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SPECIES IDENTIFICATION IN MEAT ORIGIN FARM ANIMALS THROUGH DNA TECHNOLOGY[1]

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Abstract: RAPD fingerprint technique was used on several meat sources to identify the species. The tested meat species were: Buffalo, Cattle, Goat and Sheep. Eighteen primers as a single, short oligonucleiotides were used to detect the species by fingerprint and genetic similarity as band sharing (BS) among these four species. By comparison between all four species, the band sharing average values were [51.0, 45.0, 59.0, 41.0, 48.0 and 70.0%] respectively. In respect of comparison, the comparison between Goat and Sheep showed high similarities while between Cattle and Goat showed low similarities. The genetic similarity as BS values ranged from 41.0 to 70.0 % respectively. The results showed that RAPD analysis provided a rapid and effective method to detect the genetic variation of different species. Also the results showed that RAPD analysis produced clear fingerprints from the products analyzed for which the species could be easily identified.

Keywords: Buffalo, cattle, goat, sheep, RAPD-PCR, phylogeny and genetic similarity.

Introduction and literature review

The conventional methodology used for the determination of species origin in meat and meat products have been predominantly based on the immunochemical and electrophoretic analysis of proteins. More modern techniques now allow the identification of species-specific markers RAPD-PCR or other techniques, which has two major advantages over protein analysis: samples heated to as high as 120° C can still be analyzed and discrimination between related species, such as sheep, goat or chicken and turkey…e.g., is possible.

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Consumers nowadays very seldom can identify the species in the products they purchase: Carcasses or whole fish are rarely in display while cuts ether fresh or frozen, more or less processed (souse-vide, marinated, dried, smoked, salted, etc.) and prepared ready-to-eat products are increasingly available. This opens the possibility of fraudulent adulteration and substitution of the expected species with others of less value (*Malmheden Yman and Emanuelsson, 1998*). To safeguard consumer rights, the legislation of each country should therefore impose a labeling of food products declaring the species used in their manufacture, and food laboratories need to have available techniques to ascertain the species used in the manufacture of those products.

The advent of molecular technology has greatly enhanced animal genetic studies. A polymorphism assay, based on the amplification of random DNA fragments using an arbitrary nucleotide sequenced primer, this technology has developed by *Williams et al. (1990*) and *Welsh and McClelland (1990*). The randomly amplified polymorphic DNA (RAPD) assay, which is simple, fast, comparatively low-cost, labor intensive, and quickly became the method of choice for genotype identification, population and pedigree analysis, phylogenic studies and genetic mapping in a wide range of genotypes including animal species (*Kemp and Teale, 1994, Kantanen et al. 1995 and Appa Rao et al. 1996).*

The recently developed techniques, based on the polymerase chain reaction (PCR), offer a new tool for construction of linkage maps. The arbitrarily primed PCR (AP-PCR) (*Welsh and McCleland 1990*) as well as the random amplified polymophic DNA (RAPD) technique (*Williams et al. 1990*) utilize**s** arbitrary primers for the amplification of template DNA. The (RAPD) technique utilizes decamer primer arbitrary sequence with GC content > 50%. As time consuming and expensive synthesis of special primers can be avoided. A set of commercially available primers can be used for different species (*Klein-Lankhorst et al. 1991*).

RAPD- PCR method could be used to identify animals including bovine, goat, pig, dog, rat, rabbit, chicken, duck, human and others species by comparing their RAPD- PCR fingerprints. This study provides a simple, fast and sensitive fingerprinting method in species identification for crime scene evidence or food products of endangered species, *Lee and Chang (1994), Koh et al. (1998*) and *Martinez and Yman (1999).*

Bednarczyk et al. (2002) reported that resulted in genetic variation among goose lines as detected by RAPD. Selection for meat traits caused greater genetic diversity than selection for egg production. *Ali et al. (2003*) studied molecular differences between five chicken strains for different production (eggs, meat and both together) using RAPD-PCR. The data showed that there is a great difference between different chicken strains used. In addition, similarity among strains for the same product was observed. These studies indicated the potential use of RAPD markers for a wide range of applications in poultry breeding.

