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Polymorphism Study of *POU1F1* Gene in Mandya and NARI-Suwarna Sheep

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ABSTRACT

Background: The mutations in *POU1F1* gene result in deficiency of GH, PRL and TSH-β expression and thus have an indirect effect on development and reproduction. The present study was conducted to determine and compare the polymorphism of *POU1F1* gene in Mandya and NARI-Suwarna Sheep.

Methods: Blood samples were collected from 50 each of Mandya and NARI-Suwarna sheep and genomic DNA was isolated following Miller's high salt method. PCR-RFLP analysis was performed for exon 3 and exon 4 regions of *POU1F1* gene, whereas, PCR-SSCP analysis was performed for exon 6 region of *POU1F1* gene.

Result: The PCR products of sizes 365 bp, 508 bp and 501 bp for exon 3, exon 4 and exon 6 regions of *POU1F1* gene, respectively were successfully amplified. PCR-RFLP analysis of exon 3 and exon 4 regions of *POU1F1* gene with *Alu*I and *Eco*RI restriction enzymes, respectively revealed monomorphism in both Mandya and NARI-Suwarna sheep. The PCR-SSCP analysis of exon 6 region of *POU1F1* gene revealed two patterns, P1 and P2 with frequencies of 98 and 2 per cent, respectively in Mandya sheep and 90 and 10 per cent, respectively in NARI-Suwarna sheep. The alignment of P1 and P2 pattern sequences revealed two SNPs (109 G>C and 112 T>A) in Mandya sheep, whereas 3 SNPs (218 T>G, 225 G>A and 264 T>A) were revealed in NARI-Suwarna sheep. Polymorphism was successfully established at exon 6 region of *POU1F1* gene.

Key words: Genetic polymorphisms, Mandya sheep, NARI-Suwarna sheep, *POU1F1*.

INTRODUCTION

Mandya sheep is considered as a best meat breed with respect to its conformation (Acharya, 1982). Its meat is regarded as the most nutritious and high quality mutton in human diets at national and international spectrum of sheep products (Dinakar *et al*., 2019). These animals are predominantly distributed in Mandya and adjacent districts of Karnataka. NARI-Suwarna sheep with more than 60 per cent ewes having twin was developed by Nimbkar Agriculture Research Institute (NARI), Phalton, through introgression of *FecB* gene from the prolific Garole breed of Sunderban, West Bengal into non-prolific locally adapted Deccani breed of Maharashtra (Nimbkar *et al*., 2002).

Identification of genetic markers in relation to growth rate and its possible use in Marker Assisted Selection (MAS) will speed up the genetic progress. In general, the crucial step to ascertain a MAS system is to fishing out and validating the genetic markers of growth traits (Allan *et al*., 2007). For growth traits, growth hormone (*GH*), growth hormone receptor (*GHR*), Insulin like growth factor 1 (*IGF1*), leptin, pituitary specific transcription factor - 1 (*POU1F1*), myostatin (*MSTN*) and bone morphogenetic protein (*BMP*) genes are necessary for bone formation, birth weight, weaning weight, body condition and muscle growth (Supakorn, 2009).

The *POU1F1* belongs to the large family of POU domain proteins. The *POU1F1* (also known as PIT-1 or GHF-1) is a tissue-specific transcription factor which is mainly expressed in the anterior pituitary. This protein has a certain role in the transcriptional adjustment of growth hormone (*GH*) and prolactin (*PRL*) genes. Additionally, *POU1F1* is also involved in the activation of thyroid-stimulating hormone, *POU1F1* itself and growth hormone-releasing hormone receptor (*GHRH*-R) genes. Moreover, besides its role in gene activation, *POU1F1* is essential for differentiation, reproduction and survival of somatotrope and lactotrope as well as thyrotrope cells. The ovine *POU1F1* gene contains five introns and six exons and is located on chromosome 1 (Bastos *et al*., 2006).

