Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2018; 7(3): 323-325
Received: 12-03-2018
Accepted: 16-04-2018

**K Swathi**
Ph.D., Department of animal genetics and breeding, College of veterinary science, Rajendranagar, Telangana, India

**B Ramesh Gupta**
Associate Dean (Retd.), College of veterinary science, Korutla, Telangana, India

**S Sai Reddy**
Associate professor, Department of animal genetics and breeding, college of veterinary science, Rajendranagar, Telangana, India

**M Kishan Kumar**
Professor, Department of Livestock Production and Management, College of Veterinary Science, Koratla, Telangana, India

**Correspondence**
**K Swathi**
Ph.D., Department of Animal Genetics and Breeding, College of Veterinary, Science, Rajendranagar, Telangana, India

# Assessment of genetic variability in murrah buffaloes using microsatellite markers

**K Swathi, B Ramesh Gupta, S Sai Reddy and M Kishan Kumar**

**Abstract**
In the present study, a total of 30 microsatellite markers selected from the list suggested by FAO were screened to explore genetic variability in Murrah buffaloes. All the target loci were successfully amplified by polymerase chain reaction and observed number of alleles ranged from 4 (CSSM032 and ILSTS005) to 12 (CSSM033 and ILSTS028) with a total of 239 alleles across the 30 loci. The overall mean observed heterozygosity, expected heterozygosity and unbiased expected heterozygosity values were 0.439, 0.826 and 0.841, respectively, and ranged from 0.000 to 0.967; 0.709 to 0.899; and 0.712 to 0.915, respectively. All the 30 primer-pairs used in the present study were found to be polymorphic and highly informative with the PIC values ranging from 0.656 to 0.879 with overall mean PIC 0.803. The overall mean inbreeding coefficient ($F_{IS}$) and the outcrossing rate obtained in the present study were 0.483 and 0.464, respectively. Out of 30 microsatellite marker primers studied, 29 markers showed significant deviation from Hardy - Weinberg equilibrium which might be due to selection of animals over the generations. The sufficiently high mean values of observed number of alleles, observed heterozygosity and PIC for various microsatellites in the present study supported their suitability for genetic diversity studies. Results of the present study have contributed to the knowledge of genetic information of Murrah buffaloes.

**Keywords:** murrah buffalo, microsatellite, genetic variability, inbreeding coefficient, hardy - weinberg equilibrium

## Introduction
India has 105.1 million buffaloes, comprising 56.7% of World buffalo population (Sethi, 2012) [17]. India is the World's highest milk-producing country with an average sectoral growth rate of 1.5%. Mechanization, unplanned and indiscriminate breeding among native stocks and human bias in favour of certain breeds are directly or indirectly responsible for the dilution of Indian livestock breeds. The Murrah buffalo is one of the finest genetic resource as a milch animal world-wide. It is extensively used for grading up of non-descript buffaloes in India and abroad. Molecular characterization of Murrah breed is important for prevention of germplasm erosion. Microsatellites are highly polymorphic, dispersed throughout genome at a frequency of one at every 6 kb sequence (Bruford and Wayne, 1993) [2] and amenable to PCR amplification. Hence, the present study was carried out with an objective of assessing the molecular genetic variability of Murrah buffaloes of Telangana region using microsatellite markers.

## Materials and Methods
A total of 35 unrelated Murrah buffaloes (9 males and 26 females) were utilized for the present study. The blood samples were obtained from Institutional Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad and Livestock Research Station, Mamnoor, Warangal and the animals chosen were unrelated by ancestry. The genomic DNA was isolated and assessed for purity following standard molecular biology protocols (Sambrook and Russell, 2001) [16]. The quality and quantity of DNA was evaluated on spectrophotometer and through 0.8% agarose gel electrophoresis. A set of 30 dinucleotide microsatellite markers were chosen from the list recommended by FAO (http://www.fao.org/docrep/meeting/022/am652e.pdf)
Polymerase chain reaction (PCR) amplification of microsatellite loci was carried out in a 12.5µl reaction volume containing 1.5 mM $MgCl_2$, 2.5 mm dNTPs, 60 pM of forward/reverse primer, approximately 100 ng of genomic DNA and 0.5 U of Taq DNA polymerase. PCR was carried out using cycling conditions as: 5 min at 95 °C, followed by 34 cycles of 1 min at 94°C, 30 s at annealing temperature (depending upon locus), 30 s at 72°C and final extension at 72°C for 5 min. PCR amplification was confirmed on 2% agarose gel containing ethidium bromide. PCR products were then resolved by running on 8% polyacrylamide gel electrophoresis and visualized by silver staining (Cominicini *et al*., 1995). Allele sizes were estimated by running a 10 bp DNA ladder (GeneRuler, Fermentas) along with the PCR products and genotypes scored manually.

