

Full Length Research Paper

Genetics similarity among four breeds of goat in Saudi Arabia detected by random amplified polymorphic DNA marker

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Phylogeny analysis using random amplified polymorphic DNA (RAPD) markers was performed for studying genetic variation in four Saudi Arabia goat breeds, namely: Harri, Ardi, Habsi and Masri. Six goats from Harri breed, four each from both Ardi and Habsi breeds and five from Masri breed were used for the experiment. Four different 10 bp RAPD primers were employed for the polymerase chain reaction (PCR). The results obtained showed that Harri and Ardi goat breeds lay in the same group and share about 73.5% genetic similarity, while Habsi and Masri goat breeds were closer to each other more than the previous two breeds, where they share about 82.5% of genetic similarity.

Key words: Goats, breeds, RAPD, genetic similarity.

INTRODUCTION

Goats were among the first farm animals to be domesticated. As indicated by the archaeological evidence, they have been associated with man in a symbiotic relationship for up to 10,000 years (Ensminger and Parker, 1986). Goats disseminated all over the world because of their great adaptability to varying environmental conditions and the different nutritional regimes under which they were evolved and subsequently maintained. They proved useful to man throughout the ages due to their productivity, small size and non-competiveness with him for food. In the developing countries, goats make a very valuable contribution, especially to the poor in the rural areas. The importance of this valuable genetic resource is underestimated and its extent of contribution to the livelihood of the poor is inadequately understood. They are often neglected in comparison with cattle and sheep. Part of this attitude towards them can probably be due to

the recognition of their capability, rather any prejudice against them, as it is believed that goats are intelligent, independent, agile and tolerant to many diseases and parasites and can look after themselves much better than other livestock species (Abdul Aziz, 2010).

Goats have been raised in various areas of the world for their production of meat, milk skin and fiber as well. This species is well known for its high ability to adaptation in the tropical and subtropical regions and especially in the arids. In Saudi Arabia where the climate is suitable for goats, the number of these animal is believed to exceed 2.5 million (Salah et al., 1989) mainly of the Masri (Egyptian) and Ardi (Baldi) breeds. Also, there are two other breeds, however, with less numbers (Harri and Habsi). The Ardi goats are more adapted to the arid region than Masri. Although, the latter produces more milk (Al Saily et al., 2007) while the first produces milk steadily and therefore, is greatly appreciated by desert dwellers, where it is widely spread. The contribution of goat as a source of meat to total meat income of Saudi Arabia is about 30%. Although, goats have a large and substantial contribution to the total meat income of Saudi

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Table 1. Sequence of primers and annealing temperature used in RAPD study.

Primer	Sequence	Annealing temperature (°C)
1	5`- GTG GGC TGAC -3`	35
2	5`- GTC CAT GCCA- 3`	35
3	5`- ACA TCG CCCA- 3`	35
4	5`-AAGGGCGAGT-3`	35

Arabia, this species remain neglected and rearing is exclusively in the hand of the nomadic people. Little information is available about the phylogeny and genetic relations between Saudi Arabia goat breeds. Also, few studies were conducted for genotyping of most important economic traits of Saudi Arabia goats and few steps were taken in the area of genetic improvement of these local adaptive genetic resources. Therefore, the productive performance of this animal has great lack of information.

Breed characterization requires the knowledge of genetic variation that can be effectively measured within and between populations (Hetzl and Drinkwater, 1992). Genetic markers may provide useful information at different levels: Population structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness (Feral, 2002). During the last few years, the great strides of molecular biology virtually gave access to the entire genome, but their complexity and high cost limited their use to precisely targeted projects in population biology. However, the polymerase chain reaction (PCR) which induced a methodological revolution (Mulis and Faloona, 1987; Sakai et al., 1988; Erlich, 1989) has been applied for genetic variations studies. The PCR technique is basically a primer extension reaction for amplifying specific nucleic acids *in vitro*. The use of a thermostable polymerase referred to as Taq allows a short stretch of DNA to be amplified to about a million fold so that one can determine its size, nucleotide sequence, etc. The extensive genetic polymorphism revealed by DNA markers may be used as an advantage to resolve genetic difference of even closely related goats. The main interest for population biology is that it is now possible to work with a very small initial amount of DNA (virtually, one cell is sufficient).