Ozmen *et al*. (2014) studied the association of SNP in exon 6 region of *POU1F1* gene with milk productive traits in Turkish sheep breeds (Sakiz, White Karaman and Awassi). They reported a significant association of milk yield, fat and

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lactose with TT and CC genotypes in Sakiz sheep. Jalil-Sarghale *et al*. (2014) investigated the SNPs at exon 3 region of *POU1F1* gene and its association with growth and biometric traits in two Iranian sheep breeds (Zel and Lori-Bakhtiari). They found that AA genotype had a higher weaning weight than those with GG genotype and opined that *POU1F1* polymorphism can be used as molecular marker for production traits.

The present study was conducted to find out and compare the polymorphism of *POU1F1* gene in Mandya and NARI-Suwarna sheep, if any.

MATERIALS AND METHODS

Blood samples were collected from 50 Mandya sheep from farmers' flock distributed over villages of Malavally taluk, Mandya district, Karnataka and 50 NARI-Suwarna sheep from the flock maintained at Department of Veterinary Gynaecology and Obstetrics, Veterinary College, KVAFSU, Hebbal, Bengaluru. Genomic DNA was isolated from venous blood by following high salt method as described by Miller *et al*. (1988) with a few modifications.

The primers previously published by Ekegbu *et al*. (2018) and Al-Khuzai and Al-Anbari, (2018) were utilized for amplification of exon 3 and 4 regions of *POU1F1* gene, respectively. Whereas, for amplification of exon 6 region of *POU1F1* gene, the primer was designed using PRIMER 3 PLUS software by referring the *Ovis aries* sequences available in NCBI website. The details of the primers used for amplification of *POU1F1* gene are presented in Table 1.

The amplification of exon 3, 4 and 6 regions of *POU1F1* gene were performed in a total volume of 25 µl which consisted of 12.5 µl of Red PCR master mix, 1 µl (10 pmol/ µl) each of forward and reverse primer, 9.5 µl of PCR grade water and 1 µl of template DNA. The PCR reaction was carried out with an initial denaturation temperature of 95°C (4 min), 35 cycles of 94°C (45 sec), 55.8°C (45 sec) and 72°C (60 sec) for exon 3 and 4 regions and 34 cycles of 94°C (60 sec), 60°C (60 sec) and 72°C (60 sec) for exon 6 region of *POU1F1* gene followed by final extension at 72°C (10 min). The PCR amplified products were resolved on 1.5 per cent agarose in parallel with 100 bp DNA ladder and photographed under gel documentation system (Bio rad Molecular imager Gel Doc XR+, USA).

The PCR products of the exon 3 and 4 regions of POU1F1 gene were digested with *Alu*I and *Eco*RI restriction enzymes, respectively. The digestion was done in a total volume of 30 µl which consisted of 2 µl of 10X buffer, 10 µl of PCR amplicon, 1 µl of RE and 17 µl of Nuclease free water (NFW) with incubation at 65°C for 3 hours. The RE digested products were resolved on two per cent agarose gel agarose in parallel with 100 bp DNA ladder and photographed under gel documentation system (Bio rad Molecular imager Gel Doc XR+,USA). Based on visualization of different band patterns, genotypes were determined.

The PCR product of exon 6 region of *POU1F1* gene was subjected to single-strand conformation polymorphism (SSCP) by using 12 per cent polyacrylamide gel. The 12 per cent polyacrylamide gel comprised of 15 ml of Acrylamide:Bisacrylamide (29:1), 5 ml of 10x TBE, 32.6 ml of autoclaved triple distilled water, 330 µl of 10 per cent ammonium per sulphate and 70 µl of TEMED. Approximately 12 µl of PCR product was mixed with 8 µl of formamide dye and properly mixed. The mixture was denatured at 95°C for 10 minutes and snap cooled on ice for 3 minutes. The product was carefully loaded into the gel. The electrophoresis was performed for 5 hours at 150 volts at 16°C. The gel was stained with silver staining after the run in order to visualize the banding pattern.

The PCR products showing different patterns in RFLP and SSCP were custom sequenced by double pass sequencing method using primers used for amplification of different products.

