

Full Length Research Paper

Genetic diversity of indigenous chicken ecotypes in Jordan

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DNA polymorphism of 4 indigenous chicken ecotypes was assessed in Jordan using random amplified polymorphic DNA (RAPD) markers. 10 RAPD markers showed high genetic diversity values in the 4 ecotypes located in the northern, eastern, western and southern provinces of Jordan. The effective number of alleles per locus ranged from 1.47 to 1.7 (mean 1.65). The expected heterozygosity varied from 0.28 to 0.41 (mean 0.39) and Shannon's index from 0.42 to 0.60 (mean 0.58). The Western ecotype showed higher levels of effective allele number, expected heterozygosity and Shannon's index than the others. The genetic similarity between the northern, eastern and western ecotypes ranged from 0.95 to 0.97, while it ranged from 0.69 to 0.85 between the southern ecotype and the others. The largest genetic distance was found between the northern and southern ecotypes (0.37), whereas the smallest (0.04) was between the northern and eastern ecotypes, the southern ecotype was found to be the most genetically distant among all ecotypes. The study revealed that RAPD markers were effective in detecting genetic diversity in the chicken ecotypes. These results may prove to be valuable for the future conservation of genetic resources of indigenous chicken ecotypes in Jordan.

Key words: Polymorphism, conservation, allele numbers, genetic distance, RAPD markers.

INTRODUCTION

DNA diversity is continuously suffering erosion in several fields of animal genetic resources. This is especially true for the chicken industry where very few genotypes provide the breeding basis for the industrialized production. Since this type of production is world widely increasing because of its commercial efficiency, indigenous breeds on the other hand can hardly compete. They are excluded from the competition in spite of their occasionally unique values as egg and meat quality, disease resistance and adaptation to local environment. Therefore, there is possibility for extinction. It is recommended to establish the uniqueness of these genetic resources for conservation purposes.

In Jordan, genetic diversity of indigenous chickens has been accumulating since a long time and as a consequence many populations, hybrids and/or strains differences are found (Abdelqader et al., 2008). They were rather described as distinct ecotypes assigned to their geographical areas (Abdelqader et al., 2008). A complete description of each ecotype would entail ascertaining all genes that contribute to any phenotypic trait (Barker et

al., 1993). Different sources of the chicken breeds were assumingly used to produce the indigenous chickens that are currently raised as well adapted ecotypes in all rural places of Jordan (Al-Fataftah, 1987; Alshawabkeh and Tabbaa, 2001, Abdelqader and Wollny, 2007). Therefore, Jordan indigenous chicken breed, resulting from centuries of breeding are now at the risk of being lost as a result of intensive indiscriminate crossing with exotic breeds and lines (Abdelqader et al., 2008) in addition to the current worldwide threat of avian influenza.

Before starting any breeding or conservation program, phenotypic and genetic characterization is a prerequisite (Hammond, 1994). Therefore, the evaluation of indigenous chickens as genetic resources includes records of phenotypes and breeding history as well as determination of genetic polymorphisms. The latter can be achieved by DNA molecular technology that has provided new opportunities to assess genetic polymorphism at the DNA level. Such technology is random amplified polymorphic DNA (RAPD) marker that provides reliable information on genetic diversity, polymorphisms and relationships of po-

