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Molecular characterization of Alexandria rabbit line using DNA markers

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Abstract. Alexandria line is a synthetic paternal rabbit line which was established and progressing at the nucleus breeding rabbit unit of the Poultry Research Center, Egypt. Molecular genetic characterization of rabbit's lines is a prerequisite towards effective utilization of selection and breeding program. Ten RAPD and SSR primers were used in this study to characterize Alexandria line. A total of 143 bands were detected. Non-specific RAPD markers were detected at MW ranged from 400 to 1800 bp, these markers were generated from primers (1, 3, 4, 5, 7, 9 and 10). While specific SSR markers were detected at MW ranged from 550 to 950 bp, these markers were generated from primers (1, 2, 6, and 8). Molecular markers including RAPD and SSR fingerprints represent reliable tools which may have a great impact in rabbit breeding programs and genetic improvement of rabbits.
Key Words: Rabbit fingerprint, rabbit breeding program, RAPD-PCR, SSR-PCR.

Introduction. Domestic rabbit (*Oryctolagus cuniculus*) has its importance as supplier of meat and it is widely accepted through out the world for human consumption (Colin & Lebas 1996). Recently, rabbits have attracted more attention from the biotechnology experiments (El-Bayomi et al 2013). Molecular genetic markers have been widely used as powerful tools for characterization and analyzing the genome. DNA markers data provide useful information on the origins, characterization, relationships, genetic similarity and diversity of domestic animal breeds. Characterization at the molecular level is important to explore genetic diversity within and between animal populations and to determine genetic relationships among such populations (Rahimi et al 2005). PCR-based multi-locus DNA fingerprinting represents one of the most informative and cost effective measures of genetic diversity (Bagley et al 2001). El-Raffa (2005) reported that some countries of hot climate as Egypt have started a programme of founding several synthetics between exotic lines and local breeds intended to reach a compromise between the performance of the exotic line and the adaptation to the heat stress. Finally we had reached to Alexandria line which combines several important economical traits.

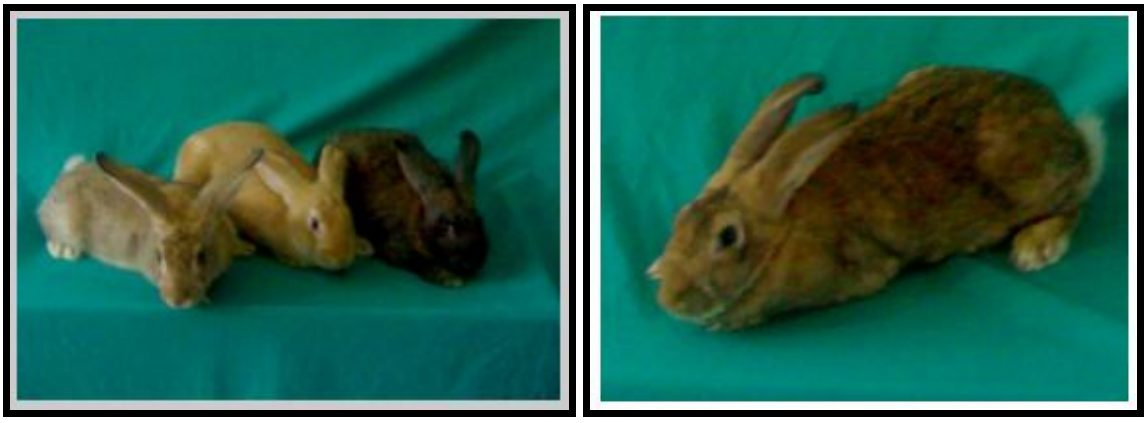

Figure 1. Alexandria line. Left: progeny; Right: adult individual (El-Raffa 2007).

Consequently, the main object of the present study is to genetically characterize Alexandria line (Figure 1) by studying DNA fingerprinting using: (i) RAPD analysis, (ii) SSR analysis.

Random amplified polymorphism DNA (RAPD) becomes an important technique for identifying lines of rabbits and the markers linked to economical traits of interest without the necessity for mapping the entire genome (Bardakci 2001). Furthermore, SSR markers have practical applications in breeding systems because they can be used in high-throughput analyses for genetic mapping, heritable diversity, purity analysis, and marker assisted selection.

Material and Method

Experimental animals. The experimental rabbits involved in this study were Alexandria line which is a synthetic paternal rabbit line originated by crossing a V-line with a Baladi Black rabbits. Individual selection for daily gain from weaning (28 days) to slaughter age (63 days) was used as a selection criterion of genetic improvement for this line. This line was established and progressing at the nucleus breeding rabbit unit of the Poultry Research Center, Faculty of Agriculture, Alexandria University, Egypt (El-Raffa 2005, 2007).

Housing and management. Rabbits of this study were housed in a rabbitry and were fed commercial pelleted diet containing 18% crude protein, 13% crude fiber and 2600 Kcal/Kg. By using high standard hygiene and good management, the happening of dangerous diseases was largely avoided and rabbits have never been treated with any kind of systematic vaccination.

