length (BP)	131	133	137	139	141	143	145	149	151	153	155
	Count	5	16	24	42	11	5	108	9	8	11	8
	Frequency	0.0127551	0.040816	0.061224	0.107143	0.028061	0.012755	0.27551	0.022959	0.020408	0.028061	0.020408
	SD	0.00563103	0.012677	0.012251	0.017113	0.008977	0.007599	0.023724	0.00905	0.008716	0.010937	0.009433
ETH225 (cont.)	Fragment Length (BP)	161	163	165	167	169	171	173				
	Count	7	15	6	5	8	3	5				
	Frequency	0.017857	0.038265	0.015306	0.012755	0.020408	0.007653	0.012755				
	SD	0.007546	0.011925	0.006152	0.005631	0.007067	0.004385	0.007599				
BM1824	Fragment Length (BP)	173	175	181	185	191	211	217	227			
	Count	1	1	3	1	3	350	25	2			
	Frequency	0.00255102	0.002551	0.007653	0.002551	0.007653	0.892857	0.063776	0.005102			
	SD	0.0025445	0.002545	0.004385	0.002545	0.004385	0.017489	0.011914	0.005089			

Table 4: Allele sizes and absolute count of 11 microsatellite loci of Nili Ravi Buffalo.

Marker	BM2113	ETH10	SPS115	TGLA126	TGLA122	INRA23	BM1818	ETH3	ETH225	BM1824
BM2113	0.1114	0.0819	0.0726	0.0829	0.1057	0.0662	0.0946	0.0562	0.1010	0.0854
ETH10		0.0717	0.0837	0.2601	0.1285	0.0665	0.1035	0.1015	0.1705	0.1043
SPS115			0.0975	0.0800	0.1587	0.0674	0.0538	0.1250	0.1253	0.1225
TGLA126				0.1055	0.1113	0.0782	0.0695	0.0796	0.1344	0.1076
TGLA122					0.1775	0.0996	0.0924	0.0589	0.1567	0.1312
INRA23						0.2343	0.0777	0.0967	0.1542	0.1297
BM1818							0.0898	0.0559	0.0986	0.1539
ETH3								0.0610	0.1232	0.0526
ETH225									0.1009	0.1516
BM1824										0.1012

Table 5: LD matrix for Nili Ravi buffalo population

Details of polymorphic allele size, count and frequency at each locus is shown in Table 4. Linkage disequilibrium (LD) is association of alleles at two different loci and shows dependence of gene frequencies. LD Matrix for Nili Ravi Buffalo is shown in Table 5.

Discussion

Genetic diversity of Nili Ravi Buffalo breed based on FAO approved STR markers was reported in this study which will serve as a baseline to understand genetic dynamics of breed. Highly polymorphic nature of STR markers will help in understanding effects of various breed improvements efforts on genetic diversity of Nili Ravi breed in future studies.The use of FAO approved markers will be helpful for future studies on same breed as well as comparative studies on other breeds.

Authors' Contributions

AB designed and conducted the study. NA and FK helped in lab work. NA conducted field work and write up. FK, NA and MS helped in data compilation and analysis. AB and ASS reviewed and approved the draft.

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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