RESULTS AND DISCUSSION

About 200 - 300 µg of good quality DNA per 10 ml of venous blood was obtained in the present study by following Miller's high salt method.

The PCR products of sizes 365, 508 and 501 bp (Fig 1, 2 and 3) were successfully amplified for exon 3, exon 4 and exon 6 region of *POU1F1* gene, respectively in the studied population.

The restriction enzyme (RE) digested products of exon 3 region of *POU1F1* gene resolved on agarose gel electrophoresis revealed fragments of sizes of 112, 105 and 95 bp in one band and 31 and 22 bp fragments in another band. The RE have cut the PCR amplicons to give the above mentioned fragments indicating AA genotype in both Mandya and NARI-Suwarna Sheep (Fig 4). Contrast to the present study, Bastos *et al*. (2006) observed polymorphism in 'Churra da Terra Quente' sheep. Sumantri *et al*. (2009) reported polymorphism in local sheep from Jonggol Animal Science

Table 1: Details of primers used for amplification of Ovine *POU1F1* gene.

Region	Primer sequence	Expected product size (bp)	References
Exon 3	F: 5′ACTGGCCTTCACAGAACAATC3′	365	Ekegbu *et al*., 2018
	R: 5′GACTTTGCAGATGGGGTTGT3′		
Exon 4	F: 5′ATACCAGGCAATTCTACACTG3′	508	Al-Khuzai and Al-Anbari, 2018
	R: 5′GGCCTTGCTTTTCTTTATAG3′		
Exon 6	F: 5′CAATAACCTGAGTTCTGGGGGA3′	501	-
	R: 5′TCAGGCTTGTTTTCACCCGT3′		

Teaching and Research Unit (JASTRU) farm in Bogor using PCR-RFLP with *Hinf*1 restriction enzyme. By employing PCR-RFLP with *Aci*I restriction enzyme, polymorphism was reported in Iranian sheep breeds (Zel and Lori-Bakhtiari) by Jalil-Sarghale *et al.* (2014). But, by following PCR-SSCP analysis many authors *viz*., Ekegbu *et al*. (2018) in New Zealand sheep breeds (NZ Romeny and Merino), Bahrami *et al.* (2014) in Mehraban sheep, Sadeghi *et al*. (2014) in Iranian sheep breeds (Zel and Lori-Bakhtiari) and Negahdary *et al*. (2013) in Makooei sheep reported polymorphism in exon 3 region of *POU1F1* gene.

The restriction enzyme digested products of exon 4 region of *POU1F1* gene resolved on agarose gel electrophoresis revealed fragments of sizes 275 and 233 bp. The RE have cut the PCR amplicons to give rise to two bands indicating AA genotype in both Mandya and NARI-Suwarna Sheep (Fig 5). This is in agreement with the reports of Sudhakar (2009) in Nilagiri and Mecheri sheep breeds and Bastos *et al*. (2006) in 'Churra da Terra Quente' sheep, who also reported absence of polymorphism in exon 4 region of *POU1F1* gene. Contrast to the present study, AL-Khuzai and Al-Anbari, (2018) reported polymorphism in Iraqi Awassi sheep by following PCR-RFLP. They reported two genotypes, MM and NN with frequency of 0.58 and 0.42, respectively and two alleles with frequency of 0.58 and 0.42, respectively. Similarly, Ansari *et al*. (2008) reported three genotypes (AA, AB and BB) in Baluchi Sheep.

