GENETIC POLYMORPHISMS WITHIN EXON 8, 9, AND 10 OF HEAT SHOCK PROTEIN (HSP) 90AA1 IN DEONI CATTLE

Shahid Ahmad Shergojry\(^1\), K.P. Ramesha\(^2\), Ovais Aarif\(^3\), Nazir Ahmad Mir\(^4\)

ABSTRACT

HSP 90, a member of heat shock proteins (HSP) plays key role in moderating the heat stress of the animal. Zebu cattle (Bos indicus) possessing this gene display better heat resistance. This study details the single stranded conformational polymorphism (SSCP) pattern of exon 8, 9, and 10 of HSP90AA1 gene in Deoni cattle, a premier dual purpose (dairy and draught) breed in peninsular India, which was not investigated earlier. The study was conducted on Deoni cows maintained at the institute farm at Bangalore. The genomic DNA was isolated by Miller’s High Salt method, and the purity was confirmed on the basis of OD ratio (260nm/280nm). Primers, specific to coding sequence of HSP90AA1 gene were designed using Primer 3 software and amplified by polymerase chain reaction (PCR). PCR products were subjected to Single-Strand Conformation Polymorphism (SSCP) analysis, to determine the genetic variants. Exon 8 revealed three unique SSCP patterns, labeled as pattern I, pattern II and pattern III, which showed one, two, and six distinct DNA bands, in the genotypic frequency of 0.250, 0.639, and 0.111, respectively. Exon 9 revealed two unique SSCP patterns, labeled as pattern I and pattern II, which showed two and three distinct DNA bands, in the genotypic frequency of 0.153 and 0.847, respectively. Exon 10 revealed two unique SSCP patterns, labeled as pattern I and pattern II, which showed two and three distinct DNA bands, in the genotypic frequency of 0.236 and 0.764, respectively. This study concludes that SSCP polymorphism with in exon 8, exon 9, and exon 10 of HSP90AA1 gene in Deoni cattle, can be further exercised to explore their linkage with economically important traits in this breed.

KEY WORDS

Deoni cattle, Exon, HSP90AA1 gene, Polymorphism, PCR-SSCP

Author attribution: \(^1\)PhD scholar, Dairy Cattle Breeding Division, \(^2\)PhD scholar, Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal, Haryana, India- 132001, \(^3\)Principal Scientist, Dairy Production Division, National Dairy Research Institute, Southern Campus, Bangalore, Karnataka, India- 560030. Corresponding author: drshahid21uk@gmail.com Date of Receipt: 04/ 02/ 2013, Acceptance: 17/ 09/ 2013. pp. 26-30
INTRODUCTION

HSP90, a member of heat shock proteins (HSP) plays key role in moderating the heat stress of the animal. Tropical cattle (Bos indicus) bearing this gene survive and perform better as compared to temperate breeds or their zebu crossbreds due to their resistance to heat stress (Collier et al., 2008). There is very little information on the polymorphism of HSP90AA1 gene in Indian zebu cattle. The single stranded conformational polymorphism (SSCP) of exon 5, 6, and 7 of HSP90AA1 gene, investigated earlier in Deoni cattle, a dual purpose (dairy and draught) breed in India (Figure-1), did not reveal polymorphism (Shergojry et al., 2012). This paper depicts the SSCP pattern of exon 8, 9, and 10 of HSP90AA1 gene in Deoni cattle.

DNA extraction and quality evaluation:

The genomic DNA was extracted from the white blood cell (WBC) pellets, from the blood of the cows using Miller’s High Salt method, with minor modification (Miller et al., 1988). They yield of DNA ranged between 375 and 1330 µg/ ml of blood, with a mean yield of 596.50 ± 36.50 µg / ml. The purity of DNA (determined as OD ratio at 260nm/ 280nm) ranged between 1.7 and 1.9, with a mean of 1.80 ±0.01, indicating high purity of the extracted DNA. The DNA samples were diluted to get the final concentration of 100 ng/ µl in low Tris EDTA (TE) buffer, and were utilized later as DNA templates for further studies.

Thermal cycling conditions:

Three sets of primers (Table-1) were designed for HSP90AA1 gene using Primer 3 software based on the 5332 bp sequence for HSP90AA1 gene (NCBI GenBank NC-007319). The polymerase chain reaction (PCR) conditions were optimized for each primer. The combinations giving the best amplification were used for further studies.

The PCR amplifications were carried out in a 0.2 ml PCR tube in a Master cycler (Eppendorf, Germany). In the PCR reactions, DNA template (100 ng/ µl) was amplified in a final volume of 25 µl containing 20 pmol/ µl of each primer, 2.5mM of dNTPs (100 µM each), 2.5µl of 10× buffer with 1.5mM Mgcl₂ (Chromous), and 1U Taq polymerase (Chromous).

MATERIALS AND METHODS

The present investigation was undertaken on 72 Deoni cows, maintained at National Dairy Research Institute (NDRI), Southern Campus, Bangalore, India.
PCR conditions involved an initial denaturation at 94°C for 5 minutes, followed by 30 cycles with initial denaturation at 94°C for 1 minute, primer specific annealing temperatures of 55°C for 1 minute to specifically amplify Exon-8, 56°C for 1 minute to specifically amplify Exon-9 and 56°C for 1 minute to specifically amplify Exon-9 respectively, extension at 72°C for 1 minute followed by a final extension at 72°C for 5 minutes.