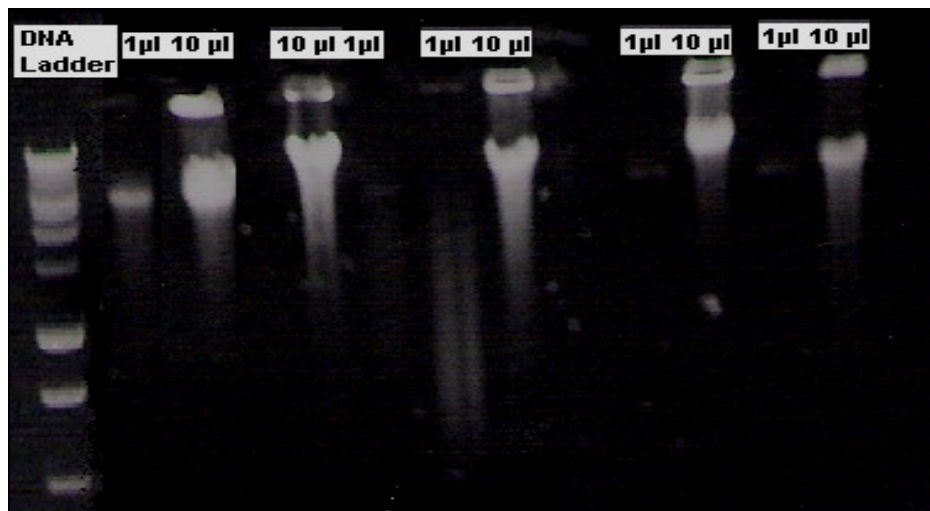


Figure 1. Agarose gel of 1 % loaded with DNA ladder of 1 kb, and DNA samples of 1 μ l and 10 μ l.

pulations of different origins (Williams et al., 1990). The main advantages of the RAPD method lie in its rapidity and applicability to any organism without prior knowledge of the nucleotide sequence. However, the RAPD assay suffers from few drawbacks, particularly the issue of reproducibility and difficulty to determine whether a band is actually present or not. So far the RAPD assay has demonstrated a powerful approach for identifying polymorphism in different animal populations (Cushwa et al., 1996). The effectiveness of RAPD in detecting polymorphism in chicken populations and their applicability in population studies and establishing genetic relationships among chicken populations has been recently reported by many researchers (Sharma et al., 2001; Ali and Ahmed, 2001; Ali et al., 2003). Ali et al. (2003) studied the genetic polymorphisms between some Egyptian chicken strains. The use of RAPD marker technology was recently reviewed in poultry research, especially in some genetic resources of economically important species such as chickens, quails, ducks, goose, turkey and other birds (Salem et al., 2005).

In Jordan, a developing country in Asia, there is no scientific study so far on the state of the genetic polymorphisms of indigenous chicken breeds. Therefore, the present study was conducted to assess genetic diversity among indigenous chickens of Jordan.

MATERIALS AND METHODS

Indigenous chicken population

100 indigenous chickens were collected from northern, eastern, western and southern provinces of Jordan. To avoid bias, 25 non-related indigenous chickens were collected from each area in order to cover a wide range of indigenous chicken ecotypes. Where relevant throughout this study, indigenous chicken population will be referred to as indigenous chicken ecotype of each geographical

area.

Blood and tissue samples collection

Samples were collected in 2 forms, blood and tissue. The blood sample was collected from the wing vein and transferred into vacutainer tube. This method was used to sample all young chickens, while tissue sampling was applied to the chicken of old ages by taking a punch of 0.5 cm of Wattle tissue using an animal punch applicator and then transferred into Eppendorf tube. Blood and tissue samples were immediately stored at a recommended temperature till the DNA has been extracted. All work for this research using animals was performed with the permission of and in accordance with the guidelines set by Animal Ethics Committee of Mutah University.

DNA extraction

A commercial kit, Wizard[®] Genomic DNA purification kit-Promega[®], was used as a simple and convenient technique to isolate high quality genomic DNA from blood and tissue samples (Technical Manual, 2007). The recommended protocols in the technical manual were used for isolation genomic DNA from 10 ml blood volume and 0.5 cm animal tissues. After extraction step, precipitation of DNA pellet was dried for thirty minutes in a 37°C incubator, resuspended in 100 μ l TE buffer and then incubated at 65°C for 5 min to aid solubilisation. Finally, the DNA sample was stored at 4°C (Sambrook et al., 1989).