The aim of this study was to employ DNA analysis at the molecular level for meat species identification in meat products and know the genetic relationships of four meat species Buffalo, Cattle, Goat and Sheep.

Materials and methods

The present study was carried out at the Nucleic Acid Research Dept., (GEBRI), Mubarak City For Scientific Research and Technology Applications, Alexandria, Egypt.

Meat samples

Meat samples of four species, Buffalo, Cattle, Goat and Sheep from muscle Purchase were collected in New Borg El-Arab City, Alexandria, Egypt.

DNA extraction

DNA was extracted from muscle sample following the method described by *Bardakci and Skibinski (1994*) with some modifications. Approximately 0.5 g of the tissue was cut into small pieces and suspended in 1000 µl STE (0.1 M Na Cl, 0.05 M Tris and 0.01 M EDTA, pH 8). After adding 30 µl SDS (10%) and 30 µl proteinase K (10 mg/ ml). The mixture was incubated at 50 °C for 30 min. DNA was purified by successive extraction with phenol, phenol: chloroform: isoamyl alcohol (25:24:1) and chloroform: isoamyl alcohol (24:1) respectively. DNA was precipitated with ice-cold absolute ethanol and washed with 70 % ethanol. The pellet was dried and resuspended in 200µl mill Q water.

PCR primers

Ten and twenty meter long oligonucleotide primers were used to initiate PCR amplifications. Primers were randomly (arbitrarily) selected on the basis of GC content and annealing temperature for RAPD-PCR amplification. The nucleotide sequences of the primers are given in table 1.

PCR amplification and agarose gel electrophoresis

PCR amplifications were performed following the procedure of Williams et al. (1990). The reaction were carried out in (25 µL) consisted of 1.0 U of Taq DNA polymerase (Promga), 25 pmol dNTPs, 25 pmol of random primer, 2.5 µL of 10X Taq DNA polymerase buffer and 50 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycle (Ependorff). The PCR program included an initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 30 seconds for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72 °C for 10 minute. The samples were cooled at 4°C. The amplified DNA fragments were separated on 3% agarose gel and stained with ethidium bromide. ΦX174 DNA marker (bp 1353, 1078, 872,….., 72) was used in this study. The amplified pattern was visualized on an UV transilluminator and photographed by Gel Documentation system.

Scoring and data analysis of rAPDs

The DNA bands were scored for their presence (1) or absence (0) in the RAPD profile of the four species. The index of similarity between each two species was calculated using the formula: Bab =2 Nab/ (Na + Nb), where Nab is the number of common fragments observed in individuals a and b, and Na and Nb are the total number of fragments scored in a and b respectively (Lynch, 1990). The BS values were calculated for each primer separately and average for all primers was carried out with each comparison. Dendrogram was constructed using the Average Linkage Between Groups statistical system.

Results and discussion

Each species produced a species-specific RAPD fingerprint (Figure 1). Samples (1-4) produced the fingerprint of four species Buffalo, Cattle, Goat and Sheep, respectively. Among the twenty primers screened, eighteen primers were selected, that gave the best product for RAPD analysis (Table 2). The nucleotide sequence of each primer arbitrarily was within the constraints. These primers were ten and twenty nucleotides in length and possessed 60-80% G + C content and only one primer (primer No. 7) possessed 40 % G + C content (Table 1). A series of several DNA fragments of four species were amplified with all primers (Figure 1). All the amplification products were found to be

reproducible when reactions were repeated using the same reaction conditions.