In the present study, PCR-SSCP technique was employed to determine the polymorphism at exon 6 region of *POU1F1* gene. The PCR-SSCP analysis revealed two patterns, P1 and P2 both in Mandya and NARI-Suwarna sheep (Fig 6). The frequency of P1 and P2 in Mandya was 98 and 2 per cent, respectively whereas, in NARI-Suwarna it was 90 and 10 per cent, respectively. The proportion of

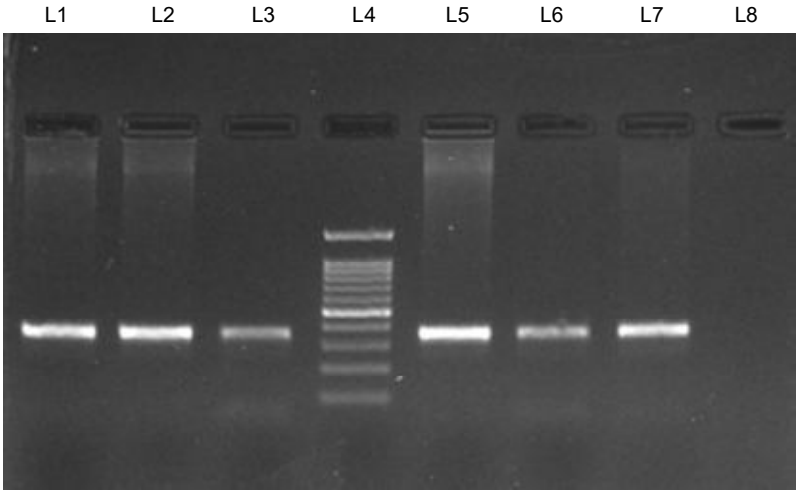

Fig 1: Agarose electrophoresis gel showing PCR amplicons of exon 3 (365 bp) region of *POU1F1* gene.
Lanes 1-3: PCR Amplicons of Mandya.
Lane 4: 100 bp DNA ladder.
Lanes 5-7: PCR Amplicons of NARI-Suwarna.
Lane 8: No template control (NTC).

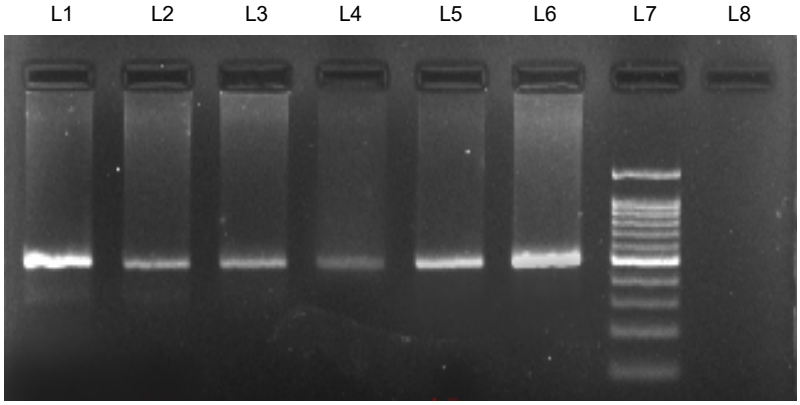

Fig 2: Agarose electrophoresis gel showing PCR amplicons of exon 4 (508 bp) region of *POU1F1* gene.
Lanes 1-3: PCR Amplicons of Mandya.
Lanes 4-6: PCR Amplicons of NARI-Suwarna.
Lane -7: 100 bp DNA ladder.
Lane 8: No template control (NTC).

animals with P1 was more in both Mandya and NARI-Suwarna population. In agreement to the current study, Ozmen *et al.* (2014) reported two genotypes (TT and CC) in Sakiz sheep by PCR-RFLP technique with *Alu*I restriction enzyme. Similarly, Bai *et al*. (2016) conducted PCR-RFLP analysis and reported polymorphism in five sheep breeds (large-tailed Han, small-tailed Han, Yuxi fat-tailed, Lanzhou large-tailed and Mongolian sheep) of China. In contrast to the present study, Bastos *et al*. (2006) reported no polymorphism at exon 6 region of *POU1F1* gene in 'Churra da Terra Quente' sheep.