The genotype data was analysed by using Excel Microsatellite Tool kit (Park, 2001) [14] and GenAlex 6.1 (Peakall and Smouse, 2006) [15]

**Results and Discussion**

In the present study, effort was made to characterize the extent of genetic variability in Murrah buffaloes. The overall mean optical absorbance ratio of isolated DNA was 1.664, indicating good quality. The mean concentration of DNA was 1.281 µg/µl, which was higher than the mean quantity of 0.472 µg/µl reported by Gayathri (2005) in Murrah buffaloes. Various within breed diversity estimates, viz. mean number of alleles ($N_a$), effective number of alleles ($N_e$), Shannon's information index (I), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$) and unbiased expected heterozygosity ($uH_e$), polymorphism information content (PIC), fixation indices ($F_{IS}$) at each microsatellite marker analysed in Murrah buffaloes are presented in Table 1.

A total of 239 alleles were amplified across 30 loci. The allele size varied from 88 (RM099) to 274 (BM1818) bp. The number of alleles obtained at a locus varied from 4 to 12, which was slightly higher number of alleles than the report of Navani *et al*. (2002) [13] for the Murrah, Nili-Ravi and Mehsana breeds of buffaloes in India. The average number of alleles per locus of 7.967 obtained in the present study was in line with the recommendation by FAO suggesting at least 5 different alleles per locus for estimation of genetic diversity between the breeds (Muralidhar *et al*., 2004) [12]. The high mean number of alleles implies great allelic diversity which can be exploited for selection and indicates their suitability for the diversity analysis (Mishra *et al*., 2009b) [11]. The mean number of alleles per locus in the present study was similar to the reports of Kathiravan *et al*. (2010) [6] in South Kanara buffaloes and Marques *et al*. (2011) in Brazilian buffaloes; and high, when compared to the reports in Murrah buffaloes by Martinez *et al*. (2006) [9] and Bhuyan *et al*. (2010) [1]. The effective number of alleles ranged from 3.435 to 9.945 with an overall mean of 6.117. This value was higher than the reports made on Banni (Mishra *et al*., 2009b) [11], Nagpuri (Kataria *et al*., 2009) [5], Marathwada (Kathiravan *et al*., 2009) [7] and Chilika buffaloes (Mishra *et al*., 2009a) [10]. The overall mean Shannon's information index was 1.880 and ranged from 1.301 (CSSM032) to 2.340 (CSSM013). The overall mean observed heterozygosity, expected heterozygosity and unbiased expected heterozygosity obtained in the present study was 0.439, 0.826 and 0.841, respectively and varied from 0.000 to 0.967; 0.709 to 0.899; and 0.721 to 0.915, respectively. The observed heterozygosity values at all the loci studied were lower than the expected heterozygosity which might be due to the fact that samples collected from the animals which were maintained as closed populations, even though samples selected were from unrelated individuals and also due to the presence of more homozygosity in the individual samples analyzed.

The overall mean PIC was 0.803 and ranged from 0.656 (CSSM032) to 0.879 (ILSTS029), comparable with the reports of Martinez *et al*. (2006) [9] in Murrah buffaloes. All the 30 loci studied were found to be polymorphic with a PIC more than 0.5 and regarded as highly informative. The mean inbreeding coefficient ($F_{IS}$) obtained in the present study was 0.483. A majority (80%) of the loci had positive $F_{IS}$ values indicating considerable heterozygosity deficit within the population. Out of 30 microsatellite markers studied, 29 markers (except BM1818) showed significant deviation from Hardy-Weinberg equilibrium, which might be because of heterozygote deficiency due to selective breeding practiced in the herds over several generations under closed herd conditions.

Sufficiently high mean values of observed number of alleles, observed heterozygosity and PIC for various microsatellites in the present study supported their suitability for further genetic diversity studies. Results of the present study have contributed to the knowledge of genetic information of Murrah buffaloes.