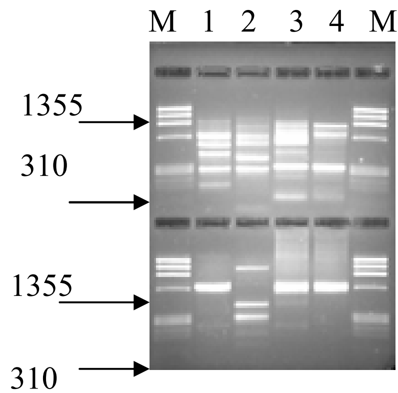
The ability of RAPD fingerprint to discern among four species, Buffalo, Cattle, Goat and Sheep were included in this study to ascertain the genetic distance between goat breeds. Eighteen oligonucleotide primers or random sequences (10 and 20 mer) were tested to amplify the DNA from all four species. The genomic DNA was successfully amplified from all samples of goat breeds (Figure 1). Table 2 showed the genetic similarity estimated as band sharing (BS) for each prime with all four species. The average values by comparison between four species were [51.0, 45.0, 59.0, 41.0, 48.0 and 70.0%] respectively. In respect of comparison and the high similarities between Goat and Sheep, low similarities were detected between Cattle and Goat. The genetic similarity as BS values ranged from 41.0 to 70.0 % respectively. In addition, results declared the similarity between the four species with all primers used the all comparison over 41.0 %. RAPD analysis has been used for constructing a tree as shown in figure 2 using average linkage between groups rescaled distance cluster combine.

Table 1. The primer sequence, G+C content % and annealing temperatures of the primers used
Tabela 1. Sekvenca prajmera, G+C sadržaj % i temperature prekalenja korišćenih prajmera

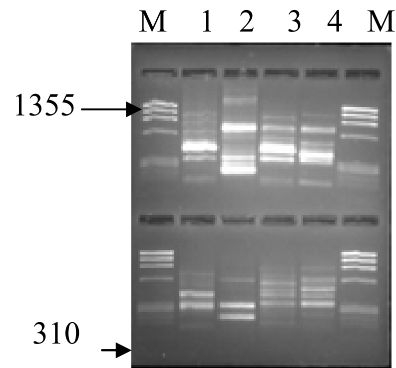
Primer	Sequence 5`- 3`	G + C content %	Annealing Tem., / Sec.
1	TGG TGG ACC A	60	47/30
2	GAA TGC GAC G	60	47/30
3	CGC TGT CGC C	80	47/30
4	AGT CCT CGC C	70	47/30
5	AAA GCT GCG G	60	30/30
6	ACC GCC GAA G	70	30/30
7	ATG ACG TTG A	40	47/30
8	CTG AGG AGT G	60	47/30
9	AGG CCC CTG T	70	30/30
10	ATG CCC CTG T	60	30/30
11	GGA CTG GAG TGG TGA CGC AG	65	56/30
12	AGC AGG TGG A	60	45/30
13	GGT GAC GCA GGG GTA ACG CC	70	54/30
14	GGA CTG GAG TGT GAT CGC AG	60	54/30
15	CAG GCC CTT CCA GCA CCC AC	70	52/30
16	GAA ACG GGT GGT GAT CGC AG	60	52/30
17	GGG CTA GGG T	70	45/30
18	ACC GGG AAC G	70	45/30

Tabela 2. Genetic similarities estimated as band sharing (BS) for each primer between all four species
Tabela 2. Genetske sličnosti ocenjene kao BS za svaki prajmer između sve šetiri vrste