Application of the random amplified polymorphic DNA (RAPD) technique has greatly increased the ability to understand the genetic relationships within species at the molecular level. Information on genetic relationships in livestock within and between species has several important applications for genetic improvement and in breeding programmes (Appa Rao et al., 1996). Domestic animal genetic diversity will meet current and future production needs under various environments for allowing sustainable genetic improvement and facilitating rapid adaptation to change breeding purpose (El-Hanafy and El-Saadani, 2009; El-Hanafy et al., 2010).

Since little information are available about the genetic relationships between Saudi Arabia local goats, the aim of this research was to study genetic diversity of these breeds in an attempt to have a clear image about phylogeny and genetic relations between these local adaptive breeds which will aid in the future for genetic improvement of these local valuable genetic resources.

MATERIALS AND METHODS

Blood samples were collected from four goat breeds namely Harri, Ardi, Habsi and Masri raised in Hada AL Sham farm, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia. Approximately, 10 ml venous blood was collected from each animal using 0.5 ml of 2.7% ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. Genomic DNA was isolated from blood using DNA extraction kit (GF-1, Vivantis) according to the manufacturer's instructions. The quality of DNA was checked by spectrophotometry taking ratio of optical density (OD) value at 260 and 280 nm. Good quality DNA having OD ratio between 1.7 and 1.9 was used for further work. The poor quality DNA was re-extracted with phenol-chloroform.

Six goats from Harri breed, four from both Ardi and Habsi and five from Masri breed were used for the experiment. Four 10 bp RAPD primers were used for the PCR as described by Williams et al. (1990). The PCR was carried out in a 25 µL reaction mixture containing: 100 to 150 ng genomic DNA, 0.5 µM of each primer 1.00 U of Taq DNA polymerase, 2.5 µL of 10 × PCR assay buffer (1.5 mM MgCl₂), deoxynucleotide triphosphates (dNTPs) each at 100 µM. The amplification was carried out using a pre-programmed thermal cycler (Eppendorf Mastercycler). The sequence of the four primers and their annealing temperature are shown in Table 1.

The thermal cycle profile is as follows: 4 min initial denaturation at 95°C, 45 cycles of 1 min at 95°C, 1 min at 35°C, 1 min at 72°C, followed by a final extension at 72°C for 10 min. PCR product were analyzed in 2% agarose gel stained with ethidium bromide. Gels were photographed by Gel Documentation system (Syngene). PCR products were scored across the lanes as variables. The presence of a band of amplified DNA was scored as '1' and absence as '0'. The phylogeny tree of goat breeds was made according to statistical program analysis (Statistica® Version 5).

RESULTS AND DISCUSSION

To ensure that the amplified DNA bands originated from genomic DNA and not primer artifacts, negative control was carried out with each of the four primers and no amplification was detected in control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction

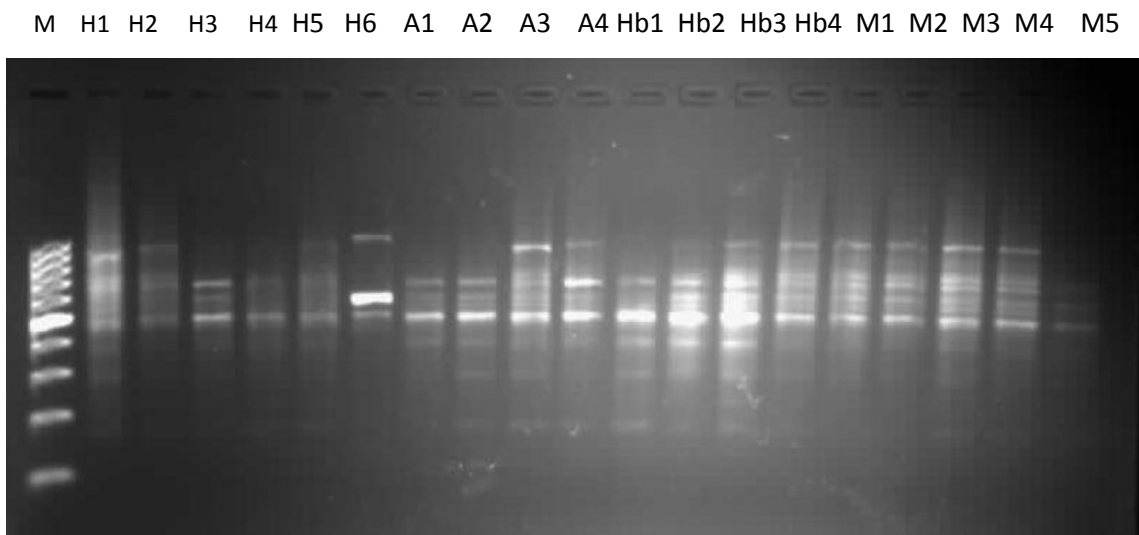


Figure 1. RAPD profiles obtained with primer 1 from the DNA of Harri (lanes H1-6), Ardi (lanes A1-4), Habsi (lanes Hb1-4) and Masri (lanes M1-5) goat breeds. Lane 1 M, Molecular size marker (100 bp DNA ladder).

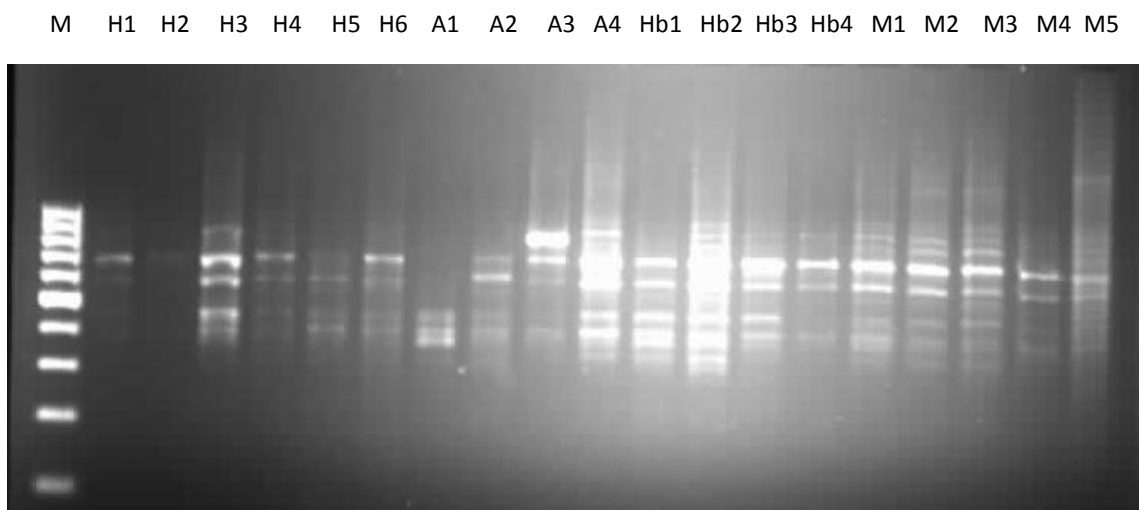


Figure 2. RAPD profiles obtained with primer 2 from the DNA of Harri (lanes H1-6), Ardi (lanes A1-4), Habsi (lanes Hb1-4) and Masri (lanes M1-5) goat breeds. Lane 1 M, 100 bp DNA.

conditions. All of the four primers used in this study were successfully amplified with polymorphic bands among the goats of four breeds studied as shown in Figures 1 to 4.

RAPD analysis was used for constructing parsimony tree depicting relationships among the four goat breeds studied (Figure 5). Data presented in Figure 5 showed that at linkage distance 35% there are two groups descended from one cluster, one of this group share 73.5% of genetic similarity and contains three goats of Harri breed and all the four goats of Ardi breed. The other group shared 82.5% of genetic similarity and contains three goats from Habsi breed and four of five goats of Masri breed. However, these two groups share only 65%

of genetic similarity. Also, as it is shown in dendrogram that there are odd values, these odd values would be refer to the goat variation within samples.