DNA quantification

Agarose gel electrophoresis of 1% was used to quantify DNA and check the integrity of genomic DNA. Figure 1 is an example of quantifying DNA by using 1% agarose gel for some DNA samples. All DNA samples were also quantified by spectrophotometer. The measurements were taken at λ 260 nm and λ 280 nm. The purity of DNA samples was ranged from 1.2 up to 1.9. Samples were then diluted to 10 ng/ μ l for use in subsequent PCR (PTC-200 programmable Thermal Controller, MJ Research Inc.) reactions.

Table 1. Operon codes and DNA sequences and fragment size of the ten Random Amplified Polymorphic DNA primers used in polymorphic analysis of Jordan Indigenous chicken ecotypes.

Primer	Sequence (5'-3')	Fragment size (bp)
OPZ-11	CTCAGTCGCA	250-2000
OPA-05	AGGGGTCTTG	500-4000
OPN-16	AAGCGACCTG	500-1500
OPF-14	TGCTGCAGGT	250-1000
OPC13	AAGCCTCGTC	1000-2500
OPT-07	GGCAGGCTGT	250-850
OPR-04	CCCGTACGCAC	500-1500
OPR-09	TGAGCACGAG	250-750
OPR-14	CAGGATCCC	500-2000
OPR-20	ACGGCAAGGA	200-750

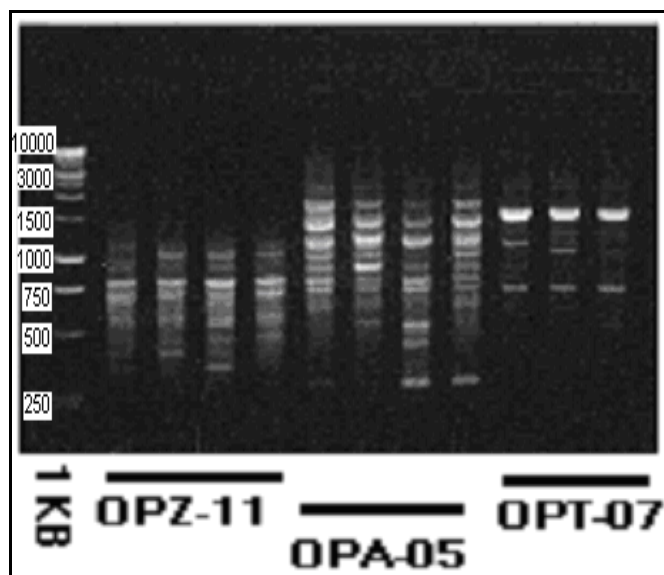


Figure 2. Agarose gel of 1% loaded with DNA ladder of 1 kb and three RAPD primers (OPZ-11, OPA-05 and OPT-07, consequently).

RAPD markers genotyping

10 RAPD primers, shown in Table 1, have been selected from operon technologies company-USA for their ability to be useful in studies of biodiversities, taxonomic identities and systematic relationships. At the beginning, 20 RAPD primers of genotyping process were screened to find high polymorphic primers and utilized further for this project. Only 10 highly polymorphic identified RAPD primers were used in PCR reaction and further study (Table 1). In general, PCR reaction of 20 μ l volume conducted under the following conditions, 10 ng template DNA, 250 nM of each primer, 200 nM dNTPs, 1 U Taq polymerase and 1.5 mM $MgCl_2$. The reaction was performed for each type of primer pair following the programs recommended in protocols of Sambrook et al. (1989). As an example, allele information collected from genotyping of some RAPD markers are shown in Figure 2. All gel photographs were scored for the presence or absence of RAPD bands.

Population genetic parameters

Population genetic parameters of the studied chicken ecotypes were investigated using genotypic data of RAPD by utilizing POPGENE[®] software (Yeh and Boyle, 1996). Using the calculated allele frequencies for RAPD markers in each chicken ecotype, POPGENE calculated effective number of alleles (A_e) (Kimura and Crow, 1964), expected heterozygosity (H_e) (Nei, 1973), Shannon's Information index (I) or gene diversity (Shannon and Weaver, 1949), and measures of genetic identity and genetic distance. This program also constructed UPGMA dendrogram that showed the genetic distance among ecotypes constructed according to Nei (1978).

