Analysis of Genetic Diversity among Different Egyptian Chicken Strains Using RAPD and SSR Markers

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Abstract: Molecular markers including random amplified polymorphism DNA (RAPD) and simple sequence repeats (SSR) fingerprints represent reliable tools which may have a great impact in chickens breeding programs and genetic improvement. Twenty primers (10 RAPD and 10 SSR) were examined to study the genetic variation among four Egyptian chicken strains (Fayoumi, Alexandria, Matrouh and Golden-Montazah); Eight RAPD primers were screened and yielded distinct polymorphic RAPD profiles at MW ranged from 600 to 1800 bp with a total of 86 of polymorphic band patterns and nine monomorphic band patterns. The primers also detected 2, 4 and 2 unique bands specific for Fayoumi, Alexandria and Golden-Montaza strain respectively. While six SSR primers detected specific markers at MW ranged from 850 to 1750 bp, these markers were generated from primers (2, 3, 7, 8, 9 and 10). A total of 62 alleles were found across 10 loci, with overall mean number of alleles per locus of 6.2. Polymorphism results demonstrate the efficiency of the studied primers to assess the genetic specificity and analysis the genetic diversity among strains. Phylogenetic analysis using Nei and Li method generally produced two clusters which were completely distinguished based on the locality of the chicken strains.
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1. Introduction

Local chicken strains have important genetic attributes like adaptability to local conditions, besides its resistance against some diseases. Local chicken strains in developing countries are still out of competition with commercial ones which benefit from the technology advantages and economic of scale (Hoffmann, 2005). Information about the genetic characterization of these strains and the amount of genetic diversity among them are minimal. Molecular genetics knowledge providing modern tools for chicken breeding and enhanced selection progress with fast and precise identification and selection at gene level with better performance (Fulton, 2008). Development strategies of chicken breeds should be based on molecular genetics identification and characterisation of these breeds that are important to maintain genetic variation for adaptation to local environment and unexpected breeding requirements in future (Romanov *et al*., 1996). Genetic variation found in chicken strains permits breeders to improve new characteristics in response to environmental changes (Ola *et al*., 2013). Molecular analysis of genetic diversity and relatedness among chicken strains are important prerequisite for the recognition of genetic resources that are economically important (Nikkhoo *et al*., 2011).

Hence, the objectives of this study were to genetically characterize four Egyptian chicken strains named (Fayoumi, Alexandria, Matrouh and Golden-Montazah) and to estimate the genetic diversity among them in order to enhance selection and breeding programs by using random amplified polymorphism DNA (RAPD) and simple sequence repeats (SSR) techniques.

2. Materials and methods

The experimental work of the present study was conducted at Poultry Research Center of the Poultry Production Department on four Egyptian chicken strains: Fayoumi (Fay), Alexandria (Alex), Matrouh (Mat) and Golden-Montazah (GM).

Molecular genetics analyses

DNA extraction

Individual blood samples were collected from 40 birds (10 birds/strain), the blood sample (approximately 2 ml) was taken via the brachial vein from each individual of Fayoumi, Alexandria, Matrouh and Golden Montazah chickens, randomly chosen at 11 months of age under vacuum tubes containing EDTAK3 as anticoagulant for molecular genetic analysis, All chickens used were normal, healthy and sexually fertile. 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 and agarose gel electrophoresis.

PCR amplification and electrophoresis analysis

To resultant RAPD profiles from chicken DNA, 10 decamer primers (OPA1, OPA7, OPA8, OPA10,

OPA12, OPA15, OPA16, OPA18, OPA19 and OPA20) obtained from Biosearch Technologies in this study (Table 1). The selection of primers was based on the level of polymorphism detected between the tested samples, the specificity and the reproducibility of amplified products. Equal amounts of individual DNA from samples of studied genotypes were used. PCR program conditions consisted of an initial denaturation step at 94°C for 3 minutes followed by 40 cycles, each one consisted of 94°C for 1 minute, followed by annealing at 34°C for 1 minute and lasted by extension at 72°C for 2 minutes and finished with a final extension cycle at 72°C for 6 minutes. PCR products were separated on 1.5% agarose gel stained with 1 µl of ethidium bromide at 100 V for 60 minutes and visualized under U.V. transilluminator (El-Sabrout *et al*., 2014). PCR reaction mixture contained 75 ng genomic DNA, 1.5 µl 10X enzyme buffer containing MgCl2, 0.2 µl Taq DNA polymerase, 2 µl dNTPs, 0.5 µl primer (10 pmol) and sdH2O was added to the mix to reach a total volume of 15 µl.