**Table 1:** Mean number of alleles ($N_a$), Effective number of alleles ($N_e$), Shannon's Information Index (I), Observed heterozygosity ($H_o$), Expected heterozygosity ($H_e$) and Unbiased Expected heterozygosity ($uH_e$), Polymorphism Information content (PIC) and Fixation Indices ($F_{IS}$) at various microsatellite loci in Murrah buffaloes.

| Locus | $N_a$ | $N_e$ | I | $H_o$ | $H_e$ | $uH_e$ | PIC | $F_{IS}$ |
|---|---|---|---|---|---|---|---|---|
| BMC1013 | 8 | 5.873 | 1.907 | 0.036 | 0.830 | 0.845 | 0.809 | 0.957 |
| BM1818 | 8 | 5.809 | 1.846 | 0.912 | 0.828 | 0.840 | 0.805 | -0.101 |
| BRN | 9 | 6.149 | 1.959 | 0.357 | 0.837 | 0.853 | 0.818 | 0.573 |
| CSSM013 | 11 | 9.945 | 2.340 | 0.967 | 0.899 | 0.915 | 0.819 | -0.075 |
| CSSM019 | 11 | 7.347 | 2.129 | 0.433 | 0.864 | 0.879 | 0.849 | 0.498 |
| CSSM029 | 7 | 4.900 | 1.723 | 0.938 | 0.796 | 0.809 | 0.768 | -0.178 |
| CSSM032 | 4 | 3.435 | 1.301 | 0.000 | 0.709 | 0.721 | 0.656 | 1.000 |
| CSSM033 | 12 | 7.945 | 2.217 | 0.912 | 0.874 | 0.887 | 0.861 | -0.043 |
| CSSM022 | 5 | 4.512 | 1.547 | 0.097 | 0.778 | 0.791 | 0.742 | 0.876 |
| CSSM047 | 6 | 4.765 | 1.659 | 0.000 | 0.790 | 0.813 | 0.759 | 1.000 |
| CSSM057 | 11 | 7.891 | 2.183 | 0.848 | 0.873 | 0.887 | 0.860 | 0.028 |
| CSSM061 | 8 | 6.828 | 1.965 | 0.788 | 0.854 | 0.867 | 0.836 | 0.077 |
| CSSM062 | 6 | 4.960 | 1.664 | 0.114 | 0.798 | 0.810 | 0.768 | 0.857 |
| CSRM060 | 9 | 6.671 | 2.031 | 0.281 | 0.850 | 0.864 | 0.833 | 0.669 |
| CSSME070 | 9 | 7.180 | 2.085 | 0.882 | 0.861 | 0.874 | 0.846 | -0.025 |
| DRB3 | 6 | 5.951 | 1.788 | 0.000 | 0.832 | 0.845 | 0.809 | 1.000 |
| ETH003 | 8 | 5.240 | 1.806 | 0.414 | 0.809 | 0.823 | 0.783 | 0.489 |
| ETH121 | 7 | 6.149 | 1.871 | 0.000 | 0.837 | 0.850 | 0.816 | 1.000 |
| HMH1R | 10 | 5.873 | 2.005 | 0.500 | 0.830 | 0.845 | 0.812 | 0.397 |
| RM099 | 6 | 4.767 | 1.655 | 0.000 | 0.790 | 0.802 | 0.759 | 1.000 |
| ILSTS017 | 9 | 6.844 | 2.020 | 0.600 | 0.854 | 0.868 | 0.837 | 0.297 |
| ILSTS019 | 5 | 3.806 | 1.444 | 0.000 | 0.737 | 0.754 | 0.696 | 1.000 |
| ILSTS028 | 12 | 7.670 | 2.201 | 0.688 | 0.870 | 0.883 | 0.856 | 0.209 |
| ILSTS029 | 10 | 9.058 | 2.250 | 0.960 | 0.890 | 0.908 | 0.879 | -0.079 |
| ILSTS030 | 10 | 7.377 | 2.110 | 0.414 | 0.864 | 0.880 | 0.850 | 0.521 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ILSTS034 | 7 | 5.333 | 1.801 | 0.833 | 0.813 | 0.830 | 0.788 | -0.026 |
| ILSTS005 | 4 | 3.948 | 1.380 | 0.000 | 0.747 | 0.760 | 0.699 | 1.000 |
| ILSTS008 | 6 | 5.297 | 1.730 | 0.000 | 0.811 | 0.826 | 0.785 | 1.000 |
| ILSTS058 | 7 | 6.240 | 1.887 | 0.516 | 0.840 | 0.854 | 0.820 | 0.385 |
| ILSTS087 | 8 | 5.734 | 1.885 | 0.680 | 0.826 | 0.842 | 0.804 | 0.176 |
| Mean | 7.967 | 6.117 | 1.880 | 0.439 | 0.826 | 0.841 | 0.803 | 0.483 |