Molecular genetic analysis

Blood collection. Approximately 2 mL of blood sample was collected from central artery vein of the ear under vacuum in centrifuge tubes containing EDTA as anticoagulant for molecular genetic analysis. Blood samples were taken from ten rabbits and all rabbits used were normal, healthy and sexually fertile.

DNA extraction. DNA was extracted from whole blood following the instruction of Thermo Scientific GeneJET Genomic DNA Fermentas Purification Kit. The quantity and quality of the isolated DNA was determined by spectrophotometer (at 260 nm to that of 280 nm) and agarose gel electrophoresis.

RAPD-PCR analysis. To resultant RAPD profiles from rabbit DNA, 10 different decamer oligonucleotide RAPD markers from the Operon Technologies were used for identifying Alexandria line (Table 1).

Table 1

List of 10 RAPD primers and their sequences employed for rabbits

N	*Primer code*	*Nucleotide sequence (5'-3')*
1	OPA1	CAGGCCCTTC
2	OPA2	TGCCGAGCTG
3	OPA3	AGTCAGCCAC
4	OPA4	AATCGGGCTG
5	OPA5	AGGGGTCTTG
6	OPA6	GGTCCCTGAC
7	OPA7	GAAACGGGTG
8	OPA8	GTGACGTAGG
9	OPA9	GGGTAACGCC
10	OPA10	GTGATCGCAG

Equal amounts of DNA of the individual samples were drawn and mixed together to get a mixed DNA sample. PCR reaction mixture contained 1.5 µL 10X enzyme buffer containing $MgCl_2$, 75 ng genomic DNA, 0.2 µL Taq DNA polymerase (2 units per µL), 2 µL dNTPs (2

mM), 0.5 µL primer (10 pmol) and sdH_2O was added to the mix to reach a total volume of 15.0 µL. Amplification of DNA fragments was carried out in Piko™ thermal cycler (Thermo Scientific). PCR program included three steps. Step 1, was an initial denaturation step at 94°C for 10 minutes. Step 2, was running 34 cycles, each starting with denaturation at 96°C for 30 seconds followed by annealing at 35°C for 30 seconds and lasted by extension at 72°C for 45 seconds. Step 3, was the final extension at 72°C for 5 minutes. Amplification results were separated on 1.5% agarose gel, stained with Ethidium Bromide and visualized under U.V. light.

SSR-PCR analysis. To resultant SSR profiles from rabbit DNA, 10 SSR primer pairs from Biosearch Technologies were tested (Table 2). Microsatellites were selected according to the number of alleles, the allele size and the chromosome localization. PCR reactions were set up in a 15 µL of reaction volume containing 7.5 µL of 2× Thermo Multiplex PCR Master mix, 0.50 µL of 10 µM of each primer pair, 1 µL of DNA elutant (~ 20 ng) and 3.5 µL of DNase-free water. Samples were subjected to a PCR program consisted of an initial denaturation step at 5 minutes initial heat activation of Hot Start Taq DNA polymerase at 95°C, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds and extension at 72°C for 45 seconds with a final extension at 72°C for 5 minutes. Amplification was checked on 1.5% agarose gel.

Table 2

List of 10 SSR primers and their sequences employed for rabbits

N	*Primer code*	*Nucleotide sequence (5'-3')*
1	ADL0112	GGCTTAAGCTGACCCATTAT ATCTCAAATGTAATGCGTGC
2	ADL0268	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT
3	ADL0278	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG
4	MCW0016	ATGGCGCAGAAGGCAAAGCGATAT TGGCTTCTGAAGCAGTTGCTATGG
5	MCW0123	CCACTAGAAAAGAACATCCTC GGCTGATGTAAGAAGGGATGA
6	MCW0165	CAGACATGCATGCCCAGATGA GATCCAGTCCTGCAGGCTGC
7	MCW0183	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC
8	MCW0222	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC
9	MCW0248	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC
10	LEI0234	ATGCATCAGATTGGTATTCAA CGTGGCTGTGAACAAATATG

Scoring and analysis of RAPD and SSR patterns. The resolved DNA bands were documented and processed for data analysis. The amplified products were scored as 1 and 0 for presence and absence of bands (for RAPD) or alleles (for SSR) respectively. PCR bands separated on gels were calculated by analyzing gel profiles with GelAnalyzer software version 2010a.

Results and Discussion. Usage of biotechnology in rabbit's field can subscribe significantly to the development and enforcement of genetic improvement programs (El-Sabrout et al 2014). The study suggests that RAPD and SSR can be successfully utilized for detecting specific molecular genetic markers for rabbit's lines such as Alexandria line. These specific markers (fingerprints) providing an easy and rapid tools for characterization, identification and sustainable use of these lines in breeding programs. Molecular markers were used in the present study to obtain fingerprints for Alexandria

line rabbits. Ten RAPD and SSR primers were used in this study to characterize Alexandria line. Seven out of ten RAPD primers (1, 3, 4, 5, 7, 9 and 10) were employed to assess Alexandria line genetically (Figure 1), while only four out of ten SSR primers (1, 2, 6, and 8) were employed (Figure 3).