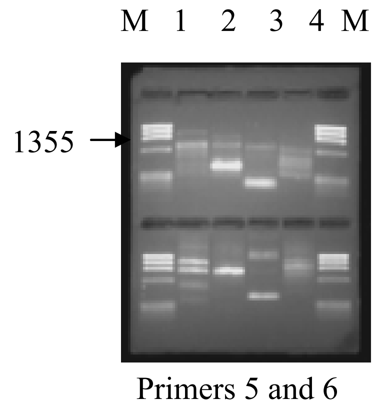
Primer No.	Comparisons/Poređenje					
	1X2	1X3	1X4	2X3	2X4	3X4
1	0.50	0.00	0.50	0.33	0.67	0.67
2	0.86	0.28	0.28	0.28	0.28	0.67
3	0.44	0.57	0.44	0.40	0.33	0.67
4	0.67	0.57	0.57	0.28	0.28	1.00
5	0.00	0.50	0.33	0.40	0.57	0.33
6	0.40	0.00	0.50	0.00	0.40	0.00
7	0.73	0.73	0.60	0.80	0.67	0.89
8	0.00	1.00	1.00	0.00	0.00	1.00
9	0.60	0.67	0.91	0.86	0.67	0.85
10	0.67	0. 73	0.73	0.44	0.44	1.00
11	0.50	0.75	0.80	0.50	0.80	0.80
12	0.67	0.00	0.50	0.00	0.00	0.67
13	0.28	0.33	0.33	0.57	0.57	1.00
14	0.80	0.50	0.80	0.67	1.00	0.67
15	0.86	0.67	0.67	0.57	0.57	1.00
16	0.76	0.80	1.00	0. 73	0.67	0.80
17	0.50	0.00	0.67	0.50	0.67	0.00
18	0.00	0.00	0.00	0.00	0.00	0.50
Average/ Prosek	0.51	0.45	0.59	0.41	0.48	0.70



