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Original Research

Single Strand Conformation Polymorphism in Exon C of Estrogen Hormone Receptor Gene (ER α) in Murrah / Graded Murrah Buffaloes

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Abstract

Buffaloes' productivity is affected mainly due to many inherent disorders. Estrogen hormone is found to be an important hormone directly linked with reproductive functions of buffalo. The present study was performed with 203 Murrah / Graded Murrah buffaloes maintained at different locations. The Polymerase Chain Reaction- Single Strand Conformation Polymorphism (PCR-SSCP) of ER- α gene revealed monomorphic pattern for all the tested animals, which indicates the fixation of allele in tested buffalo population. As a conclusion, monomorphic pattern of Estrogen Receptor- α (ER α) gene is considered a unique feature that may be related to the characteristic of buffaloes.

Keywords: Buffalo, ER α gene, Monomorphism, PCR-SSCP

Introduction

Livestock plays a major role in the socio-economic status of rural community in India, India ranks first in buffalo population as well as milk production. India is an excellent reservoir for livestock biodiversity in the form of species, breed and strains. India has 57.83 percent of world's buffalo population. As per 19th Livestock Census, the total number of milch animals in the country was 116.77 million and the buffalo contributes around 31 per cent. When compared to previous census, the milch buffalo population has increased from 48.64 million to 51.05 million with an increase of 4.95 per cent. Milk plays an important role in human health. In milk, total proteins content is around 3.5% (Miller *et al*. 2007). Casein and whey proteins (ß-lactoglobulin and alpha- actalbumin) are major protein groups with a share of about 80% and 20% respectively (Hoffman and Falvo, 2004).

The reproductive efficiency of animal has been influenced by number of genetic, environmental, nutritional and management factors. Animals reared under similar environmental and management conditions also have reproductive problems, that indicating genetic factors may also have a key role in animal reproductive efficiency (Kumar *et al*., 2014). The reproductive performance of buffaloes are commonly affected due to late maturity, poor expression of estrous, anestrous, inactive ovaries, prolonged postpartum interval, seasonal cyclicity and silent estrous (Mishra, 1997). Many genes are involved in the physiological and endocrine functions of the inherent fertility and account for the genetic association of reproduction and growth traits, milk and overall productivity (Meyer *et al.,* 1990).

The endocrine system is a major regulator of the reproductive functions through the hypothalamic–pituitary–gonad axis and its interactions. The reproductive performance of buffaloes are affected by many hormones (Estrogen, Melatonin, Follicle-stimulating hormone, Luteinizing hormone, Progesterone, Prolactin, Cortisol, etc.) coupled with their respective receptors. Estrogen is found to be an important hormone directly linked with reproductive functions and it influences growth, differentiation and functions of ovary, uterus and mammary glands (Sarla *et al*., 2015).

In livestock, candidate gene approaches are successful tools to identify the molecular genetic markers associated with production traits (Rothschild and Soller, 1997). Candidate gene approach can predict more accurate breeding values and it accelerates the genetic gain achieved by selection (Parmentier *et al*., 1999). Now a days, researchers and breeders focus on marker-assisted selection (MAS) and genome analysis for selection of livestock. MAS have higher annual rate of genetic gain in livestock by 15 to 30 per cent without increasing the risk involved in breeding schemes (Ge *et al*., 2001).

Few numbers of polymorphism studies has been reported in Indian buffaloes. Realising the importance of the functions played by estrogen hormones in reproduction, the present study of ERα genes polymorphism for reproductive traits was undertaken in Murrah / graded Murrah buffaloes.