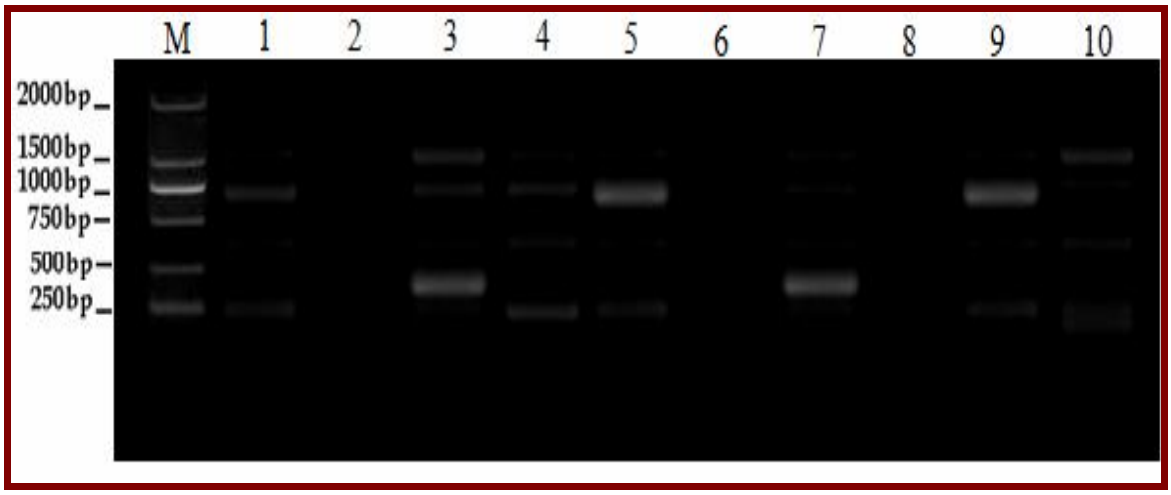

Figure 2. Fingerprint of Alexandria line using 10 RAPD primers.

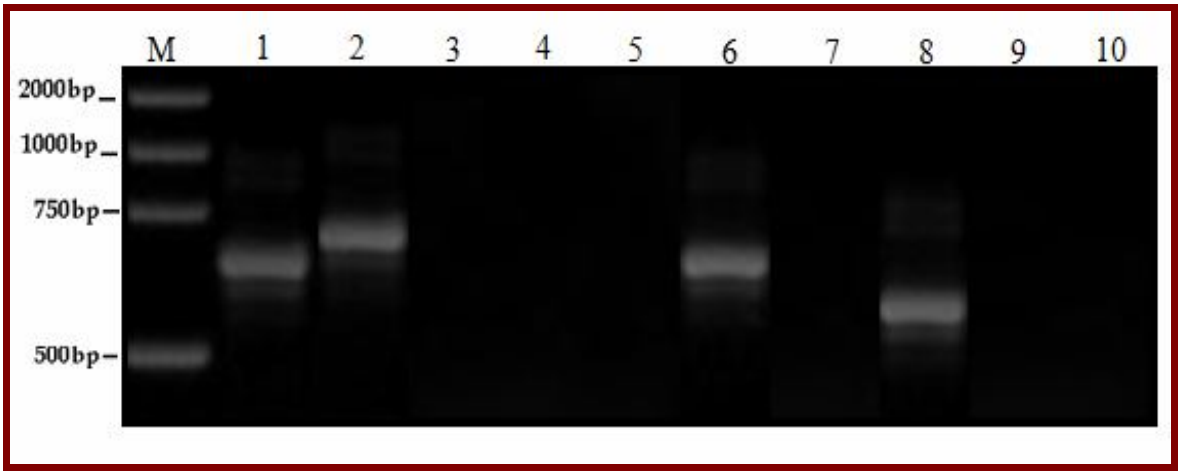

Figure 3. Fingerprint of Alexandria line using 10 SSR primers.

A total of 143 bands were detected. Alexandria non-specific RAPD markers were detected at MW ranged from 400 to 1800 bp, these markers were generated from primers (1, 3, 4, 5, 7, 9 and 10). The maximum number of fragment bands was produced by the primers 3, 4 and 5. While the results demonstrated that Alexandria specific SSR markers were detected at MW ranged from 550 to 950 bp, these markers were generated from primers (1, 2, 6, and 8). The results of present study can provide basic molecular information for future researches. These results indicated efficiency of RAPD and SSR techniques in the characterization of rabbit genotypes.

Conclusions. The use of highly discriminatory methods for the identification and characterization of genotypes is essential for rabbit protection and appropriate use. This study supplies comprehensive approaches for studying the genetically molecular characterization of Alexandria line rabbits which can help to the genetic develop of rabbits and showed that RAPD and SSR techniques are effective methods for detecting specific DNA markers in Alexandria rabbits. These markers are useful for estimating genetic distances and relationships among other rabbit's lines and productive traits.

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