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Original Research

Genetic Polymorphism of Melatonin Receptor (MTNR1A) Gene in Swamp Buffalo of Assam and Manipur, India

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Abstract

The most important environmental factor that affects the regulation of reproductive seasonality in buffaloes is photoperiod which biologically acts through its effect on melatonin hormone secretion. The melatonin receptor, MTNR1A appears to have marked involvement in this regulation of seasonal reproductivity along with milk production during the period. The aim of this study was to identify genetic polymorphism in the MTNR1A gene in the swamp buffalo population of Assam (Luit) and Manipur (Manipuri eroi) states of India using PCR-RFLP methods. Three genotypes CC, CT and TT have been detected in the Luit buffalo population having frequencies 0.37, 0.47 and 0.16, respectively while the genotype TT was found to be absent in the Manipuri eroi buffalo. In regards to MTNR1A locus the two populations do not show much variation as indicated by gene frequency. The presence of variability in the population indicates the potential of increasing the production performance of the population by implying proper selection methods and breeding systems.

Keywords: Genotypes, Genetic Polymorphism, Genetic Variability, MTNR1A Gene, Swamp Buffalo, PCR-RFLP

Introduction

Buffaloes belonging to state of Assam and Manipur of India are swamp type in nature, mainly reared for land preparation in wet paddy fields as they are more adaptable to the sub-tropical climate of Assam and Manipur. The farmers with poor resources usually prefer these multipurpose swamp buffaloes as they provide many benefits like milk, meat and drought power to farmers under zero input system and thus play an important role in farmers' economy.

The reproductive seasonality in buffaloes causes low reproductive efficiency during specific seasons of the year and thereby leading to seasonal shortage of milk and milk products causing huge economic loss to the farmers. The melatonin hormone secretion pattern has been seen to be involved in providing information of photoperiod to the brain cells, which have the necessary receptors to control reproductive functions (Zetouni *et al.,* 2013). Two types of Melatonin receptors have been reported in mammals (MTNR1A and MTNR1B) out of which only the MTNR1A has been seen to be associated with regulation of seasonal reproductive pattern (Dubocovich *et al.,* 2003). The melatonin receptor (MTNR1A) gene has been found in the chromosome 1 of buffalo which is a fusion of *Bos taurus* chromosome 1 and 27 (Iannuzzi *et al*., 2003; Miziara *et al*., 2007). Therefore, investigation of polymorphism in the MTNR1A gene is very important for buffalo farming and dairy industry as a major limitation of buffalo reproduction is its seasonal breeding nature.

The main aim of this study was to study the polymorphism in MTNR1A receptor gene in swamp buffalo population of Assam (Luit) and Manipur (Manipuri eroi) and to observe the genetic variability and heterozygosity present within and between the two populations and possibility of making genetic improvement thereafter with the help of the information obtained in the present study.

Materials and Methods

Animals and Blood Samples

The present study was carried on a total of 30 swamp buffaloes each from the states of Assam and Manipur. The animals were randomly chosen irrespective of their age and sex from the fields of Nagaon, Morigaon, Hojai, Sivsagar, Cachar and Jorhat districts of Assam and Imphal east, Imphal west and Senapati districts of Manipur. Blood samples had been collected aseptically from the jugular vein of the selected animals. About 2 ml of blood was collected in EDTA coated vials. Blood samples were then kept immediately in ice after collection and after bringing to the laboratory were stored at -20˚C till further use.

Genomic DNA Isolation

Genomic DNA from the collected samples was extracted using the GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) following the protocols provided with the kit. The quantity and quality of DNA extracted from the samples were checked using a Nano Spectrophotometer (Thermo Scientific, USA). DNAs having OD_{260}/OD_{280} ratio of 1.7 to 1.9 were subjected to further molecular analysis. The primers and restriction enzyme used for PCR-RFLP analysis are given in Table 1.

Table 1: Gene, primer sequence, restriction endonuclease (RE) enzyme used for RFLF analysis, PCR amplicon size and annealing temperature for PCR

Gene	Name of Primers	Primer sequence (5´ -3´)	RE	Product size (bp)	T^A (°C)	Reference
MTNR1A	F	TGTGTTTGTGTTGAGCCTGG	*Hpa*I	824	58	Zetouni *et al.,* 2013
	R	ATGGAGAGGGTTTGCGTTTA				

PCR and RFLP

The PCR amplification was carried out in a 25 µl reaction mixture of 10X PCR buffer, 200 µM of each dNTPs, 2mM of $Mgcl_2$, 2U Taq DNA polymerase, 5 pM of each primers and 60 ng genomic DNA. The following PCR cycles were applied: Initial Denaturation at 95ºC for 5 min, followed by 30 cycles of 95ºC for 45 sec, 58ºC for 65

sec, 72ºC for 45 sec and final extension at 72ºC for 5 min. The amplified DNA of 824 bp was subjected to RE digestion using *Hpa*I enzyme by incubating at 37ºC for 1 hour. The digested products were separated for visualisation of the bands using 2% agarose gel in 0.5 X TAE containing 1.0 µM ethidium bromide and observed under UV trans-illuminator after which photographs were taken using Gel Doc system for further analysis.

Statistical Analysis

The allele frequencies, genotypic frequencies, observed and expected heterozygosity along with F-statistics were calculated using the POPGENE 32 software (Yeh *et al.*, 1997) for population genetics analysis.