In other words, it can be concluded that Harri and Ardi goat breeds have 73.5% of genetic similarity, while share about only 65% genetic similarity with both Habsi and Masri goat breed. This can be explained on the basis that Harri and Ardi breed may be descended from common ancestor differ from the other two breeds. On the other hand, Habsi and Masri goat breeds are closer to each other than previous breeds and share about 82.5% of genetic similarity which may reflect that these two goat breeds may be imported to Saudi Arabia from convergent

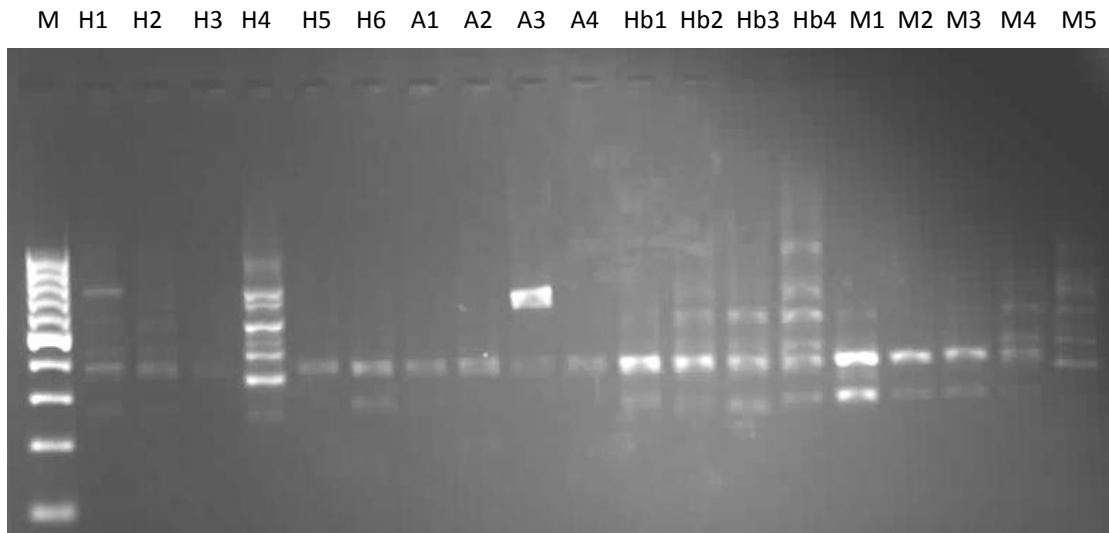


Figure 3. RAPD profiles obtained with primer 3 from the DNA of Harri (lanes H1-6), Ardi (lanes A1-4), Habsi (lanes Hb1-4) and Masri (lanes M1-5) goat breeds. Lane 1 M, Molecular size marker (100 bp DNA ladder).

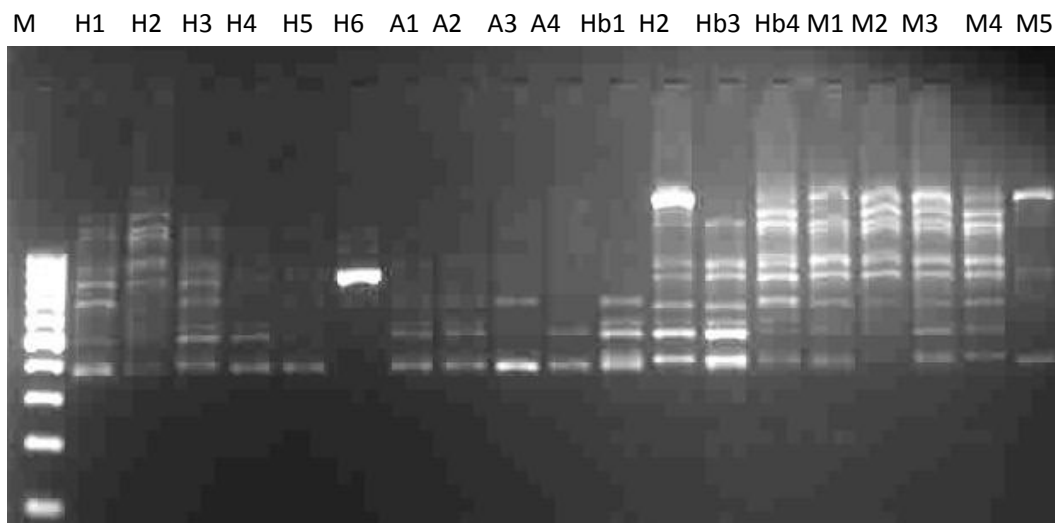


Figure 4. RAPD profiles obtained with primer 4 from the DNA of Harri (lanes H1-6), Ardi (lanes A1-4), Habsi (lanes Hb1-4) and Masri (lanes M1-5) goat breeds. Lane 1 M, Molecular size marker (100 bp DNA ladder).

geographic regions, for example, near East and Egypt. The RAPD technique has also been used for constructing trees in other animals such as buffalo, cattle, goat and sheep (Appa Rao et al., 1996), tilapia fish (Baradakci and Skibinski, 1994), bacteria (EL Hanafy et al., 2007) and date palm (Soliman et al., 2003).