Table 1: List of 10 RAPD primers and their sequences employed for chickens.

N	Primer code	Nucleotide sequence (5'-3')
1	**OPA1**	**CAGGCCCTTC**
2	**OPA7**	**GAAACGGGTG**
3	**OPA8**	**GTGACGTAGG**
4	OPA10	GTGATCGCAG
5	**OPA12**	**TCGGCGATAG**
6	**OPA15**	**TTCCGAACCC**
7	**OPA16**	**AGCCAGCGAA**
8	**OPA18**	**AGGTGACCGT**
9	OPA19	CAAACGTCGG
10	**OPA20**	**GTTGCGATCC**

To resultant SSR profiles from chicken DNA, 10 SSR primer pairs (ADL0112, ADL0268, ADL0278, MCW0016, MCW0123, MCW0165, MCW0183, MCW0222, MCW0248 and LEI0234) obtained from Biosearch Technologies were tested (Table 2). These ten microsatellite primers were recommended for the Measurement of Domestic Chickens Diversity and these primers set recommended by the ISAG-FAO Standing Committee for genetic diversity study of the chicken (Tadano and Kataoka, 2014).

Microsatellites were selected according to the number of alleles, the allele size and the chromosome localization. In this way, only those microsatellites with more than four alleles and well distributed along the whole genome were used. 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 (3.0 µl for six loci), 1 µl of DNA elutant (20 ng) and 3.5 µl of RNase-free water. Samples were subjected to a PCR program consisted of an initial denaturation step at 1 minute initial heat activation of Hot Start Taq DNA polymerase at 95°C, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55-60° for 30 seconds and extension at 72°C for 50 seconds with a final extension at 72°C for 3 minutes. Amplification was checked on 1.5% agarose gel.

Table 2: List of 10 SSR primers and their sequences employed for chickens.

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**

Data analysis

The amplified products were scored as 1 and 0 for presence and absence of bands represent across the DNA samples (for RAPD) or alleles (for SSR) respectively. The determined DNA bands on the agarose gel were processed for data analysis using GelAnalyzer software version 2010. Numbers of alleles were calculated with software FSTAT 2.9.3.2 (Goudet, 2002).

Genetic similarity and Dendrogram

The values of similarity were estimated across all possible pair wise comparisons of individuals among strains following the method of Nei and Li (1979). The similarity matrix was subjected to cluster analysis by un-weighted pair group method for arithmetic mean (UPGMA) cluster analysis algorithm and the dendrogram was generated. The dendrogram of genetic distance was constructed using the software PAST version 1.34 (Hammer *et al*., 2001) and was drawn on the basis of shared bands according to nearest neighbor analysis and the generated data were used for calculation of similarity matrix for all primers.

3. Results

Many local chicken strains (non-commercial) are at risk of extinction, mainly due to its low productivity compared to commercial breeds. To avoid this, it is necessary to define strategies for the preservation and

conservation of these strains that exhibit unique characteristics of adaptability to difficult environments and diseases. Therefore, the selection use and optimization of molecular markers are considered to be a fundamental step towards full success in genetic studies. Both RAPD and SSR markers are powerful tools in characterization and estimation of relatedness among genotypes (Al-Jallad *et al*., 2012); these markers were used in the present study to analyse genetic diversity among four chicken genotypes (Fayoumi, Alexandria, Matrouh and Golden-Montazah) and to characterize genetically the selected chicken genotypes under study. Twenty primers (10 RAPD and 10 SSR) were examined; fourteen out of them (8 RAPD and 6 SSR) were amplified and produced DNA bands (Figure 1). Eight RAPD primers were screened and yielded distinct polymorphic RAPD profiles at MW ranged from 600 to 1800 bp with a total of 86 of polymorphic band patterns and nine monomorphic band patterns. The primers also detected 2, 4 and 2 unique bands specific for Fayoumi, Alexandria and Golden-Montaza strain respectively. These markers were generated from primers (1, 2, 3, 5, 6, 7, 8 and 10). The highest number of RAPD markers was scored for Alexandria which was consisted from a crossing system among four breeds including Fayoumi and Plymouth Rocks (Kosba, 1966). The results also indicated that primers 1, 2, 5 and 7 can be used as specific DNA markers which enable to differentiate among the four strains of chickens. While six SSR primers detected specific markers at MW ranged from 850 to 1750 bp, these markers were generated from primers (2, 3, 7, 8, 9 and 10). A total of 62 alleles were found across 10 loci, with overall mean number of alleles per locus of 6.2. A total of sixteen chicken populations specific DNA markers were identified (Table 3). Four of them were Fayoumi strain specific DNA markers at MW (1600, 1550, 1750, 1500 bp). These markers were generated from primers (OPA7, OPA16, LEI0234, MCW0248) respectively. Six DNA markers were Alexandria strain specific markers at MW (1650, 1250, 1550, 1450, 1700, 1500 bp). These markers were obtained from primers (OPA1, OPA7, OPA16, OPA18, ADL0278, MCW0222) respectively. Two DNA markers were Matrouh specific markers at MW (950, 1100 bp) and these markers were obtained from primers (ADL0268, MCW0183) respectively. Four DNA markers were Golden-Montazah specific markers at MW (1800, 1250, 1600, 1000 bp) and these markers were obtained from primers (OPA7, OPA18, ADL0278, MCW0183) respectively.