Results and Discussion

The melatonin receptor protein has a 360 - 420 amino acids long chain and is composed of two exons and a long (>2 kb) intron between them (Kokkola and Laitinen, 1998). The polymorphism in the Exon 2 of MTNR1A gene was detected by PCR-RFLP analysis which was carried out by the digestion of PCR product (824 bp) with restriction endonuclease *Hpa*I for 1 hr at 37°C. Restriction digestion of amplicon yielded three genotypes *i.e.*, TT (824 bp), CT (824, 745 and 79 bp) and CC (745 and 79 bp) showing different band pattern for each genotypes (Fig. 1).

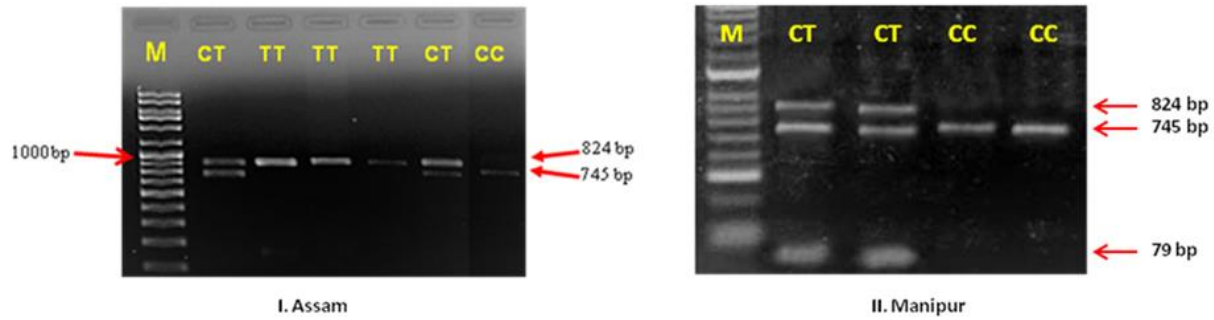

Figure 1: Genotypes of MTNR1A gene digested with RE *Hpa*I in 3% agarose gel

The genotype frequency along with observed and expected heterozygosity of Luit and Manipuri eroi buffaloes has been presented in Table 2.

Table 2: Genotypic frequency distribution in Swamp buffaloes of Assam (Luit) and Manipur (Manipuri eroi)

Genotypes	Assam (Luit) (n=30)	Manipur (Manipuri eroi) (n=30)
CC	0.37 (11)	0.43 (13)
CT	0.47 (14)	0.57 (17)
TT	0.16 (5)	0.00 (0)
Observed Heterozygosity	0.47	0.57
Expected Heterozygosity	0.49	0.41
χ^2 value	0.06 ^{NS}	4.37 ^{NS}

n = Number of animals; NS = Not significant

It was observed that the genotype TT was completely absent in the population of Manipuri eroi while 5 animals showed the TT genotype in the buffalo population of Luit with a genotype frequency of 0.16. The genotype CT was found higher in both the populations with frequencies of 0.47 and 0.57 in Luit and Manipuri eroi, respectively. The CC genotype was prevalent with the frequencies of 0.37 and 0.43, respectively. The calculated Chi-square value revealed that both the populations were in Hardy-Weinberg equilibrium with respect to the MTNR1A genotypes. The observed heterozygosity was found to be slightly higher in Manipuri eroi (0.57) as compared to the Luit buffalo population (0.47). The difference between the observed and expected heterozygosity in the Luit buffalo was found to be very less as compare to Manipuri eroi population. The C allele was found to be the predominant allele for the MTNR1A locus in both the populations as it had a higher frequency of 0.60 in Luit and 0.72 in Manipuri eroi with an overall frequency of 0.658 in both the populations (Table 3).

Table 3: Allele frequency of MTNR1A locus in Swamp buffalo of Assam and Manipur

Locus	Allele	Allelic frequency		Overall frequency
		Assam	Manipur	
MTNR1A	C	0.6	0.72	0.658
	T	0.4	0.28	0.342

Similar to present findings, polymorphism was detected in the MTNR1A locus but T allele was found to be more prevalent in Mediterranean buffalo population (Carcangiu *et al*., 2011) with a frequency of 0.56. The CT genotype was also seen to be most frequent in the population which was also seen in our findings. The higher frequency of CT genotype was also reported in Brazilian commercial buffalo population where it showed a frequency of 0.52 (Zetouni *et al.*, 2013). In another Italian buffalo population, it was found that the CC, CT and TT genotypes were present with a frequency of 0.26, 0.40 and 0.34, respectively. In contrast to our finding, T allele was reported to be more prevalent in Italian buffalo population (Luridiana *et al*., 2012).

In Murrah buffaloes, a similar observation with Luit buffalo was reported by Gunwant *et al*. (2018) where the frequencies of T and C alleles were 0.59 and 0.41, respectively. The existence of polymorphism in the MTNR1A locus of Murrah buffaloes was also reported by Cheema *et al*. (2016).

Conclusion

The presence of polymorphisms in the MTNR1A locus suggests the presence of variability in the population and indicates the potential of increasing the production performance of the population by implying proper selection methods and breeding systems. In regards to MTNR1A locus the two populations do not show much variation as indicated by gene frequency. The population following the Hardy-Weinberg equilibrium can be a sign of lack of any selection done in the population as most of the North eastern buffaloes are not reared in organised manner and random natural breeding occurs between the individuals in the fields.

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Conflict of Interests

There is no conflict of interest.

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