RESULTS

The data of RAPD markers show the A_e , H_e and I in the indigenous chicken ecotypes for each studied locus (Table 2). The mean A_e locus was the lowest for the southern chicken ecotype (1.47), when compared with the other populations 1.54, 1.64 and 1.7 for eastern, northern and western chicken ecotypes, respectively. On the other hand, OPZ-11 marker showed the highest value (1.86) of A_e for all chicken ecotypes. Despite the OPZ-11 showed low A_e (1.47) in the northern chicken ecotypes, it showed relatively very high A_e for the southern, eastern and western chicken ecotypes (ranged from 1.86 to 2.00). Therefore, the OPZ-11 can be considered more effective in assessing chicken genetic polymorphism in the present populations than other studied RAPD markers. This can be also concluded from its highest H_e and I across all chicken ecotypes over the other markers (Table 2). In fact, the 10 studied RAPD markers showed very large number of bands within wide range of molecular size from 200 to 4000 bp across all studied chicken ecotypes (Table 1).

Large variation range of H_e and I was detected for all chicken ecotypes at different loci, from 0.28 to 0.41 (mean = 0.39) and from 0.42 to 0.60 (mean = 0.58), respectively (Table 2). The lowest H_e and I values were found in the southern chicken ecotype (H_e of 0.28 and I of 0.42) and the greatest in the Western ecotypes (H_e of 0.41 and I of 0.60). The results also indicated that similar genetic diversity values were shown for the northern (H_e = 0.36, I = 0.53) and eastern (H_e = 0.33 and I = 0.51) ecotypes. On the other hand, there were large differences in the level of genetic diversity for the other 2 ecotypes; being the western ecotype of highest and the south ecotypes of lowest. Particularly, the western chicken ecotype showed higher levels of A_e , H_e and I than the other ecotypes. These may be partly due to that the western ecotype carried the highest genetic variation between its individuals indicating higher level of genetic polymorphism, whereas the southern ecotype showed the least genetic diversity or higher similarity among its individuals indicating lower genetic polymorphism. It can be observed that the ten RAPD markers showed an average H_e of 0.41, which is considered very high, ranging from 0.32 (OPC-13) to 0.46 (OPZ-11) for all indigenous chicken ecotypes.

Table 2. The effective number of alleles, expected heterozygosity and Shannon's index in the Indigenous chickens ecotypes using RAPD markers.

Primer	Northern ecotype			Southern ecotype			Eastern ecotype			Western ecotype			All ecotypes		
	A _e	H _e	I	A _e	H _e	I	A _e	H _e	I	A _e	H _e	I	A _e	H _e	I
OPZ-11	1.47	0.32	0.50	2.00	0.50	0.69	1.92	0.48	0.67	1.81	0.45	0.64	1.84	0.46	0.65
OPA-05	1.92	0.48	0.67	1.04	0.04	0.10	1.92	0.48	0.67	1.74	0.43	0.62	1.72	0.42	0.61
OPN-16	1.47	0.32	0.50	1.47	0.32	0.50	1.18	0.15	0.29	1.74	0.43	0.62	1.71	0.41	0.60
OPF-14	2.00	0.50	0.69	1.09	0.08	0.17	1.54	0.35	0.53	1.87	0.47	0.66	1.64	0.39	0.58
OPC-13	1.23	0.19	0.34	1.67	0.40	0.59	1.35	0.26	0.43	1.60	0.38	0.56	1.45	0.31	0.49
OPT-07	1.97	0.49	0.69	1.09	0.08	0.17	1.67	0.40	0.59	1.67	0.40	0.59	1.59	0.37	0.56
OPR-04	1.29	0.22	0.38	1.83	0.45	0.65	1.29	0.22	0.38	1.60	0.38	0.56	1.62	0.38	0.57
OPR-09	1.99	0.50	0.69	1.04	0.04	0.10	1.81	0.45	0.64	1.54	0.35	0.53	1.59	0.37	0.56
OPR-14	1.09	0.08	0.17	1.68	0.41	0.60	1.35	0.26	0.43	1.47	0.32	0.50	1.55	0.36	0.54
OPR-20	1.92	0.48	0.67	1.81	0.45	0.64	1.35	0.26	0.43	1.97	0.49	0.69	1.77	0.44	0.63
Mean	1.64	0.36	0.53	1.47	0.28	0.42	1.54	0.33	0.51	1.70	0.41	0.60	1.65	0.39	0.58

A_e = Effective number of alleles, H_e = Expected heterozygosity, I = Shannon's Information index or gene diversity.