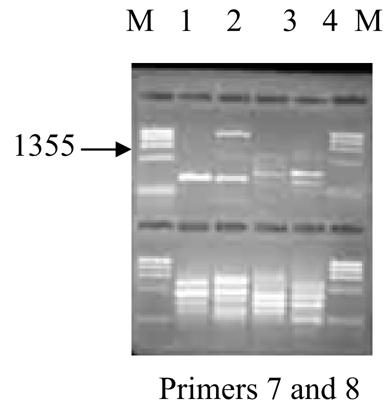
Primers 1 and 2



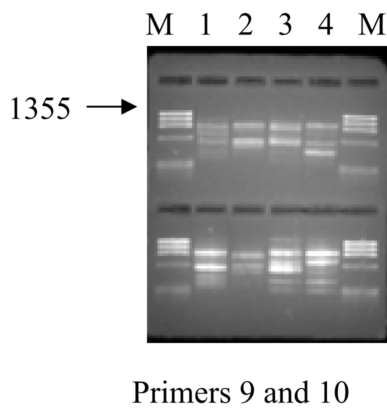
Primers 3 and 4



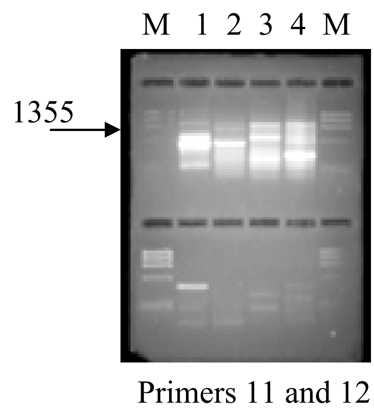
Primers 5 and 6



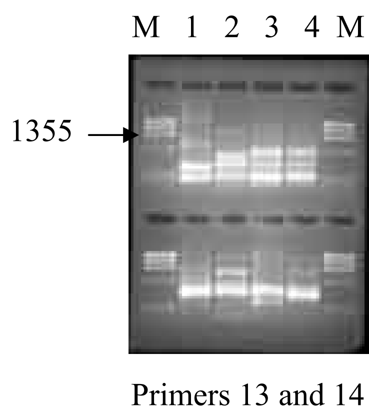
Primers 7 and 8



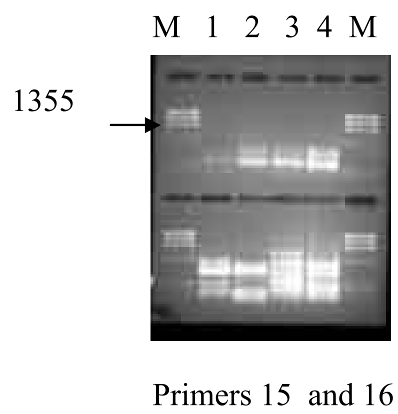
Primers 9 and 10



Primers 11 and 12



Primers 13 and 14



Primers 15 and 16

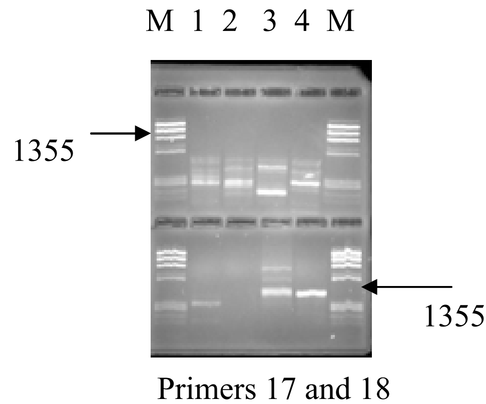


Figure 1. RAPD amplification products generated by different random primers (1-18). Lane M: DNA marker, Lane 1: buffalo, Lane 2: cattle, Lane 3: goat Lane 4: sheep
Slika 1. Proizvodi RAPD amplifikacije stvoreni od strane različitih slučajnih prajmera (1-18). M: DNK marker, 1: bivo, 2: govedo, 3: koza, 4: ovca

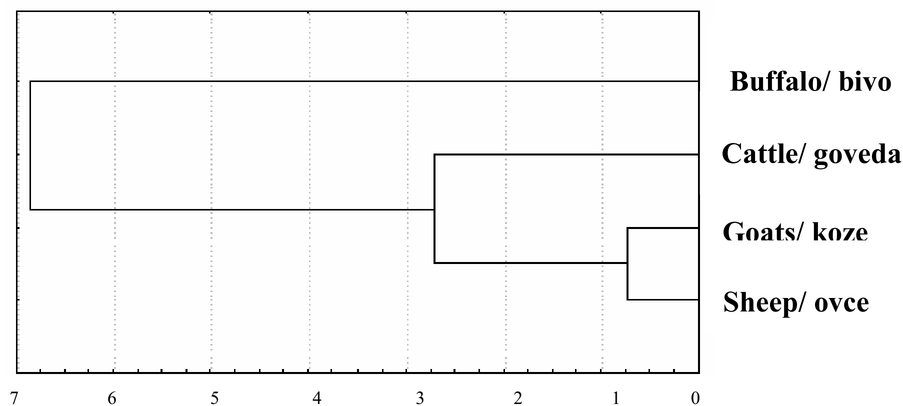


Figure 2 Phylogenetic tree constructed using statistical analysis Euclidean distances similarity between four species
Slika 2. Filogenetsko drvo konstruisano korišćenjem statističke analize Euklidovih razmaka i sličnosti između četiri vrste

The DNA fingerprint analysis, in our results demonstrate the usefulness of the RAPD approach for detecting DNA similarity or

polymorphisms in farm animals and establishing its relationships between four Species. The majority of arbitrary primers used gave distinctly reproducible patterns in all four Species studied. Thus, the RAPD profile generated for each species can be effectively used as a supporting marker for taxonomic identification, as taxonomic relationship of four Species. In taxonomic and molecular systematics, four Species relationship markers could be a tool for Species verification and in establishing the status of organism's systematics and its evolution, *Appa Rao et al. (1996); Koh et al. (1998); Martinez and Yman (1999)* and *Bednarczyk et al. (2002)*. The RAPD technique utilizes decamer primer arbitrary sequence with GC content > 50%. As time consuming and expensive synthesis of special primers can be avoided. A set of commercially available primers can be used for different species *(Klein-Lankhorst et al. 1991)*.

It is observed that the small alterations in PCR parameters or quality of target DNA can radically affect RAPD patterns (*Williams et al. 1993* and *Baradakci and Skibinski 1994*). Thus there may be reason to view with caution systematic conclusions based on RAPD analysis alone. On the other hand, the possibility of carrying out compatibility analysis with unlimited numbers of primers, each detecting variation at several regions in the genome, provides an advantage over other techniques. Even if some primers amplify identical regions of the genome or if the technique itself is noisy, it should be possible to build up quickly a consensus from patterns of interpopulation variation.