**Table-1. Description of primers used for PCR amplifications.**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Location</th>
<th>Amplicon length (bp)</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon-8</td>
<td>3241-3780</td>
<td>539</td>
<td>55°C</td>
</tr>
<tr>
<td>Exon-9</td>
<td>3871-4281</td>
<td>410</td>
<td>56°C</td>
</tr>
<tr>
<td>Exon-10</td>
<td>4430-4680</td>
<td>250</td>
<td>57°C</td>
</tr>
</tbody>
</table>

PCR amplification: The PCR conditions were optimized for fragment specific amplification of HSP90AA1 gene. The PCR products were electrophoresed in 1.5% agarosegel at 90V for 45 minutes along with 100 bp DNA ladder as molecular weight marker and visualized under Gel Doc System (BIO RAD, USA). The amplified HSP90AA1 PCR products were subjected to single-strand conformation polymorphism (SSCP) analysis to determine the genetic variants.

**SSCP analysis:** For single strand conformation polymorphism (SSCP) analysis, each PCR product was diluted in denaturing solution, denatured at 95°C for 8 minutes, chilled on ice and resolved on optimized concentration of non-denaturing polyacrylamide gels. The electrophoresis was carried out in a vertical electrophoresis chamber (SCIE-PLAS, U.K) in 1x TBE buffer. The optimized conditions for SSCP analysis of Exon 8, Exon 9, and Exon 10 of HSP90AA1 gene, using vertical electrophoresis is summarized in Table-2.

The gels were silver stained. Silver stained SSCP gels were dried and documented for detecting mobility shifts in different fragments of HSP90AA1 gene. The different band patterns/ variants were characterized by the number of bands and mobility shifts identified for the different fragments of HSP90AA1 gene. Each pattern was represented by a code.

**Table-2 Optimized conditions for SSCP analysis.**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Acryl : Bisacryl ratio</th>
<th>Acrylamide (%)</th>
<th>DNA (µL)</th>
<th>Denaturing solution (µL)</th>
<th>Duration (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 8</td>
<td>19:1</td>
<td>6</td>
<td>10</td>
<td>15</td>
<td>10.00</td>
</tr>
<tr>
<td>Exon 9</td>
<td>29:2</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td>06:00</td>
</tr>
<tr>
<td>Exon 10</td>
<td>29:2</td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>05:00</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The amplified HSP90AA1 PCR products (Figures: 1-3), subjected to single-strand conformation polymorphism (SSCP) analysis, revealed varying degree of genetic polymorphisms with respect to each of the HSP90AA1 gene fragments analyzed (Figures: 4-6).

The Exon 8 in HSP90AA1 gene revealed three unique PCR-SSCP patterns with different mobility shifts, marked as Pattern I, Pattern II, and Pattern III. Pattern I showed one distinct DNA band, Pattern II showed two distinct DNA bands, and Pattern III showed six distinct DNA bands. The genotypic frequency of Pattern I, Pattern II, and Pattern III was 0.250, 0.639, and 0.111, respectively (Table-3).

The Exon 9 of HSP90AA1 gene revealed two PCR-SSCP patterns with different mobility shifts, marked as Pattern I and Pattern II. Pattern I showed two distinct DNA bands, while Pattern II showed three distinct DNA bands. The genotypic frequency of Pattern I and Pattern II was 0.153 and 0.847, respectively (Table-3).

The Exon 10 of HSP90AA1 gene revealed two PCR-SSCP patterns with different mobility shifts, labeled as Pattern I and Pattern II. Pattern I revealed two distinct DNA bands, while Pattern II revealed three distinct DNA bands. The genotypic frequency of pattern I and pattern II was 0.236 and 0.764 respectively (Table-3). No earlier reports are available to compare or contrast the present findings.

Table-3. Distribution of genotypic frequencies of exon 8, 9 and 10 of HSP90 AA1gene.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Pattern I</th>
<th>Pattern II</th>
<th>Pattern III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 8</td>
<td>0.250 (18)</td>
<td>0.639 (46)</td>
<td>0.111 (8)</td>
</tr>
<tr>
<td>Exon 9</td>
<td>0.153 (11)</td>
<td>0.847 (61)</td>
<td>——</td>
</tr>
<tr>
<td>Exon 10</td>
<td>0.236 (17)</td>
<td>0.764 (55)</td>
<td>——</td>
</tr>
</tbody>
</table>

Note: The figures with in parentheses are the number of observations.

Figure-1. PCR amplification of Exon 8.

Figure-2. PCR amplification of Exon 9.
CONCLUSIONS

Three exons of HSP90AA1 gene were amplified by PCR using three sets of primers and the genetic variants were determined by PCR-SSCP technique. The polymorphism was confirmed by direct sequencing. The genotype frequencies observed in the present investigation suggest that Deoni breed of cattle have a diverse type of SSCP pattern for HSP90AA1 gene in the sampled population indicating the existence of variability.

REFERENCES