Table 3. Genetic Identity* and distances of the 4 indigenous chicken ecotypes.

Ecotypes ID	Northern	Southern	Eastern	Western
Northern	0	0.69	0.96	0.95
Southern	0.37	0	0.78	0.85
Eastern	0.04	0.24	0	0.97
Western	0.05	0.16	0.03	0

*Genetic Identities are above the diagonal and genetic distances are below the diagonal.

The same was observed for the I with the lowest (0.49) found for OPC-13 and the greatest for (0.65) for OPZ-11 (Table 2). The most notable result, regarding interecotype comparisons, was that the highest A_e, H_e and I found at 5 RAPD markers (OPA-05, OPN-16, OPF-14, OPT-07 and OPR-09) in northern chicken ecotype. In contrast, the same markers detected the lowest values in the southern ecotype.

The inter-ecotype similarity indices were expressed as genetic identity and shown in Table 3. The largest value of genetic identity (0.97) was between the eastern and the western ecotypes followed by 0.96 between the northern and the eastern ecotypes and 0.95 between the northern and the western ecotypes. The lowest value of genetic identity (0.69) was between the northern and the southern ecotypes. Table 3 also shows the values of genetic distance between the studied ecotypes that ranged from 0.04 between the eastern and the northern to 0.37 between the southern and the northern. The UPGMA dendrogram, based on the genetic distances, constructed the genetic relationship among the studied indigenous chicken ecotypes (Figure 3). The dendrogram grouped the ecotypes genotypes into 2 main clusters. The first cluster was divided into 2 sub-clusters; the first included the

eastern and the western ecotypes, while the second sub-cluster included only the northern ecotypes. The second cluster included the only the southern

DISCUSSION

The A_e, H_e, and I values were calculated to estimate the level of genetic polymorphisms in indigenous chicken ecotypes. The results were indicating that there were high genetic polymorphism in the studied indigenous chickens. Previous reports, using RAPD markers, indicated high gene diversity within Chinese, Russian, European, Indian, Egyptian and Asian indigenous chicken populations (Zhang et al., 2002; Semenova et al., 2002; Sharma et al., 2001; Ahlawat et al., 2004; El-Gendy et al., 2006). Heterozygosity estimates in those studies ranged from 0.02 to 0.48. Gene polymorphism was also detected in these studies within a range from 0.32 to 0.65. In general, the gene diversity and polymorphism estimates found in the present study were within the reported range in the previous reports. Furthermore the range of molecular size of detectable RAPD bands (from 200 to 4000 bp) might be in agreement with the range (200 to 2000

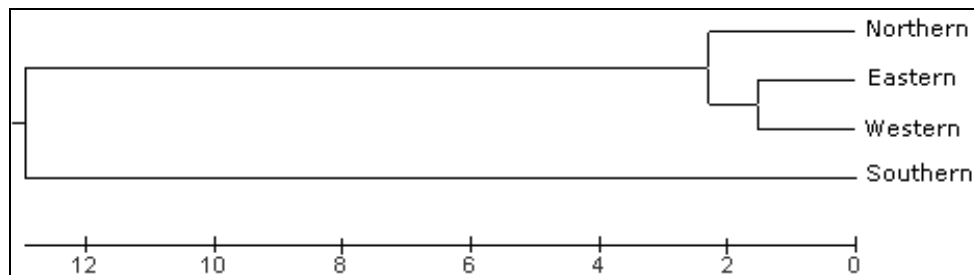


Figure 3. Dendrogram of genetic relationships among the four Jordan indigenous chicken ecotypes.

bp) found by Ahlawat et al. (2004) and (128 to 5467 bp) by Chatterjee et al. (2007).