The genetic variation among the species may be due to differences in species Genotypes, phenotypes, growth rate and high temperature tolerance of each species. These results reflect relationship between RAPD patterns and quantitative trait loci (QTL). The obtained results are in agreement with the findings of several authors in their researches (*Feral, 2002; Ali et al. 2002 and Ali et al. 2003*). Also, may be due to differences in phenotype and growth of each Species resulted from difference at the molecule level of DNA among them. Also, the sensitivity of RAPD technique played a good role in detection of these differences. This observation is agreed with *Bardakci and Skibinski (1994); Martinez and yman (1999)*.

RAPD- PCR method could be used to identify animals including bovine, goat, pig, dog, rat, rabbit, chicken, duck, human and others species by comparing their RAPD PCR fingerprints. This study provides a simple, fast and sensitive fingerprinting method in species

identification for crime scene evidence or food products of endangered species, *Lee and Chang (1994) Koh et al. (1998); Martinez and yman (1999) and Bednarczyk et al. (2002).*

RAPD analysis has been used for constructing a tree (Figure 2) using average linkage between groups rescaled distance cluster combine. This method has been used for constructing trees in other organisms: farm animals as Buffalo, Cattle, Goat and sheep, tilapia fish and Plant as mentioned by *Appa Rao et al. 1996); Koh et al. (1998); Martinez and yman (1999); Bednarczyk et al. (2002); Lee and Chang (1994); Baradakci and Skibinski (1994) and Soliman et al. (2003).*

Conclusion

The present results confirmed the ability of RAPD analysis for meat species identification of samples of different origin and submitted to different manufacturing conditions. Although the technique to be used is greatly a matter of preference if the DNA is not severely degraded to fragments of under a few hundred base pairs. For species used in this work, RAPD was reliable, easier, faster and cheaper than other DNA-based techniques.

IDENTIFIKACIJA VRSTE ŽIVOTINJE PREKO MESA DOMAĆIH ŽIVOTINJA KORIŠĆENJEM DNK TEHNOLOGIJE

M. M. M. Ahmed

Rezime

Tehnika RAPD je korišćena na nekoliko vrsta mesa kako bi se identifikovale vrste životinja od kojih meso potiče. Ispitivane vrste mesa su od: bivola, goveda, koza i ovaca. Osamnaest prajmera kao pojedinačni, kratki oligonukleotidi su korišćeni za otkrivanje vrste i to “fingerprint” tehnikom i genetskim sličnostima kao BS između četiri vrste. Poređenjem sve četiri vrste, vrednosti BS su bile 51.0, 45.0, 59.0, 41.0, 48.0 i 70.0%, respektivno. U vezi sa tim, poređenje između koze i ovce je pokazalo visoku genetsku sličnost, dok je između goveda i koze utvrđena niska genetska sličnost. Genetska sličnost kao BS vrednost je varirala od 41.0 do 70.0 % respektivno. Rezultati su pokazali da je RAPD

analiza brz i efikasan metod za otkrivanje genetskih varijacija različitih vrsta. Takođe, rezultati su pokazali da je RAPD analiza proizvela jasne “fingerprint” podatke za proizvode koji su analizirani i kod kojih je vrsta mogla lako da se identifikuje.

Dobijeni rezultati su potvrdili sposobnost RAPD analize u identifikovanju vrste mesa uzoraka različitog porekla i koji su bili podvrgnuti različitim proizvodnim uslovima. Iako je pitanje tehnike koja će se koristiti stavr izbora ako DNK nije ozbiljno razgrađena na fragmente od ispod nekoliko stotian baznih prajmera. Za vrste korišćene u ovom radu RAPD je bila pouzdana, lakša, brža i jeftinija tehnika u poređenju sa ostalim tehnikama koje se baziraju na DNK.