The alignment of P1 and P2 pattern sequences of Mandya sheep revealed two SNPs, G to C transversion at 109 bp position and T to A transversion at 112 bp position (Fig 7). Whereas, alignment of P1 and P2 pattern sequences of NARI-Suwarna sheep revealed 3 SNPs, T to G transversion at 218 bp, G to A transition at 225 bp and T to A transversion at 264 bp positions (Fig 8). In concurrence to the present study, Bai *et al*. (2016) conducted PCR-RFLP analysis and detected three SNPs at exon 6 region of *POU1F1* gene in five sheep breeds (large-tailed Han, small-tailed Han, Yuxi fat-tailed, Lanzhou large-tailed and

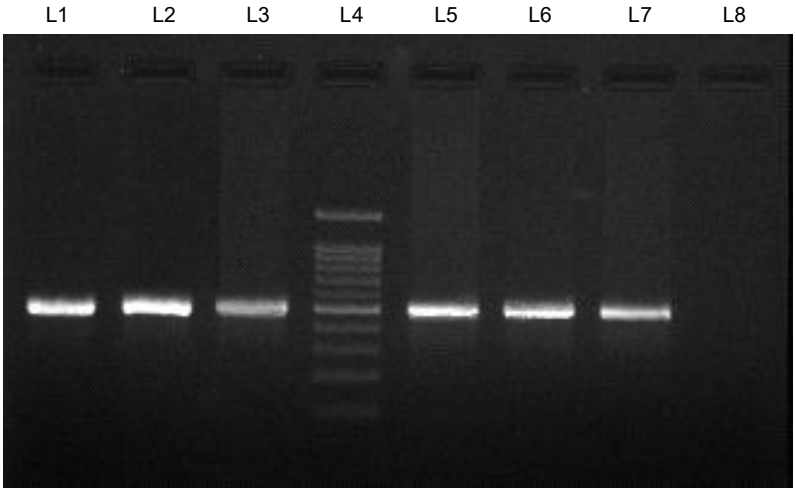

Fig 3: Agarose electrophoresis gel showing PCR amplicons of exon 6 (501 bp) region of *POU1F1* gene.
Lanes 1-3: PCR Amplicons of Mandya.
Lane 4: 100 bp DNA ladder.
Lanes 5-7: PCR Amplicons of NARI-Suwarna.
Lane 8: No Template Control (NTC).

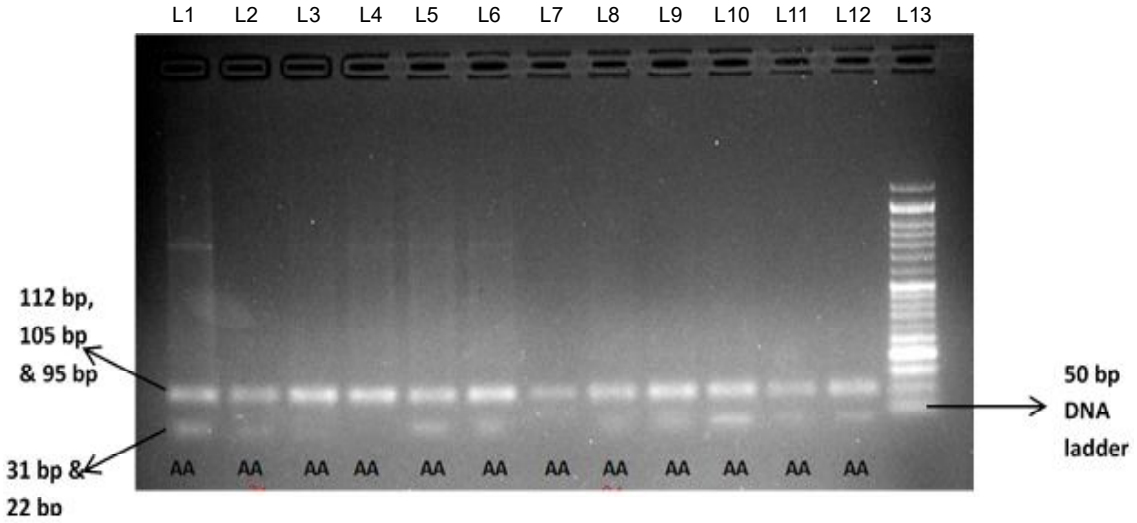

Fig 4: Agarose electrophoresis gel showing *Alu*I restriction enzyme digested PCR - RFLP pattern of exon 3 region of *POU1F1* gene.
Lanes 1-6: RFLP pattern for Mandya.
Lanes 7-12: RFLP pattern for NARI-Suwarna.
Lane 13: 50 bp DNA ladder.