The high H_e and I for the northern, eastern and western ecotypes were expected because their individuals expressed high genetic variation at the most studied loci (Table 2). On the other hand, lowest genetic diversity estimates, H_e and I , among individuals of southern ecotype was also expected because the 5 studied RAPD markers (OPA-05, OPN-16, OPF-14, OPT-07 and OPR-09) were limited in detecting polymorphisms (Table 2). In other words, southern ecotype showed the least genetic diversity or higher similarity among its individuals indicating lower genetic diversity, whereas western ecotype showed the highest genetic variation among its individuals indicating higher level of polymorphism. The greater polymorphisms in the western chicken ecotypes may be due to larger population size, random mating and a wider distribution of geographical areas.

The inter-ecotype similarity found between the pairwise comparisons ranged from 0.67 to 0.97 (Table 3). This is higher than that reported by Ahlawat et al. (2004), who reported the genetic identity between Indian chicken strains ranged from 0.77 to 0.87. The high identity values (0.96 and 0.97 between the eastern ecotypes and each of the northern and western ecotypes can be supposedly explained by their common ancestors, past gene flow and similar geographical and environmental conditions. However, the lower identity values (0.69 - 0.85) between the southern ecotypes and the others may partly due to very long time divergence, absence of gene flow and dissimilar of geographical conditions. On the other hand, the eastern, northern and western ecotypes showed the least genetic distances between each other, while the southern ecotypes appeared most distant from them (Table 3). The genetic closeness between the 3 ecotypes clustered them into one cluster, while the south ecotype was further away in the phylogenetic tree (Figure 3). The first cluster included a sub-cluster of the eastern and western chickens that were raised in similar conditions of moderate temperature in winter and mixed production system of flat areas. The northern chickens of second sub-cluster were raised in mountains where cold temperature in winter and under semi-intensive production system (Abdelqader et al., 2008). The southern ecotype was raised in the south-

ern parts of Jordan under scavenging production system of subtropical conditions. This ecotype is more isolated from other parts of the country because it has fewer cities and villages and far distant from other ecotypes.

The results in this study have confirmed the effectiveness of RAPD markers in assessing polymorphisms of indigenous chickens with the current results showing what have been reported previously by Sharma et al. (2001) and Salem et al. 2005 regarding effectiveness of RAPD markers in detecting polymorphism and in the establishment of genetic relationships between chicken populations. The results of present study may prove valuable for the future conservation of genetic resources of indigenous chicken breeds in Jordan. For more general view, Siegel et al. (1992) studied the genetic diversity among wild jungle fowl and commercial chickens, using RAPD fingerprinting technique, and provided information to assess the genetic variation and propose conservation plan. Hence, the importance of the studying genetic polymorphism of indigenous chickens as a genetic resource could support global programs to determine genetic distances among chicken populations and establish core collections of diversity within each species.

Conclusion

Genetic polymorphism of indigenous chickens was relatively high with high values of A_e , H_e and I at each studied locus. Furthermore, the genetic similarity between the pairwise comparison of northern, eastern and western ecotypes was very high. On the other hand, the largest genetic distance was found between the northern and southern ecotypes and the smallest between the northern and eastern ecotypes. As a consequence, the southern ecotype was found to be the most genetically distant among all studied ecotypes. These results may prove to be valuable for the future conservation of genetic resources of indigenous chicken breeds in Jordan. For more general view, the importance of indigenous population as genetic resources is presence of genes for better adaptive and disease resistance traits of worldwide commercial interest through out long-term genetic improvement. Hence, support global programs to assess

genetic polymorphisms between indigenous chicken breeds and to establish core collections of diversity within species. To sum up, the study revealed that RAPD markers were effective in detecting polymorphism in the chicken ecotypes of Jordan.

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