Conclusion

This study reveals that genetic diversity exist among the

four Saudi Arabia goat breeds and has demonstrated the usefulness of the RAPD approach for detecting DNA polymorphism in goats. It also establishes a relationship among the different Saudi goat breeds, especially as few information are available, until now, about their genetic relationships. This study considers the primary step for clarifying the image of the genetic diversity of these local Saudi Arabia goat breeds and should be followed by further studies using large number of animals from different geographical regions in this kingdom to get the precise estimation of the phylogeny of these local genetic

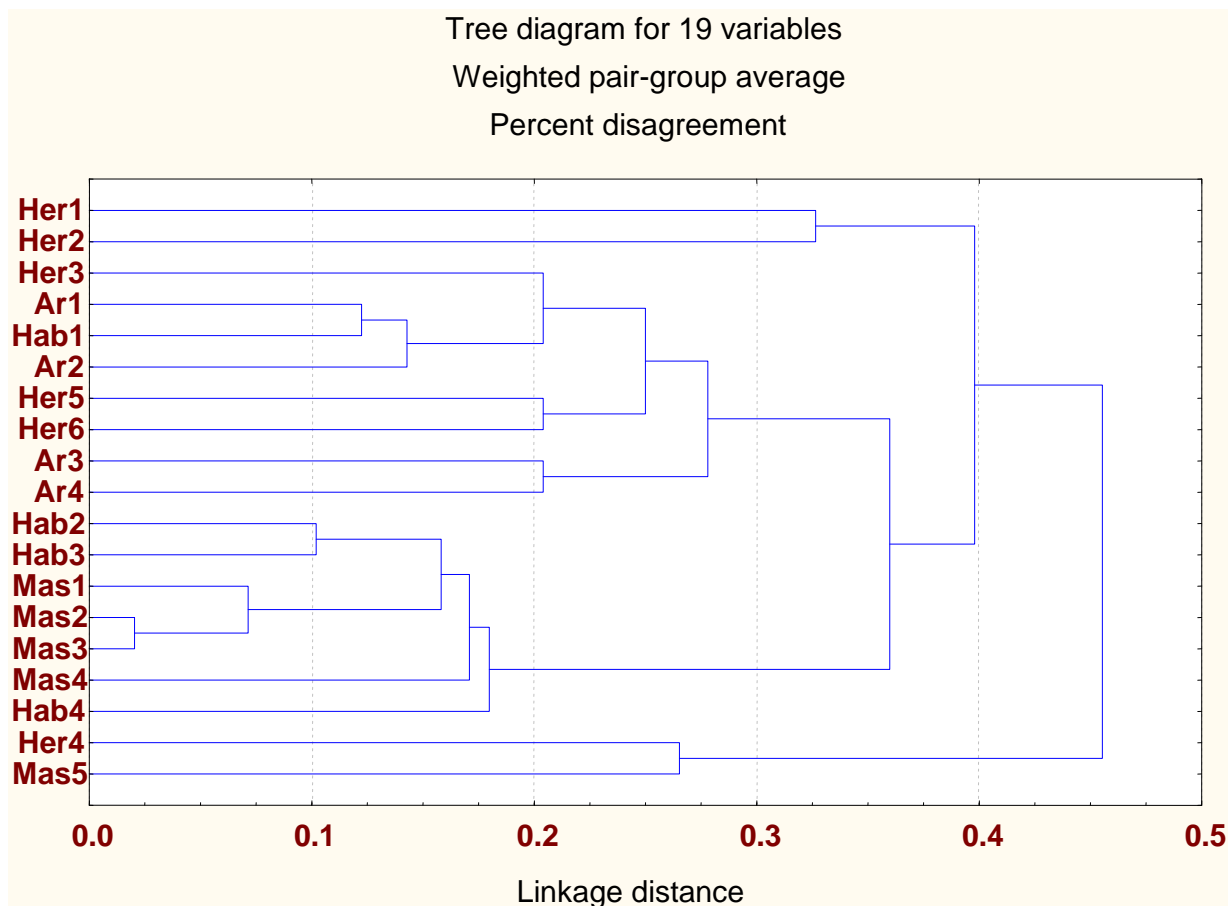


Figure 5. Phylogeny tree showing the relationships among the goats of Harri (Her), Ardi (Ar), Habsi (Hab) and Masri (Mas) goat breeds obtained by RAPD analysis using four different primers.

