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PCR–Based randomly amplified polymorphic DNA used for molecular characterization and detection of genetic diversity in sheep breeds

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Abstract
Randomly amplified polymorphic DNA (RAPD) was used to analyze blood samples of Munjal and Nali sheep breeds. In the present study, RAPD-PCR method was used to amplify DNA samples using forty arbitrary oligonucleotide primers. Only seven primers found informative. From the 7 random primers, a total of 91 bands were amplified between breeds and 71 of these (78.02%) were found to be polymorphic. In Munjal, overall percentage polymorphism of 71.73 was observed, while in Nali it was 84.44. Genetic distance between breeds was 0.95. MAPD values of 22.85 and 29.46 were observed in Munjal and Nali breeds, respectively, while MAPD 46.88 was observed between breeds. One primer (OPV-09) in Munjal and one primer (OPV-09) in Nali were found to be specific for these breeds. The scientific data presented in the study suggests that RAPD-PCR could be used as a valuable tool in the molecular characterization and genetic diversity study.

Keywords: RAPD, Sheep, PCR, polymorphism, genetic distance, MAPD

Introduction

Randomly amplified polymorphic DNA (RAPD) could be a PCR based procedure employments broadly to gauge hereditary differing qualities and have been utilized effectively in assessing hereditary relatedness among different populaces of sheep, cattle, goat, buffalo, camel and horse (Okumus A. and Kaya M, 2005; Mahfouz *et al.*, 2008) [11, 9]. The RAPD employments more due to have a few extraordinary characters such as the straightforwardness of utilizing, fast and efficient screen for DNA sequence-based polymorphism at a really expansive of loci and the major advantage is that no earlier DNA grouping data is required (Fukuoka *et al.*, 1992) [5], hereditary variety and characterization of goat breeds can give solid data for the choice of parental fabric and in this way help in breeding program (Olivier *et al.*, 2005) [12]. RAPD uses arbitrary primers to detect changes in DNA sequences at locations in the genome that are annealed by primer. It provides a faster and cheaper alternative to the RFLP analysis and was described by William *et al.* (1990). This technique was used to characterize breeds/animals.

Despite the advantages of using traditional PCR tests to detect DNA alteration, there are a number of potential difficulties, first, nucleotide sequences flanking the target upstream and downstream. The DNA has to be determined and the size of the PCR product plays a crucial role in the diction of DNA damage, as the amplification of short fragments (less than 300bp) may be slightly reduced and possibly not inhibited at all even in the event of extensive DNA damage. Third, it is conceivable that any diminishment within the intensity of PCR amplicons can be due to halfway hindrance of the PCR response caused by variables other than DNA harm such as residuals phenol reaming after DNA extraction. Then again, on the off chance that an amplicon completely vanishes, it can be contended that the PCR did not work at all, independent of the nearness of DNA harm, such drawback as portrayed can be killed by utilizing the irregular increased polymorphic DNA at first built up and still utilized for hereditary mapping and ordered distinguishing proof (Williams *et al.* 1990) [16] this consider was embraced to examine the convenience of RAPD analyzed in characterization of sheep breeds (Devrum and Kaya, 2006) [2].

Material and Methods

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Genomic DNA was isolated from blood of 20 animals of Munjal and Nali breeds using protocol of Sambrook and russel (2001) [14] with slight modifications.

The quality of isolated genomic DNA was evaluated by agrose gel electrophoresis. The concentration of DNA was also checked by UV spectrophotometer taking optical density diluated to the concentration of 25ng/µl. Forty random primers were used for screening polymorphism. Only ten random primers were given informative polymorphism.

PCR reaction mixture consisting of 2.0 mM $MgCl_2$, 10.0 mM dnTPs,1.0 taq polymerase, 40 ng of random primer with 50 ng of genomic DNA. The amplification was carried out for forty cycles with initial denaturation at 95°C for 5 minutes, second denaturation for 1.0 minute at 94°C, annealing at 37°C for 1.0 minutes and extension for 2 minutes at72°C and final extension at 72°C for 7 minutes. All the amplified products were separately by electrophoresis in 1.4% agrose gel containing 0.05% ethidium bromide and scored by gel documention (Biovis gel) system. Only clean bands of RAPD-PCR products on agrose gels were scored. Genetic similarity index was calculated on the basis of band frequencies (Lynch 1990) [8]. Mean average percentage difference (MAPD) was determined by formula of Gilbert *et al.* (1990) [6]. band sharing frequency BSF was estimated as reported by Jeffrey's and Morton (1987) [7].

Table 1: List of primers employed with GC content.

Sr. No.	Primers	Sequence (5'-3')	GC Content (%)
1	OPU-01	ACGGACGTCA	60
2	OPU-02	CTGAGGTCTC	60
3	OPU-06	ACCTTTGCGC	60
4	OPU-08	GCCGAAGGTT	60
5	OPU-14	TGGGTCCCTC	70
6	OPV-06	ACGCGCAGGT	70
7	OPV-09	TGTACCCGTC	60

The RAPD-PCR technique is highly sensitive to minor alterations in the reaction conditions. The number, intensity of amplification and reproducibility of amplified DNA fragments depend on a variety of variables such as PCR cycling conditions (Yu and Pauls, 1992; Don *et al.*, 1991) [17, 3], template and primer concentrations (Muralidharan and Wakeland, 1993) [10], and the components of the PCR buffer (Ponce and Micol, 1992) [13].