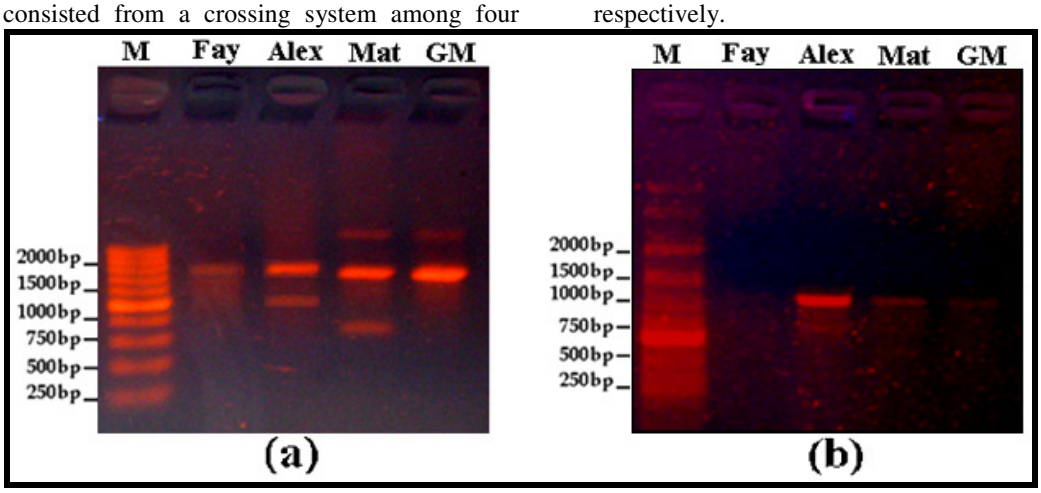

Figure 1: a) Example of RAPD profiles of four Egyptian chicken genotypes: Fayoumi (Fay), Alexandria (Alex), Matrouh (Mat) and Golden-Montazah (GM); **b)** Example of SSR profiles of four Egyptian chicken genotypes: Fayoumi (Fay), Alexandria (Alex), Matrouh (Mat) and Golden-Montazah (GM).

Table 3: Genotype specific markers for four Egyptian chicken genotypes: Fayoumi (Fay), Alexandria (Alex), Matrouh (Mat) and Golden-Montazah (GM) as determined by RAPD and SSR-PCR analysis.

Chicken genotypes	TSM	SM					
		1	2	3	4	5	6
Fay	4	OPA7	OPA16	LEI0234	MCW0248		
Alex	6	OPA1	OPA7	OPA16	OPA18	ADL0278	MCW0222
Mat	2	ADL0268	MCW0183				
GM	4	OPA7	OPA18	ADL0278	MCW0183		

TSM= total specific marker; SM= specific marker

Phylogenetic relationships among four Egyptian chicken genotypes

The values of similarity indices reflect the genetic distance among studied genotypes (Table 4). Phylogenetic relationships and genetic distance were performed to determine the relatedness among different chicken strains. Both RAPD and SSR are based on different strategies for exploring genetic diversity. RAPD primers randomly target complementary but SSR primers amplify the highly specification. The combination of both techniques will enhance the screening of diversity among genotypes. Dendrogram based on the RAPD and SSR data showed separations among studied strains. Phylogenetic analysis using Nei and Li method, generally produced two clusters which were completely distinguished based on RAPD data of the studied chickens strains (Figure 2). Simple matching genetic distances among all studied genotypes were estimated using the software PAST (Hammer *et al*., 2001).