resources even within each breed. With further experimentations, the RAPD profile generated for each breed can be effectively used as a supporting marker for taxonomic identification. In taxonomic and molecular systematic, species-specific RAPD markers could be an invaluable tool for species variation and establishing the status of organisms and its evolution (Allard et al., 1992; Dinesh et al., 1993; Appa Rao et al., 1996).

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REFERENCES

- Abdel Aziz M (2010). Present status of the world goat populations and their productivity. *Lohman Information*.45:42-52.
- Al Saidy MY, AL Shaikh MA, Mogawer HH, AL Mufarrej SI, Kraidess MS(2007). Effect of feeding different levels of Fenugreek seeds (*Trigonella foenum. graecum* L) on milk yield, milk fat and some blood hematology and chemistry of Ardi goat. *J.Saudi Soc. For. agric. Sci.* 2: 62-65.
- Allard MW, Miyamoto MM, Jarecki I, Kraus F, Tennant MR (1992). *Proc. Natl. Acad. Sci. USA* 18: 3972-3976.
- Appa Rao KBC, Bhat KV, Totey, SM (1996). Detection of species specific genetic markers in farm animals through random amplified polymorphic DNA (RAPD). *Genetic analysis: Bimolecular Engineering*, 13: 135-38.
- Baradakci F, Skibinski DOF (1994). Application of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity*, 73: 117-123.
- Dinesh KR, Lim TM, Chan WK, Phang VPE (1993). *Zool. Sci.* 10: 849-854.
- EL-Hanafy AA, Abd-Elsalam HE, Hafez EE (2007). Fingerprinting For the Lignin Degrading Bacteria from the Soil. *J. Appl. Sci. Res.* 3: 470-475
- EI-Hanafy AA, EI-Saadani M.A(2009). Fingerprinting of fecb gene in five Egyptian sheep breeds. *Biotechnol. in Anim. Husbandry.* 25: 205-212.
- EI-Hanafy AA, EI-Saadani MA, Eissa M, Maharem GM, Khalifa Z A (2010). Polymorphism of β -lacto globulin gene in Barki and Damascus and their cross bred goats in relation to milk yield. *Biotechnol. Anim. Husbandry*, 26: 1-12.
- Ensminger M E, Parker R O (1986). *Sheep and Goat Science*, Fifth Edition. Danville, Illinois: The Interstate Printers and Publishers Inc.
- Erlich HA (1989). *PCR technology*. Stockton Press, New York.

- Feral JP (2002). How useful are the genetic markers in attempts to understand and manage marine biodiversity. *J. Exp. Mar. Biol. Ecol.* 268: 121-145.
- Hetzel DJS, Drinkwater RD (1992). The use of DNA technologies for the conservation and improvement of animal genetic resources. *FAO Expert Consultation on Management of Global Animal Genetic Resources*. Rome, April.
- Mullis KB, Faloona FA (1987). Specific synthesis of DNA in vitro via polymerase catalyzed chain reaction. *Methods Enzymol.* 155: 335-350.
- Sakai RK, Delfet DH, Stoffel S, Sharf SJ, Higushi R, Horn GT, Mullis KB, Ehrlich HA (1988). Primer directed amplification of DNA with a thermostable DNA polymerase. *Science*, 239: 487-491.
- Salah M S, Bakkar M N, Mogawer (1989). Body weight of Aradi goat kids in Saudi Arabia at different ages and affecting factors. *J. King Saud Univ.* 1: 17-24.
- Soliman SS, Ali BA, Ahmed MMM (2003). Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR. *Afr. J. Biotechnol.* 2: 86-87.
- Williams JGK, Kubelic AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.* 18: 6531-6535.