Result and Discussion
Specific RAPD pattern that distinguished between 20 samples were analyzed and RAPD results have been described in Fig.1, 2, 3 and 4. To estimate the exact size of fragments 100bp ladder used. . Representative samples of each breed were screened with 40 random primers of series OPU and OPV. Out of 40 random primers screened only 7 primers were found to be informative and were used in the subsequent study.

In Munjal, all the 7 random primers also exhibited polymorphic bands. A total of 46 bands were amplified out of which 33 were polymorphic (about 56.36%). In Munjal animals, the average number of bands ranged from 4.7 to 5.4 with overall average number of bands as 5.04. In Nali breed all the 7 random primers also exhibited polymorphic bands. A

total of 45 bands were amplified out of which 38 were polymorphic in nature with overall percent polymorphism of 84.4. The average number of bands ranged from 4.3 to 5.7 with overall average number of bands as 5.03. Between Munjal and Nali with 7 random primers a total of 63 bands were amplified out of which only 60 were found to be polymorphic with overall percent polymorphism of 95.24. Al-Allak *et al.*, 2020 [1] study showed variance in the number of bands for each primer ranged between five in OPD18 to sixteen in OPAA17. Higher number of RAPD bands yield more reliable information about the genotypes of populations. The higher polymorphism information contents at the seven markers (Seventy- three bands obtained with 28.3% of polymorphism) indicate the retention of natural variation from source populations for the domestic breeds of Iraqi Awassi sheep of different geographic regions in Iraq (Al-Allak *et al.*, 2020) [1].

The overall genetic similarity in Munjal was observed as 0.69 while in Nali as 0.62. Genetic similarity between breed was 0.31. These values indicate that the genetic similarity within breed is more than between breeds.

Band sharing frequency (BSF) was calculated both within and between breeds. In Munjal value of BSF ranged from 0.52 to.91, with primers OPU-06 and OPU-01, respectively. The average BSF in Munjal was observed as 0.76. In Nali, values of BSF ranged from 0.44 to 0.84, with primers OPV-06 and OPU-01 respectively. The overall BSF value in Nali animals was 0.68. The genetic distance was observed in the range of 0.27 to 1.4, with an overall genetic distance of 0.95. This value indicates that within breed genetic distance was lower as compared to between breed genetic distances. Mean average percentage difference (MAPD) was calculated as a measure of genetic diversity within and between breeds. In Munjal, the APD values ranged from 0 to 20.43 with MAPD of 22.85. In, Nali APD ranged from 15.47 to 53.66 with random primers OPU-01 and OPV-06 respectively with MAPD of 29.46. Between breeds APD values ranged from 25.66 to 97.71 while MAPD value of 46.88% was observed between Munjal and Nali breed. This value indicates high genetic diversity between Munjal and Nali animals.

