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Polymorphism of *SLC11A1* gene in Doom pigs of Asom

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ABSTRACT

The solute carrier family 11 member 1 (*SLC11A1*) also called as *NRAMP1* gene is found to be associated with the transport of iron and other bivalent cations. To find out polymorphism in *SLC11A1* gene in Doom pigs of Asom, 55 blood samples were collected randomly from Doom pigs and genomic DNA was extracted using the rapid isolation of mammalian DNA method. A fragment of 934 bp length of *SLC11A1* gene was amplified using polymerase chain reaction (PCR) with a pair of specific primers. The amplified PCR products of *SLC11A1* gene was digested with *Sma*I restriction enzyme, which produced 3 fragments (505bp, 231bp and 198bp), 4 fragments (505bp, 429bp, 231bp and 198bp) and 2 fragments (505bp and 429bp) for AA, AB and BB genotypes, respectively. At position 736, A↔G transition was found for AB genotype. The present study revealed that the population of Doom pigs under study was polymorphic in respect of *SLC11A1* gene having 2 alleles with frequencies 0.8364 and 0.1636 for 'A' and 'B', respectively. The genotypic frequencies were 0.71, 0.26 and 0.03 for AA, AB and BB genotypes, respectively.

Key words: Doom pig, PCR-RFLP, Polymorphism, *Sma*I and *SLC11A1*

Pork has a lucrative demand in North Eastern States of India and hence there is a better prospect for expansion of pig farming, which is expected to uplift the economic status of the farmers. The doom variety of pig comprises a very small proportion of the total indigenous pig population in Asom. The animals are black with thick hairs on the neck and shoulder. This variety of pig is available mostly in the eastern region of India, in areas bordering Asom and West Bengal. They are reared in scavenging system and are considered to be quite hardy and resistant to many diseases. They produce the finest bristle that can be utilized for brush industry and so there is a need to conserve this unique germplasm.

The Solute carrier family 11 member A1 (*SLC11A1*) gene, formerly known as natural resistance-associated macrophage protein 1 (*NRAMP1*) was first isolated in mouse as a candidate gene for the Bcg/Ity/Lsh locus that conferred genetic resistance/susceptibility to inbred mouse strains to infections by *Mycobacteria*, *Salmonella* and *Leishmania* (Vidal *et al*. 1993). *NRAMP1* mainly exists in reticulo-endothelial cells and organs, such as outer white blood cells, spleen and lung, can resist several intracellular pathogenic microorganisms (Blackwell 1996, Supek *et al*. 1996). Tuggle *et al*. (1997) reported that the whole sequence of *NRAMP1* gene in swine encodes 539 amino acids. *NRAMP1* gene in swine is located on q23–26 of chromosome 15 (Sun *et al*. 1998, Xiaoling *et al*. 2014). The *SLC11A1* gene also codes for an iron/divalent cation transporter (Blackwell *et al*. 2003). The sequence length of *NRAMP1* gene in swine is about 15 kb, containing 15 extrons and 14 introns as that of human beings and *Mus musculus* (Govoni *et al*. 1995, Marquet *et al*. 2000, Wu *et al*. 2007). The present study was undertaken to identify polymorphism in the genomic sequence of *SLC11A1* gene and to estimate the gene and genotype frequencies in Doom pigs of Asom, India.

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MATERIALS AND METHODS

Blood samples from 55 Doom pigs were collected randomly from animals of different districts of Asom, India, viz. Dhubri (Agamani and Golokganj area) and Kamrup (Bamunigaon and Chaygaon area) including animals from Conservation Unit of Doom pig at Livestock Research Station, Mandira, Assam Agricultural University, Kamrup, Assam.

Isolation of genomic DNA: Blood (5 ml) was aseptically collected from the anterior vena cava of the animal in vaccutainer tube containing EDTA (2.7%) as anticoagulant. Genomic DNA was isolated from 600 µl of blood by rapid isolation of mammalian DNA method using standard protocol (Sambrook and Russell 2001).

Gene amplification by polymerase chain reaction: A pair of specific forward (F: 5'- TGAACACTTCATTTAACA GAAGA - 3') and reverse (R: 5'-GCCTCTGAGCA

GGGAAGACT-3') primers was used as per Tuggle *et al*. (2005) with slight modification and polymerase chain reaction was performed to amplify the 934 bp fragment of *SLC11A1* gene.

The PCR reactions were carried out in 25 µl reaction mixture containing 1.0 µl DNA template, 0.5 µl each of forward and reverse primers of concentration 20 pmol/µl, PCR master mix 12.5 µl and nuclease free water 10.5 µl. The PCR reaction was carried out in 0.2 ml PCR tubes in a thermal cycler under the following condition: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 60 sec, extension at 72°C for 30 sec with a final extension step at 72°C for 5 min.

Polymorphism detection by PCR-RFLP: The PCR products (8.5 µl) was digested with 1.5 µl of *Sma*1 enzyme, 3.5 µl of 10× Tango buffer and 6.5 µl of nuclease-free water. The mixture was incubated at 30°C for 4 h and inactivation was done at 65°C for 20 min in a thermocycler. The digested products were analyzed by 5% polyacrylamide gel electrophoresis (PAGE) and the gel was visualized in a GelDoc system. Identification of genotypes was done according to the restriction fragment patterns and it was confirmed by sequencing**.**

Sequencing and alignment: The PCR products from different genotypes were sequenced commercially. The nucleotide sequences obtained were confirmed through BLAST analysis. Alignments of the sequences were performed by using software BioEdit. The sequences were submitted to NCBI with Accession no KF31415, KF 934415 and KF 934416.