References

1. ALI B. A., AHMED M.M. M., BAHIE EL-DEEN M., SHALAN H. M. (2002): Genetic Variability in The 17th Generation of Japanese Quail Selected For High Eggs and Meat Production. Egyptian J. Poultry Science. 22: 59--71.
2. ALI B. A, AHMED M. .M. .M., ALY, O. M. (2003): Relationship Between Genetic Similarity and Some Productive Traits in Local Chicken Strains. African Journal Of Biotechnology. 2**:** 46-- 47.
3. APPA R.A.O, K.B. C., BHAT K.V., TOTEY, S.M. (1996): Detection of Species Specific Genetic Markers in Farm Animals Through Random Amplified Polymorphic DNA (Rapd). Genetic Analysis: Bimolecular Engineerin*.* 13: 135--38.
4. BARADAKCI F., SKIBINSKI D.O.F. (1994): Application Of The Rapd Technique In Tilapia Fish: Species And Subspecies Identification. Heredity. 73: 117--123.
5. BEDNARCZYK M., SIWEK M., MAZANOWSKI A., CZEKALSKI P. (2002): DNA Polymorphism in Various Goose Lines by Rapd-Pcr. Folia Biol.50:45—8
6. FERAL J. P. (2002): How Useful is the Genetic Markers in Attempts to Understand and Manage Marine Biodiversity. Journal Of Experimental Biology And Ecology. 268: 121- 145.
7. LYNCH M., (1990): The Similarity Index And Dna Fingerprinting. Mol. Biol. Evol**.,** 7: 478-- 484.
8. KANTANEN J., VILKKI J., ELO K., MAKI- TANILA A. (1995): Random Amplified Polymorphic DNA in Cattle and

Sheep: Application for Detecting Genetic Variation. Animal Genetics. 26: 315-- 320.

9. KELEIN –LANKHORST R. M., VERMUNT A., WEIDE R., LIHARSKA T., ZABEL P. (1991): Isolation Of Molecular Markers for Tomat (L. Esculentum) Using Random Amplified Polymorphic DNA (Rapd). Thero. Appl. Gent. 81: 661--667.
10. KEMP S.J., TEALE A.J. (1994): Randomly Primed PCR Amplification Of Pooled Dna Revealed Polymorphism in A Ruminant Repetitive DNA Sequence Which Differentiates Bos Indicus and Bos Taurus. Animal Genetics. 25: 83-88.
11. KOH M. C., LIM C. H., CHUA S. B., CHEW S. T., PHANG S. T. W., (1998): Random Amplified Polymorphic DNA (RAPD) Fingerprints for Identification Of Red Meat Animal Species. Meat Science. 48: 275-- 285.
12. LEE J.C., CHANG J.G. (1994): Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) Fingerprints in Forensic Species Identification. Forensic Sci. Int. 4: 103--107.
13. MALMHEDEN YMAN, I., EMANUELSSON, R. (1998): New Technology for Faster Disclosure of Meat of Adulteration. Var Fada. 3: 6-- 7.
14. MARTINEZ I., YMAN I. M. (1999): Species Identification in Meat Products by RAPD Analysis. Food Research International, 31: 459--466.
15. SOLIMAN S.S., ALI B. A., AHMED, M.M.M., (2003): Genetic Comparisons of Egyptian Date Palm Cultivars (*Phoenix Dactylifera L.*) by Rapd-Pcr. African Journal Of Biotechnology. 2**:** 86--87.
16. WELSH J., MCCLELLAND M. (1990): Fingerprinting Genome-Using PCR With Arbitrary Primers, Nucleic Acid Research. 8: 7213--7218.
17. WILIAMS J. G. K., HANAFEY M. K., RAFALSKI. J. A., TINGEY. S. V. (1993): Genetic Analysis Using Random Amplified Polymophic DNA Markers. Methods Enzymol., 218: 704--740.
18. WILLIAMS J.G.K., KUBLIK A.R., LIVAK K.J., RAFALSKI J.A. &TINGEY S.V., (1990): DNA Polymorphism Amplified Arbitrary Primers are Useful as Genetic Markers, Nucleic Acid Research. 18: 6531–6535.