One random primer in each was found to be specific for Munjal breed (OPV-09 with 800bp) as well as Nali breed (OPV-09 with 700bp) in figure-2. Breed specific RAPD markers for both breeds which can be used for identification of these breeds and for establishing the genetic relatedness within and between these breeds. El-Hentati *et al.*, [4] in their analysis of molecular variance (AMOVA) showed that the variation between breeds was 29.54%, and that the variation between populations within breeds and within populations was 8.47 and 61.99%, respectively. Waheed *et al.*, 2015 [15] study also confirmed that molecular techniques such as RAPD-PCR can be used economically and efficiently to establish genetic distances and similarities between and within breeds, as well as to identify breed-specific genetic markers. Seven sheep breeds in their study could be identified using a single breed RAPD-specific marker or a combination of two or more markers.
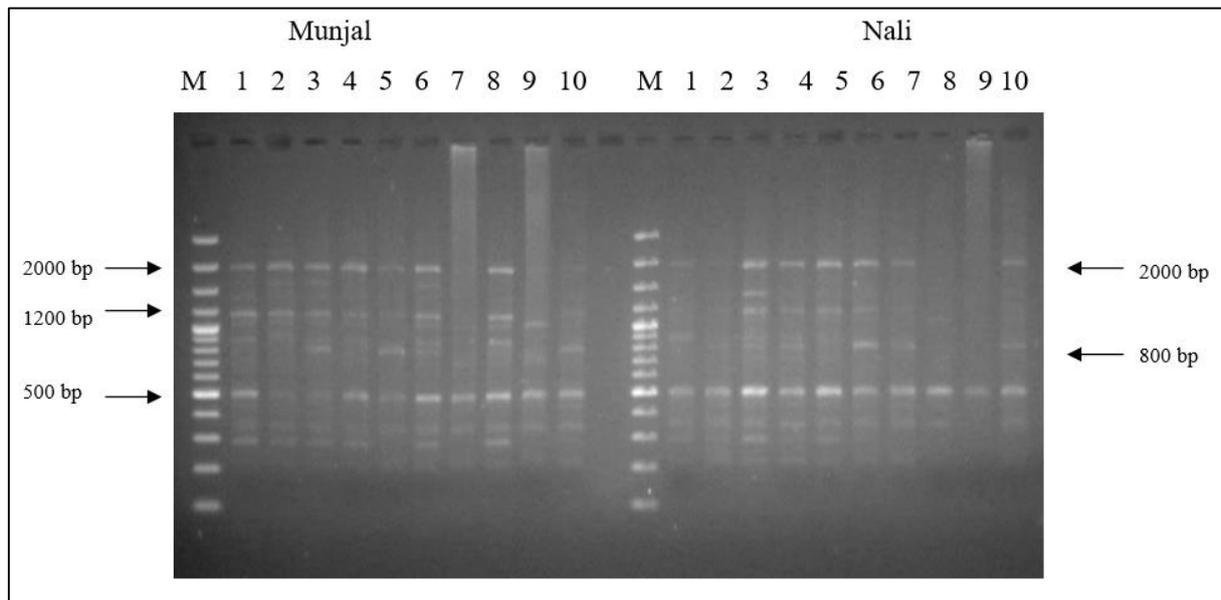
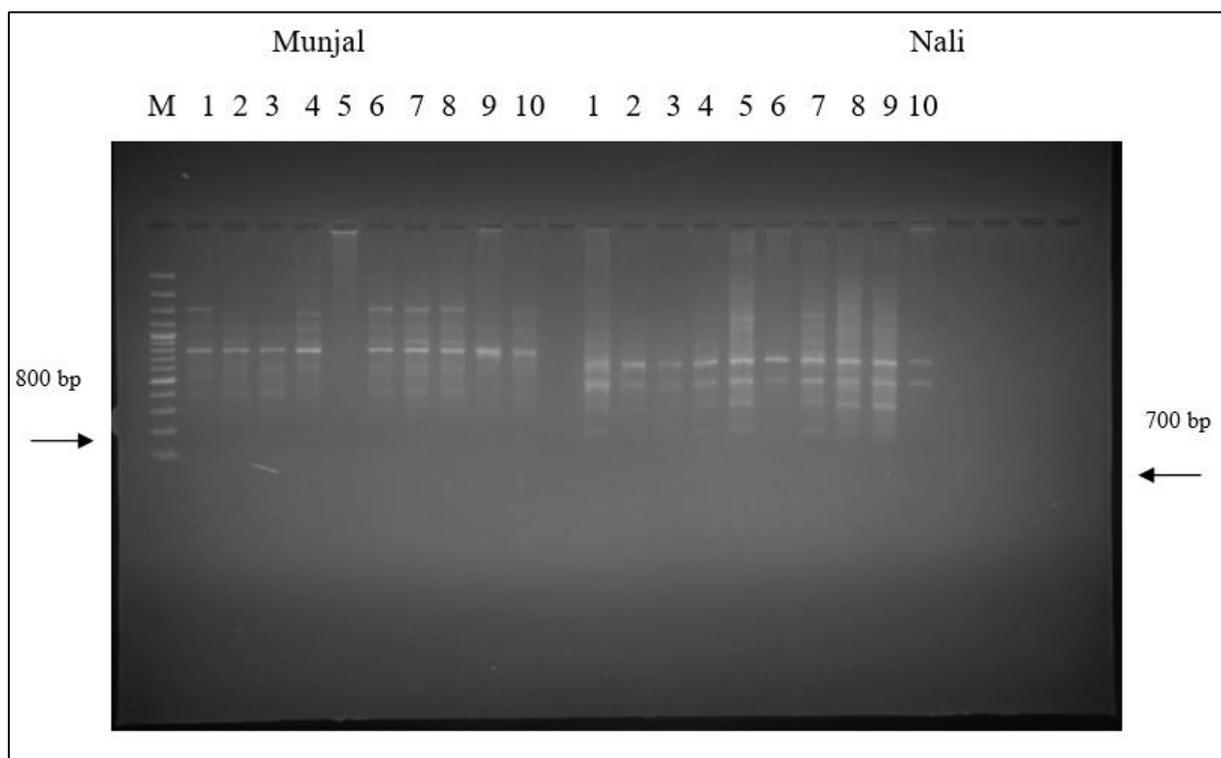**Fig 1:** RAPD Pattern in Munjal and Nali breed by OPU-6 Primer

Fig 2: RAPD Pattern in Munjal and Nali breed by OPV-09 Primer

Conclusion
In conclusion, hereditary variety may be a basic requirement for animal breeding, while a high genetic variety is required for hereditary improvement of domestic animals. Information of hereditary distance among animals and breeds, and genetic diversity/structure inside breeds may be valuable for conservation of hereditary assets. A simple RAPD-PCR is efficient in differentiating between and within breed at molecular level, however, after further analysis of more Sheep breeds may allow the identification of other breeds.

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