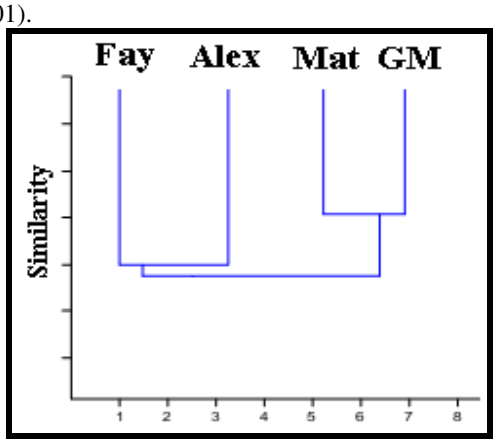

Figure 2: Dendrogram of genetic distance based on RAPD data of four Egyptian chicken genotypes: Fayoumi (Fay), Alexandria (Alex), Matrouh (Mat) and Golden-Montazah (GM).

Table 4: Genetic similarity estimated among four Egyptian chicken genotypes: Fayoumi (Fay), Alexandria (Alex), Matrouh (Mat) and Golden-Montazah (GM).

	Fay	**Alex**	**Mat**	**GM**
Fay	**1**	0.1675	0.1383	0.1310
Alex	0.1675	**1**	0.3813	0.3191
Mat	0.1383	0.3813	**1**	0.3011
GM	0.1310	0.3191	0.3011	**1**

4. Discussion

Molecular markers have been used to distinguish different strains of *Gallus gallus domesticus* (Fulton, 2008). In this study, random amplified polymorphism DNA (RAPD) technique was applied to detect genetic similarity and diversity among four Egyptian chicken strains. The effectiveness of RAPD-PCR in detecting polymor-phism among chicken strains and establishing genetic relationships among them has been reported by Sharma *et al*. (2001). Also, simple sequence repeats (SSR) is the second technique which was applied to characterize and estimate the genetic variability among these four strains by reliable and reproducible results. SSRs are highly polymorphic and abundant molecular markers that are easily typed using PCR (Polymerase Chain Reaction) technique and scored on electrophoresis gels (Rincon *et al.,* 2000). Detection of genetic variation at molecular level is necessary to develop the breeding programs for effective utilization of chicken genetic resources (Mollah *et al*., 2009). From the results of this study, we can found that Alexandria chicken strain has a great genetic variability which can be an important genetic resource for future chicken breeding programs. The Random amplified polymorphism DNA and microsatellites markers used in this study were significantly polymorphic, demonstrating high variation among strains which making them suitable for analysis of population genetic variability, characterization of breeds and assisting in programs of breeding. The microsatellite polymorphisms elucidate a clearer differentiation, even between closely related chicken strains and raise precision of the predicted divergence (Zhang *et al*., 2002 a, b). Moreover, polymorphism results of this study demonstrate the effectiveness of the used primers to estimate the genetic specificity among different genotypes. These genotypes involve the recording of the homozygous or heterozygous state of the animal. Therefore, the possibility to characterize every genotype examined submits an auspicious perspective for varietal identification. Knowledge of genetic diversity among chicken breeds and relationship between their genetic markers and performance play an important role in the conservation of poultry local breeds (Wilkinson *et al*., 2011).

Dendrogram based on the RAPD and SSR data showed a clear separation among studied strains. This might be due to the reason that these Egyptian chicken strains were originated from different structures and bred for different purposes by continuous efforts made over many years to improve these local strains. Knowledge of the genetic distances among different genotypes is very useful tool for the genetic improvement (El-Sabrout *et al*., 2014).

Conclusion

Analysis of genetic diversity among different Egyptian chicken strains is important for better adaptation to environmental changes, understanding phenotypic variability and would be a usable material to conserve chicken genetic resources which can be a

perfect guide for future breeding programs. RAPD and SSR markers permit to quick and precise identification and selection at DNA level of chicken strains. The results of this study can be of great benefit, especially for researchers working on chicken genetics to establish breeding programs for improving of productive performance of local chicken strains.

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