Materials and Methods

Experimental Animals and Sample Collection

The research study was carried out with Graded Murrah buffaloes maintained at organised farms *viz*., Saraswathi Krishi Vigyan Kendra, Karur district, Tamil Nadu; Post Graduate Research Institute in Animal Sciences (TANUVAS), Katupakkam, Tamil Nadu; Central Cattle Breeding Farm, Alamadhi, Chennai, Tamil Nadu; Buffalo Research Station, Venkataramanna Gudem, S.V.V.U, West Godavari District, Andhra Pradesh and farmers herd in Namakkal district, Tamil Nadu. A total of 203 blood samples were collected from jugular vein aseptically in the vaccutainer containing EDTA as anticoagulant. Collected samples were brought in ice to the laboratory and stored at -20°C till processed.

DNA Isolation

Genomic DNA was extracted from whole blood by using the standard high salt method (Miller *et al*., 1988). Purity and concentration of genomic DNA was determined by using spectrophotometry. Quality of genomic DNA was assessed in a one per cent agarose gel using horizontal gel electrophoresis technique. Good quality DNA samples with clear bands were selected for further study and diluted to the working concentration of 100ng/µl, which is suitable for polymerase chain reaction (PCR).

Polymerase Chain Reaction (PCR)

A region of estrogen receptor α (*ER*α) gene of exon C was amplified by using a set of forward (5′ TTT GGT TAA CGA GGT GGA G ′3) and reverse (5′ TGT GAC ACA GGT GGT TTT TC ′ 3) primers (Othman and Abdel-Samad, 2013). The reaction of PCR was carried out in 25µl volume containing 12.5 µl of 2 X PCR master mix, 9.5 µl of nuclease free water 1 µl of each primer (10 pM) and 1 µl of genomic DNA. Master Mix was prepared with one additional sample to cover pipetting error. The thermal protocol used for amplifying the *ER* gene consisted of an initial denaturation step at 94º C for 1 min followed by 30 cycles shared in denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, extension at 72 °C for 1 min and final extension 72 °C for 10 min (Bio-Rad T^{100}). To confirm the targeted PCR amplification, five µl of each of PCR amplicons were electrophoresed through 2 per cent (w/v) agarose gel containing 0.5 µg/ml ethidium bromide in 1x TAE buffer. The horizontal gel electrophoresis was carried out at constant voltage of 5 V/cm for 40 min.

Single Strand Conformation Polymorphism (SSCP)

To explore genetic polymorphism in *ER* gene, amplified PCR products were subjected for Single Strand Conformation Polymorphism (SSCP) through 8 % Polyacrylamide gel electrophoresis (acrylamide:bisacrylamide (29:1) 13.3 ml; 5 x TBE 10 ml; Ammonium persulfate (10 %) 250µl; TEMED 100µl; Triple distilled water 26.35 ml and total volume of 50 ml). The gel solution was prepared by combining all reagents and allows the gel to polymerize for at least 45 minutes. The vertical electrophoresis tank was filled with 1 x TBE and gel was given a pre-run at 200 V for 30 min to remove any polar impurity. After the pre-run was completed, denatured products were loaded in to the well without quenching. The electrophoresis was performed at 4 °C for 24 hours at 120 – 150 V based on the size of fragments. After the run was completed, gel was removed from the glass plates and silver staining was carried out according to Bassam *et al.* (1991) with certain modifications to visualize the banding patterns. The gels were visualised and the images were documented in a gel documentation system (Bio-Rad Gel Doc^{TM}).

Results and Discussion

The isolated DNA was quantified and checked for quality in spectrophotometry. The mean yield of DNA isolated was 450.88 ng / µl. The ratios of optical densities were around 1.8 indicating good deproteinisation. The lower bound and upper bound for 95 per cent confidence interval for the ratio of optical density at 260/230 of DNA isolated was 1.88 and 1.91. For most of the samples, the ratios of optical densities were around 1.8 indicating less RNA contamination. The PCR products of 248bp *ERα* (exon C) gene in Murrah / Graded Murrah buffaloes were amplified by horizontal gel electrophoresis (Fig. 1).