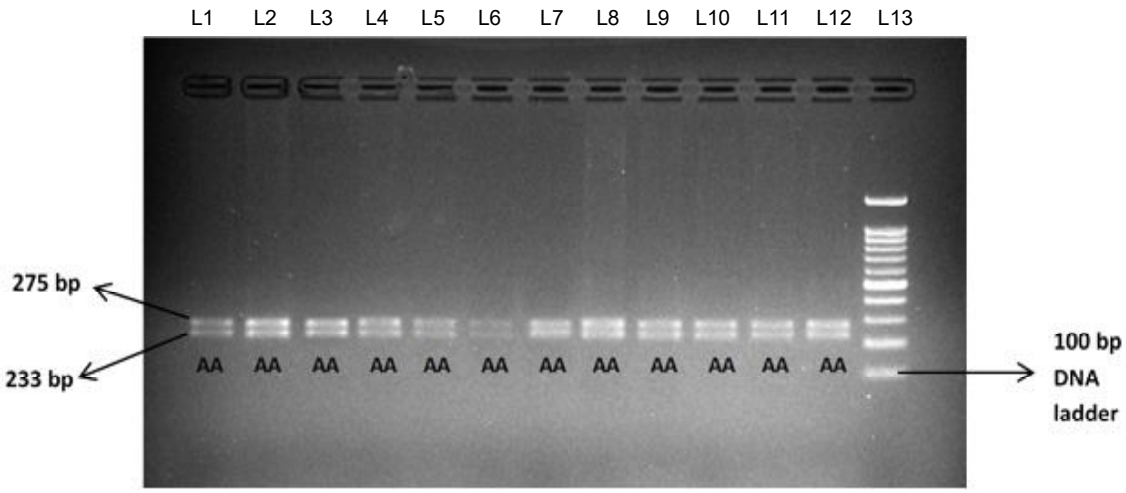

Fig 5: Agarose electrophoresis gel showing *Eco*RI restriction enzyme digested PCR - RFLP pattern of exon 4 region of *POU1F1* gene.

Lanes 1-6: RFLP pattern for Mandya.

Lanes 7-12: RFLP pattern for NARI-Suwarna.

Lane 13:100 bp DNA ladder.

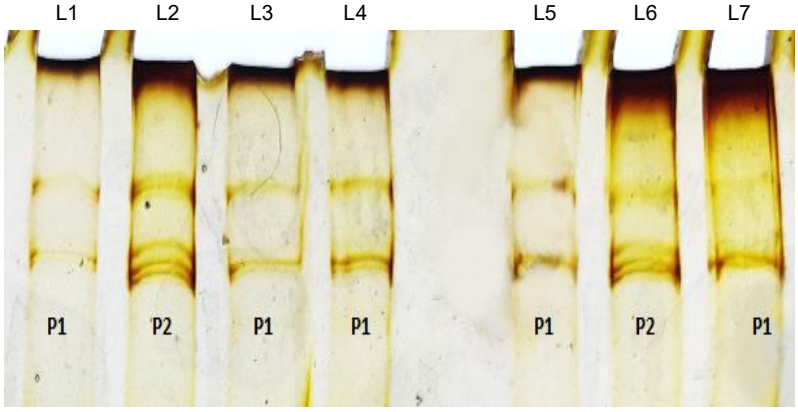

Fig 6: PAGE gel showing SSCP patterns of exon 6 region of *POU1F1* gene.

Lanes 1-3: SSCP pattern for Mandya.

Lanes 4-7: SSCP pattern for NARI-Suwarna.