**References**


1. Bhuyan DK, Sangwan ML, Gole VC, Sethi RK. Studies on DNA Fingerprinting in Murrah buffaloes using microsatellite markers. Ind. J Biotech. 2010; 9:367-37.
2. Bruford MW, Wayne RK. Microsatellites and their application to population genetic studies. Cur. Opt. Gen. and Dev. 1993; 3:939-943.
3. Cominicini S, Leone P, Redaell I, De Giuli L, Zhang Y, Feretti L. Characterization of bovine microsatellites by silver staining. J Anim. Breed Genet. 1995; 112:415-440.
4. Gayathri S, Subramanian A, Sivaselvam SN, Karthickeyan SMK, Kumarasamy P, Saravanan *et al*. Molecular characterization of murrah buffaloes using cattle microsatellite markers. Buffalo J. 2006; 1:85-90.
5. Kataria RS, Sunder S, Malik G, Mukesh M, Kathiravan P, Mishra BP. Genetic diversity and bottleneck analysis of Nagpuri breed of India based on microsatellite data. Russ. J Genet. 2009; 45:826-832.
6. Kathiravan P, Mishra BP, Kataria RS, Goyal S, Tripathy K, Sadana DK. Short tandem repeat based analysis of genetic variability in Kanarese buffalo of South India. Russ. J Genet. 2010; 46:988 to 993.
7. Kathiravan P, Mishra BP, Kataria RS, Sadana DK. Evaluation of genetic architecture and mutation drift equilibrium of Marathwada buffalo population in Central India. Livest. Sci. 2009; 121:288-293.
8. Marques JRF, Martínez AM, Costa MR, Albuquerque MSM, Quiroz J, Vega-Pla JL *et al*. Genetic diversity of Brazilian buffaloes (*Bubalus bubalis*) using DNA microsatellites. Revista arch Zootec. 2011; 60:231-239.
9. Martinez E, Tirado JF, Ceron-Munoz MF, Moreno M, Montoya A, Corrales JD *et al*. Genetic characterization of the Murrah buffalo in Columbia using microsatellite markers, 2006. www.scielo.org.co.
10. Mishra BP, Kataria RS, Bulandi SS, Prakash P, Kathiravan P, Mukesh M, *et al*. Riverine status and genetic structure of Chilika buffalo of eastern India as inferred from cytogenetic and molecular marker-based analysis. J Anim. Breed Genet. 2009a; 126:69-79.
11. Mishra BP, Kataria RS, Kathiravan P, Bulandi SS, Singh, KP, Sadana DK. Evaluation of genetic variability and mutation drift equilibrium of Banni buffalo using multilocus microsatellite markers. Trop. Anim. Health Prod 2009b; 8:301-308.
12. Muralidhar M, Sriramana K, Narasimharao G, Nagaraju J. Genetic characterization of the Indian cattle breeds, Ongole and Deoni (*Bos indicus*), using microsatellite markers–a preliminary study. BMC Genet. 2004; 5:16.
13. Navani N, Jain PK, Gupta S, Sisodia BS, Kumar S. A set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (*Bubalus bubalis*). Anim. Genet. 2002; 33:149-154.
14. Park SDE. The Excel microsatellite toolkit V 31 Animal Genomics Laboratory University College, Dublin, Ireland, 2001.
15. Peakall R, Smouse PE. GENALEX 6: genetic analysis in Excel Population genetic software for teaching and research. Mol. Ecol. Notes. 2006; 6:288-295.
16. Sambrook J, Russel DW. Molecular Cloning, Animal Laboratory manual. Cold Spring Harbor Laboratory press, Cold spring Harbor, New York, 2001.
17. Sethi RC. Buffalo breeding research in India. Compendium of national symposium on "Improvement of livestock productivity through conventional breeding and emerging technologies in changing global scenario-challenges, prospects and retrospects" held at College of Veterinary Science, Rajendranagar, 2012, 23-30.