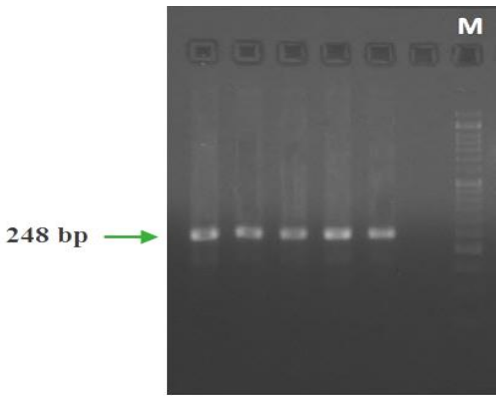

Figure 1: PCR amplified product of *ERα* gene of Murrah / Graded Murrah buffaloes

PCR-SSCP analysis of *ERα* (exon C) gene showed monomorphic pattern in all the PCR products (Fig. 2). All the tested animals showed only single band pattern for all the PCR products of *ERα* genes which indicate the fixation of allele in the studied population. There was no literature available to support the finding in buffaloes especially by PCR-SSCP method. Szreder *et al*. (2011) analyzed the *ERα* gene in Red and White cattle from Jastraebier, Poland by PCR-SSCP methods observed CC and CA genotypes and PCR-RFLP by *Cfr*I restriction enzymes observed genotypic frequency of 0.17 and 0.83 for AC and CC genotypes.

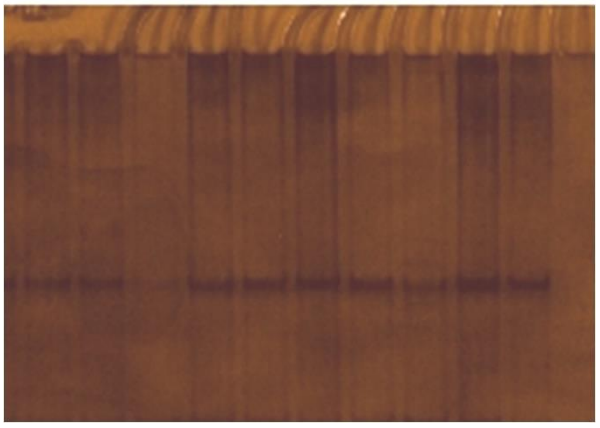

Figure 2: Monomorphic patterns of PCR products of *ERα* gene of Murrah / Graded Murrah buffaloes by PCR – SSCP

Similar to our findings Kathiravan *et al*. (2018) observed monomorphic pattern of GG genotype (171bp and 77 bp) in Graded Murrah buffaloes by PCR – RFLP method and by Sarla *et al.* (2015) in Murrah buffalo maintained at Central Institute for Research on Buffaloes, Hisar a similar monomorphic pattern of *ER*α (exon 13) was obtained with *Mbo*I restriction enzyme. Contradictory result was obtained by Othman and Abdel-Samad (2013) as two genotypes namely *AG* and *GG* with frequency of 0.18 and 0.82 respectively in Egyptain buffalo by PCR – RFLP method.

In Murrah buffaloes the genetic variation of 870 bp *ER*α (exon 13) gene by *Stu*I and *Hpa*II restriction enzymes was done by Rani *et al.* (2016) and both of these enzyme digestions exhibited monomorphic pattern in all animals. The 870 bp amplified PCR product of the ERα (exon 13) are genotyped for polymorphic pattern using *MboI* restriction enzyme revealed monomorphic pattern at 614 and 256 bp fragments representing AA genotype in Murrah / Graded Murrah buffaloes (Kathiravan *et al*., 2017).

Conclusion

The entire tested animals showed monomorphic pattern in SSCP gel electrophoresis for all the PCR products of *ER*-α gene indicates the fixation of allele in the buffalo population. Thus, we can conclude that the monomorphic pattern of Estrogen Receptor-α (*ER*α) gene can be considered a unique feature that may be related to the characteristic of Indian buffaloes.

Conflict of Interests

There is no conflict of interest.

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