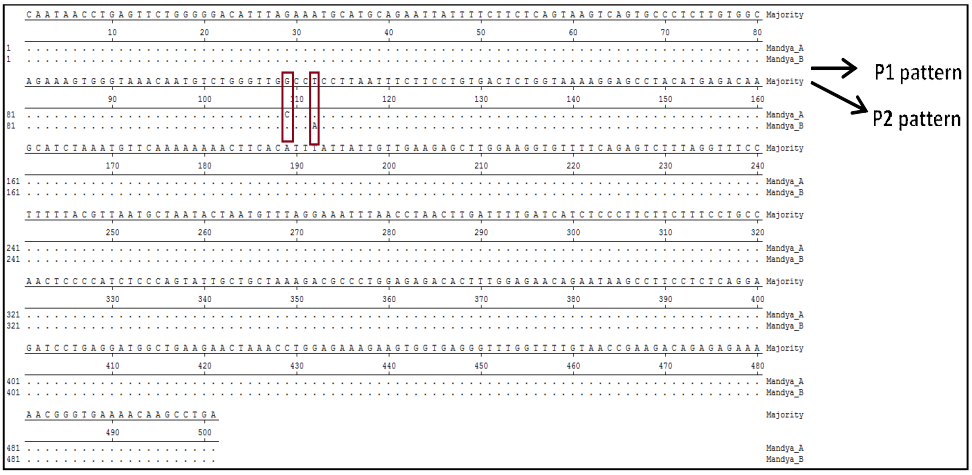

Fig 7: Alignment of P1 and P2 pattern sequences of Mandya sheep.

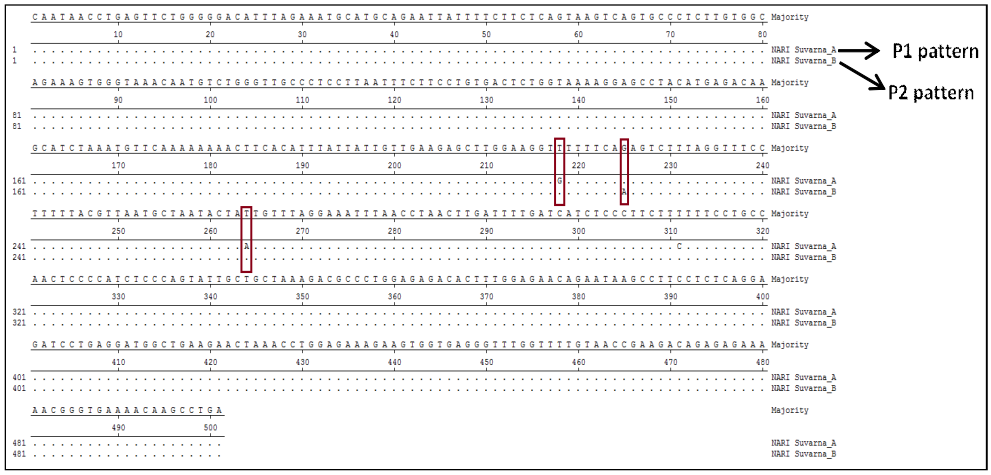

Fig 8: Alignment of P1 and P2 pattern sequences of NARI Suwarna sheep.

Mongolian sheep) of China. The three SNPs were C355T (C/T), C71G (C/G) and C330G (C/G). C allele of C355T locus was the dominant allele in all the five sheep populations.

CONCLUSION

In the present study, PCR-RFLP analysis of exon 3 and exon 4 regions of *POU1F1* gene using *Alu*I and *Eco*RI, respectively showed monomorphism in both Mandya and NARI-Suwarna sheep. Polymorphism was established at exon 6 region of *POU1F1* gene in both Mandya and NARI-Suwarna sheep by PCR-SSCP. Mandya sheep revealed two SNPs, G to C transversion at 109 bp position and T to A transversion at 112 bp position. NARI-Suwarna sheep revealed 3 SNPs, T to G transversion at 218 bp, G to A transition at 225 bp and T to A transversion at 264 bp positions.

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