Genotype and allele frequencies: To find out the restriction site of *SLC11A1* gene, WEB CUTTER 2.0 software was used. The gene and genotype frequencies were calculated by using software POPGENE 1.32 (Yeh *et al*. 1999) and are presented in Table.1.

Table 1. Gene and genotype frequency of *SLC11A1* gene in Doom pig

Gene frequency		Genotype frequency			
		Genotypes	Obs.(O)	Exp.(E)	$(O-E)^2/E$
Allele A	(0.8364)	(A,A)	(39)	(38.4037)	(0.0093)
Allele B	(0.1636)	(B, A)	(14)	(15.1927)	(0.0936)
		(B,B)	(2)	(1.4037)	(0.2533)

RESULTS AND DISCUSSION

Genotype and allele frequencies: By PCR amplification, a 934 bp amplified product was obtained in all samples from Doom pigs (Fig.1). The PCR-RFLP analysis using *Sma*I on *SLC11A1* gene in Doom pigs yielded (Fig. 2) 3 fragments for AA genotype (505 bp, 231 bp and 198 bp), 4 fragments for AB genotype (505 bp, 429 bp, 231 bp and 198 bp) and 2 fragments for BB genotype (505 bp and 429 bp). A nucleotide transition (A→G) was observed at position 736 bp (Accession no KF31415). Double peak was observed among the sequences of all the heterozygous Doom pigs having 4 unique fragments (505 bp, 429 bp, 231 bp and 198 bp), while among the homozygous single peaks were observed (Accession no KF 934415) with 3 fragments (505 bp, 231bp and 198 bp) for AA genotype and 2 fragments (505 bp and 429 bp) for BB genotypes (Accession no KF 934416). Comparable findings were also reported by Liu *et al.* (2011) in Chinese indigenous pig, Large white and Landrace pig using restriction enzyme *Sma*I. They found 3 banding patterns; 3 each for GG genotype (506 bp,232 bp and 198 bp), AG genotype (506 bp 430 bp and 232 bp) and 2 banding patterns for AA genotype (505 bp and 430 bp). Tuggle *et al*. (2005) made almost similar observations, who could find 2 banding patterns with 3 fragments (505 bp,232 bp and 198 bp) and 2 fragments (505 bp and 430 bp) with 2 alleles in intron 1 due to *Sma*1 digestion in Chinese pig. However, Sun *et al*. (1998) using the restriction enzyme *Hinf*I identified polymorphism in respect of *SLC11A1* gene with 3 alleles.

The frequency of AA, AB and BB genotype was 0.71, 0.26 and 0.03, receptively, in the population studied. Similarly, the allele frequency of A and B was 0.836 and 0.163, respectively. Liu *et al.* (2011) found that allele G is dominant in Large white, Landrace and Songalio black pig and allele A has lower frequencies and AA genotype is not detected in large white and Songalio black pig upon digestion with *Sma*I.

The animals showed 3 genotypes AA, AB and BB in

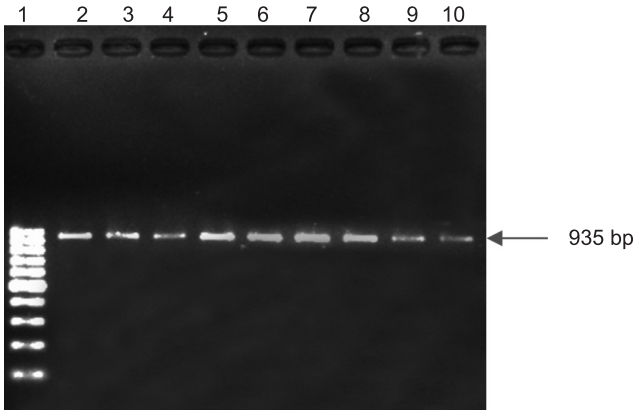

Fig. 1. Lane 1: 100 bp DNA Ladder;Lane 2–10: PCR Amplicons of *SLC11A1* gene.

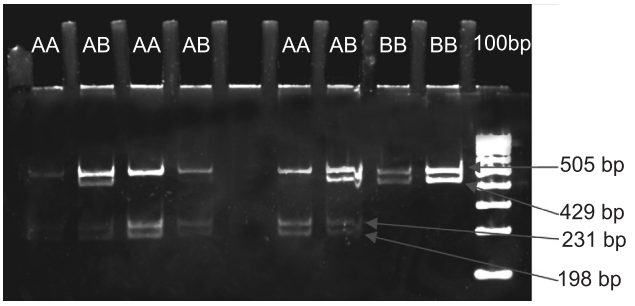

Fig. 2. PCR-RFLP OF *SLC11A1* gene in Doom Pig, DNA Ladder: 100 bp.

respect of the *SLC11A1* gene. The gene frequencies were estimated to be 0.8364 and 0.1636 respectively for A and B. The genotype frequencies for AA, AB and BB were estimated to be 0.71, 0.26 and 0.03 respectively.

In conclusion, the population of Doom pigs under study was polymorphic in respect of the *SLC11A1* gene, having 2 alleles with frequencies 0.8364 and 0.1636, respectively.

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